Received: 14 November 2022 | Accepted: 19 April 2023
DOI: 10.1002/jemt.24339

RESEARCH ARTICLE

MICROSCOPY RESEARCH & TECHNIQUE WILEY

Evaluating the effect of different irrigation activation techniques on the dentin tubules penetration of two different root canal sealers by laser scanning confocal microscopy

Fatma Kaplan[1] | Ali Erdemir[2]

[1]Department of Endodontics, Faculty of Dentistry, Bezmialem Vakif University, Istanbul, Turkey

[2]Department of Endodontics, Faculty of Dentistry, Kirikkale University, Kirikkale, Turkey

Correspondence
Fatma Kaplan, Department of Endodontics, Faculty of Dentistry, Bezmialem Vakif University 34093, Istanbul, Turkey.
Email: fatmakpln.91@gmail.com

Funding information
TUBITAK, Grant/Award Number: 318S243

Review Editor: Paolo Bianchini

Abstract

Purpose: The aim of this study is to evaluate the effect of different irrigation activation techniques on the dentin tubules penetration of two different sealers by confocal laser scanning microscope (CLSM).

Methods: A hundred premolar teeth were used in this study. The root canals were shaped and irrigated with 5% NaOCl and 17% EDTA using the following final irrigation activation techniques in each group; Group1: Conventional Syringe Irrigation (CSI), Group2: Passive Ultrasonic Irrigation (PUI), Group3: Apical Negative Pressure (EndoVac), Group4: Er:YAG laser and Group5: Er,Cr:YSGG laser. Then the teeth were separated two subgroups according to sealers (AH-Plus and Totalfill-BC). Horizontal sections were obtained at 2, 5, 8 mm distance of apex. Images were obtained with CLSM and the penetration areas of sealers were calculated with four different dentin tubule penetration assessment techniques. The data were statistically analyzed with Kruskal Wallis and Mann Whitney *U* tests.

Results: No significant difference was observed between the sealers ($p > .05$). EndoVac, Er:YAG and Er,Cr:YSGG laser activation groups were observed to have a significantly higher mean penetration depth, penetration percentage and penetration area than the Control group. There was a significant difference between all regions in all penetration parameters ($p < .05$).

Conclusions: While the use of resin or bioceramic-based root canal sealers did not affect dentin tubule penetration, the use of activation techniques positively affect the dentin tubule penetration. The average tubule penetration and penetration area assessment techniques are suitable methods for the investigation of dentinal tubule penetration.

Research Highlights

It can be stated that the use of resin or bioceramic based root canal sealers does not affect dentin tubule penetration and the use of irrigation activation techniques during removal of the smear layer positively affects dentinal tubule penetration. In addition, it has been determined that the average tubule penetration and penetration area assessment techniques are suitable methods for the investigation of dentinal tubule penetration.

KEYWORDS
confocal microscopy, dentinal tubule penetration, irrigation activation, laser

1 | INTRODUCTION

In endodontic literature, it was reported that pulpal and periapical diseases occur as a result of bacteria invading the main root canal space and dentinal tubules (Kakehashi et al., 1965; Nair et al., 2005). Therefore the goals of endodontic therapy are to completely eliminate pulp tissue, inorganic and organic debris, microorganisms and their by-products from the all root canal system (Ma et al., 2011). For successful root canal treatment, these products should be removed mechanically and chemically from the root canal system. Mechanical or chemical cleaning of root canals is not enough alone. The efficiency of irrigation solutions depends on various factors such as the presence of a complex root canal systems, the penetration depth of the irrigation solutions in the dentinal tubules and whether or not the irrigation solution activation is used (Ghorbanzadeh et al., 2016).

In spite of insufficient distribution of irrigation solutions, conventional syringe irrigation is stillpracticed widely. It has been shown that the irrigation solutions can be distributed only 1 mm distance the tip of the needle (Ls et al., 2009; Ram, 1977). To increase the distribution and the penetration depth of irrigation solutions into the dentinal tubules, different irrigation activation techniques have been developed, including the brushes, manual dynamic activation, sonic and ultrasonic systems, apical negative pressure and laser activation techniques. Passive ultrasonic irrigation (PUI), is activates the irrigation solution by acoustic streaming and microcavitation, in this way it allows the delivery of irrigation solution up to the working length of the root canal unlike conventional endodontic needle (Munoz & Camacho-Cuadra, 2012). Apical negative pressure irrigation is an alternative technique for the delivery of irrigation solution inside the root canal that was recommended in order to minimize the risk of irrigant extrusion through the apical foramen (Gutmann et al., 2014; Nielsen & Baumgartner, 2007). Erbium: yttrium-aluminum- garnet (Er:YAG) and erbium, chromium: yttrium–scandium–gallium–garnet (Er,Cr:YSGG) laser has been reported to be effective in irrigation activations (De Groot et al., 2009). Photon-induced photoacoustic streaming (PIPS) is a novel laser agitation technique used with Er:YAG laser. PIPS with a deep photoacoustic shock wave allows 3-dimensional motion of irrigation solutions (Peeters & Suardita, 2011). A diacritic feature of this method is the ability of the fiber optic tip to position only in the pulp chamber (DiVito et al., 2012). It has been reported that Er,Cr:YSGG laser is capable of removing the smear layer and developing root canal disinfection without causing damage to periapical tissues (Peeters & Suardita, 2011; Peters et al., 2011). In recent research, radial firing tip (RFT3) is laser activation used with Er,Cr:YSGG laser (Bolhari et al., 2014; Peeters et al., 2015).

Subsequent to an efficient chemomechanical preparation, three-dimensional obturation of the root canal system is required for successful endodontic treatment (Mamootil & Messer, 2007; Stoll et al., 2005). The use of a sealers is essential, as it establishes a connection between the core filling material and the radicular dentin (Nielsen et al., 2006). The penetration of sealer into dentinal tubules is a required, thus it evolves the connection of sealer with dentine and increasing of the sealing ability (Mamootil & Messer, 2007). Due to its antibacterial effect, the penetration ability of root canal sealers into the dentinal tubules may also enable prevent of the colonization of residual bacteria and reinfection of the root canal system (Bouillaguet et al., 2008).

Epoxy resin-based root canal sealers, principally AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany) is commonly used for comparison because of its good physicochemical features and adaptability to the root canal dentine (Garrido et al., 2010; Marciano et al., 2011; Marin-Bauza et al., 2010). Bioceramic-based root canal sealers have advantageous biological and physical properties and are so widely used. It was showed that bioceramic-based sealars have a high tubular penetration capacity and therefore make them suitable for use in obturation procedures (McMichael et al., 2016). TotalFill BC (FKG Dentaire, La-Chaux-de-Fonds, Switzerland) is a bioceramic-based sealar with high radiopacity (Aydın et al., 2019).

In the field of endodontics, four different dentin tubule penetration assessment techniques have been developed to examine the penetration ability of root canal sealars into dentinal tubules. Some researchers have used simple techniques that require measurement such as maximum penetration or average penetration depth, while others have used more advanced techniques that require measurement and calculation such as percentage penetration or penetration area. In addition, some researchers used a single technique for evaluation, while others used several techniques together. But so far, there has been no study in the literature that uses four different techniques and evaluates them.

The aim of this study is to evaluate the effect of different irrigation activation techniques on the dentin tubules penetration of resin and bioceramic-based root canal sealers by laser scanning confocal microscopy. The second aim is to evaluate the effect of different dentin tubule penetration assessment techniques used in endodontic literature.

2 | MATERIALS AND METHODS

Ethics Committee Approval Certificate dated June 27, 2018 and numbered 2018.06.12 of the Clinical Research Ethics Committee of

Kirikkale University was obtained for this study. Sample size was calculated at the significance level of 0.05 and power of 0.95 using G * Power v3.1 (Heinrich Heine, Universität Düsseldorf).

One hundred freshly extracted single root and single canal mandibular premolar teeth, due to orthodontic or periodontal reasons, were used in this study. The calculus and extraneous tissue were cleaned with periodontal curettes and the teeth were kept in 0.1% thymol solution at 4°C until used. The teeth length was standardized and the access cavities were prepared. A #15-K type file was inserted into the root canal until it could be seen through the apical foramen, and the working length was established by subtracting 1 mm from this length. The root canals were shaped to F4 # with Protaper Universal rotary system and irrigated with 2 mL 5% NaOCl between each file. The teeth were randomly divided into five main groups of 20 teeth, according to the final irrigation activation methods.

Group 1 (Conventional Syringe Irrigation, CSI): 3 mL 5% NaOCl and 3 mL 17% ethylene diamine tetra acetic acid (EDTA) were used for 30 s per irrigation solution. A 30-gauge needle tip was placed into the root canal within 1 mm of the working length and moved with up and down motion during the irrigation process.

Group 2 (PUI): During the irrigation with 3 mL 5% NaOCl and 3 mL 17% EDTA, activation protocol were achieved with Ultrasonic device (Minipiezon; EMS, Milano, Italy). The stainless steel ultrasonic tip (Endosoft ESI; EMS, Nyon, Switzerland) was placed in the root canal 1 mm behind the working length and moved up and down motion during irrigation. The power settings of the device were ½. Irrigation and activation process were completed in 30 s per irrigant.

Group 3 (EndoVac): Irrigation solutions were introduced into the access cavity with master delivery tip and pulled with a microcannula placed 1 mm shorter than the working length. The delivering of each solution was completed for 30 s.

Group 4 (Er:YAG): The 300 μm diameter PIPS tip attached to the 2940 nm wavelength ER: YAG laser (LightWalker AT, Fotona, Ljubljana, Slovenia) was used with parameters of 0.3 W, 20 mJ and 15 Hz, as recommended by the manufacturer. During root canal irrigation, PIPS fiber optic tip was placed access cavity. Irrigation and activation process were completed in 30 s per irrigation solution.

Group 5 (Er,Cr:YSGG): Irrigation solutions were activated by Er,Cr:YSGG laser (Waterlase MD, Biolase Technology, Inc., Irvine, CA, USA) with a length of 2780 nm with 1.5 W, 20 Hz parameters. During the activation, a 320 μm diameter RFT3 tip was positioned 1 mm shorter than the working length and applied with continuous circular movements at a speed of 2 mm/s towards the root canal mouth.

The residual effects of NaOCl and EDTA were removed and neutralized by rinsing the canal with 3 mL distilled water followed by the drying of canals with paper points after the each irrigation activation procedures. Then, the teeth were randomly separated two subgroups according to root canal sealers to be used. Resin (AH Plus, Dentsply DeTrey GmbH, Konstanz, Germany) and Bioceramic based (Totalfill BC, FKG Dentaire, La-Chaux-de-Fonds, Switzerland) root canal sealers were used. The root canals were filled with 0.1% rhodamine B (Interlab Laboratory Products San. Ve Tic. A.Ş., Istanbul, Turkey) mixed root canal sealer and 40/.06 F4# gutta percha using a single cone technique. The access cavities of the all teeth were restored with temporary filling material (Coltosol; Coltene-Whaledent) and incubated at 37°C in 100% humidity for a week to allow the root canal sealer to set.

2.1 | Confocal laser scanning microscope analysis

Horizontal sections of approximately 1 mm height were taken from the roots at 2, 5 and 8 mm, by 0.4 mm thick diamond discs rotating at low speed with cutting device (Presı Mecatome T180; Sturbridge, USA). This horizontal sections were water-polished with silicon carbide sandpaper (Presı Minitech 233; Sturbridge, USA) to obtain a smooth surface. The samples were fixed on glass slides with a transparent double-sided tape for a Leica TCS-SPE confocal laser scanning microscope (Leica, Mannheim, Germany) at ×10 with a wavelength of 565–605 nm. When the entire canal could not be examined in one image, partial images were taken to assemble these images into one image using Photoshop (Adobe Systems Inc., San Jose, CA, USA). A 200 μm scale was placed on each image.

The obtained CLSM images were analyzed according to four different tubule penetration assessment techniques in the literature.

1. The maximum penetration technique: the deepest point of penetration was measured from the root canal wall to the point of maximum sealer penetration (Generali et al., 2017; Kara Tuncer & Tuncer, 2012) (Figure 1a).
2. The average penetration technique: Four fixed points were selected on the image around the root canal and the part between the root canal wall and the part where the sealer penetration was measured. The average penetration depth was calculated with the average of four lengths (Gharib et al., 2007) (Figure 1b).
3. The penetration percentage technique: The whole circumference of the root canal wall and the areas with sealer penetration were outlined and measured. The ratio of these measurements was calculated as the percentage of sealer (Gharib et al., 2007) (Figure 1c).
4. The penetration area technique: The penetration area of the sealer was computed by subtracting the area of root canal space from the whole area with sealer penetration (Eymirli et al., 2019) (Figure 1d).

2.2 | Statistical analysis

While evaluating the data, IBM SPSS Statistics 22 (IBM SPSS, Turkey) program was used for statistical analysis. The suitability of the parameters to normal distribution was evaluated with Kolmogorov–Smirnov and Shapiro Wilks tests and it was determined that the parameters did not show normal distribution. The Kruskal Wallis test was used to compare the study data parameters between irrigation activation groups and Dunn's test was used to determine the group that caused the difference. Mann Whitney U test was used for the comparison

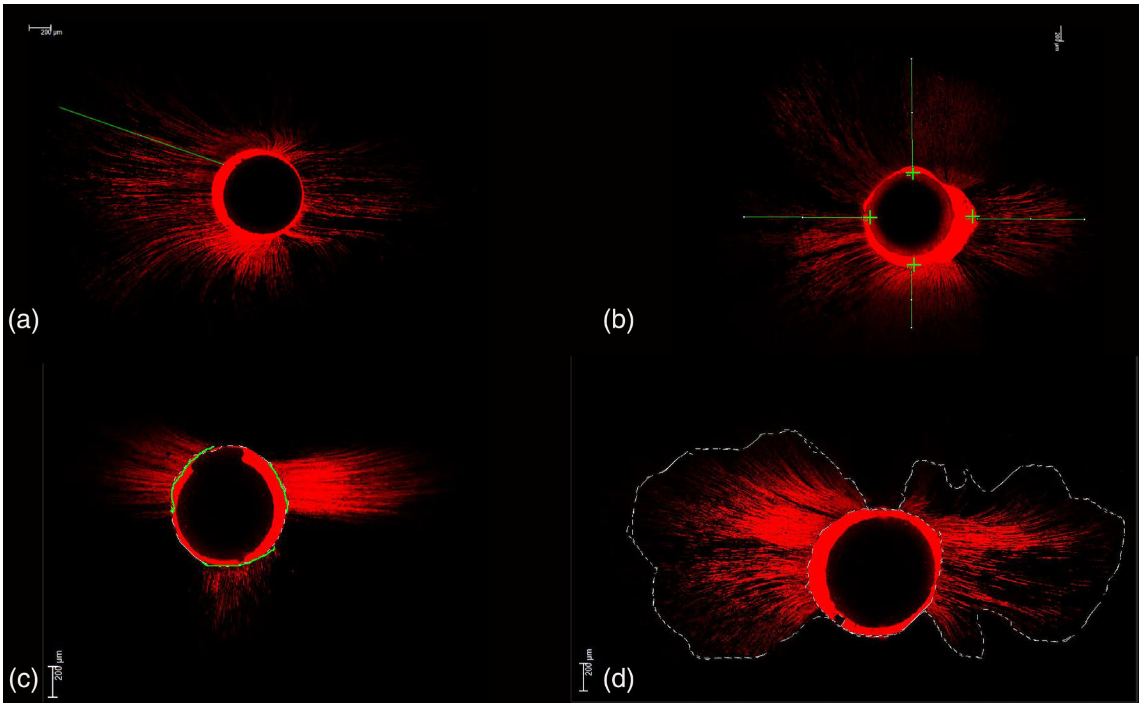

FIGURE 1 (a) Maximum penetration depth. (b) Average penetration depth. (c) Sealer penetration percentage. (d) Sealer penetration area in dentine tubules.

of the parameters between the root canal sealers groups. Friedman test and post hoc Wilcoxon sign test were used for in-group comparisons of parameters. The statistical significance level was set at 5% ($p < .05$).

3 | RESULTS

The mean and standard deviations of dentin tubule penetration of sealers regardless of the irrigation activation type and root canal regions are showed in Table 1a. There were no statistically significant differences dentin tubule penetration of sealers, used in this study, in all four tubule penetration assessment techniques ($p > .05$).

The mean and standard deviations of dentin tubule penetration of sealers according to the effects of irrigation activation techniques, regardless of the sealers type and root canal regions, are given in Table 1b. There were no statistically significant differences among the irrigation activation types when evaluated with the maximum penetration technique ($p > .05$). Endovac, Er:YAG and Er,Cr:YSGG laser activation were caused significantly higher dentin tubule penetration depts than the control group when evaluated with other penetration assessment techniques ($p < .05$). There were no significant differences between the other groups ($p > .05$).

The mean and standard deviations of dentin tubule penetration of sealers according to root canal region, regardless of the sealers types and irrigation activation techniques, are showed in Table 1c. Dentin tubule penetration depts were affected by root canal regions in all penetration assessment techniques ($p < .05$). There were statistically significant differences among the all regions. CLSM images of the root canal sealers in the apical, middle and coronal regions, respectively, in the samples where smears were removed by different irrigation activation techniques are shown (Figure 2, Figure 3 and Figure 4).

3.1 | Evaluation of maximum penetration technique

The mean and standard deviations of dentin tubule penetration of sealers when evaluated with the maximum penetration technique are shown in Table 2. In the coronal region of the group which AH Plus is used, the maximum penetration depth of the EndoVac and Er,Cr:YSGG laser activation groups were significantly higher than the PUI group ($p < .05$)., There were no statistically significant differences among the groups which Totalfill BC sealer is used ($p > .05$).

3.2 | Evaluation of average penetration technique

The mean and standard deviations of dentin tubule penetration of sealers when evaluated with the average penetration technique are shown in Table 3. In the middle region of the group which AH Plus is used, the average penetration depths of the EndoVac, Er:YAG and Er,Cr:YSGG laser activation groups were significantly higher than the control group ($p < .05$). Use of EndoVac was caused higher average penetration than control and PUI groups in coronal region.

TABLE 1 The mean and standard deviations of dentin tubule penetration of sealers used in the study, regardless of the irrigation activation types and root canal regions (a), regardless of the sealers types and root canal regions (b), regardless of the sealers types and irrigation activation techniques (c).

	(a)		(b)					(c)		
	AH plus mean ± SD	Totalfill BC mean ± SD	Control mean ± SD	PUI mean ± SD	EndoVac mean ± SD	Er:YAG mean ± SD	Er,Cr;YSGG mean ± SD	Apical mean ± SD	Middle mean ± SD	Coronal mean ± SD
Maximum penetration	1235.74 ± 714.16[a]	1374.37 ± 665.76[a]	1146.9 ± 673.94[a]	1311.99 ± 691.94[a]	1349.24 ± 744.1[a]	1359.47 ± 643.1[a]	1357.68 ± 705.87[a]	642.84 ± 405.11[a]	1371.88 ± 533.28[b]	1900.45 ± 441.83[c]
Average penetration	701.81 ± 478.08[a]	810.44 ± 486.1[a]	521.88 ± 375.49[a]	752.33 ± 481.19[ab]	824.9 ± 522.75[b]	855.53 ± 448.19[b]	825.98 ± 516.34[b]	318.7 ± 257.63[a]	767.75 ± 359.94[b]	1181.93 ± 368.88[c]
Penetration percentage	69.89 ± 32.3[a]	67.28 ± 31.94[a]	48.83 ± 30.05[a]	65.46 ± 32.20[a,b]	72.20 ± 30.83[b]	78.97 ± 27.28[b]	77.38 ± 31.25[b]	42.75 ± 31.02[a]	73.09 ± 24.94[b]	89.65 ± 19.72[c]
Penetration area	4.3 ± 4.05[a]	4.97 ± 4.67[a]	2.7 ± 2.84 a	4.54 ± 4.22[a,b]	5.46 ± 4.71[b]	4.65 ± 3.99[b]	5.81 ± 5.22 (3.9)[b]	0.97 ± 1.13[a]	4.01 ± 2.89[b]	8.88 ± 4.01[c]

Note: Different lowercase letters in the same lines show a statistically significant difference ($p < .05$).

In the group which TotalFill BC root canal sealar is used, only Er:YAG activation in the apical region and all activation techniques in the coronal region were showed better average penetration than the control group ($p < .05$). There was no significant difference in the other region.

The average dentin tubule penetration depths were increased significantly from apical to coronal in both sealers and all activation techniques ($p < .05$).

3.3 | Evaluation of penetration percentage technique

The mean and standard deviations of dentin tubule penetration of sealers when evaluated with the penetration percentage technique are shown in Table 4. When AH plus root canal sealar is used, irrigation activation techniques did not affect dentin tubule penetration percentage in any region ($p > .05$). While there were significant differences among the all regions in laser activated groups ($p < .05$), there was no significant difference between middle and coronal region in other groups. In the group which TotalFill BC root canal sealar is used, only Er,Cr:YSGG activation in the apical region, both laser activation in the middle region and all activation techniques in the coronal region showed better penetration percentage than the control group ($p < .05$). While there were significant differences among the all regions in control, PUI and EndoVac groups ($p < .05$), there was no significant difference between apical and middle region in laser activated groups.

3.4 | Evaluation of penetration area technique

The mean and standard deviations of dentin tubule penetration of sealers when evaluated with the penetration area technique are shown in Table 5. In the group which AH Plus root canal sealar is used, while EndoVac and Er,Cr:YSGG activation were caused higher penetration area than the Control group in the middle region, Er,Cr:YSGG laser activation showed better penetration area compared with control, PUI and Er:YAG laser activation in the coronal region ($p < .05$). While TotalFill BC root canal sealar penetration area in apical region was higher with Er:YAG activation compared with control and PUI group ($p < .05$), Control group caused lower penetration area than PUI, EndoVac and Er,Cr:YSGG laser activation in coronal region ($p < .05$).

Except for the Er: YAG activation group filled with TotalFill BC root canal sealer, there were statistically significant differences among the all regions in all groups filled with both sealers ($p < .05$). There was no significant difference between apical and middle region in the Er:YAG activation group filled with TotalFill BC root canal sealer ($p > .05$).

4 | DISCUSSION

A successful endodontic treatment aims to hermetically filling the root canal in three dimensions after removal of pulp residues in the root

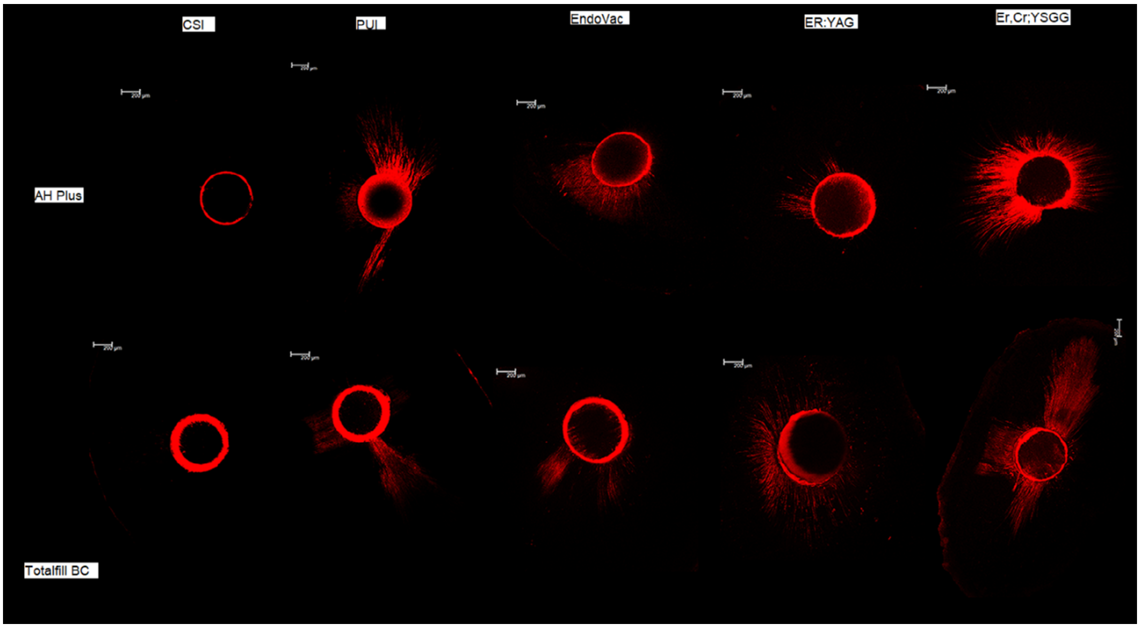

FIGURE 2 Dentin tubule penetration of sealers in the apical region.

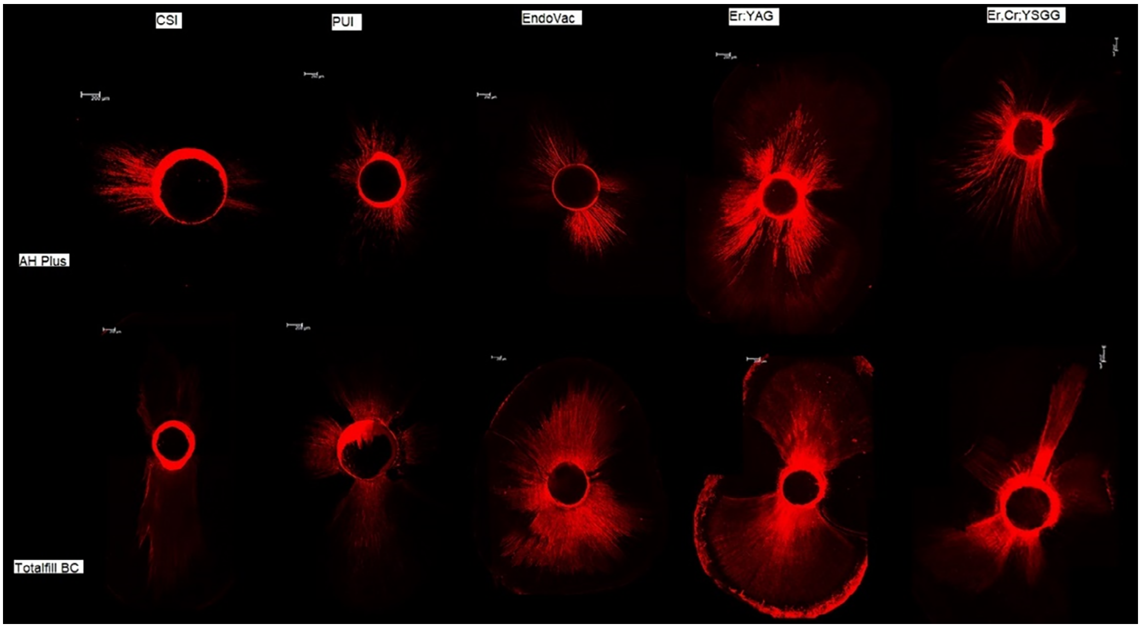

FIGURE 3 Dentin tubule penetration of sealers in the middle region.

canal system and the smear layer formed during chemomechanical procedures (Kuci et al., 2014). Bacteria are one of the main causes of pulp and periapical tissue pathologies (Kakehashi et al., 1965). Therefore, it has been suggested to use various irrigation activation techniques to remove bacteria and by products from the root canal system and increase disinfection (DiVito & Lloyd, 2012; Ls et al., 2009).

Some of the important factors determining the sealing property of the root canal filling are the adhesion of the root canal filling material to the dentin surfaces and its penetration into the dentin tubules (Limkangwalmongkol et al., 1991; Oguntebi & Shen, 1992). The dentinal tubule penetration in the sealer depends on the diameter of the dentinal tubules and the particle size of the materials (Bird et al., 2012). Tubules in radiculer dentin are wide enough to allow penetration of endodontic sealers (Gharib et al., 2007; Kara Tuncer & Tuncer, 2012).

There are many studies in the literature examining dentinal tubule penetration of sealers (Akcay et al., 2016; Mamootil & Messer, 2007; Patel et al., 2007). In some studies, examinations were performed after applying different irrigation activation techniques (Chaudhry

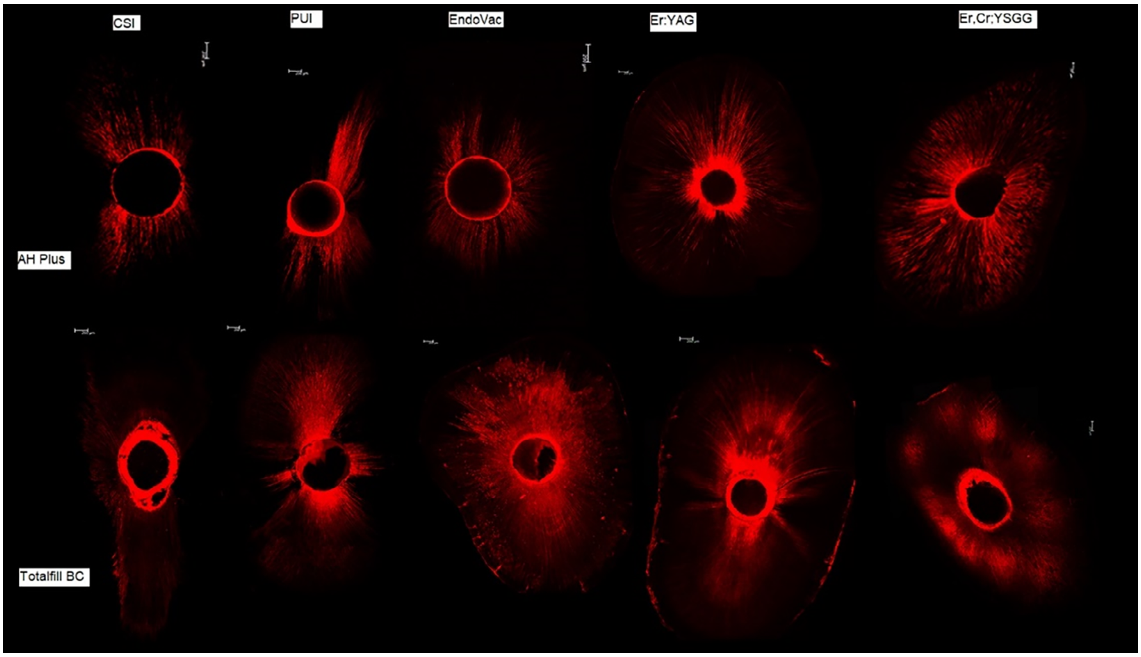

FIGURE 4 Dentin tubule penetration of sealers in the coronal region.

TABLE 2 The mean and standard deviations of dentinal tubule penetration of sealers when evaluated with the maximum penetration technique.

	μm	Control mean ± SD	PUI mean ± SD	EndoVac mean ± SD	Er:YAG mean ± SD[a]	Er,CR:YSGG mean ± SD	p[a]
AH Plus	Apical	439.12 ± 420.19^{a,A}	565.18 ± 534.26^{a,A}	441.45 ± 443.1^{a,A}	645.18 ± 418.65^{a,A}	653.89 ± 346.95^{a,A}	.402
	Middle	1123.53 ± 356.7^{a,B}	1320.37 ± 482.96^{a,B}	1471.17 ± 744.59^{a,B}	1221.49 ± 283.07^{a,B}	1419.44 ± 562.89^{a,B}	.358
	Coronal	1724.4 ± 406.72^{ab,C}	1581.1 ± 587.77^{b,B}	2058.99 ± 308.99^{a,C}	1806.25 ± 661.9^{ab,C}	2064.55 ± 258.66^{a,C}	.039*
	p[b]	0.000*	0.000*	0.000*	0.002*	0.000*	
Total fill BC	Apical	596.07 ± 276.53 ^{a,A}	716.86 ± 377.53^{a,A}	780.53 ± 460.43^{a,A}	924.15 ± 354.78^{a,A}	665.99 ± 279.77^{a,A}	.405
	Middle	1309.17 ± 791.34^{a,B}	1572.43 ± 452.08^{a,B}	1340.55 ± 407.54^{a,B}	1563.09 ± 503.2^{a,B}	1377.49 ± 604.65^{a,B}	.593
	Coronal	1689.11 ± 429.87^{a,B}	2116 ± 200.58^{a,C}	2002.77 ± 282.06^{a,C}	1996.63 ± 347.97^{a,C}	1964.7 ± 516.82^{a,C}	.136
	p[b]	.003*	.000*	.000*	.000*	.000*	

[a]*p*: Different lowercase letters in the same line show a statistically significant difference ($p < .05$).
[b]*p*: Different uppercase letters in the same column show a statistically significant difference ($p < .05$).

TABLE 3 The mean and standard deviations of dentinal tubule penetration of sealers when evaluated with the average penetration technique.

	μm	Control mean ± SD	PUI mean ± SD	EndoVac mean ± SD	Er:YAG mean ± SD	Er,CR:YSGG mean ± SD	p[a]
AH Plus	Apical	134.6 ± 177.8^{a,A}	243.25 ± 318.69^{a,A}	240.4 ± 251.19^{a,A}	339.6 ± 268.65^{a,A}	292.1 ± 232.64^{a,A}	.232
	Middle	489.8 ± 199.27^{a,B}	684.9 ± 303.41^{ab,B}	939 ± 417.76^{b,B}	780.7 ± 264.49^{b,B}	763.4 ± 302.58^{b,B}	.011*
	Coronal	891.8 ± 320.77^{a,C}	890.5 ± 384.95^{a,c,C}	1323.1 ± 158.98^{b,C}	1165.6 ± 405^{bc,C}	1348.4 ± 239.13^{b,c,C}	.002*
	p[b]	0.001*	0.000*	0.000*	0.002*	0.000*	
Total fill BC	Apical	220.8 ± 127.08^{a,A}	352 ± 219.24^{a,b,A}	377.5 ± 286.63^{a,b,A}	606.4 ± 253.25^{b,A}	380.3 ± 181.81^{a,b,A}	.025*
	Middle	571.5 ± 338.24^{a,B}	963 ± 316.41^{a,B}	706.2 ± 385.65^{a,B}	944 ± 401.29^{a,B}	835 ± 421.73^{a,B}	.078
	Coronal	822.8 ± 309.21^{a,C}	1380.3 ± 219.49^{b,C}	1363.2 ± 260.39^{b,C}	1296.9 ± 287.4^{b,C}	1336.7 ± 446.2^{b,C}	.007*
	p[b]	.002*	.000*	.000*	.001*	.002*	

[a]Different lowercase letters in the same line show a statistically significant difference ($p < .05$).
[b]Different uppercase letters in the same column show a statistically significant difference ($p < .05$).

TABLE 4 The mean and standard deviations of dentinal tubule penetration of sealers when evaluated with the penetration percentage technique.

	(%)	Control mean ± SD	PUI mean ± SD	EndoVac mean ± SD	Er:YAG mean ± SD	Er,CR:YSGG mean ± SD	p[a]
AH Plus	Apical	29.21 ± 24.64[a,A]	36.75 ± 30.55[a,A]	47.75 ± 33.03[a,A]	54.68 ± 29.23[a,A]	36.26 ± 35.05[a,A]	.226
	Middle	65.57 ± 18.43[a,B]	80.58 ± 21.49[a,B]	79.2 ± 32.26[a,B]	85.99 ± 13.51[a,B]	74.25 ± 21.54[a,B]	.151
	Coronal	85.03 ± 26.6[a,B]	81.5 ± 24.34[a,B]	91.94 ± 19.35[a,B]	99.51 ± 1.56[a,C]	96.87 ± 9.91[a,C]	.147
	p[b]	0.004*	0.001*	0.000*	0.001*	0.000*	
Total fill BC	Apical	15.66 ± 10.23[a,A]	27.71 ± 20.03[a,c,A]	41.29 ± 24.0[ab,A]	62.24 ± 30.86[b,c,A]	75.31 ± 26.32[b,A]	.000*
	Middle	42.01 ± 14.86[a,B]	71.03 ± 21.05[a,b,B]	76.50 ± 14.28[a,b,B]	74.15 ± 30.79[b,A]	81.61 ± 31.85[b,A]	.004*
	Coronal	55.51 ± 20.51[a,C]	92.34 ± 13.04[b,C]	96.53 ± 7.32[b,C]	97.26 ± 7.12[b,B]	100 ± 0[b,B]	.000*
	p[b]	.000*	.000*	.000*	.008*	.013*	

[a]p: Different lowercase letters in the same line show a statistically significant difference ($p < .05$).
[b]p: Different uppercase letters in the same column show a statistically significant difference ($p < .05$).

TABLE 5 The mean and standard deviations of dentinal tubule penetration of sealers when evaluated with the penetration area technique.

	mm[a]	Control mean ± SD	PUI mean ± SD	EndoVac mean ± SD	Er:YAG mean ± SD	Er,CR:YSGG mean ± SD	p[b]
AH Plus	Apical	0.33 ± 0.41[a,A]	0.93 ± 1.71[a,A]	0.76 ± 1.53[a,A]	1.17 ± 1.35[a,A]	0.93 ± 0.9[a,A]	.262
	Middle	1.91 ± 0.94 [a,B]	3.3 ± 1.97[a,b,B]	5.3 ± 3.49[b,B]	3.39 ± 1.34[a,b,B]	4.8 ± 2.69[b,B]	.037*
	Coronal	6.4 ± 2.82[a,C]	5.8 ± 3.86[a,C]	9.97 ± 2.35[ab,C]	7.26 ± 3.26[a,C]	11.88 ± 2.2[b,C]	.001*
	p[a]	0.000*	0.000*	0.000*	0.000*	0.000*	
Total fill BC	Apical	0.49 ± 0.41[a,A]	0.65 ± 0.42[a,A]	1.32 ± 1.1[a,b,A]	1.99 ± 1.33[b,A]	1.14 ± 0.73[ab,A]	.002*
	Middle	2.34 ± 1.56[a,B]	5.23 ± 3.02[a,B]	4.41 ± 3.27[a,B]	4.07 ± 2.96[a,A]	5.39 ± 4.42[a,B]	.250
	Coronal	4.76 ± 2.96[a,C]	10.96 ± 2.11[b,C]	11 ± 3.43[b,C]	9.99 ± 4.12[a,b,B]	10.75 ± 5.09[b,C]	.007*
	p[a]	.001*	.000*	.000*	.000*	.000*	

[a]p: Different uppercase letters in the same column show a statistically significant difference ($p < .05$).
[b]p: Different lowercase letters in the same line show a statistically significant difference ($p < .05$).

et al., 2017; Machado et al., 2018) while in others only the penetration of different root canal sealers was evaluated (Chandra et al., 2012; Kim et al., 2019). To our knowledge, although there are studies comparing dentinal tubule penetration of different root canal sealers in the literature, there is no study comparing dentinal tubule penetration of different canal filling materials as a result of removing the smear layer with PUI, Endovac, ER:YAG (PIPS) and Er:Cr:YSGG (RFT3) laser activation methods. Such a study was planned in order to provide some suggestions for successful root canal treatments and contribute to the literature by evaluating the penetration of the canal filling sealers from the images obtained with CLSM.

In the literature, light microscopy (De Deus et al., 2004) SEM (Mamootil & Messer, 2007; Shokouhinejad et al., 2011), and CLSM (Jardine et al., 2016; Kim et al., 2019) images were used to examine the penetration of the dentinal tubule of canal filling sealers. Since it is difficult to distinguish root dentin and root canal sealant from each other under light microscope, it is not preferred today (Chandra et al., 2012). Samples must be dried in the oven, treated with alcohol and high vacuum applied in order to be examined in SEM. These steps, which are necessary for the preparation of samples, may cause loss of material in the samples (De Deus et al., 2004). At the same time, gold plating can damage the specimens. The preparation process of the samples in CLSM is non-destructive and they create less artifacts than the examinations can be done under normal environmental conditions. The samples are obtained without any damage and can be reused when necessary (De-Deus et al., 2012). CLSM allows taking images from different depths, so the images can be combined later to create the final image (Tuncer & Ünal, 2014). Since the magnification ratio in SEM is higher than CLSM, it is more difficult to visualize and evaluate the total area. In CLSM, a larger area can be evaluated by making use of the ease of view provided by fluorescent materials at smaller magnifications, or the entire sample surface can be examined (Mamootil & Messer, 2007). However, CLSM allows visualization of the parts under the dentin surface without removing the smear layer (Perdigao et al., 2000). Because of all these advantages, imaging with CLSM was preferred in this study.

The fluorescent Rhodamine B dye added to the canal sealer to distinguish the canal sealer and dentin tubules with CLSM does not cause any change in the physical properties of the sealers (Gharib et al., 2007; Patel et al., 2007; Paulo et al., 2006). Gharib et al. added Rhodamine B dye in various concentrations to the root canal filling sealer and stated that a concentration higher than 0.1% caused excessive fluorescent images (Gharib et al., 2007). Therefore, 0.1% Rhodamine B dye was used as fluorescent material in this study.

In the literature, four different tubule penetration assessment techniques have been used in studies examining dentinal tubule penetration of root canal sealers. The maximum penetration depth is calculated by measuring the distance between the canal wall and the point where the furthest canal sealer penetrates (Eymirli et al., 2019; Kara Tuncer & Tuncer, 2012). The average penetration depth is calculated by determining four fixed points around the canal and measuring the distance between the canal wall and the part where the sealer penetration ends, and by taking the average of these four lengths (Ordinola-Zapata et al., 2009; Russell et al., 2013). Penetration percentage is obtained by multiplying the total length of the zones where the sealers penetrate on the canal walls by proportioning to the canal perimeter (Generali et al., 2016; Gharib et al., 2007). The penetration area is determined by drawing the area around the areas where the paste penetrates and subtracting the area where it is measured by drawing the canal circumference (Eymirli et al., 2019). There are studies in which some of these methods are used together (Aydın et al., 2019; Piai et al., 2018), as well as studies that have been conducted solely by examining the penetration area (Akcay et al., 2016). Considering the information in the literature, in this study, it was preferred to use four different evaluation criteria together, which were not included in any study before, for dentin tubule penetration examination. Thus, the maximum penetration depths, average penetration depths, penetration percentages and penetration areas of two different root canal sealers with different irrigation activation techniques were investigated in this study.

As a result of this study, when the irrigation activation techniques and regions were ignored, no significant difference was observed between AH Plus and Totalfill BC root canal sealers in all tubule penetration assessment techniques. Kim et al. (2015) compared epoxy resin based AH Plus and bioceramic based Endosequence BC root canal sealars in their study and stated that there was no significant difference in the penetration percentage, regardless of the regions (Kim et al., 2015) Turkel et al. (2017). evaluated tubule penetration of AH Plus and Totalfill BC root canal sealers after EndoVac and Er: YAG laser activation methods and reported that there was no significant difference in the maximum penetration and penetration percentage techniques. The results of this study evaluated with four tubule penetration assessment techniques are consistent with other studies (Turkel et al., 2017).

It was observed that the EndoVac, Er:YAG and Er,Cr:YSGG laser activations caused significantly higher dentin tubule penetration depth than the control group when evaluated with all penetration assessment techniques except the maximum penetration depth. The maximum penetration depth is a measurement method based on a single tubule showing the highest penetration on the entire examined dentin surface. Therefore, it may not be a reliable technique compared to other tubule penetration assessment techniques, as it is attempted to find the dentinal tubule with the highest penetration in all samples.

Akcay et al., reported that the dentinal tubule penetration area was significantly affected by irrigation activation of PIPS with Er:YAG laser and PUI compared to the conventional irrigation (Akcay et al., 2016). In the literature, it has been stated that Er,Cr:YSGG laser activation has better smear removal efficiency compared to conventional irrigation protocol (Bolhari et al., 2014; Madhusudhana et al., 2016). In a study evaluated penetration percentage and penetration area, the EndoVac achieved better dentinal tubule penetration compared with conventional irrigation (Machado et al., 2018). Since removing the smear layer positively supports the dentin tubule penetration of the root canal sealer, the results in accordance with previous studies were obtained in this study.

It was determined that, dentin tubule penetration depths were significantly affected by root canal regions in all penetration assessment techniques, when the sealers and irrigation activation types were ignored. Dentin tubule penetration depths were increased significantly from apical to coronal. In studies examining the penetration of sealers into dentinal tubules, sections taken from the apical, middle, and coronal regions were generally found to have higher sealer penetration in the coronal and middle regions than the apical (Calt & Serper, 1999; Turkel et al., 2017; Weis et al., 2004). Chandra et al., evaluated the maximum penetration depth of four different sealers containing resin, reported that they observed the lowest penetration of all root canal sealers in the apical region (Chandra et al., 2012). Akcay et al. was evaluated the different irrigation activation techniques and penetration area of sealers and reported that the lowest penetration area was in the apical region in all experimental groups (Akcay et al., 2016). Generali et al. was investigated the penetration percentage, average penetration depth and maximum penetration depth of the sealers and stated that the lowest penetration values were in the apical region (Generali et al., 2017). The diameter and number of dentinal tubule orifices increase as it progresses from the apical to the coronal region (Vasiliadis et al., 1983).

It was reported that the number of dentinal tubules began to decrease, the mineralization of dentin occurred and the mineralization of the peritubular dentin surrounding the dentinal tubules increased with aging. This situation may sometimes cause the dentinal tubules to become completely blocked (Carrigan et al., 1984) and prevent the penetration of the root canal sealers into the dentinal tubules. The data obtained in this study are consistent with the results of comparing the apical, middle and coronal regions in dentinal tubule penetration studies in the literature.

As a result of this study, it can be concluded that dentinal tubule penetration cannot be evaluated by irrigation activation techniques based on the maximum penetration technique. This may be due to the search for maximum penetration depth in each sample and indicate that the maximum penetration assessment technique is not a very reliable technique. Although there was a statistically significant difference in some regions and not in some regions, the lowest penetration values were observed in the control group when the average penetration assessment technique was used. It can be said that irrigation activation techniques cause higher dentin penetration compared to the control group. Not looking for the maximum penetration depth in the whole sample and taking the average of the penetration depth at four different points previously determined in all samples may show that the average penetration assessment technique is a more suitable method.

The whole circumference of the root canal wall and the areas with sealer penetration are marked and measured. The ratio of these measurements are calculated as the penetration percentage of root canal sealer. This measurement only helps to have an idea about the dentin tubule penetration around the root canal. Even if dentinal tubule penetration does not reach very deep, this area is included in the penetration percentage. The penetration area should be calculated to evaluate the dentinal tubule penetration in the obtained cross section. Because the penetration area of the root canal sealer is calculated by subtracting it from the non-penetrating area. A more realistic assessment can be made by calculating the penetration area compared to the penetration percentage. Considering this information, it can be said that average penetration and penetration area techniques are more reliable and preferred methods in dentinal tubule penetration studies.

There was no statistically significant difference between the average penetration depth, the apical region of the teeth where AH Plus canal filling material was used, the middle region of the teeth where Totalfill BC canal filling material was used, and the control and all other groups. In the middle and coronal region of the AH Plus, EndoVac, Er:YAG and Er,Cr:YSGG showed a statistically significantly higher average penetration depth compared to the Control and PUI activation, while in the apical region of the teeth using Totalfill BC sealer, Er:YAG laser activation and all irrigation activation systems in the coronal region showed a statistically significantly higher average penetration depth compared to the control group.

No statistically significant difference was found between the control and all other activation groups in terms of penetration percentage in all areas of the teeth where AH Plus was used. Er,Cr:YSGG laser activation in the apical region of teeth using Totalfill BC sealer provided a better penetration percentage compared to the Control and PUI groups. Er:YAG and Er,Cr:YSGG laser activations in the middle region of the teeth compared to the control group, and all activation groups in the coronal region provided a better penetration percentage than the control group. Thanks to this information, it can be said that irrigation activation methods have a positive effect on the penetration percentage of the paste when using bioceramic based canal sealer.

In terms of penetration area, no statistically significant difference was found in the apical region of the teeth where AH Plus was used, and in the middle region of the teeth where Totalfill BC sealer was used. With the activation of EndoVac and Er,Cr:YSGG laser in the middle region of the teeth in which AH Plus was used, the Er,Cr:YSGG laser Control showed a statistically significant difference compared to the control group and the PUI and Er:YAG laser groups in the coronal region. When using Totalfill BC sealer, Er:YAG laser activation according to control and PUI activation in the apical region, and PUI, EndoVac and Er,Cr:YSGG laser activation showed a statistically significant difference compared to the control group in the coronal region. Thanks to this information, it can be said that both pastes have better penetration area values than the control group with all activation techniques.

5 | CONCLUSION

Within the limitations of this study, it can be stated that the use of resin or bioceramic based root canal sealers does not affect dentin tubule penetration and the use of irrigation activation techniques during removal of the smear layer positively affects dentinal tubule penetration. In addition, it has been determined that the average tubule penetration and penetration area assessment techniques are suitable methods for the investigation of dentinal tubule penetration.

AUTHOR CONTRIBUTIONS

Fatma Kaplan: Writing – original draft; writing – review and editing; visualization; resources; data curation; software; formal analysis; validation; methodology; conceptualization; investigation; funding acquisition. **Ali Erdemir:** Project administration; supervision; conceptualization; investigation; writing – review and editing; resources; methodology; funding acquisition.

ACKNOWLEDGMENTS

This research, the doctorate thesis of Fatma Kaplan, was supported by Turkish Scientific and Technical Research Council (TUBITAK), 1002 Fast Support Program (Project number: 318S243).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ORCID

Fatma Kaplan https://orcid.org/0000-0002-4113-6727

REFERENCES

Akcay, M., Arslan, H., Durmuş, N., Meşe, M., & Çapar, I. D. (2016). Dentinal tubule penetration of AH plus, iRoot SP, MTA fillapex, and guttaflow bioseal root canal sealers after different final irrigation procedures: A confocal microscopic study. *Lasers in Surgery and Medicine*, *48*(1), 70–76.

Aydın, Z. U., Özyürek, T., Keskin, B., & Baran, T. (2019). Effect of chitosan nanoparticle, QMix, and EDTA on TotalFill BC sealers' dentinal tubule penetration: A confocal laser scanning microscopy study. *Odontology*, *107*(1), 64–71.

Bird, D. C., Komabayashi, T., Guo, L., Opperman, L. A., & Spears, R. (2012). In vitro evaluation of dentinal tubule penetration and biomineralization ability of a new root-end filling material. *Journal of Endodontics*, *38*(8), 1093–1096.

Bolhari, B., Ehsani, S., Etemadi, A., Shafaq, M., & Nosrat, A. (2014). Efficacy of Er, Cr: YSGG laser in removing smear layer and debris with two different output powers. *Photomedicine and Laser Surgery*, *32*(10), 527–532.

Bouillaguet, S., Shaw, L., Barthelemy, J., Krejci, I., & Wataha, J. (2008). Long-term sealing ability of pulp canal sealer, AH-plus, GuttaFlow and epiphany. *International Endodontic Journal*, *41*(3), 219–226.

Calt, S., & Serper, A. (1999). Dentinal tubule penetration of root canal sealers after root canal dressing with calcium hydroxide. *Journal of Endodontics*, *25*(6), 431–433.

Carrigan, P. J., Morse, D. R., Furst, M. L., & Sinai, I. H. (1984). A scanning electron microscopic evaluation of human dentinal tubules according to age and location. *Journal of Endodontics*, *10*(8), 359–363.

Chandra, S. S., Shankar, P., & Indira, R. (2012). Depth of penetration of four resin sealers into radicular dentinal tubules: A confocal microscopic study. *Journal of Endodontics*, *38*(10), 1412–1416.

Chaudhry, S., Yadav, S., Talwar, S., & Verma, M. (2017). Effect of endoactivator and Er, Cr: YSGG laser activation of Qmix, as final endodontic irrigant, on sealer penetration: A confocal microscopic study. *Journal of Clinical and Experimental Dentistry*, *9*(2), e218.

De Deus, G. A., Gurgel-Filho, E. D., Maniglia-Ferreira, C., & Coulinho-Filho, T. (2004). The influence of filling technique on depth of tubule penetration by root canal sealer: A study using light microscopy and digital image processing. *Australian Endodontic Journal*, *30*(1), 23–28.

De Groot, S., Verhaagen, B., Versluis, M., Wu, M. K., Wesselink, P., & Van Der Sluis, L. (2009). Laser-activated irrigation within root canals: Cleaning efficacy and flow visualization. *International Endodontic Journal*, *42*(12), 1077–1083.

De-Deus, G., Brandao, M., Leal, F., et al. (2012). Lack of correlation between sealer penetration into dentinal tubules and sealability in non-bonded root fillings. *International Endodontic Journal*, *45*(7), 642–651.

DiVito, E., & Lloyd, A. (2012). ER: YAG laser for 3-dimensional debridement of canal systems: Use of photon-induced photoacoustic streaming. *Dentistry Today*, *31*(11), 124–127.

DiVito, E., Peters, O. A., & Olivi, G. (2012). Effectiveness of the erbium: YAG laser and new design radial and stripped tips in removing the smear layer after root canal instrumentation. *Lasers in Medical Science*, *27*(2), 273–280.

Eymirli, A., Sungur, D. D., Uyanik, O., Purali, N., Nagas, E., & Cehreli, Z. (2019). Dentinal tubule penetration and retreatability of a calcium silicate–based sealer tested in bulk or with different Main Core material. *Journal of Endodontics*, *45*(8), 1036–1040.

Garrido, A., Lia, R., França, S., Da Silva, J., Astolfi-Filho, S., & Sousa-Neto, M. (2010). Laboratory evaluation of the physicochemical properties of a new root canal sealer based on Copaifera multijuga oil-resin. *International Endodontic Journal*, *43*(4), 283–291.

Generali, L., Cavani, F., Serena, V., Pettenati, C., Righi, E., & Bertoldi, C. (2017). Effect of different irrigation systems on sealer penetration into dentinal tubules. *Journal of Endodontics*, *43*(4), 652–656.

Generali, L., Prati, C., Pirani, C., Cavani, F., Gatto, M., & Gandolfi, M. (2016). Double dye technique and fluid filtration test to evaluate early sealing ability of an endodontic sealer. *Clinical Oral Investigations*, *21*(4), 1267–1276.

Gharib, S. R., Tordik, P. A., Imamura, G. M., Baginski, T. A., & Goodell, G. G. (2007). A confocal laser scanning microscope investigation of the epiphany obturation system. *Journal of Endodontics*, *33*(8), 957–961.

Ghorbanzadeh, A., Aminsobhani, M., Sohrabi, K., Chiniforush, N., Ghafari, S., Shamshiri, A. R., & Noroozi, N. (2016). Penetration depth of sodium hypochlorite in dentinal tubules after conventional irrigation, passive ultrasonic agitation and Nd: YAG laser activated irrigation. *Journal of Lasers in Medical Sciences.*, *7*(2), 105–111.

Gutmann, J. L., Zehnder, M., & Levermann, V. M. (2014). Historical perspectives on the roots of the apical negative pressure irrigation technique in endodontics. *Journal of the History of Dentistry*, *62*(1), 32–40.

Jardine, A. P., Da Rosa, R. A., Santini, M. F., Wagner, M., Só, M. V. R., Kuga, M. C., Pereira, J. R., & Kopper, P. M. P. (2016). The effect of final irrigation on the penetrability of an epoxy resin-based sealer into dentinal tubules: A confocal microscopy study. *Clinical Oral Investigations*, *20*(1), 117–123.

Kakehashi, S., Stanley, H., & Fitzgerald, R. (1965). The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surgery, Oral Medicine, and Oral Pathology*, *20*(3), 340–349.

Kara Tuncer, A., & Tuncer, S. (2012). Effect of different final irrigation solutions on dentinal tubule penetration depth and percentage of root canal sealer. *Journal of Endodontics*, *38*(6), 860–863.

Kim, H., Kim, E., Lee, S.-J., & Shin, S.-J. (2015). Comparisons of the retreatment efficacy of calcium silicate and epoxy resin-based sealers and residual sealer in dentinal tubules. *Journal of Endodontics*, *41*(12), 2025–2030.

Kim, Y., Kim, B.-S., Kim, Y.-M., Lee, D., & Kim, S.-Y. (2019). The penetration ability of calcium silicate root canal sealers into dentinal tubules compared to conventional resin-based sealer: A confocal laser scanning microscopy study. *Materials*, *12*(3), 531.

Kuci, A., Alaçam, T., Yavaş, Ö., Ergul-Ulger, Z., & Kayaoglu, G. (2014). Sealer penetration into dentinal tubules in the presence or absence of smear layer: A confocal laser scanning microscopic study. *Journal of Endodontics*, *40*(10), 1627–1631.

Limkangwalmongkol, S., Burtscher, P., Abbott, P. V., Sandler, A. B., & Bishop, B. M. (1991). A comparative study of the apical leakage of four root canal sealers and laterally condensed gutta-percha. *Journal of Endodontics*, *17*(10), 495–499.

Ls, G., Kim, J. R., Ling, J., Choi, K. K., Pashley, D. H., & Tay, F. R. (2009). Review of contemporary irrigant agitation techniques and devices. *Journal of Endodontics*, *35*(6), 791–804.

Ma, J., Wang, Z., Shen, Y., & Haapasalo, M. (2011). A new noninvasive model to study the effectiveness of dentin disinfection by using confocal laser scanning microscopy. *Journal of Endodontics*, *37*(10), 1380–1385.

Machado, R., Cruz, A. T. G., de Araujo, B. M. M., et al. (2018). Tubular dentin sealer penetration after different final irrigation protocols: A confocal laser scanning microscopy study. *Microscopy Research and Technique*, *81*(6), 649–654.

Madhusudhana, K., Swapna, S., Suneelkumar, C., Lavanya, A., & Deepthi, M. (2016). Effect of endoactivator and Er, Cr: YSGG laser irradiation in removing the smear layer after root canal instrumentation: An in vitro study. *Journal of Dr NTR University of Health Sciences.*, *5*(1), 24.

Mamootil, K., & Messer, H. (2007). Penetration of dentinal tubules by endodontic sealer cements in extracted teeth and in vivo. *International Endodontic Journal*, *40*(11), 873–881.

Marciano, M. A., Guimarães, B. M., Ordinola-Zapata, R., Bramante, C. M., Cavenago, B. C., Garcia, R. B., Bernardineli, N., Andrade, F. B., Moraes, I. G., & Duarte, M. A. H. (2011). Physical properties and interfacial adaptation of three epoxy resin–based sealers. *Journal of Endodontics*, *37*(10), 1417–1421.

Marin-Bauza, G. A., Abi Rached-Junior, F. J., Souza-Gabriel, A. E., Sousa-Neto, M. D., Miranda, C. E. S., & Silva-Sousa, Y. T. C. (2010). Physicochemical properties of methacrylate resin–based root canal sealers. *Journal of Endodontics*, *36*(9), 1531–1536.

McMichael, G. E., Primus, C. M., & Opperman, L. A. (2016). Dentinal tubule penetration of tricalcium silicate sealers. *Journal of Endodontics*, *42*(4), 632–636.

Munoz, H. R., & Camacho-Cuadra, K. (2012). In vivo efficacy of three different endodontic irrigation systems for irrigant delivery to working length of mesial canals of mandibular molars. *Journal of Endodontics*, *38*(4), 445–448.

Nair, P., Henry, S., Cano, V., & Vera, J. (2005). Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, Endodontology.*, *99*(2), 231–252.

Nielsen, B. A., & Baumgartner, J. C. (2007). Comparison of the EndoVac system to needle irrigation of root canals. *Journal of Endodontics*, *33*(5), 611–615.

Nielsen, B. A., Beeler, W. J., Vy, C., & Baumgartner, J. C. (2006). Setting times of Resilon and other sealers in aerobic and anaerobic environments. *Journal of Endodontics*, *32*(2), 130–132.

Oguntebi, B. R., & Shen, C. (1992). Effect of different sealers on thermoplasticized gutta-percha root canal obturations. *Journal of Endodontics*, *18*(8), 363–366.

Ordinola-Zapata, R., Bramante, C. M., Graeff, M. S., del Carpio Perochena, A., Vivan, R. R., Camargo, E. J., Garcia, R. B., Bernardineli, N., Gutmann, J. L., & de Moraes, I. G. (2009). Depth and percentage of penetration of endodontic sealers into dentinal tubules after root canal obturation using a lateral compaction technique: A confocal laser scanning microscopy study. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics*, *108*(3), 450–457.

Patel, D., Sherriff, M., Ford, T., Watson, T., & Mannocci, F. (2007). The penetration of RealSeal primer and Tubliseal into root canal dentinal tubules: A confocal microscopic study. *International Endodontic Journal*, *40*(1), 67–71.

Paulo, H. D., Pereira, J. C., Svizero, N. R., Rueggeberg, F. A., & Pashley, D. H. (2006). Use of fluorescent compounds in assessing bonded resin-based restorations: A literature review. *Journal of Dentistry*, *34*(9), 623–634.

Peeters, H. H., De Moor, R. J., & Suharto, D. (2015). Visualization of removal of trapped air from the apical region in simulated root canals by laser-activated irrigation using an Er, Cr: YSGG laser. *Lasers in Medical Science*, *30*(6), 1683–1688.

Peeters, H. H., & Suardita, K. (2011). Efficacy of smear layer removal at the root tip by using ethylenediaminetetraacetic acid and erbium, chromium: Yttrium, scandium, gallium garnet laser. *Journal of Endodontics*, *37*(11), 1585–1589.

Perdigao, J., Lambrechts, P., & Vanherle, G. (2000). Microscopy investigations: techniques, results, limitations. *American Journal of Dentistry*, *13*, 3D18D.

Peters, O. A., Bardsley, S., Fong, J., Pandher, G., & DiVito, E. (2011). Disinfection of root canals with photon-initiated photoacoustic streaming. *Journal of Endodontics*, *37*(7), 1008–1012.

Piai, G. G., Duarte, M. A. H., Nascimento, A. L., Rosa, R. A., Só, M. V. R., & Vivan, R. R. (2018). Penetrability of a new endodontic sealer: A confocal laser scanning microscopy evaluation. *Microscopy Research and Technique*, *81*(11), 1246–1249.

Ram, Z. (1977). Effectiveness of root canal irrigation. *Oral Surgery, Oral Medicine, and Oral Pathology*, *44*(2), 306–312.

Russell, A. A., Chandler, N. P., Hauman, C., Siddiqui, A. Y., & Tompkins, G. R. (2013). The butterfly effect: An investigation of sectioned roots. *Journal of Endodontics*, *39*(2), 208–210.

Shokouhinejad, N., Sabeti, M., Gorjestani, H., Saghiri, M. A., Lotfi, M., & Hoseini, A. (2011). Penetration of epiphany, epiphany self-etch, and AH plus into dentinal tubules: A scanning electron microscopy study. *Journal of Endodontics*, *37*(9), 1316–1319.

Stoll, R., Betke, K., & Stachniss, V. (2005). The influence of different factors on the survival of root canal fillings: A 10-year retrospective study. *Journal of Endodontics*, *31*(11), 783–790.

Tuncer, A. K., & Ünal, B. (2014). Comparison of sealer penetration using the EndoVac irrigation system and conventional needle root canal irrigation. *Journal of Endodontics*, *40*(5), 613–617.

Turkel, E., Onay, E. O., & Ungor, M. (2017). Comparison of three final irrigation activation techniques: Effects on canal cleanness, smear layer removal, and dentinal tubule penetration of two root canal sealers. *Photomedicine and Laser Surgery*, *35*(12), 672–681.

Vasiliadis, L., Darling, A., & Levers, B. (1983). The amount and distribution of sclerotic human root dentine. *Archives of Oral Biology*, *28*(7), 645–649.

Weis, M. V., Parashos, P., & Messer, H. (2004). Effect of obturation technique on sealer cement thickness and dentinal tubule penetration. *International Endodontic Journal*, *37*(10), 653–663.

How to cite this article: Kaplan, F., & Erdemir, A. (2023). Evaluating the effect of different irrigation activation techniques on the dentin tubules penetration of two different root canal sealers by laser scanning confocal microscopy. *Microscopy Research and Technique*, *86*(7), 791–802. https://doi.org/10.1002/jemt.24339