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Influence of apical preparation size and final irrigation protocol on the debridement of oval root canals

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This study assessed the influence of apical preparation size and final irrigation protocol on the debridement of the apical third of oval root canals of mandibular molars. Seventy-seven distal roots were divided into 7 groups (n = 11): Control: without instrumentation or irrigation; Group 30CI: ProTaper Next (up to size 30; PTN) + conventional irrigation (CI); Group 30UAI: PTN + ultrasonically activated irrigation (UAI); Group 30XPF: PTN + XP-endo Finisher (XPF); Group 40CI: PTN + ProDesign Logic (up to size 40; PDL) + CI; Group 40UAI: PTN + PDL + UAI; and Group 40XPF: PTN + PDL + XPF. The total volume of irrigating solutions used per root canal in all the experimental groups was 33 mL of 2.5% sodium hypochlorite (NaOCl) and 6 mL of 17% ethylenediaminetetraacetic acid (EDTA). After specimen processing and histological analysis under a digital microscope (100x), the percentages of untouched canal walls (UCW) and remaining debris (RD) were assessed using Image J software. A descriptive and exploratory analysis was conducted, indicating that the data failed to meet the assumptions of an analysis of variance. Therefore, generalized linear models were used to assess the effects of the different instrumentation and irrigation protocols, as well as the interaction among them, on the percentage of UCW and RD. No significant difference was found among the irrigation protocols regarding the percentage of UCW, irrespective of apical preparation size ($p>0.05$). However, UCW and RD were significantly lower in groups 40CI, 40UAI and 40XPF than in groups 30CI, 30UAI and 30XPF ($p<0.05$). The percentage of RD was significantly lower in the UAI and XPF groups than in the CI groups, irrespective of apical preparation size ($p<0.05$). The difference between preparation sizes 30 and 40, with respect to RD, was higher when CI was used ($p<0.05$). In conclusion, instrumentation up to apical preparation size 40 resulted in lower percentages of UCW and RD than up to apical preparation size 30. Use of UAI or XPF resulted in lower percentages of RD than CI.

Key Words: Molar, root canal irrigants, root canal preparation, sodium hypochlorite, ultrasonic surgical procedures.

Introduction

The anatomical complexity of the root canal system (RCS) precludes complete removal of all organic and inorganic contents using mechanical instrumentation alone (1). Instruments would have to touch the entire surface of all the root canal walls to ensure such effective debridement (2). However, anatomical regions inaccessible to intracanal instruments may harbor microorganisms, or hide pulp and dentin debris that may potentially impair successful endodontic treatment. This challenge is even more significant when instrumenting oval canals or isthmuses, because the design of the instruments also fails to match that of the root canal anatomy (3). Therefore, mechanical instrumentation must be complemented by irrigation to enhance the degree of debridement and disinfection (1). Sodium hypochlorite (NaOCl) solutions are the most widely used substances for this purpose, owing to their antimicrobial and tissue dissolution properties (1). However, NaOCl has no effect on the inorganic portion of the smear layer, and is therefore generally associated with 17% ethylenediaminetetraacetic acid (EDTA) in the final step of root canal irrigation (4). Furthermore, conventional syringe-and-needle irrigation (CI) is incapable of delivering the irrigant to the entire length of the root canal, thus increasing the likelihood of microorganisms remaining in the RCS after treatment, especially in accessory canals and anatomic irregularities (5).

The apical third of root canals poses yet another challenge to debridement, owing to the limited action of instruments and irrigants in these regions. Thus, the choice of apical preparation size becomes a relevant factor. Some authors have associated larger apical diameters with more effective debridement and disinfection (6,7), whereas others have reported that over-instrumentation might be associated with postoperative pain and treatment failure (8).

Several instrumentation systems with different tip and taper designs, alloys, and surface treatments have become available, and offer the promise of enhancing both safety and instrument effectiveness for root canal debridement, shaping, and disinfection. The instrumentation systems used in this study were ProTaper Next (PTN; Dentsply Maillefer, Ballaigues, Switzerland) and ProDesign Logic (PDL; Easy Equipamentos Odontológicos, Belo Horizonte, MG, Brazil), the latter consisting of files specifically developed for the apical preparation of the root canal.

In order to be mechanically and biologically valid in endodontics, the minimally invasive approach must be able to combine a slighter preparation of the cervical and middle thirds with an ampler preparation of the apical third, so as to ensure increased irrigant volume and enhance the likelihood of the irrigants reaching the entire apical region. This goal can be achieved more easily by using specific devices, and by increasing apical debridement (7,9,10).

To this end, several irrigation protocols and devices have been introduced to enhance the action of irrigants, and promote more effective debridement and disinfection of the RCS (7,11). Ultrasonically activated irrigation (UAI) is one of the techniques most widely cited in the literature for enhancing the action of irrigants, and is based on the physical phenomena of cavitation and microacoustic streaming (12). In contrast, the XP-endo Finisher (XPF) instrument (FKG Dentaire, La Chaux-de-Fonds, Switzerland), size 25/.00, performs additional scraping of the root canal walls, and causes turbulence of the irrigant, as part of a supplementary irrigation step. The nickel-titanium (NiTi) alloy of its composition (MaxWire) undergoes a molecular phase change at body temperature, causing the instrument to take on a "spoon" shape inside the root canal, thereby increasing its ability to adapt three-dimensionally to the root canal walls (13).

Despite the availability of numerous studies evaluating the effectiveness of different instrumentation systems and protocols in cleaning the root canal, studies are scarce on the effectiveness of different final irrigation protocols in teeth submitted to broader apical preparation, while being minimally prepared in terms of taper (7).

Debridement of the RCS is widely reported in the existing scientific literature; however, to the best of our knowledge, no previous study has compared the combined effect of apical preparation size, whether 30 or 40, and final irrigation protocol, whether CI, UAI or irrigation performed with the XPF instrument using 2.5% NaOCl and 17% EDTA. In view of the mechanical and chemical challenges to ensuring effective debridement and disinfection of the RCS, further investigation of the potential associations of the mechanical effects of instrumentation with the chemical effects of irrigation is needed. Therefore, the aim of this study was to compare the influence of apical preparation size and final irrigation protocol on the debridement of the apical third of oval root canals of mandibular molars. The null hypotheses tested were [1] that the two apical preparation sizes tested (30 and 40) would be equivalent in terms of the percentages of untouched canal walls (UCW) and remaining debris (RD); [2] that the three final irrigation protocols tested (CI, UAI and XPF) would be equivalent in terms of the percentage of UCW and RD; and [3] that there would be no interaction between the different apical sizes and the final irrigation protocols regarding UCW and RD percentages.

Materials and methods

This study was approved by the local research ethics committee (register no. 3.499.445). The 77 specimens used were donated expressly by patients whose mandibular molars were indicated for extraction. They were disinfected in a 0.1% thymol solution for 24 h, and then kept in a vial containing 10 mL of a 10% neutral buffered formalin solution.

The study sample size of 11 specimens per group provided a test power of 80%, with a type I error probability of 0.05, and an effect size of 0.40. The G*Power program (14) was used to calculate the sample size. The inclusion criteria were a fully formed distal root, a root canal foramen with an initial diameter corresponding to a #15 K-type file (Dentsply Maillefer), and a root curvature of up to 10°. In addition, specimens were required to have a single, oval-shaped distal root canal, ascertained by confirming that their buccolingual diameter was at least twice as large as their mesiodistal diameter, as measured at 3 mm short of the apex (15). These root-shape measurements were performed on standardized buccolingual and mesiodistal digital radiographs (CDRelite; Fona, Assago, MI, Italy), using Image J software (National Institute of Health, Bethesda, MD, USA), whereas the root-curvature measurements were performed on the same buccolingual digital radiographs using CDR DICOM software

(Fona). The exclusion criteria were teeth with calcifications, dilacerations, pathological root resorption (internal, external or apical), perforations in the furcation region (internal or external), root caries, and previous endodontic treatment.

Subsequent to accessing each tooth, a #15 K-file (Dentsply Maillefer) was inserted into the distal root canal until its tip was visible at the apical foramen under a stereomicroscope (Stemi 508; Carl Zeiss, Jena, Germany) at 40x magnification. This measurement was recorded as the patency length (15). A silicone stop was adjusted to the tip of the corresponding cusp to obtain the initial measurement of the specimen. The occlusal aspect was abraded with a sintered diamond disc (Tri Hawk, Morrisburg, Ontario, Canada) coupled to a micromotor and straight handpiece, operated at low speed and under refrigeration, to establish a standard length of 18 mm. The working length (WL) for instrumentation was established at 1.0 mm short of the apical foramen (15).

The mesial root of the specimen was sectioned with a sintered diamond disc (Tri Hawk) under refrigeration and discarded, and the surface of its distal root was scraped with periodontal curettes (Hu-Friedy, Chicago, IL, USA). The specimens were then stored in 10 mL of 10% neutral buffered formalin.

Well-balanced study groups with respect to root angle were ensured by first dividing the 77 specimens of the study sample into two preliminary groups of 0° to 5° and 6° to 10°, and then randomly distributing the specimens (www.random.org) into seven study groups (n = 11). Group homogeneity with respect to this feature was confirmed by the Kruskal-Wallis test (p = 1.000). In addition, oval-shaped distal root canals were ensured by analyzing the buccolingual and mesiodistal measurements at 3 mm short of the apex on digital radiographs using Image J software (National Institute of Health); group homogeneity with respect to this feature was confirmed by the Kruskal-Wallis test (p = 1.000).

Root canal instrumentation and final irrigation protocols

Prior to chemical-mechanical preparation, a pellet of utility wax was fixed on the tip of each root to simulate a closed system. The roots were embedded in small plastic vials containing condensation silicone (Zetaplus; Zhermack, Badia Polesine, RO, Italy) up to the level of the cementoenamel junction, to simulate the periodontal ligament. The root canals were instrumented by the same operator, experienced in all the systems used, and were divided into the different study groups, described below according to instrumentation and final irrigation protocol (the root canals were neither instrumented nor irrigated in the control group). Table 1 shows the characteristics of the instruments and devices used for chemical-mechanical preparation in each group.

Table 1. Characteristics of the instruments and devices used for the chemical-mechanical preparation of the specimens of the study groups.

Group	Instrumentation	Tip of the final instrument used	Taper of the final instrument used	Final irrigation	Tip of the instrument	Taper of the instrument
Control	None	-	-	None	-	-
30CI	ProTaper Next	30	.07	Conventional irrigation (NaviTip needle)	28	.00
30UAI	ProTaper Next	30	.07	Ultrasonically activated irrigation (Irrisonic insert)	20	.01
30XPF	ProTaper Next	30	.07	XP-endo Finisher	25	.00
40CI	ProTaper Next + ProDesign logic	40	.01	Conventional irrigation (NaviTip needle)	28	.00
40UAI	ProTaper Next + ProDesign logic	40	.01	Ultrasonically activated irrigation (Irrisonic insert)	20	.01
40XPF	ProTaper Next + ProDesign logic	40	.01	XP-endo Finisher	25	.00

Study groups according to the instrumentation protocol

In Groups 30CI, 30UAI and 30XPF (*PTN up to size 30/.07*), the root canals were instrumented with the PTN system (Dentsply Maillefer) in continuous rotation, operating at 300 rpm, with a torque of

2 N.cm. The X1 instrument was used initially for cervical preparation. Subsequently, the X2 and X3 instruments were used up to the WL, thus achieving apical preparation size 30. All 3 files were applied using an in-and-out motion, with a brushing action on the withdrawal stroke.

In Groups 40CI, 40UAI and 40XPF (*PTN up to size 30/.07 + PDL up to size 40/.01*), the root canals were instrumented with the PTN system (Dentsply Maillefer) in continuous rotation, at 300 rpm and with a torque of 2 N.cm. The X1 instrument was used initially for cervical preparation. Subsequently, the X2 and X3 instruments were used up to the WL, thus achieving apical preparation size 30. All 3 files were applied using an in-and-out motion, with a brushing action on the withdrawal stroke. The apical third of the root canals was then prepared up to size 40, by applying an in-and-out motion to PDL files sizes 35/.01 and 40/.01 (Easy Equipamentos Odontológicos) in continuous rotation, and with speed and torque settings at 350 rpm and 1 N.cm, respectively.

In all the groups mentioned above, specimens were irrigated during instrumentation with a total volume of 15 mL of 2.5% NaOCl. This solution was renewed at each instrument change using a 31-gauge Navitip needle with two lateral vents and closed tip (Ultradent, South Jordan, UT, USA), which was positioned 1 mm short of the WL (11,16) and submitted to vertical motion with an amplitude of 2 mm.

Study groups according to irrigation protocol

In groups 30CI and 40CI (PTN up to size 30/.07 or PTN + PDL up to size 40/.01 and conventional irrigation), the final irrigation protocol consisted of one rinse with 9 mL of 2.5% NaOCl, followed by 6 mL of 17% EDTA, and then a final rinse with 9 mL of 2.5% NaOCl, using a 31-gauge Navitip needle (Ultradent), positioned 1 mm short of the WL (11,16) and submitted to vertical motion with an amplitude of 2 mm.

In groups 30UAI and 40UAI (PTN up to size 30/.07 or PTN + PDL up to size 40/.01 and ultrasonically activated irrigation), the final irrigation protocol was performed using UAI. The 2.5% NaOCl solution was delivered into the root canal 1 mm short of the WL with a 31-gauge needle (Ultradent), and agitated in 3 cycles of 20 s using an E1 Irrisonic insert size 20/.01 (Helse Dental Technology, Santa Rosa de Viterbo, SP, Brazil), coupled to a piezoelectric ultrasonic unit (ENAC, Osada Electric, Tokyo, Japan) set to operate at 10% power. The insert was also positioned 1 mm short of the WL, moved vertically with an amplitude of 2 mm, and the solution was renewed after each cycle. The canal was dried with a 0.36-mm capillary tip (Ultradent), and the same 3-cycle procedure was performed with the canal filled with 17% EDTA, and then again with 2.5% NaOCl. The total volumes used for the final irrigation protocol were 18 mL of 2.5% NaOCl and 6 mL of 17% EDTA.

In groups 30XPF and 40XPF (PTN up to size 30/.07 or PTN + PDL up to size 40/.01 and irrigation with XPF), the final irrigation protocol was performed with the XPF instrument (FKG Dentaire) coupled to an X-Smart Plus motor (Dentsply Maillefer) set to operate at 800 rpm, with a torque of 1.5 N.cm. Initially, the instrument was cooled (Endo-Frost; Roeko, Langenau, Germany), and then inserted into the root canal and operated in continuous rotation, using slow and gentle movements with an amplitude of 7–8 mm in the longitudinal direction, until reaching the WL. Irrigants were renewed after each instrument change. The canal was dried with a 0.36-mm capillary tip (Ultradent), and the same 3-cycle procedure was performed with the canal filled with 17% EDTA, and then again with 2.5% NaOCl. The total volumes used for the final irrigation protocol were 18 mL of 2.5% NaOCl and 6 mL of 17% EDTA. The specimens were prepared inside a container, immersed in water at 37°C up to the cementoenamel junction, and the temperature was confirmed with a digital thermometer. Each XPF instrument (FKG Dentaire) was used in two canals and then discarded.

Irrespective of apical preparation size, the total volumes of irrigating solutions used per root canal in all the experimental groups were 33 mL of 2.5% NaOCl (15 mL during instrumentation and 18 mL during the final irrigation protocol) and 6 mL of 17% EDTA (during the final irrigation protocol). Final aspiration was performed with a 0.36-mm capillary tip (Ultradent), followed by drying of the canals with absorbent paper points (Dentsply Maillefer). An X-Smart Plus motor (Dentsply Maillefer) was used for all the instrumentation systems. Each instrument was used in four root canals, and then discarded. Apical patency was maintained at each instrument change with a #15 K-type file (Dentsply Maillefer).

Histological evaluation

Once the instrumentation and irrigation protocols were completed, the specimens were removed from their vials, and each root canal was filled passively with 10% buffered formalin, using a 10 mL disposable hypodermic syringe and a 31-gauge Navitip needle (Ultradent), immersed in 10% buffered formalin, and then fixed in the same solution for 24 h (17). Afterwards, the specimens were demineralized in 20% formic acid (Merck, São Paulo, SP, Brazil) for 15 days, and subsequently washed under running water for 8 h. They were then dehydrated in graded ethanol (70%–100%), cleared in xylol, and embedded in paraffin (Synth, Diadema, SP, Brazil), using the Leica TP 1020 processor (Leica Biosystems, Nussloch, Germany). Serial cross sections were obtained (0.5 μm) at every 0.2 mm of a 2 mm segment of the apical region, extending from 1 to 3 mm short of the root apex, totaling 10 sections per specimen. All of the 770 sections thus obtained were mounted on slides and stained with hematoxylin and eosin (H&E; 17).

Each section was photographed with a digital camera (Tucsen Prime HD, Tucsen Photonics, Fujian, China) coupled to a trinocular digital microscope (Nikon Eclipse 100-LED, Nikon Instruments, Kawasaki, Japan) under 100x magnification. The images were transferred to a workstation and saved in TIFF format. The percentage of UCW was determined by calculating the relation between the length of the root canal contour that remained untouched by the instruments and the total length of the canal contour, using Image J software (National Institute of Health). The criterion used to identify UCW was surface irregularity, as characterized by an abrupt change in the continuity of the root canal wall, and partial removal of pre-dentin. The percentage of RD (dentin chips, residual necrotic pulp, and particles loosely attached to the canal wall) was determined by dividing the area occupied by RD by the total area of the canal lumen (in mm^2), using Image J software (National Institute of Health). All of the 1,540 assessments (770 for UCW and 770 for RD) were performed by a single calibrated and blinded examiner (7). Figure 1 contains a flowchart illustrating the experimental procedures performed in the study.

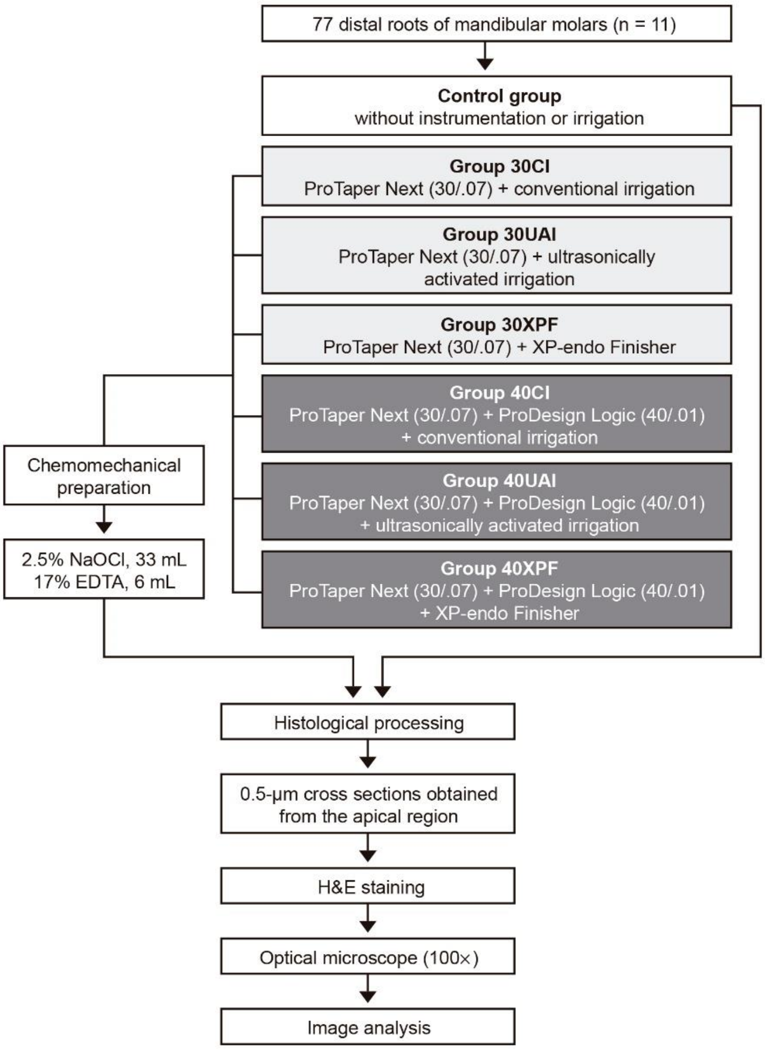

Figure 1. Flowchart of the experimental procedures performed in the study.

Statistical analysis

A descriptive analysis of the data was performed, followed by an exploratory analysis that showed that the data did not meet the assumptions of an analysis of variance (ANOVA). Owing to this asymmetric distribution of the data, generalized linear models with a gamma distribution were applied to assess the effects of the instrumentation protocol, the irrigation protocol, and the interaction between them. All the analyses were performed using the R program (18). The level of significance adopted was 5%.

Results

Figures 2 through 5 show photomicrographs representative of the cross sections of the apical region (1 to 3 mm short of the apex) of the distal root of the mandibular molars from the control and experimental groups, after conducting the different instrumentation (PTN up to size 30, or PTN + PDL up to size 40) and final irrigation protocols (CI, UAI or XPF). The percentages of UCW and RD were significantly lower in all of the experimental groups than in the control group ($p < 0.05$; Tables 2 and 3). No significant difference was observed among the irrigation protocols used for the percentage of UCW ($p > 0.05$), irrespective of apical preparation size. However, the percentage of UCW was significantly lower for apical preparation size 40 than for size 30 ($p < 0.05$), irrespective of the final irrigation protocol used (Table 2).

Table 2. Mean (standard deviation) and median (minimum and maximum value) percentages of untouched root canal walls (UCW) according to the instrumentation and final irrigation protocols.

Irrigation protocol	Instrumentation protocol			
	ProTaper Next (up to 30/.07)		ProTaper Next + ProDesign Logic (up to 40/.01)	
	Mean % (standard deviation)	Median % (minimum and maximum)	Mean % (standard deviation)	Median % (minimum and maximum)
Conventional	*40.54 (1.39) Aa	41.00 (38.50–42.50)	*11.13 (1.44) Ba	11.10 (9.10–14.50)
Ultrasonic	*40.54 (1.23) Aa	40.50 (39.00–42.50)	*11.11 (1.44) Ba	11.20 (9.10–14.50)
XP-endo Finisher	*40.50 (1.48) Aa	41.00 (38.00–42.50)	*11.10 (1.38) Ba	11.20 (9.10–14.50)

p-value: p (instrumentation) = < 0.0001; p (irrigation) = 0.9976; p (interaction) = 0.9984; Control group (no instrumentation or irrigation); p (group) < 0.0001
Mean % (standard deviation): 100.00 (0.00); Median % (minimum and maximum) 100.00 (100.00–100.00)

*: Value significantly different from that of the control group ($p \leq 0.05$). Means followed by different letters differ significantly ($p \leq 0.05$). Uppercase letters in rows refer to a comparison between the different instrumentation protocols, and lowercase letters in columns refer to a comparison among the different irrigation protocols.

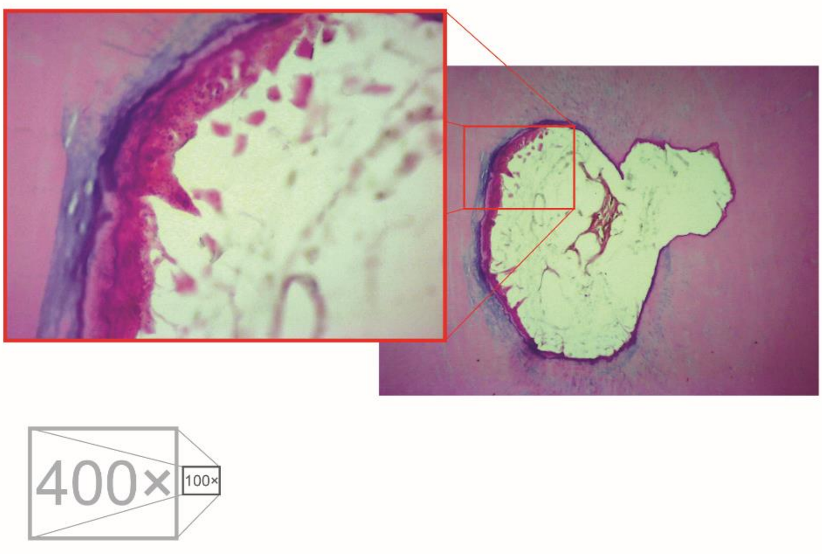

Figure 2. Representative photomicrograph of the cross sections of the apical region of the distal root of mandibular molars from the control group (no instrumentation or irrigation; H&E; 100x and 400x): note the presence of both organic and inorganic debris, and untouched root canal walls in the magnified inset (400x).

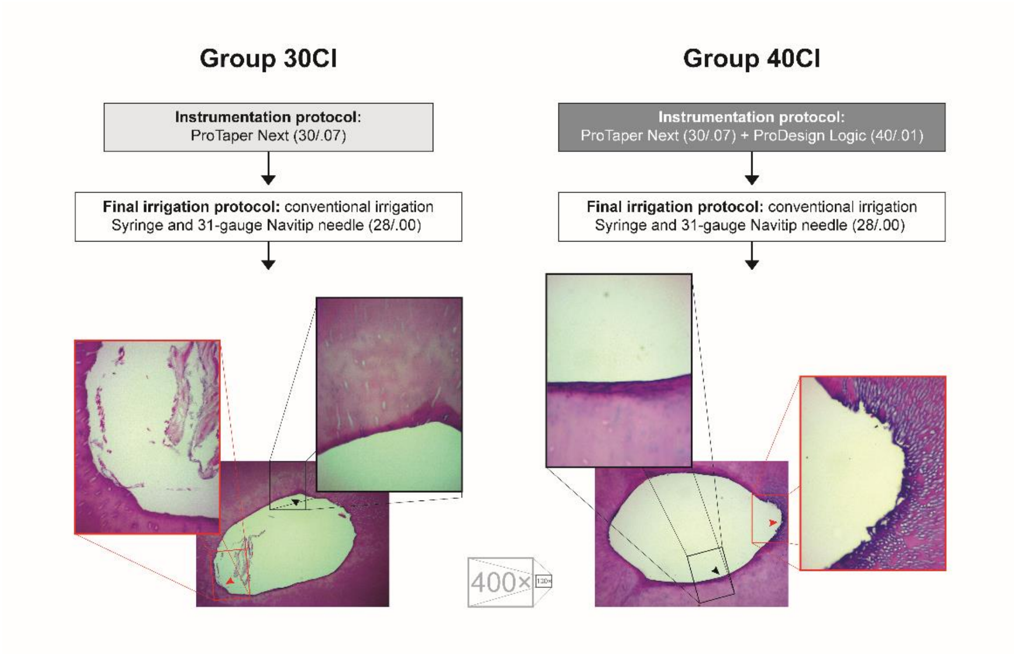

Figure 3. Representative photomicrographs of the cross sections of the apical region of the distal root of mandibular molars from the different study groups (H&E; 100 and 400). 30CI: ProTaper Next (up to size 30/.07) and conventional irrigation (LEFT); 40CI: ProTaper Next, ProDesign Logic (up to size 40/.01), and conventional irrigation (RIGHT); The magnified insets (400) depict root canal segments with touched walls without any debris (black arrow heads) versus untouched walls with the occasional residual debris (red arrow heads).

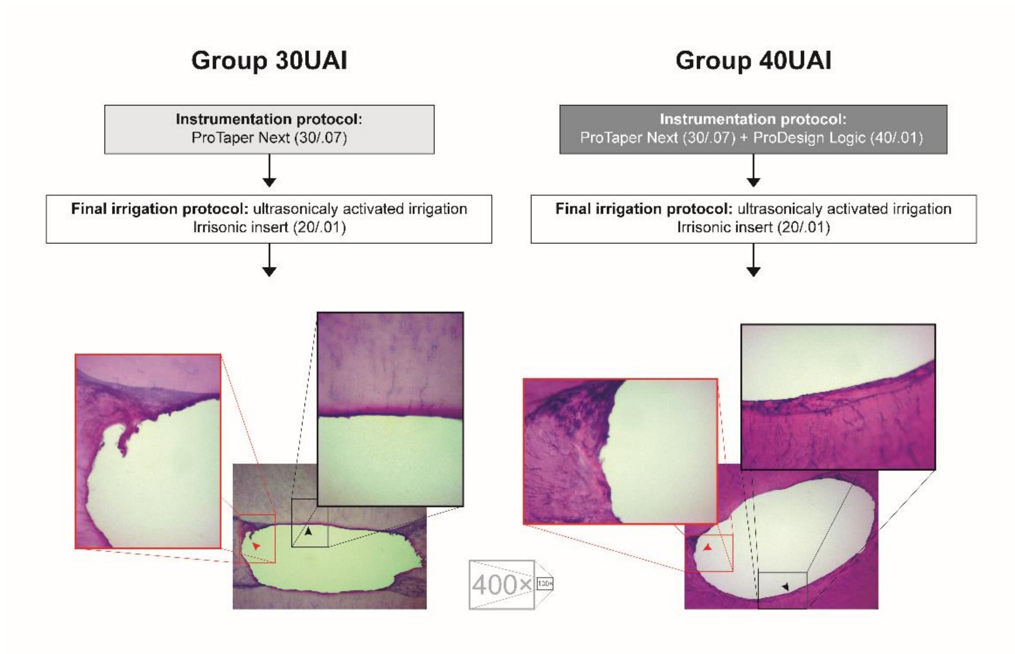

Figure 4. Representative photomicrographs of the cross sections of the apical region of the distal root of mandibular molars from the different study groups (H&E; 100 and 400). 30UAI: ProTaper Next (up to size 30/.07) and ultrasonically activated irrigation (LEFT); 40UAI: ProTaper Next (up to size 30/.07), ProDesign Logic (up to size 40/.01), and ultrasonically activated irrigation (RIGHT); The magnified insets (400) depict root canal segments with touched walls without any debris (black arrow heads) versus untouched walls with the occasional residual debris (red arrow heads).

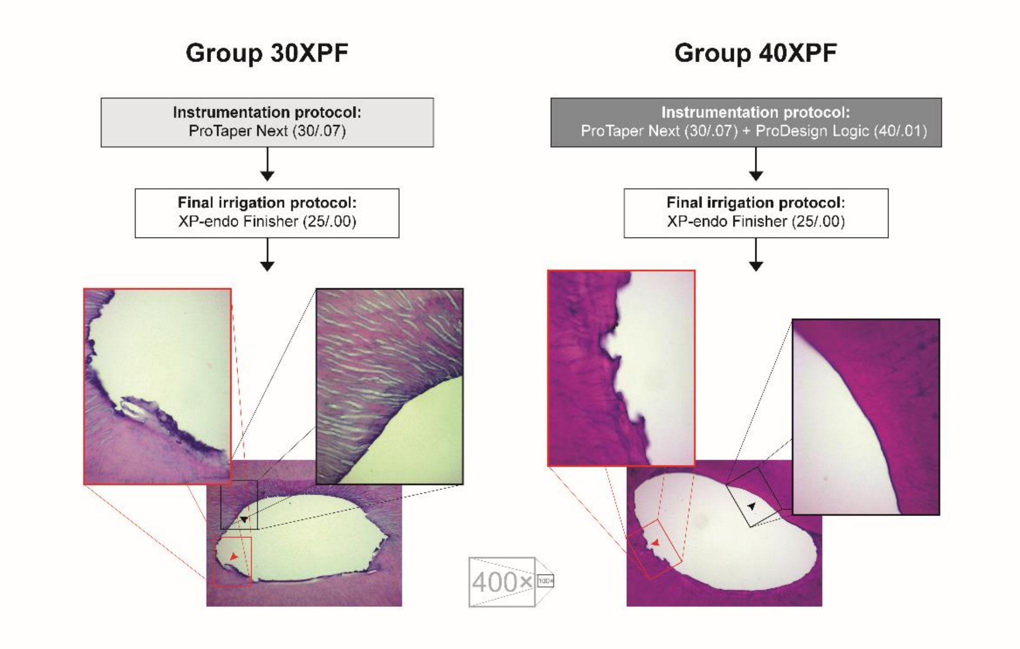

Figure 5. Representative photomicrographs of the cross sections of the apical region of the distal root of mandibular molars from the different study groups (H&E; 100 and 400). 30XPF: ProTaper Next (up to size 30/.07) and XP-endo Finisher (LEFT); 40XPF: ProTaper Next (up to size 30/.07), ProDesign Logic (up to size 40/.01), and XP-endo Finisher (RIGHT). The magnified insets (400) depict root canal segments with touched walls without any debris (black arrow heads) versus untouched walls with the occasional residual debris (red arrow heads).

The percentage of RD was also significantly lower for apical preparation size 40 than for size 30 ($p < 0.05$), irrespective of the final irrigation protocol used. In addition, the percentage of RD was significantly lower for UAI and XPF than for CI ($p < 0.05$), irrespective of the apical preparation size, and there was a significant interaction between final irrigation and instrumentation protocols for RD ($p < 0.05$), in that the difference between apical preparation sizes 30 and 40 was higher when CI was used (Table 3).

Table 3. Mean (standard deviation) and median (minimum and maximum value) percentages of remaining debris (RD) according to the instrumentation and final irrigation protocols.

Irrigation protocol	Instrumentation protocol			
	ProTaper Next (up to 30/.07)		ProTaper Next + ProDesign Logic (up to 40/.01)	
	Mean % (standard deviation)	Median % (minimum and maximum)	Mean % (standard deviation)	Median % (minimum and maximum)
Conventional	*3.49 (1.79) Aa	3.00 (2.00–8.50)	*0.55 (0.23) Ba	0.50 (0.05–0.75)
Ultrasonic	*1.00 (0.90) Ab	1.00 (0.30–3.60)	*0.16 (0.16) Bb	0.10 (0.01–0.50)
XP-endo Finisher	*1.00 (0.90) Ab	1.00 (0.30–3.60)	*0.16 (0.16) Bb	0.10 (0.01–0.50)

p-value: p (instrumentation) = < 0.0001; p (irrigation) < 0.0001; p (interaction) < 0.0001; Control group (no instrumentation or irrigation); p (group) < 0.0001
Mean % (standard deviation): 42.23 (14.12); Median % (minimum and maximum) 37.50 (32.00–82.50)

*: Value significantly different from that of the control group ($p \leq 0.05$). Means followed by different letters differ significantly ($p \leq 0.05$). Uppercase letters in rows refer to a comparison between the different instrumentation protocols, and lowercase letters in columns refer to a comparison among the different irrigation protocols.

Discussion

UCW and RD were lower for apical preparation size 40 than for size 30; therefore, the first null hypothesis was rejected. No difference was observed among the final irrigation protocols with respect to UCW, but RD was lower for UAI and XPF than for CI; therefore, the second null hypothesis was rejected for RD alone. No significant interaction was found between instrumentation and final irrigation protocols for UCW, but one was observed for RD, in that the difference between apical preparation sizes

30 and 40 was higher when CI was used; therefore, the third null hypothesis was also rejected for RD alone.

These results show that when the apical preparation was performed using the PDL system up to instrument size 40/.01, the instruments were able to reach a greater percentage of the irregular areas inherent in oval-shaped root canals than when using the PTN system up to the X3 file, size 30/.07. This observation of remaining untouched areas after instrumentation corroborates the results found in the related literature, which has no report of a system capable of touching all of the root canal surfaces (1,7,15,19).

A wider apical preparation can boost disinfection of the RCS by enhancing the antimicrobial action of NaOCl (20). Wider preparations also increase the likelihood of the instrument touching ampler areas of the canal surface (6,20,21), thus increasing their efficiency in removing adhered biofilm and infected dentin. Furthermore, the greater the apical preparation size, the greater the likelihood of incorporating anatomical irregularities. The mechanical and chemical efficacy of irrigation is also enhanced because larger preparations sizes allow deeper penetration of the irrigation needle and, as a result, a greater volume of irrigant can reach the apical portion of the RCS through improved hydrodynamics (5,6).

Several studies have evaluated the influence of apical preparation size on microbial reduction and/or on the degree of root canal cleanliness; however, methodological differences preclude precise comparisons (7,22). Lorencetti *et al.* (6) and Lee *et al.* (7) concluded that an enlargement of the apical third helped reduce the amount of debris in this region, corroborating the results of the present study, and disproving the hypothesis according to which the greater the number of instruments used to prepare the canal, the greater the amount of debris produced and impacted therein. In fact, the greater number of instruments used in the present study seem to have favored the flow of irrigants, and promoted a reduction in debris accumulation, as also reported by Boutsioukis *et al.* (5).

Despite some disagreement regarding the ideal size of the apical preparation, there is a consensus that this is specific to each tooth, and is contingent on anatomical, microbiological and mechanical factors (23-25). The initial apical diameter of the specimens used in the present study corresponded to a #15 K-type file (Dentsply Maillefer), and the selection of a minimum apical preparation size corresponding to a size 30 instrument was based on two important pieces of evidence related to achieving successful primary endodontic treatment: that size 30 is the bare minimum required to promote irrigant penetration in the apical third of the root canal (24), and that enlargement of the apical preparation must be performed progressively by using three instruments larger than the instrument compatible with the baseline apical diameter (25). However, other studies (7,20,21) observed superior results when using a wider apical preparation size than that suggested by Saini et al. (25), and these were corroborated by the results of the present study.

Although it could be argued that wider apical preparations could allow deeper penetration of the irrigation needle (6,16), care must be taken to avoid apical extrusion of irrigant and debris. To this end, the needle selected in the present study had two lateral vents and a closed tip to minimize this clinical risk. The needle insertion level was also standardized at 1 mm short of the WL in all groups to ensure appropriate irrigant renewal, as suggested by Boutsioukis et al. (5). Furthermore, the needle was moved within the canal with an amplitude of 2 mm during chemical-mechanical preparation both to avoid taper-lock (16) and to promote enhanced flow of the irrigant.

The present study was designed to assess oval canals of distal roots of mandibular molars, considering that these pose a major challenge for any instrumentation system and/or irrigation protocol, and to assess only the apical third of these root canals, considering that this region is the most difficult to clean, owing to its anatomical complexity (1,15). Siqueira (8) emphasized that endodontic instruments must touch most of the root canal walls to ensure removal of substantial amounts of pulp tissue and microorganisms. To this end, the author suggested that the apical preparation size should ideally incorporate difficult-to-access anatomical irregularities, albeit observing that this could be especially difficult to achieve in root canals with a non-circular cross section.

Several studies have shown that UAI was superior to syringe-needle irrigation in terms of root canal cleanliness and disinfection (7,11), irrespective of the evaluation method, thus corroborating the results of the present study. In addition, when CI was used in the present study, the difference between apical preparation sizes 30 and 40 was higher with respect to the amount of RD. This seems to suggest that performing an apical preparation up to size 40 is even more strongly indicated when no additional debridement technique is used. Conversely, it also suggests that performing a supplementary cleaning step is especially warranted when an apical preparation size 40 is not an option, considering the comparatively less effective action of CI for smaller sizes, at least as far as RD is concerned.

Furthermore, the results of the present study showed that combining apical preparation size 40 with UAI or XPF removed significantly more RD than a size 30 with the same final irrigation protocols, thus confirming the importance of a wider apical preparation. However, Lee *et al.* (7) found that mandibular premolar root canals prepared to size 40 were not significantly cleaner than those prepared to size 20 when using UAI, but were cleaner when using CI. This discrepancy requires further investigation, considering the numerous methodological differences that could be involved, either individually or combined. In the present study, activation was performed using a size 20/.01 instrument, positioned 1 mm short of the WL, in oval canals of mandibular molars, whereas Lee et al. (7) used a size 15/.02 instrument, positioned 2 mm short of the WL, in oval or round canals of mandibular premolars. In addition, the present study used a total of 33 mL of 2.5% NaOCl and 6 mL of 17% EDTA per root canal, and the ultrasonic unit was set to operate at 10% power, whereas Lee et al. (7) used 18 mL of 3% NaOCl, 3 mL of 17% EDTA, and an unspecified power setting.

Although lacking the ability to promote cavitation and acoustic streaming, the XPF instrument was equivalent to UAI in terms of debridement effectiveness, corroborating the results of recent previous studies (26,27). This could be explained by its ability to take on a "spoon" shape within the root canal at body temperature, and thus gain access to areas untouched during instrumentation (13).

Apical enlargement performed with highly tapered instruments may remove more dentin in the middle and cervical thirds of the root canal, predisposing the tooth to vertical root fracture, which may be one of the most frequent complications observed in endodontically filled teeth (10,28,29). Minimally invasive treatment strategies were devised to address this issue, particularly by performing preparations using smaller-taper instruments and instruments designed specifically to produce larger apical diameters, while preserving dentin in the middle and cervical thirds of the root canal (9,29). In this sense, Plotino *et al.* (9) found that a 4% to 6% increase in taper did not significantly reduce the amount of RD and smear layer in the apical third. Should this evidence be confirmed, clinicians could choose to reduce the taper of their root canal preparations without running the risk of reducing cleanliness, and this could be particularly helpful when preparing curved root canals (9). Bearing this in mind, the present study used a basic instrumentation protocol using an instrument size 30/.07 of the PTN system, which has a progressively decreasing taper, and compared it to a protocol using an instrument size 40/.01 of the PDL system, which has a smaller and continuous taper, and is specifically developed for the apical preparation of the root canal.

The presence of pulp tissue in the root canals of specimens from the control group confirmed the validity of the histological method used. However, fixed pulp tissue undergoes some degree of shrinkage, leaving it at risk to become detached from certain areas of the root canal wall. This explains why the calculations performed for control specimens failed to indicate 100% presence of pulp tissue (7). Another limitation of the histological method is its two-dimensional nature, where very thin cross sections of the root may not reflect the degree of cleanliness of the entire volume of each root canal segment under assessment. Nonetheless, this method has been used extensively in the related literature, and this limitation can be partially addressed by using serial apical cuts, whose individual readings are then combined to form a more accurate overall picture (1,7), a procedure which was also carried out in the present study. Additionally, although group homogeneity was statistically confirmed for both canal curvature and apical cross section (oval-shaped), future investigations may use computerized microtomography to standardize the baseline canal configuration of specimens, to attain a higher level of accuracy at this step.

The clinical significance of the present study is that its results seem to indicate that an increase in apical preparation size combined with a supplementary final irrigation protocol is likely to produce enhanced debridement of the RCS, particularly when oval-shaped root canals are involved. However, further investigation is warranted to assess the effect of other irrigant activation/agitation methods applied to different preparation sizes and tapers. In any event, enlargement of the apical preparation should not be performed indiscriminately. The use of this procedure must be scrutinized, and is contingent on the specific root anatomy of the tooth being treated, and on careful consideration of all the clinical factors involved.

In conclusion, instrumentation of the distal root canals of mandibular molars up to an apical preparation size 40 resulted in lower percentages of UCW and RD than instrumentation up to an apical preparation size 30. In addition, the final irrigation protocols using UAI or XPF resulted in lower percentages of RD than CI.

Resumo

Este estudo avaliou a influência do tamanho do preparo apical e do protocolo de irrigação final sobre o debridamento do terço apical de canais radiculares ovais de molares inferiores. Setenta e sete raízes distais foram selecionadas e divididas em 7 grupos (n = 11): Grupo controle: sem instrumentação nem irrigação; Grupo 30IC, ProTaper Next (até o tamanho 30; PTN) + irrigação convencional (IC); Grupo 30IAU, PTN + irrigação ativada ultrassonicamente (IAU); Grupo 30XPF, PTN + XP-endo Finisher (XPF); Grupo 40IC, PTN + ProDesign Logic (até o tamanho 40; PDL) + IC; Grupo 40IAU, PTN + PDL + IAU; e Grupo 40XPF, PTN + PDL + XPF. Os volumes totais dos irrigantes utilizados por canal radicular em todos os grupos experimentais foram 33 mL de hipoclorito de sódio (NaOCl) a 2,5% e 6 mL de ácido etilenodiamino tetracético (EDTA) a 17%. Após o processamento dos espécimes e a análise das secções histológicas sob um microscópio digital (100x), as porcentagens de paredes não instrumentadas (PNI) e detritos remanescentes (DR) foram avaliadas utilizando-se o software Image J. Uma análise descritiva e exploratória foi realizada, indicando que os dados não atenderam aos pressupostos de uma análise de variância. Modelos lineares generalizados foram, portanto, utilizados para avaliar os efeitos dos diferentes protocolos de instrumentação e irrigação, bem como da interação entre eles, sobre as porcentagens de PNI e DR. Não houve diferença significativa entre os protocolos de irrigação final quanto à porcentagem de PNI, independentemente do tamanho do preparo apical ($p>0,05$). Entretanto, as porcentagens de PNI e DR foram significativamente menores nos grupos 40IC, 40IAU e 40XPF do que nos grupos 30IC, 30IAU e 30XPF ($p<0,05$). A porcentagem de DR foi significativamente menor nos grupos em que se utilizou a IAU ou o XPF do que naqueles em que se utilizou a IC, independentemente do tamanho do preparo apical ($p<0,05$). A diferença entre os tamanhos de preparo apical 30 e 40 com relação aos DR foi maior quando se utilizou a IC ($p<0,05$). Concluiu-se que a instrumentação até um preparo apical de tamanho 40 resultou em menores porcentagens de PNI e DR do que até um preparo apical de tamanho 30. A utilização da IAU ou do XPF resultou em uma menor porcentagem de DR do que a utilização da IC.

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