Effect of Different Irrigation Activation Techniques on Sealer Penetration: A Confocal Laser Microscopy Study

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Objective: *To compare the efficiency of a new sonic powered irrigation system named EDDY (VDW, Munich, Germany), passive ultrasonic irrigation (PUI) and conventional needle irrigation (CNI) in root canal sealer penetration.*
Methods: *A total of 45 mandibular premolars were instrumented up to size 30, 0.9 taper and randomly divided into three groups (n = 15) depending on the final irrigation activation technique: EDDY, PUI or CNI. After the final irrigation procedures, the root canals were obturated with labelled sealer mixed with 0.1% rhodamine B. Transverse sections at 3, 5 and 7 mm from the root apex were examined using confocal laser scanning microscopy. The maximum depth and total area and percentage of sealer penetration were measured using ImageJ analysis software (National Institutes of Health, Bethesda, MD, USA).*
Results: *In the EDDY group, the penetration depth was higher compared to the CNI group in the apical and middle sections and compared to the PUI group in the apical section ($P < 0.05$). The penetration area in the EDDY group was higher compared to the CNI group in all sections and compared to the PUI group in the coronal section ($P < 0.05$). The percentage of penetration was higher in the EDDY group compared to the CNI group in all sections and compared to the PUI group in the coronal section ($P < 0.05$).*
Conclusion: *In the present study, sealer penetration was superior in the EDDY group than the CNI group in the apical section. In the middle and coronal sections, sealer penetration was similar for the EDDY and PUI groups.*
Key words: *EDDY, passive ultrasonic irrigation, sealer penetration, sonic irrigation*
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During root canal instrumentation, the formation of a smear layer containing organic and inorganic substances such as vital or necrotic pulp tissues, coagulated proteins, microbial elements, odontoblastic extensions and dentine chips is almost inevitable[1,2]. This granular film limits the penetration of irrigants, intracanal medicaments and root canal sealers into the dentinal tubules and adversely affects the adaptation of sealers to the root canal walls[3]. This may cause microleakage following root canal obturation and lead to failure of root canal treatment[1-4].

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In many studies in the literature, the importance of activating irrigation solutions in addition to mechanical preparation in removing the smear layer containing organic and inorganic tissues has been emphasised[2,5,6]. Conventional needle irrigation (CNI) is widely used in endodontics because of its ease of administration; however, contact between the irrigant and the apical region is reduced in CNI because the irrigant reaches only 1.5 to 2.0 mm beyond the needle tip[5,7,8]. In addition, during CNI, air is trapped in any part of the root canal in what is known as the vapour lock effect. The vapour lock effect has been found to limit contact of the solution with the entire root canal surface[2,7-9]. Thus, various techniques and devices have been developed for effective irrigation[2,5,6].

Passive ultrasonic irrigation (PUI) is an irrigation activation system that activates irrigation through acoustic microstreaming at ultrasonic frequencies (25 to 30 kHz) with a stainless-steel file that does not touch the canal walls. In the literature, it has been reported that PUI can impair endodontic biofilms, resulting in better penetration of irrigants along the the dentinal tubules[1,2,10].

EDDY (VDW, Munich, Germany), a sonic powered irrigation activation system, has a size 25, 0.04 taper non-cutting, flexible polyamide tip with an air scaler activated between 5000 and 6000 Hz. According to the manufacturer, EDDY creates a 3D movement that allows the formation of cavitation and acoustic flow during activation of irrigation; this effect is similar to PUI and provides superior cleaning efficiency[8,11].

This study aimed to investigate the effect of different irrigation activation techniques on penetration of root canal sealer into the dentinal tubules using confocal laser scanning microscopy (CLSM). The null hypothesis was that there would be no difference between the efficacy of different irrigation activation systems for sealer penetration.

Materials and methods

The study design was approved by the local ethics committee (Bolu Abant Izzet Baysal University No. 2019-55). Based on a previous study[12], a power calculation was performed using G*Power software (version 3.1, Heinrich Heine University, Dusseldorf, Germany). Forty-five maxillary incisors with a single root and single canal were included in this study. Periapical radiographs of each tooth and root canal anatomy were taken from buccolingual and mesiodistal angles and evaluated. Teeth with undeveloped root canals, resorption, curved roots, calcification, fractures or cracks were replaced with new ones. The teeth were stored in distilled water at 4°C until use.

Endodontic access cavities were prepared with diamond round burs (Dentsply Maillefer, Ballaigues, Switzerland). The apical patency was controlled with a size 10 K-file (Dentsply Maillefer) and the working length was determined to be 1 mm shorter than the apical foramen. For root canal preparation, ProTaper Universal (Dentsply Maillefer) SX, S1, S2, F1 and F2 files were used, in accordance with the manufacturer's instructions. During each file change, the canals were irrigated with 2 ml of 2.5% NaOCl (CanalPro; Coltene-Whaledent, Allstätten, Switzerland). Following preparation, each canal was irrigated with 10 ml of 17% EDTA and 10 ml of 2.5% NaOCl, respectively, and dried with paper points (DiaDent, Chongju, Korea). According to the irrigation activation technique, the teeth were randomly divided into three groups (n = 15) as follows:

- CNI: A 31-gauge double sideport irrigation needle (NaviTip Double Sideport irrigator tip, Ultradent Products, South Jordan, UT, USA) was placed in the canal to be 1 mm shorter than working length. The irrigating needle was moved back and forth and 9 ml of 2.5% NaOCI was used for irrigation with a flow rate of approximately 0.1 ml/second.
- EDDY: A size 25, 04 taper polyamide tip was adapted to the TA-200 (Micron, Tokyo, Japan) and placed in the canal 1 mm shorter than working length. The canals were irrigated with 3 ml of 2.5% NaOCI applied at a flow rate of approximately 0.1 ml/second and irrigant activated at 6000 Hz for 30 seconds. This irrigation activation cycle was repeated three times and 9 ml irrigant was used in total.
- PUI: A size 25 ultrasonic tip (Irrisafe, Satelec-Acteon, Merignac, France) was placed to canal 1 mm shorter than the working lengh. The canals were irrigated with 3 ml of 2.5% NaOCI applied at a flow rate of approximately 0.1 ml/second and irrigant activated using the ultrasonic device at power setting 4 (Newtron P5 Unit, Satelec-Acteon) for 30-second activations. This irrigation activation cycle was repeated three times and 9 ml irrigant was used in total.

For the CLSM analysis, 0.1% Rhodamine B (Batch121K3688, RITC/Rodamine B R6626, Sigma, St Louis, MO, USA) was added to root canal sealer (Dentsply DeTrey, Konstanz, Germany). The canals were obturated with a sealer–Rhodamine B mixture and ProTaper Universal F2 gutta percha (Dentsply Maillefer) using to the single cone technique. The teeth were kept at 37°C at 100% humidity for 7 days to set the root canal sealer.

Using a microtome (Buehler IsoMet, Buehler, Lake Bluff, IL, USA) with a 0.3-mm diamond disc at 200 rpm under continuous water cooling, 1-mm-thick horizontal sections were obtained from the roots at 3, 5 and 7 mm. Two-dimensional images were obtained from these sections under CLSM. Each sample was mounted on glass slides and examined with CLSM (Nikon Eclipse C1, Nikon Canada, Mississauga, ON, Canada) at a wavelength of 560 to 600 nm and 10× magnification. Three parameters were calculated: maximum penetration depth, penetration area and penetration percentage of root canal sealer. Measurement analyses of these three parameters were performed by a single operator using ImageJ software (version 1.41; National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

All statistical analyses were performed using SPSS Statistics (version 21.0, IBM, Armonk, NY, USA). A Shapiro-Wilk test was used to confirm the normal distribution of the data. The data were analysed using one-way analysis of variance (ANOVA) and post-hoc Tukey tests. The level of statistical significance was set at $P < 0.05$.

Results

Penetration depth of root canal sealer

In the PUI and EDDY groups, the penetration depth of the root canal sealer was not significantly different between sections (Fig 1) ($P > 0.05$). In the CNI group, the penetration depth was significantly higher in the coronal section than the apical section ($P < 0.05$), whereas in the EDDY group, it was significantly higher in the apical section than it was in the CNI and PUI groups ($P < 0.05$). In the apical section, the penetration depth was not significantly different between the CNI and PUI groups ($P > 0.05$), and in the coronal and middle sections, it was not significantly different between the PUI and EDDY groups ($P > 0.05$) (Table 1).

Penetration area of root canal sealer

In the PUI and EDDY groups, the penetration area of the root canal sealer was not significantly different between sections ($P > 0.05$). In the CNI group, it was significantly lower in the apical section ($P < 0.05$). In all sections, the penetration area was not significantly different between the CNI and PUI groups ($P > 0.05$), but in the apical and coronal sections, it was significantly higher in the EDDY group than the CNI group ($P < 0.05$) (Table 2).

Penetration percentage of root canal sealer

In all groups, the penetration percentage of the root canal sealer was significantly higher in the coronal section than the apical section ($P < 0.05$). In the middle and coronal sections, the penetration percentage was significantly higher in the PUI and EDDY groups than the CNI group ($P < 0.05$) (Table 3).

Discussion

To increase the penetration of root canal sealer into the dentinal tubules, it is advisable to activate irrigation solutions using different techniques and devices[2,12]. In the present study, the effect of different irrigation activation systems on the penetration of root canal sealer was investigated.

Scanning electron microscopy (SEM), light microscopy, stereomicroscopy and CLSM are used to evaluate the penetration of root canal sealer[13-15]. As it offers a two-dimensional evaluation, SEM can only assess the dentine surface and cannot give detailed information about sealer penetration[8,16]. It also allows only limited areas of the canal wall to be evaluated due to high magnification[8,16]. The fact that more processing steps are required for sample preparation in the SEM analysis is disadvantageous as this process can lead to loss of the root canal sealer from the dentine surface and therefore the inability to accurately assess penetration[14,17]. In light microscope examination, it is difficult to distinguish sealer from dentine[18]. In this study, CLSM was used to evaluate the penetration of the root canal. In contrast to SEM, CLSM allows the penetration of the root canal sealer to be evaluated in three dimensions, resulting in less artefacts in images than other methods[14]. Tedesco et al[19] reported that CLSM allows better evaluation of the penetration of root canal sealer compared to SEM.

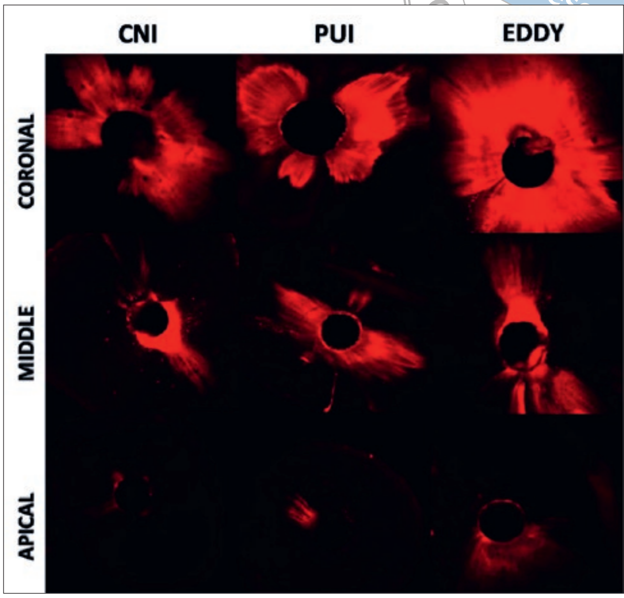

Fig 1 CLSM images displaying sealer penetration in the apical, middle and coronal sections after different means of irrigation activation.

In CLSM analysis, a fluorescent dye is needed to ensure the sealer is visible. Previous studies have reported that Rhodamine B does not change the physical properties of sealer[14,20]. Therefore, in this study, Rhodamine B was added to the sealer to show its penetration into the dentinal tubules under CLSM.

In this study, the penetration percentage of root canal sealer was higher in the coronal sections than the apical sections in all groups. Consistent with these findings, Oliveira et al[21], who used SEM and CLSM, and other studies that used CLSM[7,12,22-24] also found that the

Table 1 Mean ± standard deviation of dentinal tubule penetration depth (mm).

Section	CNI	PUI	EDDY
Apical	0.176 ± 0.119^{Ax}	0.362 ± 0.191^{Ax}	0.431 ± 0.188^{Ay}
Middle	0.284 ± 0.149^{ABx}	0.348 ± 0.202^{Axy}	0.492 ± 0.192^{Ay}
Coronal	0.339 ± 0.170^{Bx}	0.285 ± 0.136^{Ay}	0.434 ± 0.179^{Axy}

Different superscript letters (ABC columns, xyz rows) indicate statistically significant differences between groups ($P < 0.05$).

Table 2 Mean ± standard deviation of dentinal tubule penetration area (mm^2).

Section	CNI	PUI	EDDY
Apical	894.64 ± 543.22^{Ax}	2100.30 ± 1697.03^{Axy}	3136.82 ± 3621.83^{Ay}
Middle	2754.96 ± 2038.90^{Bx}	3309.27 ± 3207.66^{Ax}	4750.42 ± 2189.64^{Ax}
Coronal	3394.29 ± 2146.29^{Bx}	2517.93 ± 1667.04^{Ax}	5177.95 ± 2825.36^{Ay}

Different superscript letters (ABC columns, xyz rows) indicate statistically significant differences between groups ($P < 0.05$).

Table 3 Mean ± standard deviation of dentinal tubule penetration percentage (%).

Section	CNI	PUI	EDDY
Apical	60.80 ± 6.98^{Ax}	63.46 ± 3.96^{Ax}	69.53 ± 5.78^{Ay}
Middle	63.13 ± 6.16^{Ax}	69.80 ± 5.87^{By}	73.40 ± 5.96^{Ay}
Coronal	72.40 ± 8.32^{Bx}	80.20 ± 5.63^{Cy}	80.53 ± 6.35^{By}

Different superscript letters (ABC columns, xyz rows) indicate statistically significant differences between groups ($P < 0.05$)

sealer penetration percentage was higher in the coronal sections than apical sections. The regional differences between apical and coronal divisions can be explained by the increasing complexity of the root canal anatomy, the decrease in the number of dentinal tubules, the narrowing of the dentinal tubules towards the apical, and the increase in tubular sclerosis in the apical part[14,22,25]. Research using SEM analysis also revealed that the apical section contained a greater amount of smear layer than the coronal section after various irrigation activation methods[1,8,26]. Therefore, further removal of the smear layer and debris in the coronal region may have allowed more sealer to penetrate into the coronal tubules[7,27]. Another possible explanation for the regional differences may be that air can be compressed in the dentine surfaces when gutta-percha is inserted into the channel so that the sealer moves in the reverse direction and contact between the sealer and dentine is reduced[14,28].

In the present study, greater sealer penetration was observed in the EDDY group than in the CNI and PUI groups, but there was no significant difference between the PUI and and CNI groups in the apical section. In the EDDY group, the depth and percentage of sealer penetration were higher than in the CNI group in all sections and higher in the PUI group in the apical sections. Penetration depth was greater in the PUI group than in the CNI group in the coronal and middle sections, but there were no significant differences in the apical sections. Donnermayer et al[29] reported that EDDY and PUI were significantly more effective in the removal of calcium hydroxide than CNI. Swimberghe et al[30] found that EDDY resulted in the greatest amount of hydrogel removal and performed better than PUI. Gu et al[24] reported that sealer was higher in the sonic activation group than in the PUI group in the apical sections and the syringe group in all sections. Machado et al[12] reported that in the Endo Activator (EA, a sonic irrigation activation technique) group, sealer penetration was higher than in the PUI group in all sections, and sealer penetration was higher in the EA and PUI groups than in the CNI group in all sections. In contrast to the results of our study, Rödig et al[26] found that there was no difference between the PUI and CNI groups in terms of sealer penetration, and in the apical section the PUI group was superior to the EA group. Grischke et al[31] reported that EA showed similar efficacy with manual irrigation in the removal of residual filling materials by microscopic analysis. Similarly, Bolles et al[7] reported that the penetration of root canal sealer was similar in the EA and needle irrigation groups. Generali et al[23] reported that needle irrigation, EA and the ultrasonic method Irrisafe showed no difference in the penetration of root canal sealer. The differences between the results of these different studies can be explained by differences in volume of irrigant, duration of activation, the power setting of the activator and tip size.

A limitation of the present study is the fact that the root canals were filled immediately after canal preparation because this is not always possible in a clinical setting, and intracanal medicament may be necessary in intermediate sessions. Over the course of several treatment visits, sealer penetration may reduce as it is not possible to remove all the medicament from the root canal[14,32].

Conclusion

Irrigation is crucial for disinfection and the quality of obturation. Within the limitations of this study, it was determined that EDDY provides superior sealer penetration compared to CNI.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Dr Zeliha UĞUR AYDIN designed the study and performed the statistical work; Drs Sevim KOŞUMCU and Büşra MEŞECİ prepared and evaluated the specimens; all authors contributed to the writing of the manuscript.

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