# PAPER DETAILS

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Techniques by Confocal Laser Microscopy Analysis

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DOGAN

PAGES: 274-278

ORIGINAL PDF URL: https://dergipark.org.tr/tr/download/article-file/1547059



# Evaluation of Residual Root Canal Sealer Removal Efficacy of Different Irrigation Activation Techniques by Confocal Laser Microscopy Analysis

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#### ABSTRACT

**Objective:** The purpose of this study was to use confocal laser microscopy analysis to evaluate the effectiveness of conventional needle irrigation (CNI), EndoActivator (EA), and EDDY during endodontic retreatment.

**Methods:** This study included 45 maxillary incisor teeth with a single root and canal. Root canals were prepared with ProTaper Universal files (Dentsply Sirona, Ballaigues, Switzerland) and obturated with labeled sealer mixed with 0.1% Rhodamine B and gutta percha according to single cone techniques. Initial root canal filling material was removed using ProTaper Universal Retreatment files and F4 files. Teeth randomly were divided into 3 groups (n = 15) depending on the activation technique: CNI, EA (Dentsply, Tulsa Dental Specialties, Tulsa, OK), and EDDY (VDW, Munich, Germany). Confocal laser microscopy was used to evaluate the penetration area, depth, and percentage of the residual sealer in the apical, middle, and coronal sections after irrigation activation.

**Results:** In all sections, the EDDY group had a lower penetration area of residual sealer than the CNI group (P<.05). In comparison to the coronal section, the penetration percentage of the CNI and EA groups was lower in the apical section (P<.05). In the CNI group, the penetration depth was higher at the coronal section than at the apical and middle sections (P<.05), and it was higher at the coronal section than at the apical section.

**Conclusion:** Within the limitations of this study, none of the activation systems tested could completely remove the residual sealer. However, the lowest residual sealer was seen after using EA and EDDY.

Keywords: Confocal laser microscopy, EDDY, EndoActivator, residual sealer

#### **1. INTRODUCTION**

Inadequate disinfection and obturation of root canals may adversely affect the prognosis and lead to the failure of endodontic treatment (1). In cases where endodontic treatment fails, usually non-surgical retreatment is preferred as the first treatment option (2). The purpose of the retreatment procedure is to recover the health of the periapical tissues by disinfection, shaping, and obturation of the root canals after the complete elimination of residual filling material (3). It has been reported that if previous root canal filling material is not completely removed by the retreatment procedure, periapical inflammation and destruction of surrounding tissues may occur or may persist when present (4). Irrigation of root canals is an essential part of endodontics (5). Because of the complex anatomy of the root canal, it is recommended to use various activation methods and devices to allow the irrigation solution to contact more surfaces of the canal and to increase antimicrobial activity (6). Although conventional needle irrigation (CNI) is a widely used irrigation technique, the irrigation solution cannot reach the 0-1.1 mm of the needle tip in this technique (7-9). Different irrigation systems have been developed to overcome this disadvantage. EndoActivator (EA; Dentsply, Tulsa Dental Specialties, Tulsa, OK) is a sonic device that uses 2-3 kHz frequencies for the activation of irrigation solutions. Irrigation solutions activated by this device have been reported to reach the root canal system with hydrodynamic

Clin Exp Health Sci 2023; 13: 274-278 ISSN:2459-1459 Copyright © 2023 Marmara University Press DOI: 10.33808/clinexphealthsci.871991



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activation, as well as morphological irregularities such as the lateral canal and apical delta (10). EA is a portable and cordless device with a battery-operated hand motor and a disposable flexible polymer tip of 3 different sizes (15/02, 25/04, and 35/04) (8, 11). EDDY (VDW, Munich, Germany) has been recently introduced and is another sonic device that has a tip made of flexible polyamide that is activated using an air-driven handpiece (5000-6000 Hz) (12).

Compared to scanning electron microscopy (SEM) and histological methods, the confocal laser scanning microscope (CLSM) has the benefit of providing detailed information on root canal sealer penetration under low magnification, such as X50-X100, with the use of a marker fluorescent dye (13). Root canal sealers marked with dyes, such as rhodamine, allow the sealer to be stimulated by certain wavelengths under the CLSM and transformed into a visible spectrum. Thus, the topographic characteristics of the root canal sealer can be evaluated (14). In addition, analyzing the samples at different depths and in a three-dimensional form makes CLSM more sensitive to intratubular penetration measurement than SEM (14). This study aimed to assess the effectiveness of CNI, EA, and EDDY in removing residual sealer using CLSM.

# 2. METHODS

The design of this study was approved by the Bolu Abant Izzet Baysal University Clinical Researches Ethics Committee (No: 2019/55 – date: 11.04.2019). Based on a previous study (13), the sample size was determined (G\*Power 3.1 software; Heinrich Heine University, Dusseldorf, Germany). Forty-five maxillary incisor teeth with a single root/ canal that were extracted for orthodontic and periodontal reasons were included in this study. For each tooth, periapical radiographs were obtained from buccolingual and mesiodistal directions, and root canal anatomy was evaluated. Teeth completed root canal development and without calcification, fractures, resorption, and curvature were included. The teeth were stored at 4 °C in distilled water.

The access cavities were created using diamond round burs (Dentsply Maillefer, Ballaigues, Switzerland). The working length (WL) of the canals was determined to be 1 mm shorter than the length at which the # 10 K-file (Dentsply Maillefer) emerges from the apical foramen. The apical patency was controlled using the # 15 K-file (Dentsply Maillefer). Root canal preparation was carried out using ProTaper Universal (Dentsply Maillefer) rotary files up to size F3. The root canals were irrigated with 2 ml of 2.5% NaOCI (CanalPro; Coltene-Whaledent, Allstetten, Switzerland) after each file. After the preparation, the canals were irrigated with 10 ml 17% EDTA (CanalPro) and 10 ml 2.5% NaOCl, respectively, and dried with a paper point. Root canal sealer was given a 0.1% Rhodamine B addition for the CLSM examination (Batch 121K3688, RITC/Rhodamine B R6626 Sigma, St. Louis, MO, USA) (Dentsply De-Trey, Konstanz, Germany). Using AH Plus root canal sealer with 0.1% Rhodamine B added and ProTaper Universal F3 gutta-percha (Dentsply Maillefer), the root canals were filled using the single cone method. For 7 days,

the teeth were maintained at 37 °C with 100% humidity to allow the root canal sealer to set. All teeth were retreatment with ProTaper Universal Retreatment Files (D1, D2, and D3) and a low-torque motor (VDW Silver; VDW) using the crowndown technique. In the cervical, middle, and apical sections, respectively, D1 (size 30/.09, 550 rpm, 200 g/cm torque), D2 (size 25/.08, 550 rpm, 200 g/cm torque), and D3 (size 20/.07 taper, 250 rpm, 150 g/cm torque) files were used. Root canals were irrigated with 2 ml of 2.5% NaOCl at each file change. The ProTaper Universal F4 file was used for the final apical preparation at WL. Each file was used in only three canals and then excluded from the study. Following the retreatment procedure, the samples were randomly divided 3 groups (n = 15) according to the final irrigation activation method:

**CNI**: The 30-gauge double-sided needle (Fanta Dental, Shanghai, China) was inserted into the canal at a length 1 mm shorter than the WL. The irrigation needle was moved back and forth, and the root canals were irrigated with 2.5% NaOCl in a 3 ml volume for 90 sec.

**EA**: In this group, the root canals were irrigated in 3 cycles with a 3 ml volume of 2.5% NaOCl irrigation solution for 30 sec (1 ml per 30 sec) at a 2 ml/min flow rate. After each irrigation cycle, a flexible polymer tip (25/.04) was inserted into the canal at a distance of 1 mm shorter than the WL and activated at 1000 rpm for 30 sec. with 2-3 mm vertical strokes.

**EDDY:** The irrigation activation procedure was the same as in the EA group. After each irrigation cycle, the polyamide tip (25/.04) was adapted to TA-200 (Micron, Tokyo, Japan) and activated at 6000 Hz. The EDDY tip was placed in the canal to be 1 mm shorter than the WL and activated for 30 sec.

1 mm thick, 3 horizontal sections were obtained from all teeth at 2, 4, and 6 mm levels under continuous water cooling using the microtome with a 0.3 mm diamond disc (Isomet, Buehler Ltd. Illinois, IL, USA) at 200 rpm. Each section was examined with CSLM (Fig. 1), and 3 parameters, including maximum penetration depth, maximum penetration area, and maximum penetration percentage, were calculated by using Image J (version 1.41; National Institutes of Health, Bethesda, MD) on images.

# 2.1. Statistical Analysis

The obtained data were analyzed with the statistical software package SPSS version 20 (IBM Corp., New York, NY, USA). Equality of variance and normal distribution were analyzed using the Shapiro-Wilk test. A one-way ANOVA and posthoc Tukey's tests were used to analyze the data. The level of significance was set at P<.05.

#### Original Article



**Figure 1.** CLSM images representative of residual sealer penetration in apical, middle, and coronal regions after irrigation activation. A; CNI group, B; EA group, C; EDDY group.

## **3. RESULTS**

The penetration area, depth, and percentage values of the residual root canal sealer in dentin tubules after retreatment are shown in Tables 1, 2, and 3. No difference was observed between the apical, middle, and coronal sections in all three groups in terms of the penetration areas (P>.05). The penetration area of residual sealer was lower in the EDDY group than in the CNI group at all sections (P<.05). There was no difference between EA and CNI, EA and EDDY groups in terms of penetration area in all sections (P>.05).

 
 Table 1. Mean penetration area (mm²) and standard deviation of the residual sealer in each group after endodontic retreatment.

	Apical	Middle	Coronal
CNI	3723.72±1423.54 Aa	4694.87±1065.47 Aa	5774.50±1800.34 Aa
EA	2474.68 ± 2192.56 ABa	2886.41±1943.78 ABa	3771.78±2263.92 ABa
EDDY	1102.37±8064.69 Ba	2049.65±1383.18 <sup>Ba</sup>	3168.09±1966.26 <sup>Ba</sup>

In the same column, different superscript uppercase letters indicate a statistically significant difference (P<.05).

In the same row, different superscript lowercase letters denote statistically significant difference (P<.05).

In the CNI group, the penetration depth of the residual sealer was higher at the coronal section than at the apical and middle sections (P< .05). In the EA group, there was no difference in the penetration depth of the residual sealer between all sections (P> .05). In the EDDY group, there was a significant difference between the apical section and the

coronal section (P< .05). At all sections, the penetration depth of the residual sealer was statistically higher (P< .05) in the CNI group than in the EA and EDDY groups, but there was no difference between the EDDY and EA groups (P> .05).

**Table 2.** Mean penetration depth (mm) and standard deviation of the residual sealer in each group after endodontic retreatment.

	Apical	Middle	Coronal
CNI	0.489± 0.026 Aa	0.563±0.019 Aa	0.738±0.013 Ab
EA	0.289±0.080 <sup>Ba</sup>	0.299±0.011 <sup>Ba</sup>	0.256±0.080 <sup>Ba</sup>
EDDY	0.129±0.040 <sup>Ba</sup>	0.234±0.056 Bab	0.306±0.020 Bb

In the same column, different superscript uppercase letters indicate a statistically significant difference (P<.05).

*In the same row, different superscript lowercase letters denote statistically significant difference (P< .05).* 

In the CNI group, the penetration percentage of the residual sealer was significantly lower at the apical section when compared to the coronal section (P< .05). In the EA group, the percentage of penetration at the apical section was significantly low than compared to the coronal section (P< .05). No significant differences were observed between the sections in the EDDY group in terms of the percentage of residual sealer. (P> .05). In all sections, the penetration percentage of the residual sealer was significantly higher (P< .05) in the CNI group compared to the EA and EDDY group, but there was no significant difference between the EDDY and EA groups (P> .05).

**Table 3.** Percentage of residual sealer (%) by section in each group after endodontic retreatment.

	Apical	Middle	Coronal
CNI	19.4±6.02 Aa	23.8±5.49 Aab	28.40±3.35 Ab
EA	11.46±4.37 <sup>Ba</sup>	15.06±4.47 Bab	17.53±4.42 <sup>Bb</sup>
EDDY	10.93±2.63 <sup>Ba</sup>	11.40±4.32 <sup>Ba</sup>	13.86±3.64 <sup>Ba</sup>

In the same column, different superscript uppercase letters indicate a statistically significant difference (P<.05).

*In the same row, different superscript lowercase letters denote statistically significant difference (P< .05).* 

# 4. DISCUSSION

CLSM is an evaluation method that does not require a special procedure for the preparation of samples, such as SEM, and gives quantitative information. Fluorescent dyes are commonly used in CLSM analysis. In this study, Rhodamine B was used at a concentration of 0.1%. Studies in the literature have reported that low-dose Rhodamine B does not affect the properties of the paste (1-3).

In order to remove the residual sealer during the retreatment process, the preparation process should be performed as much as possible with larger files than the initial treatment. However, over instrumentation of the root canals should be avoided in this preparation process (2, 3). In view of this situation, in the present study, the file with a large size (F4)

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from the initial preparation size (F3) was used for the final preparation of the retreatment.

In some studies, it was stated that the use of additional irrigation activation methods (sonic and ultrasonic) did not increase the removal of residual root canal filling materials significantly (15, 16). However, in other studies, it has been stated that passive ultrasonic irrigation provides more clean root canal walls and significantly reduces the amount of residual filling material (7, 17, 18). Grischke et al. (19) reported that passive ultrasonic irrigation (PUI) was more successful than EA, RinsEndo, and CanalBrush activation systems, and syringe irrigation in the removal of residual root canal filling materials. Also in the study, it was reported that EA was more effective than CanalBrush and had similar efficacy with manual irrigation. In the current study, the penetration depth and percentage of residual sealer were significantly lower in the EA and EDDY groups than the CNI group. In terms of the penetration area of residual sealer, only EDDY was found to be significantly superior to CNI. No significant difference was found between the EDDY and EA activation systems in all three parameters. Similar to the present study, Ugur Aydın et al. (20) compared the effect of EDDY, PUI, and CNI on the penetration of root canal sealer and reported that EDDY was superior to CNI in the apical part. However, Urban et al. (12) reported no difference between EDDY, EA, and PUI in removing debris and the smear layer. Differences between study results may be due to methodological differences, including activation system, activation time, sealer, and filling technique.

Although it was not statistically significant in our study, EDDY generally showed lower residual sealer penetration values than EA. In addition, in terms of the penetration area of the sealer, it was observed that EDDY was statistically more effective than CNI and that there was no significant difference between EA and CNI. Urban et al. (12) showed that PUI and EDDY were more effective in removing debris and the smear layer from the root canal compared to manual irrigation, and there was no difference between EA and manual irrigation. Researchers reported (12) that this result could be attributed to acoustic streaming and cavitation. The EA acts at 10000 cpm, or approximately 167 Hz, whereas the EDDY acts at 6000 Hz. As a result, it may be assumed that the irrigant flow rate will be significantly reduced and that the sonic instrument oscillation patterns may be different.

In the present study, while there was no difference in the penetration depth of the residual sealer between all sections in the EA group, the percentage of penetration was found to be significantly lower in the apical section compared to the coronal section. We think that the reason for this inconsistency is that the penetration percentage is more significant since the penetration depth of the residual sealer is measured only from a certain point. This is a limitation due to the nature of this study.

In the results of our study, there was no difference among the sections in any group in terms of the penetration area of residual sealer. In the groups where the difference between the sections was determined in terms of penetration depth and percentage, it was observed that sealer penetration of the apical section was mostly less than the coronal section. This may be due to low initial sealer penetration in the apical region. Because the number and length of the dentin tubules in the apical section is usually less than the coronal section. In addition, due to the occurrence of more tubular sclerosis in the apical section, penetration of the sealer during root canal filling is less in the apical section than in the middle and coronal sections (21, 22).

# 5. CONCLUSION

In the present study, none of the irrigation methods was able to completely eliminate the residual root canal sealer after retreatment. However, since EDDY and EA were more successful than traditional irrigation methods, they can be used as an activating method to effectively remove residual sealer for endodontic retreatment.

**Funding:** The author(s) received no financial support for the research.

**Conflicts of interest:** The authors declare that they have no conflict of interest.

*Ethics Committee Approval:* This study was approved by Bolu Abant İzzet Baysal University Clinical Research Ethics Committee (Approval date: 11.04.2019 and number:2019/55).

Peer-review: Externally peer-reviewed. Author Contribution: Research Idea: ZUA Design of the study: ZUA, DA Acquisition of data for the study: SKA, BM Analysis of data for the study: ZUA, SKA, BM Interpretation of data for the study: ZUA, TDÇ Drafting the manuscript: SKA, BM, TDÇ Revising it critically for important intellectual content: ZUA, DA Final approval of the version to be published: ZUA

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**How to cite this article:** Aydın ZU, Altunbaş D, Koşumcu Akdere S, Meşeci B, Doğan Çankaya T. Evaluation of Residual Root Canal Sealer Removal Efficacy of Different Irrigation Activation Techniques by Confocal Laser Microscopy Analysis. Clin Exp Health Sci 2023; 13: 274-278. DOI: 10.33808/clinexphealthsci.871991