Abstract

The aim of this study was to use micro-computed tomography (CT) analysis to evaluate the efficiency of the XP-endo finisher (XPF) and passive ultrasonic irrigation (PUI), alone or in combination, in reducing accumulated hard tissue debris (AHTD) in the mesial canals of mandibular molars. The teeth were micro-CT scanned preoperatively (S1), after instrumentation (S2), after final irrigation (S3), and after a supplementary activation (S4). The mesial roots were prepared with BT Race sequence to apical size 35/.04 and divided in two groups according to the final irrigation method and the sequential supplementary activation (PUI + XPF group, n = 12; XPF + PUI group, n = 12). The data of percentages of reduction of AHTD after the first irrigation and supplementary activation were statistically analyzed (RM ANOVA). In the apical third, XPF reduced the percentage of remaining AHTD (66.49%) more significantly than the PUI (35.27%) (P < 0.05). The supplementary activation promoted a statistically significant reduction of AHTD in the apical-thirds of both groups (P < 0.05). In the XPF group the middle (90.85%) and apical (80.02%) thirds were affected by the supplementary activation with ultrasonic irrigation (P < 0.05). Both PUI and XPF were efficient in the reduction of AHTD, but in the apical third XPF was more effective. The supplementary activation with both systems reduced the AHTD significantly. The order of application of irrigation systems influences the reduction of accumulated AHTD and the use of XPF followed by PUI proved to be more efficient.

INTRODUCTION

The primary goal of endodontic therapy is the reduction or elimination of microorganisms and their by-products from the root canal system. To achieve this goal, chemo-mechanical procedures should be performed in order to clean, disinfect, and eliminate bacteria and any source of bacterial nutrients. If these objectives are not achieved, the outcome of the endodontic treatment will be compromised. One of the factors that can affect mechanical preparation is the complex anatomy of the root canal system. Despite the current advances in the development of nickel-titanium (NiTi) endodontic instruments, no system presents the capacity to completely prepare the root canal walls and eliminate pulp tissue and intra-radicular biofilm. Therefore, the instrumentation techniques may lead to accumulation of smear layer in the root canal system. The removal of accumulated hard tissue debris (AHTD) should enhance canal disinfection. Thus various techniques involving the activation of irrigants such as sonic, ultrasonic, or laser technologies have been proposed and have shown better results as compared to conventional irrigation techniques.

Passive Ultrasonic Irrigation (PUI) is one of the most widely used supplementary methods aiming to improve the extent of the effect of irrigation solutions, achieving deeper cleaning and disinfection in the complex root canal system. During PUI, the ultrasonic insert should oscillate freely without contacting the canal walls,
limiting its action to the straight part of the canal.\textsuperscript{10,11} The cavitation and acoustic flow generated by PUI are limited up to 3 mm from the tip of the insert.\textsuperscript{12} The contact of the insert with the canal walls can generate uncontrolled wear of radicular dentin even with the use of smooth and blunt ultrasonic tips.\textsuperscript{13,14}

The XP-Endo Finisher file (XPF; FKG Dentaire, La Chaux-de-Fonds, Switzerland) introduced a new concept in NiTi alloys. Its exclusive MaxWire\textsuperscript{®} NiTi alloy technology (Martensite-Austenite electropolish-fleX) and the flexible small core (#25/0.00) enable expansion inside the root canal. This expansion allows the file to contact oval areas and clean deep areas of the dentinal walls, while maintaining the original shape of the internal root canal anatomy.\textsuperscript{15} The mechanical action of the XPF file combined with the irrigants agitation seems to provide greater bacterial reduction,\textsuperscript{7} better biofilm removal in the main canal and dentinal tubules,\textsuperscript{16} and lower the levels of AHTD.\textsuperscript{17,18}

Although cavitation and acoustic flow generated by PUI improve disinfection and cleaning,\textsuperscript{19} their action is limited up to 3 mm from the tip of the insert.\textsuperscript{12} On the other hand the mechanical action of XPF enables contacting the irregular areas throughout the working length of the root canal. Thus, the combination of PUI and the XPF could enhance the removal of AHTD. Nonetheless, there is still limited information about the efficacy of XPF files and no study has evaluated the supplementary irrigation of PUI and XPF combined.

Therefore, this study aimed to evaluate, by means of micro-computed tomography (micro-CT), the efficiency of the XPF files and PUI (either alone or in combination) in the reduction of AHTD in the root canal system. The null hypothesis was that the PUI and XPF irrigation or their combination has no influence on the reduction of AHTD in the mesial root canals of mandibular molars.

**MATERIAL AND METHODS**

**Sample selection and specimen preparation**

The present study was approved by the Research Ethics Committee (protocol no. 15598). Sample calculation was performed using the OpenEpi software-specific calculator (http://www.openepi.com/Menu/OE_Menu.htm) based on the pilot study data. The results of the sample calculation showed that 10 cases per group were adequate to demonstrate a difference of 5\% (\(P < 0.05\)) with a power of 0.8. A total of 24 extracted human mandibular molars were selected from the Tooth Bank at the University of Sao Paulo, Brazil. All teeth were preoperatively scanned (S1) using a micro-CT scanner (SkyScan 1176; Bruker microCT, Kontich, Belgium) to confirm the internal root canal anatomy. The selected teeth presented type-II configuration according to Vertucci’s classification\textsuperscript{20} and type-III configuration according to Fan’s isthmus classification,\textsuperscript{21} and had intact pulp chambers, mesial roots with a fully formed apex, and a curvature of 25-35\degree according to Schneider.\textsuperscript{22}

The teeth were transversely sectioned using a precision sectioning cutter (Isomet 1000; Buehler, Lake Bluff, IL) to obtain a specimen of size 17 mm. Coronal access was performed with spherical diamond burs #1014 (KG Sorensen, Sao Paulo, Brazil) and Endo Z burs (KG Sorensen) at high-speed rotation under refrigeration. Mesial canals were explored by using #10 K-files (Dentsply Sirona, Ballaigues, Switzerland), and working length was established 1 mm short of the point where the tip of the instrument was visible at the apical foramen. The apical position of the file was verified with the aid of an operating microscope at a magnification setting of 8X. All procedures were conducted by the same endodontist (E.F.I.).

**Endodontic preparation**

After establishing the glide path with a #15 K-file (Dentsply Sirona), both mesial canals were prepared with BT Race (FKG Dentaire) NiTi sequence to apical size 35/.04 (10/.06, 35/.00, 35/.04). The BT NiTi files were used at 600 rpm adjusted to 1 N torque with the help of a rotary torque control motor (VDW Silver motor, VDW GmbH, Munich, Germany). Each root was instrumented using a new file.

The canals were rinsed with 2 mL of 2.5\% sodium hypochlorite (NaOCl) solution before insertion of each file, delivered by a 30-gauge NaviTip needle (Ultradent Products Inc., South Jordan, USA) and positioned as apically as possible without binding to the walls of the canal, up to the 3 mm short the apical foramen. A
total of 6 mL of NaOCl per canal was used (12 mL per tooth). The apical patency was maintained throughout the procedure using #10 K-file (Dentsply Sirona). After preparation, the canals were irrigated with 2 mL of 17% EDTA followed by 2 mL of 2.5% NaOCl, after which the roots were submitted for the second micro-CT scanning (S2).

**Experimental groups**

The specimens were pair-matched on the basis of micro-CT analysis of the morphologic and anatomic aspects of the mesial root canal, in order to distribute the teeth in a more equal manner into the two experimental groups.

Condensation silicone was placed in each well of a culture plate and the teeth were placed in this material to simulate the periodontal ligament. This set was kept warm during the procedures, using 37 °C water to simulate body temperature. The culture plate was kept in a container that was supported by an electric water heating base, responsible for maintaining the temperature. A thermometer was fitted to allow temperature control throughout the experiment.

In order to evaluate the possible benefits of the combination of these two techniques, the groups were submitted to the final irrigation method and the sequential supplementary activation (PUI + XPF group, n = 12; XPF + PUI group, n = 12), according to figure 1.

**PUI + XPF group**: The teeth previously irrigated with the aid of ultrasonic activation were then submitted to XPF irrigation.

**XPF + PUI group**: The teeth previously irrigated with the aid of XPF irrigation were then submitted to ultrasonic activated irrigation.

**PUI protocol**: Each canal was irrigated with 2 mL of 2.5% NaOCl pre-heated to 37 °C for 30 seconds and activated for 30 seconds using a #20.01 ultrasonic tip (Irrisonic, Helse Dental Technology, Santa Rosa de Viterbo, Brazil) coupled to an ultrasound device (Suprasson P5, Satelec Acteon Group, Merignac, France). The power level used for Irrisonic was 10%, according to manufacturer’s recommendations. The ultrasonic tip was used within 2 mm of the working length using in-and-out movements up to the most apical extent where it could vibrate. The same procedure was repeated with 2 mL of 17% EDTA for 30 seconds, also pre-heated to 37°C, and activated for 30 seconds using an ultrasonic tip, followed by irrigation with 10 mL of 2.5% NaOCl for 60 seconds.

**XPF protocol**: All canals were irrigated with 2 mL of 2.5% NaOCl pre-heated to37°C for 30 seconds. The XPF was inserted and rotated for 30 seconds per canal coupled to the VDW Silver motor (VDW GmbH). The speed was adjusted for 1000 rpm and an up-and-down movement within approximately 6-7 mm of the full length of each canal. The same protocol was repeated with 2 mL of 17% EDTA for 30 seconds, also pre-heated to37°C and activated for 30 seconds using XPF. The canals were then irrigated with 10 mL of 2.5% NaOCl for 60 seconds.

After, the specimens were scanned following final irrigation (S3), and after a supplementary activation(S4).

**Micro-CT imaging**

In all scans (S1 to S4), the teeth were positioned in a sample holder and brought to the carbon fiber bed of the micro-CT scanner. Specimens were scanned at 90 kV, 278 μA, 360° rotation, and a 0.5° rotation step, resulting in a voxel size of 17.42 μm. The filter used was made of copper and aluminum (Cu 0.04 mm + Al 0.05mm).

Images were reconstructed with a modified Feldkamp cone-beam reconstruction algorithm using the NRecon software version 1.6.6.0 (Bruker microCT), which yielded 800-900 slices per specimen. Reconstruction parameters were adjusted for noise suppression using the fine-tuning function as follows: Gaussian smoothing filter (kernel = 2), beam hardening correction of 40%, post-alignment of 0.50 to compensate possible misalignment during acquisition, and ring artifact correction of 10.
Image analysis

The resulting images from the four scans were geometrically aligned by using the three-dimensional (3-D) registration function of the DataViewer version 1.5.1 software (Bruker microCT). The CTAn (version 1.14.4) and CTVol (version 2.2.1.0) software packages (Bruker microCT) were used to calculate quantitative parameters to create and visualize 3-D models.

The volume of interest for each specimen was determined from the furcation region to the apex of the mesial root. The gray scale range required to recognize the dentin was determined in a density histogram using a global threshold method. Comparisons between the original and segmented scan were performed to ensure accuracy of the segmentation. As dentin mineralization may not be constant for all specimens, the segmentation values ranged from 85 - 105. The arithmetic and logical operations between the superimposed cross-sectional images were then used to identify the AHTD. Any material with a density similar to that of dentin, in regions previously occupied by air in the preoperative canal space, was identified as AHTD on the post preparation images and was quantified by the overlap between the images obtained before and after canal preparation, according to Freire et al. 8

The volume of AHTD that remained after final irrigation with XPF or PUI and after supplementary activation with XPF or PUI was calculated. The percentage AHTD reduction after final irrigation (Rd1) was calculated considering the volume of AHTD after preparation (A) and after final irrigation (B) according to the following formula: \( \%Rd1 = \frac{(B * 100)}{A} - 100 \). The percentage AHTD reduction after supplementary activation (Rd2) was calculated considering the volume of AHTD after preparation (A) and after the supplementary activation (C) according to the following formula: \( \%Rd2 = \frac{(C * 100)}{A} - 100 \).

All analyses were conducted separately for the cervical, middle, and apical thirds of the canal.

Statistical analysis

Statistical analyses were performed using the GraphPad Prism 7 software (GraphPad Inc, La Jolla, CA). The data showed normal distribution and homogeneous variance; therefore, the mean percentage of AHTD reduction obtained was analyzed using the two-way repeated measures analysis of variance (RM ANOVA). All analyses were performed with a significance level of 5%.

RESULTS

There was no statistical difference between both groups with respect to the canal volumes after preparation with the BT Race system and the volume of AHTD produced by these instruments (\( P > 0.05 \)), thus confirming the homogeneity among specimens (Table 1).

After the first irrigation step, activation of the irrigant with PUI or XPF reduced the percentage of AHTD volume within the root canal system in a similar manner (Reduction 1 - PUI: 50, 53%, XPF: 65,41%; \( P > 0.05 \)). Analyzing only the apical third where the AHTD was greater in both groups, was observed that the XPF reduced 66.49% of the AHTD against 35.27% of reduction after the protocol with PUI (\( P < 0.05 \)).

The second activation promoted a statistically significant reduction of AHTD in all thirds of root canals of both groups (Reduction 2), when compared to the first irrigation approach (\( P < 0.05 \)). Considering the whole canal, both groups, regardless of which activation was performed first, presented considerable mean percentage of reductions: 75.65% (PUI + XPF group) and 88.31% (XPF + PUI group) (\( P > 0.05 \)). In the XPF + PUI group, the remaining amount of AHTD was significantly lower as compared to the PUI + PUI group in the middle and apical thirds (\( P < 0.05 \)). In the cervical third, the supplementary activation had no influence in the remaining amount of AHTD (\( P > 0.05 \)).

The 3-D reconstructions are showed in Figure 2.

DISCUSSION

This study aimed to compare the effectiveness of a well-established method of irrigant activation, the PUI, with the new XPF rotary file, and the combination of both. Several studies have assessed the action of
PUI and XPF in the removal of AHTD or bacterial reduction, and in biofilm removal. Although a protocol with abundant irrigation/aspiration after XPF use could be efficient enough to remove AHTD from the root canal system, no study as yet has evaluated the combination of PUI and XPF as a supplementary irrigation protocol.

The supplementary activation with PUI or XPF, irrespective of the performance order, was significantly more effective in reducing AHTD than by the final irrigation approach alone. Therefore, the null hypothesis was rejected. Both groups (PUI + XPF group and XPF + PUI group) presented mean values of percentage reductions more than 75%, showing that the final irrigation step combined with a supplementary irrigation method influences the final cleaning of the root canal system. These results are in accordance with the previous study by Leoniet al., who found similar percentage of AHTD volume reduction after activation of the irrigant with PUI or XPF.

The assessments of root canal cleanliness should be preferably accomplished through longitudinal evaluation, and micro-CT permits this analysis with high reliability, according to previous researches. This study along with others that used 3-D reconstructions to compare different irrigation protocols, showed greater accumulation of AHTD in retentive anatomic areas, highlighting the difficulty of effectively cleaning the entire root canal system. The all selected teeth presented type-II configuration according to Vertucci’s classification and type-III configuration according to Fan’s isthmus classification. These double classification was used because teeth type-II, according to Vertucci, may present different isthmus configurations.

Considering only the apical third of the roots, the XPF removed significantly more AHTD than PUI (66.49% versus 35.27%). These results were probably because of the new XPF instrument that was designed to touch the root canal walls, as long as it was used after a root canal preparation of minimum apical diameter ISO 25. In the present study, the apical size preparation was established using BT-3 (35.04), which seems improve the irrigation efficiency and the activation of the solutions inside the root canal, according to previous researches. Thus, XPF promotes mechanical cleaning without changing the original shape of the canal. These features are attributed to the metallurgy of the instrument, which is based on the shape-memory principles of the NiTi alloy. The file is straight in its martensitic phase but at temperatures equal to or greater than 37°C (body temperature) it shifts from the martensitic to the austenitic phase, which in the rotation mode expands up to 6 mm in diameter giving the instrument a semi-circular shape. This allows it to project against the walls of the root canal. Thus, in vitro studies with XPF require heating of the irrigation solutions, simulating body temperature. For that reason, the irrigation solutions in both groups were heated to 37°C for standardization. Moreover, well plates were used in a water bath at 37°C where the teeth were maintained during the experiment to ensure that the temperature remained stable up until the end. Although several studies use no heated solutions with XPF, some authors pointed out the importance of this step for the correct evaluation of this instrument because temperature directly influences the austenitic phase.

On the other hand, ultrasonic devices should not touch the root canal walls when used for final irrigation. In curved canals, the physical effects of acoustic streaming and cavitation of the irrigant solution might be attenuated, because their free vibratory movement is restricted and possibly also their cleaning efficacy. Additionally, Retsas et al. recently demonstrated that PUI could result in uncontrolled dentin removal of curved canals, and consequently in a more extensive dampening of the ultrasonic oscillation.

The final irrigation with PUI or XPF significantly reduced the AHTD resulting from root canal preparation. Nevertheless, the results demonstrated that the order of application influenced the final cleaning of the root canal system. When XPF was used first and then supplemented with PUI, significantly more AHTD were removed from the middle and apical thirds. This was probably because the mechanical action of XP-Endo Finisher files may displace the AHTD from areas that were difficult to reach. Some researchers also compared these two supplementary procedures with respect to their disinfecting ability. Alves et al. concluded that XPF were effective to reduce the bacterial counts in the main canal, while Azimet et al. reported that XPF was efficient to disinfect the main canal space and space up to 50 mm deep into the dentinal tubules. Furthermore, the results of this study show that the action of PUI after XPF reduced
significantly AHTD, which may be explained by the acoustic flow and cavitation transmitted by the ultrasonic tip responsible for the final cleaning and removal of the AHTD that remained in suspension inside the root canal system.

In the present work, although the area of the isthmus has not been analyzed separately, it can be seen in 3D reconstructions that the final irrigation sequence XPF + PUI promoted greater cleaning of this region. Further works may evaluate the action of this final irrigation sequence in this area.

AHTD may also contain bacteria and their by-products\(^\text{31}\) that could prevent the penetration of intracanal medicaments into dentinal tubules and influence the adaptation of filling materials to the canal walls,\(^\text{4}\) as well as act as a substrate for bacterial remnants. The greater removal of hard tissue debris may improve the disinfection and the quality of root canal filling. The idea to perform the final irrigation with XPF associated to PUI, as the results of the present study suggest, may improve the clinical success rate of endodontic treatment. Thus, a protocol that combines the XPF with PUI should be encouraged, though further studies are needed to confirm the clinical significance of the supplementary activation techniques to optimize the root canal cleaning.

CONCLUSION

Within the limitations of this ex vivo study, the micro-CT analysis allowed to draw the following conclusions:

The final irrigation of the mesial canals of mandibular molars with PUI or XPF decreased significantly the amount of AHTD, while the XPF was more efficient than PUI in the apical third.

The supplementary activation with XPF or PUI significantly reduced the AHTD in all thirds of both groups; however, the order of application (XPF + PUI) influenced significantly with the overall reduction in the middle and apical third areas.

FIGURE LEGEND

Figure 1. Flow chart of the groups and operative times.

Figure 2. Three-dimensional reconstructions of micro-CT scans of the mesial root canal of the PUI + XPF group (1) and the XPF + PUI group (2). (A) Superposition of AHTD after instrumentation (black areas) over the postoperative anatomy (red). (B) Superposition of AHTD (black areas) over the postoperative anatomy (red) after first activation with PUI (1) or XPF (2) (Reduction 1). (C) Superposition of AHTD (black areas) over the postoperative anatomy (red) after second activation with PUI (1) or XPF (2) (Reduction 2).

4. ACKNOWLEDGMENTS

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5. REFERENCES


Figure 1. Flow chart of the groups and operative times.
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