ACTA ODONTOLOGICA SCANDINAVICA
2020, VOL. 78, NO. 6, 409–416
https://doi.org/10.1080/00016357.2020.1733076

ORIGINAL ARTICLE

Effect of different irrigation methods in the presence of a separated instrument: an *in vitro* study

Emel Uzunoglu-Özyürek[a], Cigdem Dik Güzel[b] and Hatice Dogan Buzoglu[a]

[a]Department of Endodontics, Faculty of Dentistry, University of Hacettepe, Ankara, Turkey; [b]Private Practice, Ankara, Turkey

ABSTRACT

Objective: The aim of this study was to evaluate the effect of different irrigation methods on sodium hypochlorite (NaOCl)/debris extrusion and dentinal tubule penetration of 2.5% NaOCl in the presence of an apically separated instrument.

Materials and methods: Sixty root canals of freshly extracted mandibular single-rooted premolars were chemomechanically prepared up to ProTaper F2. Manual needle irrigation (30-gauge/side-vented) with 2.5% NaOCl was performed between files and a smear layer was removed with 17% ethylenediaminetetraacetic acid. Three mm of notched ProTaper F3 files were separated in the apical third of the roots. Samples were then randomly divided according to the final irrigation systems as follows: EndoActivator, EndoVac, manual needle irrigation, passive ultrasonic activation, and RinsEndo. Samples were mounted to Eppendorf tubes and root canals were irrigated with 3-mL Rhodamine B-labelled 2.5% NaOCl, which was applied using one of the above-mentioned methods. The extruded NaOCl/debris weight was calculated by extracting pre-irrigation weights of tubes from post-irrigation ones. Samples embedded in acrylic resin were sectioned transversely in 1-mm thicknesses at apical 1- and 3-mm levels. NaOCl's penetration depth and percentage into the dentinal tubules were evaluated with the aid of a confocal laser scanning microscope. Data were analysed statistically with Kruskal–Wallis and *post hoc* Siegel–Castellan tests ($p < .05$).

Results: EndoVac significantly decreased the extruded NaOCl/debris compared to RinsEndo, passive ultrasonic activation, and EndoActivator in the presence of a separated instrument ($p < .05$). The penetrability of NaOCl significantly increased with the use of RinsEndo and EndoVac compared to the remaining groups ($p < .05$).

Conclusion: In the presence of an irretrievable separated instrument, it could be suggested that devices with apical negative pressure such as EndoVac may improve the penetrability of irrigation solutions to the apical part of the root while preserving periapical tissues from NaOCl/debris extrusion.

ARTICLE HISTORY
Received 3 September 2019
Revised 14 February 2020
Accepted 15 February 2020

KEYWORDS
Confocal laser scanning microscopy; root canal obturation; root canal preparation; root canal therapy

Introduction

Complete chemomechanical preparation and three-dimensional obturation of root canals are *sine qua non* for successful endodontic treatment. Nickel–titanium (NiTi) files are widely preferable to stainless steel files during chemomechanical preparation. Although NiTi files have several advantages such as the ability to create well-centered and minimally transported root canals with minimal procedural errors [1,2], they might be unintentionally separated during different stages of the root canal treatment [3,4]. Several factors such as inappropriate use (not considering the manufacturers' instructions about usage time, torque, rpm, etc.), torsional and cyclic fatigue stresses, calcified root canals, and inexperienced practitioners might result in file breakage [3]. This fractured file segment might prevent microbial control beyond the obstruction, especially if it is separated at the onset of treatment without further intervention. Furthermore, the existence of necrotic pulp in addition to the separated file might worsen the clinical success and, hence, the healing of the periapical lesion [5,6] even if breakage takes place in the latter stages of treatment. Flutes of files are the areas for debris removal. However, when a file is separated, these areas might contribute to microleakage [7]. In addition, clinicians would not have knowledge of the microbial pattern and the quantity of debris remaining between the flutes at the time of file separation.

Different devices are designed and manufactured to retrieve the separated file from the root canal and to improve the quality of the root canal treatment [3,5,8]. Although these devices work efficiently, some complications such as root perforation, the formation of microcracks, extrusion of the file beyond the root apex, excessive loss of dentine, and a decrease in fracture resistance might occur during their use. Due to these drawbacks, filling a root canal up to the separated fragment could be another treatment option, especially when retrieval efforts are unsuccessful [8,9]. In this scenario, the intracanal location of the fractured file, the canal preparation stage, and the microbial

CONTACT Emel Uzunoglu-Özyürek emel_dt@hotmail.com Department of Endodontics, Faculty of Dentistry, Hacettepe University, Sihhiye, Ankara 06100, Turkey

contamination level are some of the factors that could influence the long-term prognosis of an endodontically treated tooth [8] as well as the efficiency of root canal irrigation solutions. An irrigation solution should reach the apical third by passing the blockage, to improve dentinal tubule penetration and antimicrobial control in the root canal, in the presence of the separated instrument. The activation of irrigation solutions or the use of different devices during irrigation might be essential for preventing the negative effects that a separated instrument has on microbial control.

The combination of sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) is widely used during chemomechanical preparation with NiTi files to improve antimicrobial activity [10]. NaOCl is preferred mostly because of its antimicrobial effect, tissue dissolution capacity [10,11], and acceptable biologic compatibility when confined to the root canal [12]. Various devices – such as EndoVac, EndoActivator, RinsEndo, etc. – are manufactured to increase the penetration depth of irrigants into the dentinal tubules and to improve their smear layer removal capacity [13]. Previous studies reported that ultrasonic activation [14,15], EndoVac [16], and RinsEndo [14,15] have the potential to ensure better microbial control compared to manual needle irrigation. These devices are also considered safe because they limit the extrusion potential of irrigants into the periapical tissues. It is especially important to prevent apical extrusion of NaOCl because of its toxic and caustic effects. It has been reported that EndoVac prevents apical extrusion of NaOCl [13,17–19]. It applies negative pressure, while RinsEndo applies positive pressure and causes apical extrusion *in vitro* during root canal irrigation [13,20]. However, the capacity of these devices in terms of irrigant penetration into the dentinal tubules and the prevention of the extrusion of irrigants is unknown in the presence of a separated instrument.

Therefore, this study evaluated the penetrability of NaOCl into the dentinal tubules and the NaOCl/debris extrusion potential with different irrigation systems in the presence of an apically fractured NiTi file. The null hypothesis was that there would be no differences among irrigation devices and activation techniques considering the NaOCl/debris extrusion potential and penetrability of NaOCl into the dentinal tubules in the presence of an apically separated instrument.

Materials and methods

Sixty extracted human mandibular premolar teeth with single roots and canals were used following sample size calculation that was performed *via* G*Power software version 3.1.9.4 at 0.05 alpha with 80% power and ethics committee approval (GO 17/862-38). The teeth were stored in 0.1% thymol solution at 4 °C until use. Teeth with obliterated root canal systems, caries/cracks/fractures on the root surface, root fillings, internal or external resorption, and an apical curve $>10^{\circ}$ according to the Schneider's [21] technique were excluded following radiographic evaluation. Samples were decoronized to form standardized root samples with 15-mm lengths. Mesiodistal and buccolingual radiographs were taken to check the root canal space and measure the thickness of the root dentine walls of the decoronated specimens for sample standardization. The patency of the root canals was obtained and the working lengths for each sample were determined by 10 K-file (Dentsply Maillefer, Zurich, Switzerland) at 1 mm short of radiographic apex. Specimens with an initial apical diameter corresponding to a size 15 K-file were also selected. During preparation of the root canals using ProTaper rotary NiTi files (Dentsply Maillefer, Ballaigues, Switzerland) up to F2 rotary file (size 0.25, 0.08 taper), 3 mL of 2.5% NaOCl solution was used between each file. Manual needle irrigation was done with a 30-gauge (size 0.30, length 25 mm, Steri Irrigation Tips, Diadent, Cheongju, Korea) notched, side-vented needle and syringe. The needle was advanced as apically as possible inside the root canal during irrigation. All samples were flushed with 3 mL of 2.5% NaOCl and 3 mL of 17% EDTA before instrument separation. Figure 1 shows the experimental steps of this study.

ProTaper F3 rotary NiTi files (Dentsply Maillefer, size 0.30, 0.09 taper) were notched to the depth of half of the instrument with a No. 21 round bur (Meisinger LLC, Colorado, USA) 3 mm from the instrument tip to facilitate breakage of the file at this point. The notched instrument was inserted into the canal until resistance was felt and then was rotated clockwise with pressure to fracture the instrument [7]. Each of the teeth in the experimental groups was radiographed again to ensure that the instrument had fractured in the apical third of the canal (Figure 2(A)).

The amount of apically extruded NaOCl/debris and the penetration depth of the NaOCl into the dentinal tubules were determined by using the same samples with the following experimental setups.

NaOCl/debris extrusion evaluation

A similar experimental model described by Myers and Montgomery [22] was used for purposes of determining the extruded NaOCl/debris. Briefly, stoppers were separated by a diamond disc from the Eppendorf tubes. An analytical balance (Radwag, Radom, Poland) with an accuracy of 10^{-4} g was used to measure the initial weights of the tubes, and the mean of three sequential weights of tubes was obtained. Each tooth was mounted in the holes created in the rubber stoppers, up to the cementoenamel junction. A 27-gauage needle was placed through the stopper for use as a drainage cannula and to equalize the air pressure inside and outside the tubes. Then, each stopper with the sample and the needle was attached to its Eppendorf tube. The tubes were fitted into vials which were numbered from 1 to 60 and divided randomly into five groups by randomlists.com ($n = 12$). Similarly, irrigation techniques were randomly assigned to those groups with the help of randomlists.com. Three mL of 0.1% Rhodamine B-labelled 2.5% NaOCl was continuously delivered to enable evaluation of the extruded NaOCl/debris and the depth to which NaOCl penetrated the dentinal tubules. The final irrigation in each group was

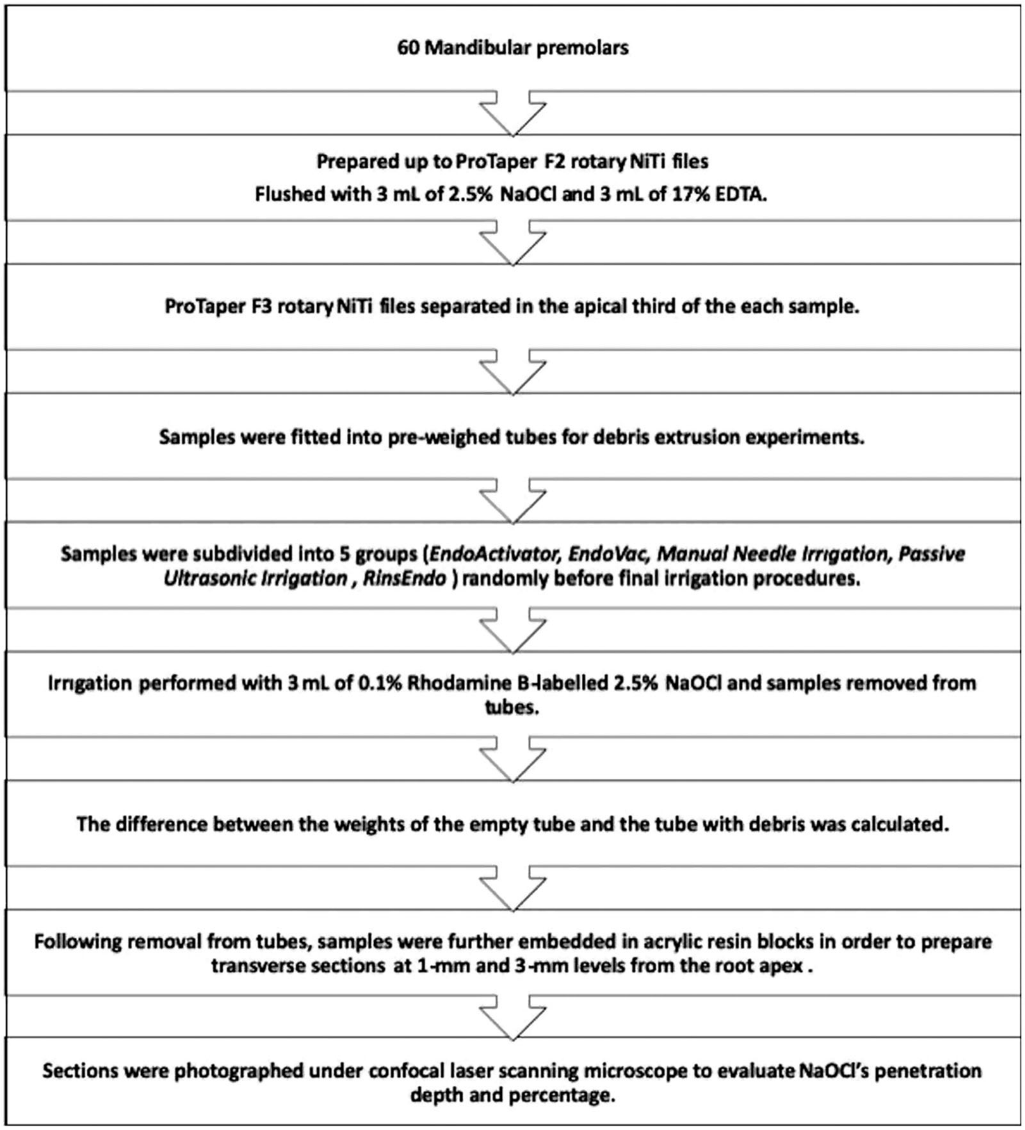

Figure 1. Flow chart showing the experimental steps of the study.

performed according to the manufacturers' instructions as follows:

1. *EndoActivator (EA, Sonic irrigation) (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA):*

One mL of labelled 2.5% NaOCl was placed into the root canal with Steri Tips, a red tip (size 25, 0.04 taper). Then, the sonic handpiece (EndoActivator) was used for 30 s at 10,000 cycles/min, which translates to 0.166 kHz in short 2–3-mm vertical strokes. This irrigation cycle was repeated 3 times.

2. *EndoVac (EV) (Discus Dental, Culver City, CA, USA):*

One mL of labelled 2.5% NaOCl was delivered using a master delivery tip placed at the pulp chamber for 30 s. Then the microcannula (28-gauge needle-0.32 mm) was placed in the canal as deep as possible and moved apico-coronally until 30 s irrigation was completed. After 30 s, the microcannula was withdrawn from the canal and the root canal was totally fulfilled with irrigant for 30 s [16]. This cycle was repeated three times. At the end of the third cycle, the microcannula was placed as deep as possible to remove the excess irrigant.

3. *Manual Needle Irrigation (MNI) (Steri Irrigation Tips, Diadent, Cheongju, Korea):*

A 30-gauge (0.30 mm × 25 mm) notched needle was placed in the root canal up to the separated file with the aid of a syringe and was moved with a slight up-and-down motion to prevent the needle from becoming locked in the canal. The root canal of each sample was irrigated manually with 3 mL of Rhodamine B-labelled 2.5% NaOCl for 90 s.

4. *Passive Ultrasonic Irrigation (PUI) (Suprasson PMax, Satelec Acteon Group, Merignac, France):*

One mL of labelled 2.5% NaOCl was placed in the canal with Steri Tips. A stainless-steel file IRR20/25 (diameter:

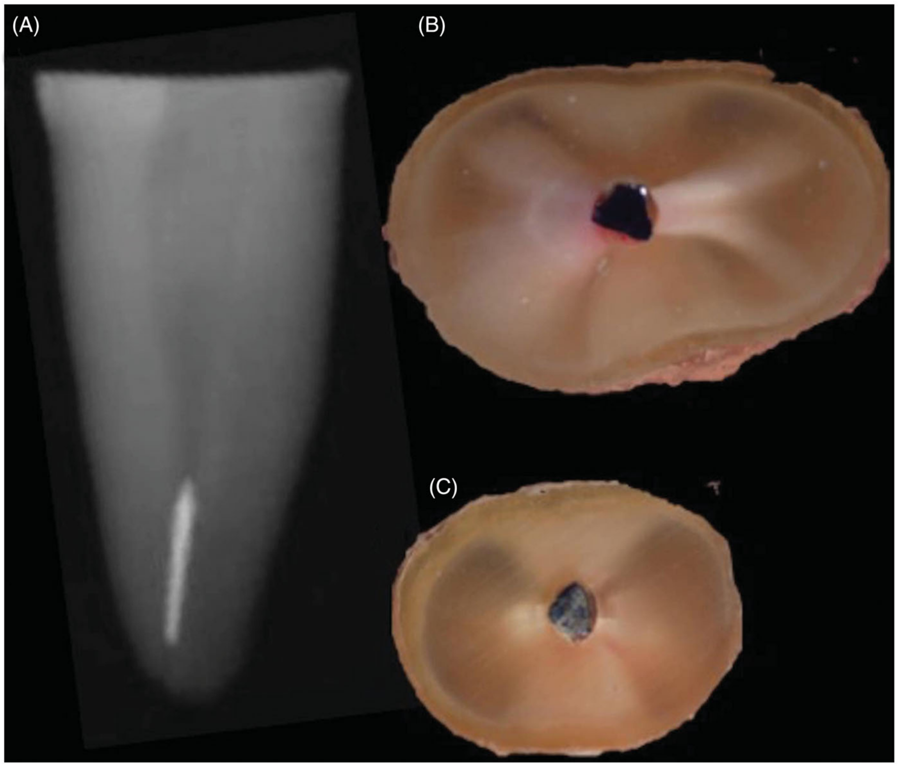

Figure 2. Radiographic (A) and stereomicroscopic (B and C) images of representative samples. A familiar cross-section of the ProTaper file could be seen at the centre of the root canal of both stereomicroscopic images from 3- (B) and 1- (C) mm levels from the apex.

0.20 mm, length: 25 mm; IrriSafe tip, Satelec Acteon Group, Merignac, France) was placed in the root canal as deep as possible in an up-and-down motion and the ultrasonic device was activated for 30 s at the recommended power setting of 5. This irrigation cycle was repeated 3 times.

5. *RinsEndo (RE) (Dürr Dental, Bietigheim, Germany):*

For 30 s, each sample was irrigated with 3 mL of labelled 2.5% NaOCl delivered *via* the RinsEndo handpiece and a 30-gauge open-ended needle provided by the manufacturer. This needle was placed as apically as possible inside the root canal.

Root canals were dried with paper points (Dentsply Maillefer) and then the stopper, needle, and sample were removed from the Eppendorf tube following irrigation. All of the tubes were incubated at 37 °C for 7 days so that the irrigant would evaporate in the Eppendorf tubes. Following this period, the Eppendorf tubes were weighed using the same analytical balance to obtain the final weight of the tubes including the extruded NaOCl/debris. The tubes were weighed again 3 times. The average of these measurements was considered to be the weight of the tube plus the NaOCl/debris. The difference between the weights of the empty tube and the tube with NaOCl/debris was calculated.

Penetration observation via CLSM

For purposes of evaluating the penetrability of 0.1% Rhodamine B-labelled 2.5% NaOCl into the dentinal tubules, each root was embedded in acrylic resin (Imicryl, Konya, Turkey) blocks and transverse sections were prepared perpendicularly to the long axis with 1-mm-thick sections with a slow-speed, water-cooled diamond saw (Isomet Low-Speed Saw; Buehler, Lake Bluff, IL, USA) at 1-mm and 3-mm levels from the root apex (2 specimens per tooth) (Figure 2(B,C)). The sections were mounted onto glass slides and photographed under a confocal laser scanning microscope (CLSM; LSM 510, Carl Zeiss, Oberkochen, Germany). The pinhole was kept at 1.5 AU in all recordings. The optical image was obtained by using a 543-nm line from a helium-neon laser as an excitation light source through a 10× objective (NA: 0.25). Observation of the entire section in a single frame was achieved with this magnification. The surfaces of the sections were covered with debris due to the sectioning procedure. Therefore, to prevent such contamination, the images were recorded at an optical section plane about 100 μm deeper than the surface. The thickness of optical sections under the given recording configurations was <115 μm. During the experiments, gain adjustments of the photomultiplier tube and digitizing process were kept at a constant setting.

The images were then evaluated *via* CLSM Image Examiner Software (Carl Zeiss) to measure the irrigant penetration depth and percentage into the dentinal tubules [23]. In brief, each image was imported into the Image Browser. The maximum irrigant penetration depth was measured as the distance from the tubule orifice at the canal wall to the deepest point of penetration along the dentinal tubule. The percentage of penetrated irrigant was calculated as follows: the amount of irrigant that penetrated into the canal wall circumference divided by the total canal wall circumference × 100 (%). All measurements were conducted by 2 different observers (EUO and CDG) and repeated 2 times for intra- and inter-rater reliability.

Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) software program, version 23.0 (IBM, Armonk, NY, USA).

Intra- and inter-rater reliability were evaluated using paired and independent *t*-tests, respectively. Data were analysed using the Shapiro–Wilk test for homogeneity. According to the results of the Shapiro–Wilk test, the data distribution was not uniform. The amount of extruded NaOCl/debris was analysed using the Kruskal–Wallis and *post hoc* Siegel–Castellan tests. Furthermore, the penetration depth and percentage of penetration into the dentinal tubules were analysed using the Mann–Whitney *U*, Kruskal–Wallis, and *post hoc* Siegel-Castellan tests at $p < .05$.

Results

NaOCl/debris extrusion

Table 1 shows the results of the *post hoc* test evaluating the amount of NaOCl/debris extrusion in each group.

Table 1. Amount of apically extruded debris (g) in experimental groups ($n = 12$).

Metho irrigation systems	Mean*	Std. deviation	Minimum	Maximum
EndoActivator	0.0015^{ab}	0.0010	0.0002	0.0027
EndoVac	0.0000^{bcd}	0.0000	0.0000	0.0001
Manual needle irrigation	0.0004^{ef}	0.0001	0.0002	0.0006
Passive ultrasonic activation	0.0134^{cf}	0.0090	0.0024	0.0279
RinsEndo	0.0484^{ade}	0.0230	0.0158	0.0958

*The same superscripts show statistical difference ($p < .05$).

The Kruskal–Wallis test revealed a significant difference among the experimental groups ($p < .05$). *Post hoc* analyses indicated that the EV irrigation system significantly decreased the amount of NaOCl/debris extrusion as compared to the RE, PUA, and EA methods ($p < .05$). Moreover, the amount of NaOCl/debris extrusion was decreased with MNI as compared to RE and PUA ($p < .05$) and with EA as compared to RE ($p < .05$). The ranking of the extruded NaOCl/debris weights was as follows: RE > PUA > EA > MNI > EV.

Dentinal tubule penetration of NaOCl

Tables 2 and 3 show the results in terms of the penetration depth and penetration percentage of NaOCl, respectively. Figure 3 shows the representative CLSM images of the experimental groups. The P values were >0.05 for both intra- and inter-rater reliability. Thus, the average values of measurements, which were conducted by two independent observers, were used for statistical analysis.

The penetration depth and penetration percentage of NaOCl with RE and MNI at 3 mm were statistically higher than they were at 1-mm measurements ($p < .05$).

The Kruskal–Wallis test showed a statistically significant difference among the groups in terms of the penetration depth and penetration percentage of NaOCl ($p < .05$). The results of MNI at the 1-mm level were significantly lower in comparison to RE and EV ($p < .05$). The penetration depth and penetration percentage of NaOCl with RE at the 3-mm level were significantly higher in comparison to PUA and EA ($p < .05$). Moreover, the penetration percentage of NaOCl

Table 2. Descriptive statistics of the maximum penetration depth of NaOCl in each group ($n = 12$) in microns*.

Irrigation systems	Section level (mm)	Mean	Std. deviation	Median	Minimum	Maximum
Manual needle irrigation	1^{acd}	0.00	0.00	0.00	0.00	0.00
	3^{a}	324.45	354.30	178.32	0.00	796.38
EndoVac	1^{d}	233.78	287.94	164.61	0.00	795.27
	3	476.54	358.25	548.39	0.00	1023.23
RinsEndo	1^{bc}	254.13	261.07	232.05	0.00	691.60
	3^{bef}	680.48	250.51	730.03	272.70	1006.25
EndoActivator	1	83.67	137.27	0.00	0.00	410.88
	3^{f}	220.70	353.06	0.00	0.00	916.89
Passive ultrasonic activation	1	77.76	182.30	0.00	0.00	503.39
	3^{e}	231.37	354.18	0.00	0.00	958.93

*The same superscripts show a statistical difference; p values were as follows: $a = 0.039$; $b = 0.001$ according to Mann–Whitney *U* test; $c = 0.023$; $d = 0.042$; $e = 0.017$; $f = 0.020$, according to *post hoc* Siegal–Castellan tests. Three comparisons were performed: 1 mm vs. 3 mm in the same group, 1 mm vs. 1 mm and 3 mm vs. 3 mm in other groups.

Table 3. Descriptive statistics of the percentage of canal wall circumference penetrated by NaOCl ($n = 12$) and percentage of sections penetrated by NaOCl*.

Irrigation systems	Section level (mm)	Mean	SD	Median	Min.	Max.	Percentage of samples (%) penetrated with NaOCl
Manual needle irrigation	1^{acd}	0.00	0.00	0.00	0.00	0.00	0
	3^{ag}	10.62	15.14	2.50	0.00	48.50	50
EndoVac	1^{d}	17.00	22.10	9.50	0.00	72.00	58.33
	3	33.71	34.61	18.25	0.00	98.00	75
RinsEndo	1^{bc}	21.54	24.56	11.00	0.00	65.50	58.33
	3^{befg}	57.54	33.35	62.25	7.00	100.00	100
EndoActivator	1	8.04	13.72	0.00	0.00	42.50	33.33
	3^{f}	13.46	23.76	0.00	0.00	73.50	33.33
Passive ultrasonic activation	1	6.71	15.75	0.00	0.00	44.00	16.67
	3^{e}	16.95	29.11	0.00	0.00	78.00	33.33

*The same superscripts show a statistical difference; the *p*-values were as follows: $a = 0.039$; $b = 0.007$ according to Mann–Whitney *U* test; $c = 0.022$, $d = 0.020$, $e = 0.011$, $f = 0.005$, $g = 0.011$ according to *post hoc* Siegal–Castellan tests. Three comparisons were performed: 1 mm vs. 3 mm in the same group, 1 mm vs. 1 mm and 3 mm vs. 3 mm in other groups.

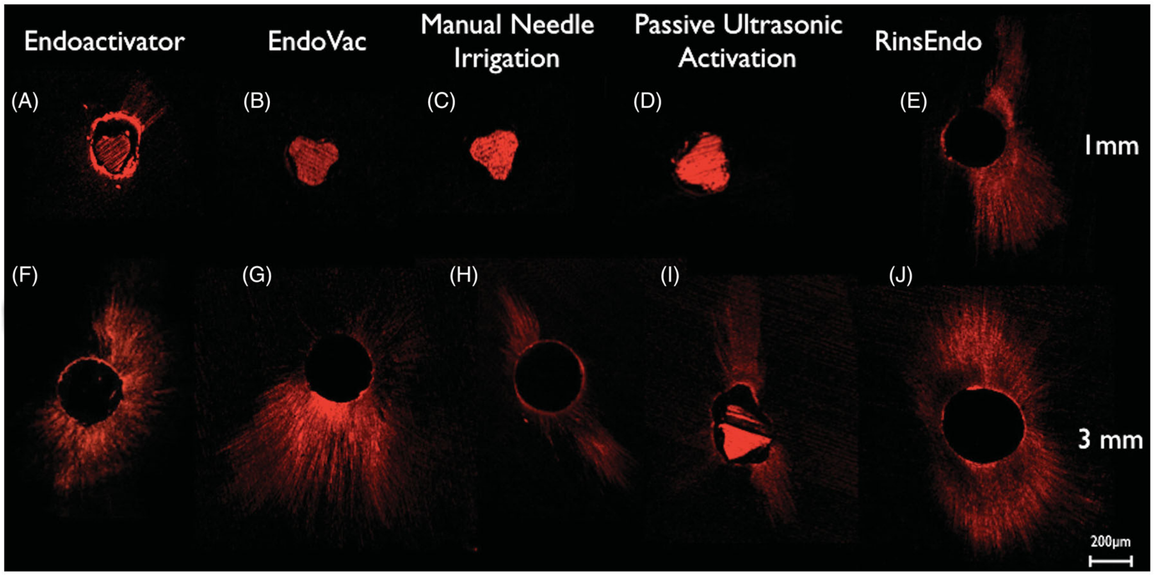

Figure 3. Representative CLSM images of experimental groups. Images were obtained by using a 10× objective. (A–E) Represents rhodamine B-labelled 2.5% NaOCl penetration at the 1-mm level after use of the Endoactivator, EndoVac, Manual Needle Irrigation, Passive Ultrasonic Activation, and RinsEndo systems, respectively, in the presence of an apically separated file. Limited NaOCl penetration could be observed with the use of EndoActivator at the 1-mm level from the apex in (A). A familiar cross-section of the ProTaper file could be seen at the centre of the root canal and there was no NaOCl penetration into the dentinal tubules in (B–D). A significant amount of NaOCl penetration into the dentinal tubules could be seen with the use of RinsEndo at the 1-mm level from the apex in (E). (F–J) represents rhodamine B-labelled 2.5% NaOCl penetration at the 3-mm level after use of the endoactivator, EndoVac, Manual Needle Irrigation, Passive Ultrasonic Activation, and RinsEndo systems, respectively, in the presence of an apically separated file. Different penetration depths and percentages could be observed with the use of these devices at the 3-mm level from the apex in (F–J).

with RE at the 3-mm level was significantly higher in comparison to MNI ($p < .05$).

Discussion

This study evaluated the final irrigation step of endodontic treatment by using different activation devices in the presence of an apically separated file as a treatment complication. According to the current results, the NaOCl/debris extrusion potential of these devices, even in the presence of a separated instrument in the root canals, was consistent with the literature [13,17–20,24]. The RE group extruded more NaOCl/debris than did all the experimental groups, as in previous studies [13,20], and there was almost no extrusion with EV, which has already been reported as being one of the safest irrigation methods [13,17–19]. Moreover, PUA created a greater amount of NaOCl/debris extrusion compared to MNI. On the contrary, some of the previous studies [13,17] reported that MNI created more extrusion as compared to EV, EA, or PUA. The most important factor affecting the extrusion amount during MNI is the penetration of the needle in the root canal [25,26]. In this study, the presence of a separated file might have limited the penetration depth of MNI, thereby reducing its extrusion potential.

The effectiveness of irrigation systems might depend on different parameters such as the activation of the system [24], the pressure of the device [13], the penetration depth of the used tip in the root canal [25,26], the application duration [27], etc. It was reported that the leakage of samples with separated ProTaper fragments was significantly lower than in samples with separated ProFile instruments [7]. Because this study used the ProTaper system, the cross-sectional design of the preferred file system might also have impacted the effectiveness of the irrigation systems.

It is well accepted that the presence of a separated instrument challenges microbial control beyond the obstruction, which can lead to apical pathosis [5,6]. Furthermore, a decreased prognosis – and, hence, survival – is observed in the presence of a periapical lesion when the apical patency is not maintained and when the root canal cleaning is not performed as close as possible to the apical terminus [28]. The depth to which irrigants penetrate the dentinal tubules is potentially an important factor for eliminating bacteria and contributes to the outcome of the root canal treatment [14–16,29]. In this study, consistent with the literature, the irrigation-activated/delivered techniques have a predominantly positive effect on the penetration of the irrigation solution into the dentinal tubules [20,30–32] as compared to MNI. While NaOCl did not penetrate into the tubules at 1 mm from the apex with MNI, it did penetrate into dentinal tubules in 58.3% of the samples of both with the RE and EV groups in the presence of a separated instrument. This value increased to 100% with RE and to 75% with EV at the 3-mm level. According to recent studies, EV also increased the penetration of NaOCl [33] and root canal sealer [34] into the dentinal tubules as compared to MNI. Therefore, it can be speculated that in cases in which the separated instrument cannot be removed, irrigation with EV or RE instead of MNI can provide better microbial control despite setbacks that prevent proper cleaning as close as possible to the apical terminus. However, it is also important to mention that increased NaOCl/debris extrusion in the RE group and decreased NaOCl/debris extrusion in the EV group were observed. Considering the fact that excess extrusion may cause postoperative pain and periapical tissue irritation (and, hence, prevent periapical healing), the current results suggest that the use of EV might be more advantageous in the presence of a separated instrument as compared to RE.

In this study, NaOCl penetration following smear layer removal by 17% EDTA was evaluated with CLSM *via* Rhodamine B dye. Mixing NaOCl with Rhodamine B could be one of the limitations of this study because the molecular weight of Rhodamine B (479.02 g/mole) is much higher than the molecular weight of NaOCl dissolved in distilled water (74.45 g/mole) – a difference that might affect NaOCl's surface tension as well as penetrability. Another limitation regarding the use of Rhodamine B mixed with NaOCl is that NaOCl might slowly inactivate Rhodamine due to its strong oxidizing properties. On the contrary, CLSM has several advantages, such as being a non-destructive approach and having the ability to control the depth of field, to eliminate or reduce background information away from the focal plane, and to collect serial optical sections even from thick specimens [23]. Hence, this methodology was the preferred means of evaluating the penetration depth and percentage penetration of NaOCl, as in previous studies [30,31]. In this study, NaOCl/debris extrusion and the penetration ability of NaOCl were evaluated simultaneously in the same samples, so the Rhodamine B-labelled NaOCl was used during the extrusion test. It was speculated that the Rhodamine B addition might also affect the amount of NaOCl/debris extrusion apically. Furthermore, NaOCl irrigation could result in sodium crystal precipitates following evaporation, which might increase the amount of extruded debris as compared to distilled water irrigation. However, to reflect clinical conditions, the utilization of clinically widely-used irrigation solutions during *ex vivo* apical extrusion studies might be more logical [18,35].

The absence of negative control groups is another limitation of this laboratory study. On the contrary, preparing samples for negative controls for each irrigation group would double the number of needed samples and control of variables might complicate the interpretation of the results. It is also important to mention that the results of this study should not be directly extrapolated to the clinical situation. Only mature roots without periodontal ligament simulation were used. These results might differ under *in vivo* conditions. However, studying the apically extruded amount of NaOCl/debris as well as its dentinal tubule penetration depth, especially in the presence of a separated file, was not possible *in vivo* due to practical and ethical limitations.

Conclusion

Under the above-mentioned limitations of this study, the null hypothesis was rejected. Irrigation devices and activation techniques affected both the penetrability of NaOCl into the dentinal tubules and NaOCl's apical extrusion potential. NaOCl still might extrude to the periapical area despite the presence of a separated instrument. MNI extruded a lower amount of NaOCl/debris but its penetration ability was not sufficient as compared to that of activated groups. Therefore, the use of devices with apical negative pressure such as EV could be suggested to improve the ability of irrigation solutions to penetrate the apical part of a root while preserving periapical tissues from NaOCl/debris extrusion in the presence of a separated instrument. Consequently, the activation of NaOCl in the presence of a separated file might be suggested as a means of improving clinical success. Because bacterial removal is an important factor affecting the healing process of teeth with a periapical lesion, further studies are needed to evaluate the efficiency of these devices following experimental bacterial contamination and file separation.

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

[1] Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. J Endod. 2004;30(8):559–567.

[2] Shen Y, Zhou HM, Zheng YF, et al. Current challenges and concepts of the thermomechanical treatment of nickel-titanium instruments. J Endod. 2013;39(2):163–172.

[3] McGuigan MB, Louca C, Duncan HF. Endodontic instrument fracture: causes and prevention. Br Dent J. 2013;214(7):341–348.

[4] Fishelberg G, Pawluk JW. Nickel-titanium rotary-file canal preparation and intracanal file separation. Compend Contin Educ Dent. 2004;25(1):17–18. 20-22, 24; quiz 25, 47.

[5] Panitvisai P, Parunnit P, Sathorn C, et al. Impact of a retained instrument on treatment outcome: a systematic review and meta-analysis. J Endod. 2010;36(5):775–780.

[6] Spili P, Parashos P, Messer HH. The impact of instrument fracture on outcome of endodontic treatment. J Endod. 2005;31(12): 845–850.

[7] Altundasar E, Sahin C, Ozcelik B, et al. Sealing properties of different obturation systems applied over apically fractured rotary nickel-titanium files. J Endod. 2008;34(2):194–197.

[8] Madarati AA, Hunter MJ, Dummer PM. Management of intracanal separated instruments. J Endod. 2013;39(5):569–581.

[9] Souter NJ, Messer HH. Complications associated with fractured file removal using an ultrasonic technique. J Endod. 2005;31(6): 450–452.

[10] Haapasalo M, Shen Y, Wang Z, et al. Irrigation in endodontics. Br Dent J. 2014;216(6):299–303.

[11] Mohammadi Z. Sodium hypochlorite in endodontics: an update review. Int Dent J. 2008;58(6):329–341.

[12] Kerbl FM, DeVilliers P, Litaker M, et al. Physical effects of sodium hypochlorite on bone: an ex vivo study. J Endod. 2012;38(3): 357–359.

[13] Desai P, Himel V. Comparative safety of various intracanal irrigation systems. J Endod. 2009;35(4):545–549.

[14] Cachovan G, Schiffner U, Altenhof S, et al. Comparative antibacterial efficacies of hydrodynamic and ultrasonic irrigation systems *in vitro*. J Endod. 2013;39(9):1171–1175.

[15] Bago Jurič I, Plečko V, Anić I. Antimicrobial efficacy of Er,Cr:YSGG laser-activated irrigation compared with passive ultrasonic irrigation and RinsEndo(®) against intracanal Enterococcus faecalis. Photomed Laser Surg. 2014;32(11):600–605.

[16] Hockett JL, Dommisch JK, Johnson JD, et al. Antimicrobial efficacy of two irrigation techniques in tapered and nontapered canal preparations: an *in vitro* study. J Endod. 2008;34(11):1374–1377.

[17] Yost RA, Bergeron BE, Kirkpatrick TC, et al. Evaluation of 4 different irrigating systems for apical extrusion of sodium hypochlorite. J Endod. 2015;41(9):1530–1534.

[18] Iriboz E, Bayraktar K, Turkaydin D, et al. Comparison of apical extrusion of sodium hypochlorite using 4 different root canal irrigation techniques. J Endod. 2015;41:380–384.

[19] Charara K, Friedman S, Sherman A, et al. Assessment of apical extrusion during root canal irrigation with the novel gentlewave system in a simulated apical environment. J Endod. 2016;42(1): 135–139.

[20] Hauser V, Braun A, Frentzen M. Penetration depth of a dye marker into dentine using a novel hydrodynamic system (RinsEndo). Int Endod J. 2007;40(8):644–652.

[21] Schneider SW. A comparison of canal preparations in straight and curved root canals. Oral Surg Oral Med Oral Pathol. 1971; 32(2):271–275.

[22] Myers GL, Montgomery S. A comparison of weights of debris extruded apically by conventional filing and Canal Master techniques. J Endod. 1991;17(6):275–279.

[23] Gharib SR, Tordik PA, Imamura GM, et al. A confocal laser scanning microscope investigation of the epiphany obturation system. J Endod. 2007;33(8):957–961.

[24] Helvacioglu Kivanc B, Deniz Arisu H, Yanar NO, et al. Apical extrusion of sodium hypochlorite activated with two laser systems and ultrasonics: a spectrophotometric analysis. BMC Oral Health. 2015; 15:71.

[25] Bronnec F, Bouillaguet S, Machtou P. Ex vivo assessment of irrigant penetration and renewal during the final irrigation regimen. Int Endod J. 2010;43(8):663–672.

[26] Perez R, Neves AA, Belladonna FG, et al. Impact of needle insertion depth on the removal of hard-tissue debris. Int Endod J. 2017;50(6):560–568.

[27] Siqueira J, Rocas I, Favieri A, et al. Chemomechanical reduction of the bacterial population in the root canal alter instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypoclorite. J Endod. 2000;26:331–334.

[28] Ng YL, Mann V, Gulabivala K. Tooth survival following non-surgical root canal treatment: a systematic review of the literature. Int Endod J. 2010;43(3):171–189.

[29] Zou L, Shen Y, Li W, et al. Penetration of sodium hypochlorite into dentin. J Endod. 2010;36(5):793–796.

[30] Akcay M, Arslan H, Mese M, et al. Effect of photon-initiated photoacoustic streaming, passive ultrasonic, and sonic irrigation techniques on dentinal tubule penetration of irrigation solution: a confocal microscopic study. Clin Oral Invest. 2017;21(7): 2205–2212.

[31] Gu Y, Perinpanayagam H, Kum DJ, et al. Effect of different agitation techniques on the penetration of irrigant and sealer into dentinal tubules. Photomed Laser Surg. 2017;35(2):71–77.

[32] Giardino L, Cavani F, Generali L. Sodium hypochlorite solution penetration into human dentine: a histochemical evaluation. Int Endod J. 2017;50(5):492–498.

[33] Venumbaka NR, Baskaran P, Mungara J, et al. Comparative evaluation of the efficacy of endovac and conventional irrigating systems in primary molars: an *in vitro* study. J Clin Pediatr Dent. 2018;42(2):140–145.

[34] Kara Tuncer A, Unal B. Comparison of sealer penetration using the EndoVac irrigation system and conventional needle root canal irrigation. J Endod. 2014;40(5):613–617.

[35] Tanalp J, Güngör T. Apical extrusion of debris: a literature review of an inherent occurrence during root canal treatment. Int Endod J. 2014;47(3):211–221.