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Effect of Different Irrigation Activation Methods on the Dentinal Tubule Penetration of Dyed Sodium Hypochlorite: Rhodamine B vs. Fluorescein Sodium Salt

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Abstract

Background: This study aimed to observe the penetration of sodium hypochlorite (NaOCl) into the dentinal tubules (DTs) with confocal laser scanning microscopy (CLSM) using fluorescein sodium salt (FSS) and Rhodamine B.

Methods: Six groups of 120 freshly extracted human maxillary central incisors were randomly divided. Both FSS and Rhodamine B were used in 3 groups (n: 20): control group, sonic irrigation (SI), and passive ultrasonic irrigation (PUI). The irrigation solution amounts and total duration were the same in all groups. Using CLSM, transverse sections (2-, 4-, and 6 mm distances to the root apex) were examined. Both the percentage (%) and maximum depth (μm) of NaOCl penetration were calculated.

Results: There was no statistically significant difference in penetration percentage (PP) and maximum depth of penetration (MPD) between Rhodamine B and FSS ($P=.635$, and $P=.869$, respectively). The maximum PP of NaOCl was seen in the 6-mm sections across all groups, including both Rhodamine B and FSS. In the same sections with 2 dyes, however, no statistically significant changes were seen between the irrigation groups. In the SI and PUI groups, a significantly higher MPD was detected with Rhodamine B in 4-mm sections than 2-mm sections. The SI group exhibited significantly more MPD than control group in the 4-mm section with Rhodamine B and FSS.

Conclusion: The FSS can be safely used in penetration studies into the DT. The SI and PUI did not significantly enhance the irrigation solution's penetration into the DT compared to conventional endodontic irrigation.

Keywords: Confocal laser scanning microscopy, fluorescein sodium, rhodamine b, root canal irrigants

What is already known on this topic?

- *Confocal laser scanning microscopy is commonly used to evaluate irrigation solution and root canal sealer penetration in dentinal tubules.*
- *Rhodamine B has been frequently preferred as a fluorescent dye in studies evaluating dentinal tubule penetration, although its moisture sensitivity may influence measurement accuracy.*
- *The evaluation of dentinal tubule penetration using alternative fluorescent dyes, such as fluorescein sodium salt, has not been comprehensively sudied.*

What this study adds on this topic?

- *This study presents a comparative assessment of fluorescein sodium salt (FSS) and Rhodamine B for evaluating dentinal tubule penetration, contributing additional data on the suitability of FSS as an alternative fluorescent dye in such analyses.*
- *This study provides insights that sonic irrigation and passive ultrasonic irrigation may offer only minimal improvements in sodium hypoclorite penetration into dentinal tubules over conventional irrigation.*
- *The results of this study contribute to a more comprehensive understanding irrigant*

INTRODUCTION

Minimizing microorganisms existing in the root canal system and avoiding any recontamination are the basic goals of root canal treatment.[1] The quantity of these pathogens is reduced by chemomechanical preparation. The anatomy of a tooth is so complicated, though, that it is very hard to eliminate all of them, particularly those positioned in the apical region.[2] Peters et al[2] declared that a maximum of 65% of the surfaces of root canals could be shaped, independent of the instrumentation method used. Sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) are

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penetration dynamics across specific depths of the root canal system (2, 4, and 6 mm from the apex), assessed using two diiferent fluorescent dyes.

the most popular chemical irrigants. They can penetrate surfaces that are mechanically untouchable, eradicate microorganisms, detach the smear layer (SL), and flush debris.[3]

The SL contains organic and inorganic tissue that form after cavity preparation or mechanical instrumentation, seals the orifice of the dentinal tubules (DTs), and coats dentin.[4] It can decrease the penetration of antibacterial dressings into the DT and alter the sealing properties of root canal fillings, thereby protecting bacteria within the DT.[4] Despite some disagreement about whether the SL must be eliminated or not, most researchers agreed upon removing it prior to the onset of the root canal filling.[5] To be sure that the root canal system is thoroughly cleaned and disinfected during the chemical preparation process, various types of irrigant delivery techniques for agitation have been suggested. It is not feasible to distribute solutions beyond the irrigation needle's tip using conventional endodontic irrigation;[6] therefore, it disinfects the coronal and middle regions effectively; however, the apical region much less so.[3] In addition, various delivery techniques have been proposed to enhance the effectiveness of the irrigation process. The EDDY (VDW GmbH, Munich, Germany) is a sonic irrigation (SI) instrument powered by an air scaler operating at around 5000 Hz. Polyamide tips are used to prevent dentin cutting and change the anatomy of root canal(s). EDDY was determined to be equally effective as passive ultrasonic irrigation (PUI) in the removal of the SL from the apical region of root canals (straight and curved).[7] Designed as a cordless PUI tool with a frequency of 40 kHz, EndoUltra (Vista, Racine, WI, USA) generates cavitation and acoustic streaming.[8] Though this technique was substantially more effective than conventional endodontic irrigation, the SL was unable to be entirely removed from the apical region of the root canal.[7] The SI and PUI efficiencies have been extensively compared in the literature; however, the findings remain inconclusive.[7,9]

Fluorescent dyes are used in studies where DT penetration is observed with confocal laser scanning microscopy (CLSM). Rhodamine B was preferred in a significant part of the studies.[9-11] Rhodamine B is extremely sensitive to moisture. The lowest moisture content in the DT may cause inaccurate results as the dye penetrates deeper.[12] Studies using a different dye[12] or double dye technique[13] are highly limited. Since it is generally harmless, fluorescein sodium salt (FSS) has been used for many years in medicine for medical diagnosis. It often used in ophthalmology and vascular neurosurgery procedures as a fluorescent dye. The FSS is slightly soluble in water, highly soluble in ethanol. Rhodamine B exhibits a red fluorescence, whereas FSS displays a green fluorescence when observed under confocal microscopy.

Generally, the success of techniques has been assessed by observing the penetration of root canal sealer (RCS) into the DT by using CLSM.[5,9,10] Although a few studies measured the amount of penetration of irrigation solutions, data regarding this are still limited.[11,14]

The objective of the current investigation was 2-fold: first, to analyze the differences in NaOCl penetration into DTs using 2 different dyes (Rhodamine B and FSS); second, to evaluate how SI and PUI techniques influence the penetration of NaOCl into DTs.

MATERIAL AND METHODS

A minimum of 18 teeth per group is required, according to data from a similar prior study using G*Power 3.1 (Heinrich Heine University, Dusseldorf, Germany). Thus, 20 teeth were included for each group. With the approval of the Clinical Research Ethics Committee of İstanbul University Faculty of Dentistry (Date: 08.02.2022; Approval no: 2022/08), 120 human upper central incisors (length: 22.5 ± 0.5 mm), which were extracted because of periodontal bone loss, were included in the present study. All procedures adhered to the STROBE guidelines for observational original research studies. Written informed consent was obtained from all participants prior to tooth extraction, allowing the use of their extracted teeth for research purposes.

Each tooth had 1 root canal, mature, straight roots, and no root canal treatment, calcification, cracks/decays/fractures on the root surface or external or internal root resorption.

The anatomical structure of teeth was evaluated by recording periapical radiographs obtained from mesiodistal and buccolingual directions. To prevent contamination, the samples were placed in 0.5% chloramine-T solution (Merck KGaA, Darmstadt, Germany) for a period of 2 days and were kept until they were required in a saline solution.

A diamond disk was used to prepare the teeth such that the total length was standardized at 21 mm. The samples were not fully decoronated in order to preserve the crown portion, which serves as a reservoir for the irrigation solutions. A size 10 K-file (Mani Inc., Tochigi Ken, Japan) was inserted into each tooth until it reached the apical foramen in order to determine the working length with a dental operation microscope (OPMI Pico, ZEISS, Oberkochen, Germany). One millimeter was subtracted and recorded from the measured length. To imitate in-vivo factors, the root surfaces of samples were coated in polyvinylsiloxane and coated with hot glue to prevent fluid passage via the apical foramen while undergoing chemomechanical preparation. The root canals were shaped using ProTaper Universal rotary files (Dentsply Maillefer, Ballaigues, Switzerland) in a modified crown-down technique, with the master apical file being up to F4. To maintain apical patency and enhance irrigation solution penetration, the recapitulation was performed following each rotary file, utilizing a K-file of size 10. After each instrument, the canals were irrigated with 1 mL of 5.25% NaOCl. Throughout the process, the root canal system were continuously filled with solution. A 5-mL disposable plastic syringe (Max-i-Probe; Dentsply Rinn, Elgin, IL, USA) with a 28-gauge side-vented needle (Ultradent Products Inc., South Jordan, UT, USA) was used for the transportation of all irrigation solutions into the canal.

For fluorescence under CLSM, fluorescent rhodamine B isothiocyanate (Bereket Chemical Industry, Istanbul, Türkiye) and FSS (CARLO ERBA Reagents GmbH, Emmendingen, Germany) were used. Rhodamine B was prepared at a concentration of 0.1% (w/v), following the procedure described by Akçay et al.[11] The dilution rates of intravenous injections used for diagnostic fluorescein angiography were taken into account, and 500 mL of 5.25% NaOCl was combined with 100 mg FSS. To prevent the dyes from being affected by light, the solutions were kept in light-proof boxes at room temperature and only taken out when needed for use.

Following chemomechanical preparation, the tooth were numbered. Using the online Research Randomizer application (https://www.randomizer.org), tooth were randomly assigned into the 6 groups (n=20). Each dye was used in 3 groups: 1 control group and 2 experimental groups. All time measurements in the study were made using a stopwatch.

In the control group, a needle was placed 2 mm short of the working length. A 17% EDTA solution (3 mL) was applied to the root canal for 60 seconds, followed by the administration of 5.25% NaOCl (3 mL) for 60 seconds.

In the SI group, a 25/0.04 taper polyamide tip from EDDY (VDW GmbH, Munich, Germany) was attached to a SONICflex 2003 airscaler (Kavo, Genova, Italy). The tip was placed 2 mm short of the working length and operated at maximum intensity with up-and-down movements exceeding 4 mm (frequency: 6000 Hz),[15] but not exceeding the working length. The 17% EDTA solution was activated 3 times for a total of 20 seconds, with 1 mL of the irrigation solution applied during each activation cycle. The same protocol was used to activate the labeled 5.25% NaOCl.

In the PUI group, EndoUltra handpiece (Vista, Racine, WI, USA) with a non-cutting nickel-titanium tip (15/0.02) was used at 40 kHz, placed 2 mm shorter than working length. Using the intermittent flush technique, 17% EDTA was activated 3 times for 20 seconds.[9] During each activation cycle, 1 mL irrigation solution was applied. Labelled 5.25% NaOCl was activated with the same protocol.

To dry the root canals, paper points were utilized and each sample was sectioned perpendicular to its long axis using a slow-speed, water-cooled 0.3-mm microtome saw (Isomet Buehler, Lake Bluff, IL, USA), into 1-mm-thick sections at points 2-, 4-, and 6-mm from the root apex. Silicon carbide abrasive paper was used to polish all surfaces to be examined with a solid laser (FSS: 525 nm, Rhodamine B: 546) using CLSM (Leica TCS SPE, Leica Microsystems, Wetzlar, Germany). At 10 magnification, the samples were examined through a dry lens with a numerical aperture of 0.3. The Leica Application Suite Advanced Fluorescence 3.3 software (Leica Microsystems) was used to capture and analyze images. A measuring program called ImageJ (https://imagej.nih.gov/ij/) was used to calculate the areas along the root canal where NaOCl penetrated. The measured value was multiplied by 100 and divided by the diameter of the root canal wall to determine the penetration percentage (PP) of NaOCl. The maximum depth of penetration (MPD) of NaOCl values were noted during the CLSM laboratory stage if only 1 image was sufficient for the calculation. If a single image could not accurately figure out the MPD of NaOCl along the root canal circumference, CorelDRAW Graphics Suite X5 (Corel Corporation, Ottawa, Canada) was used to acquire and process additional partial images. To determine the MPD of NaOCl, these files were transferred into the ImageJ program. The measurements were done by 2 researchers independently at various periods and without previous knowledge of each other's results.

Before statistical analysis, experimental groups and dyes were coded with different names. In this way, it was achieved that the statistician was blinded to the groups. The IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. The Kolmogorov–Smirnov and Shapiro–Wilk tests were used to determine whether the parameters were suitable for the normal distribution. The parameters were shown to be suitable for normal distribution. The effects of the irrigation group, dye and cross-section interactions on PP and MPD were examined using a 3-way analysis of variance. Tukey's Honestly Significant Difference test (HSD) was used for the post hoc analyses. Statistical significance was calculated at $P < .05$.

RESULTS

A representative CLSM image of a sample from the control, SI, and PUI groups in the 2-, 4-, and 6-mm sections for FSS is presented in Figure 1.

Dyes (Rhodamine B vs. Fluorescein Sodium Salt)
There was no statistically significant difference in PP and MPD between Rhodamine B and FSS ($P = .635$, and $P = .869$, respectively). The results indicate that both dyes behaved similarly in terms of their effects on the metrics of PP and MPD, providing consistent data across the groups.

Sections (2-, 4-, and 6 mm)
Penetration percentage: Statistically significant differences were observed between the 2-, 4-, and 6-mm sections in all groups ($P < .05$), suggesting a varying distribution of the property across different depths (Table 1).

Within the 6-mm section of all groups, NaOCl irrigation demonstrated the most significant PP for both Rhodamine B and FSS, although no significant differences were observed between the irrigation groups in the same sections.

Maximum Depth of Penetration: Higher MPD was detected in the 6-mm sections across all groups, indicating a depth-dependent trend. The SI group showed a significant increase in MPD in the 4-mm sections when compared to the control group ($P = .034$) for both dyes (Table 2).

Irrigation Groups (Sonic Irrigation, Passive Ultrasonic Irrigation, and Control)
In terms of irrigation techniques, Tukey HSD analysis revealed that the SI group exhibited significantly higher MPD than the control group in the 4-mm section for both Rhodamine B and FSS ($P = .034$ and $P = .038$, respectively). However, no significant differences were found between control and other irrigation groups in the same sections.

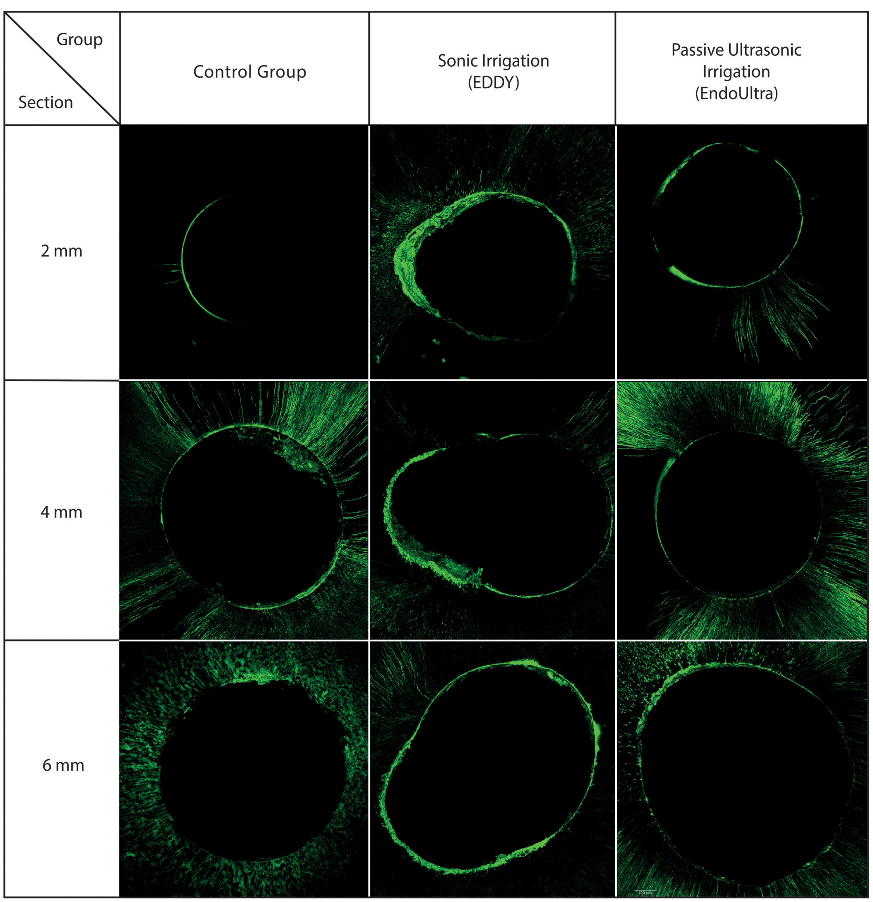

Figure 1. Representative confocal laser scanning microscopic images of fluorescein sodium salt from experimental groups at 2-, 4-, and 6-mm sections.

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Table 1. Penetration Percentage Results for Control, Sonic Irrigation, and Passive Ultrasonic Irrigation Groups for Rhodamine B and Fluorescein Sodium Salt at 2-, 4-, and 6-mm Sections

	Penetration Percentage (%)	Control Group Mean ± SD	Passive Ultrasonic Irrigation Mean ± SD	Sonic Irrigation Mean ± SD	*P*
Fluorescein sodium salt	2 mm	30.75 ± 24.67^{Aa}	35.05 ± 17.93^{Aa}	41.65 ± 21.72^{Aa}	.283
	4 mm	56.70 ± 20.36^{Ab}	71.50 ± 17.57^{Ab}	66.20 ± 21.90^{Ab}	.069
	6 mm	81.60 ± 16.11^{Ac}	90.25 ± 14.26^{Ac}	89.10 ± 17.12^{Ac}	.183
	P	.000*	.000*	.000*	
Rhodamine B	2 mm	29.4 ± 22.64^{Aa}	32.75 ± 15.07^{Aa}	39.10 ± 20.35^{Aa}	.291
	4 mm	55.0 ± 20.42^{Ab}	69.05 ± 17.07^{Ab}	65.35 ± 21.63^{Ab}	.075
	6 mm	82.50 ± 15.94^{Ac}	91.35 ± 13.11^{Ac}	89.85 ± 15.07^{Ac}	.137
	P	.000*	.000*	.000*	
Fluorescein sodium salt—Rhodamine B	2 mm	.858	.663	.704	
	4 mm	.793	.657	.902	
	6 mm	.860	.801	.884	

3-way ANOVA Test.
Lowercase letters in the columns represent differences between sections (a-b-c).
**P* < .05.

DISCUSSION

Microorganisms can easily penetrate the DT and play a crucial function in the beginning of persistent infections.[2] It is possible to gain entry to the DT with irrigation solutions and RCSs when the SL has been eliminated from the root canal surfaces.[5] The antibacterial activity and blocking effect make it a potential tool for eliminating or embedding germs inside the DT.[4]

Confocal laser scanning microscopy has been used in endodontics to assess the penetration of irrigation agents and RCSs into the DT and visualize microorganisms in the DT. It allows for the examination of the structure of DT, which cannot be examined from a single section owing to its complex anatomy, using a 3-dimensional image created by making multiple cuts from a certain depth.

The dye must be used to visualize the penetration of RCSs or irrigation solutions into the DT under CLSM. Rhodamine B has a powerful affinity for moisture. The root canal remains moist because continuous irrigation is performed during the mechanical preparation, EDTA is preferred before NaOCl during the final irrigation, and the root canal is not dried between these procedures. FSS, which is known to be slightly soluble in water, was preferred for minimizing this disadvantage of Rhodamine B. It is safely administered intravenously in various surgical operations due to its fluorescent properties. In this study, Rhodamine B and FSS were compared with each other. The reason for this is that Rhodamine B has been used in most of the studies done so far.[5,9-11,16-22] Neither Rhodamine B nor FSS showed a statistically significant difference in the present study. This result suggests that CLSM studies can use FSS as a fluorescent dye.

Table 2. Maximum Penetration Depth Results for Control, Sonic Irrigation, and Passive Ultrasonic Irrigation Groups for Rhodamine B and Fluorescein Sodium Salt at 2-, 4-, and 6-mm Sections

	Maximum Penetration Depth (μm)	Control Group Mean ± SD	Passive Ultrasonic Irrigation Mean ± SD	Sonic Irrigation Mean ± SD	*P*
Fluorescein sodium salt	2 mm	251.6 ± 163.61^{Aa}	314.05 ± 120.14^{Aa}	333.8 ± 131.57^{Aa}	.161
	4 mm	367.9 ± 170.39^{Aa}	438.3 ± 173.53^{ABa}	499.0 ± 139.46^{Bb}	.045*
	6 mm	603.0 ± 268.36^{Ab}	691.85 ± 253.42^{Ab}	760.3 ± 247.32^{Ac}	.160
	P	.000*	.000*	.000*	
Rhodamine B	2 mm	231.7 ± 160.63^{Aa}	279.25 ± 137.63^{Aa}	306.95 ± 126.39^{Aa}	.247
	4 mm	365.15 ± 164.8^{Aa}	457.9 ± 171.49^{ABb}	498.05 ± 163.45^{Bb}	.042*
	6 mm	612.95 ± 250.55^{Ab}	738.45 ± 261.5^{Ac}	799.3 ± 225.87^{Ac}	.059
	P	.000*	.000*	.000*	
Fluorescein sodium salt—Rhodamine B	2 mm	.700	.400	.514	
	4 mm	.959	.721	.984	
	6 mm	.904	.570	.606	

3-way ANOVA Test.
Capital letters in the lines represent differences between irrigation groups (A-B-C).
Lowercase letters in the columns represent differences between sections (a-b-c).
**P* < .05.

In a significant part of the penetration studies, the effectiveness of different agitation methods, irrigation solutions, root canal filling techniques, and RCSs has been evaluated, mostly using a single staining method.[9,10,14,16,17,23,24] In addition, the findings of this study are extremely similar to the previous investigations that assessed the same irrigation agitation techniques on NaOCl penetration, which may indicate that FSS is an ideal dye to be used in DT penetration studies.[20,21]

In this study, DT penetration was evaluated by labeling the irrigation solution (NaOCl) with 2 different fluorescent dyes. Gu et al[21] claimed that the patterns of RCS and irrigation solution penetration were significantly correlated for all agitation techniques. Although numerous studies have assessed the efficacy of irrigation solutions, delivery techniques, and agitation methods, most have evaluated penetration by labeling RCSs rather than directly analyzing the irrigant's infiltration into DTs.[5,9,10,16,17,23,25-27] However, several factors—including temperature, humidity, dentin tubule composition, and the chemical and physical properties of RCS—can influence their ability to penetrate DTs.[23] Ozasir et al[24] reported that different RCSs displayed statistically significant differences when using the same irrigation method. Sodium hypochlorite was labeled in this study to minimize the potential negative impacts of the chemical and physical features of RCSs. Since the penetration of NaOCl is affected by factors, such as temperature, contact time, and concentration ratio, these values were kept equal in all groups.[28] Also, the crowns were not removed to create a reservoir cavity that would allow the irrigation solutions to be refreshed and exchanged.[9]

Penetration studies were performed by evaluating 2 or 3 sections obtained from different parts of the root using CLSM. The distance between these sections and the root apex may also differ. In these studies, the section closest to the root apex was 2 mm.[5,9,17,19,29] However, some studies used 1-[10,20,25], 3-[16,26], or 4-mm[18] sections. The second section closest to the root apex was obtained at 3[10,20], 4[9], 5[5,17,19,26,30], or 8[11,18] mm. Additionally, some studies did not report the distances from which the cross-sections were obtained.[22,27] In this study, sections were obtained from 3 different depths for a more comprehensive analysis. The evaluation of the penetration efficiency with sections obtained from different depths may be one of the reasons for the different results in CLSM studies.

The combined usage of NaOCl and EDTA is efficient for SL removal.[4] As it is known that anatomical challenges of root canal anatomy exist, several irrigant delivery systems and agitation techniques were utilized to test that irrigation solutions can reach all surfaces and increase their effectiveness.[4] In this study, the efficacy of SI (EDDY) and PUI (EndoUltra) was compared by examining slices (2-, 4-, and 6-mm distance to the root apex). When the PPs were examined in every section, Rhodamine B and FSS exhibited no statistically significant differences among the tested groups. When the MPDs were assessed in every section, statistical analysis revealed no significant differences between the groups were labeled with Rhodamine B and FSS at 2- and 6-mm. At 4-mm, the MPD of EDDY was was much greater than that of the control group with 2 dyes. Uğur Aydın et al[31] examined the effects of EDDY, PUI, and conventional needle irrigation on RCS penetration and reported that RCS penetration was superior in the apical section (3 mm) in the EDDY group compared to that in the conventional endodontic irrigation group. Furthermore, penetration of RCS was similar in the EDDY and PUI groups in the 5- and 7-mm sections. Uslu et al[27] evaluated 5 different irrigation agitation methods and reported that PP and MPD of RCS were similar to conventional endodontic irrigation with PUI and EDDY, except at the coronal section. A possible explanation for this is the up-and-down movement during irrigation with EDDY. Another possible explanation may be the low taper of EndoUltra's NiTi tip (0.02). The shock-wave enhanced emission photoacoustic streaming and photon-induced photoacoustic streaming (PIPS) approaches exhibited the lowest and comparable SL reduction scores relative to PUI and EDDY in the apical area (the depth of the sections was not declared). Declared results are in agreement with this research. It is important to note that the MPD of EDDY at 4-mm was significantly higher than control group.

The PP of NaOCl into the DT was substantially greater at 6-mm than that at 2- and 4-mm in all groups with Rhodamine B and FSS. Also, ıt was significantly higher at 4-mm than that at 2-mm. These findings coincide with those of previous studies.[11,19,25] The MPD of NaOCl into the DT was substantially greater at 6-mm than that at 2- and 4-mm in all groups with Rhodamine B and FSS. This might be as a result of the DT in the apical area being smaller in diameter and less numerous, which prevents root canal irrigants from penetrating.[32] Furthermore, the dentin in the root canal's apical area was more commonly sclerosed.[32] However, it should be noted that the number and distance to the apical sections may vary in these studies.

To the best of knowledge, the penetration of irrigation agents into the DT has been evaluated in 8 studies with CLSM. Llena et al,[33] Akçay et al,[11] Gu et al,[21] and Uzunoglu-Özyürek et al[20] used NaOCl to evaluate penetration. Giardino et al,[34] Küçük and Kermeoglu,[19] and Keskin et al[35] utilized various irrigation solutions. Llena et al[33] examined the effects of PUI on DT penetration of 5.25% NaOCl, 2% chlorhexidine (CHX), and saline solutions. The NaOCl and CHX showed similar penetration rates in all the sections. Küçük and Kermeoğlu[19] evaluated the effectiveness of 3 different irrigation solutions (QMix 2 in 1 [Dentsply Tulsa Dental, Tulsa, OK], CHX, and Irritrol Two-In-One [Essential Dental Systems, South Hackensack, NJ]) and 3 different irrigation techniques on DT penetration. It was reported that penetration rates may vary as a result of using the same irrigation solution with different irrigation techniques. Keskin et al[35] used CHX and Irritrol with the EDDY, and PIPS techniques. Irritrol Two-In-One showed a greater depth of penetration than CHX in the conventional endodontic irrigation group. However, CHX provided greater penetration rates when used in combination with the EDDY and PIPS techniques. Based on these results, more detailed studies on this subject may provide insights into the determination of irrigation techniques according to the irrigation solution used.

Most research assessing DT penetration using CLSM has focused on PP and MPD. Nonetheless, these investigations include some drawbacks. Prominent instances of these shortcomings include using on a single reference point in MPD measurement, the absence of standardized protocols across all samples, and the inadequate replication of the tissue environmental factors. Additionally, extracted teeth may present limitations such as surface alterations due to the extraction process, dehydration, or changes over time, which could affect the accuracy of the confocal microscope analysis.

According the findings of this study FSS can be safely used in penetration studies into the DT. In comparison to conventional endodontic irrigation, the penetration of irrigation solutions into the DT was not substantially enhanced by SI and PUI.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul University Faculty of Dentistry (Date: 08.02.2022; Approval number: 2022/08).

Informed Consent: Written informed consent was obtained from the participants who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

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