EUROPEAN ENDODONTIC JOURNAL

ORIGINAL ARTICLE

Dentinal Tubule Penetration and Dislocation Resistance of a New Bioactive Root Canal Sealer Following Root Canal Medicament Removal Using Sonic Agitation or Laser-Activated Irrigation

Esin ÖZLEK, Prasanna NEELAKANTAN, Elif AKKOL, Hüseyin GÜNDÜZ, Yağmur Arzu UÇAR, Sema BELLI

ABSTRACT

Objective: To investigate the influence of sonic agitation or laser-activated irrigation techniques on the removal chlorhexidine (CHX) and modified triple antibiotic paste (mTAP) on the sealer penetration depth and dislocation resistance of Guttaflow Bioseal.

Methods: Single-rooted mandibular premolars (n=96) were prepared with rotary nickel titanium instruments and randomly divided into two groups (n=48) based on the intracanal medicaments used: Group 1, mTAP; Group 2, CHX gel. After 7 days, the specimens in each group were divided into three subgroups (n=16) based on the supplementary irrigation technique used to remove the medicaments: laser activated irrigation (Er, Cr: YSGG laser, Waterlase MD, Biolase Technology Inc., San Clemente, CA, USA), sonic agitation (EndoActivator, Dentslpy Sirona Endodontics, PA, USA) and syringe-and-needle irrigation (control) techniques. Canals were filled with single matched-taper gutta-percha cone and a calcium silicate-based sealer (GuttaFlow® Bioseal, Coltène/Whaledent, Langenau, Germany). At the end of three weeks, sealer penetration was investigated using confocal microscopy (n=6), and dislocation resistance was calculated by measuring the push-out bond strength (n=10). Statistical analysis was performed using three-way analysis of variance (ANOVA) and Tukey post-hoc test (P=0.05).

Results: Laser activated irrigation resulted in significantly higher depth of sealer penetration compared to sonic agitation and syringe irrigation (P<0.01). The average sealer penetration depths were recorded as 846.6 µm, 786.5 µm and 505 µm in the Er,Cr:YSGG laser, EndoActivator and control groups, respectively. The mean bond strength obtained in group 3 (syringe-and-needle irrigation) was significantly less than the other groups (P<0.05). The mean values were 9.08 in the Er,Cr:YSGG laser group, 8.44 in the EndoActivator group and 5.08 in the needle group.

Conclusion: Er,Cr;YSGG laser irrigation to remove the medicaments was advantageous to other irrigation techniques in sealer penetration and dislocation resistance of the sealer.

Keywords: Confocal, CHX gel, dentinal tubule penetration, Er,Cr:YSGG laser, EndoActivator, GuttaFlow Bioseal, mTAP

Please cite this article as: Özlek E, Neelakantan P, Akkol E, Gündüz H, Uçar YA, Belli S. Dentinal Tubule Penetration and Dislocation Resistance of a New Bioactive Root Canal Sealer Following Root Canal Medicament Removal Using Sonic Agitation or Laser-Activated Irrigation. Eur Endod J 2020; 3: 264-70

From the Department of Endodontics (E.Ö. ✉ e.ozlek@yyu.edu.tr, E.A., H.G.), Faculty of Dentistry, Van Yüzüncü Yıl University, Van, Turkey; Department of Endodontics (P.N.), Faculty of Dentistry, The University of Hong Kong, Hong Kong; Department of Endodontics (Y.A.U., S.B.), Faculty of Dentistry, Selçuk University, Konya, Turkey

Received 21 April 2020,
Accepted 22 September 2020

Published online: 15 December 2020
DOI 10.14744/eej.2020.92905

HIGHLIGHTS

- Er,Cr;YSGG laser increased the sealer penetration and dislocation resistance of the sealer
- The dentinal tubule penetration and dislocation resistance of GuttaFlow Bioseal were influenced by the irrigation technique used for removing the intracanal medicament.

INTRODUCTION

Thorough disinfection of the root canal system remains a clinical challenge, given the complex root canal anatomy (1). For this reason, inter-appointment dressings (intracanal medicaments) are frequently used in supplement instrumentation and irrigation (2). One of the key challenges following the placements of intracanal medicament is its complete removal. The most common method used to remove intracanal medicament from the root canal involves the use of hand or mechanical instruments with syringe-and-needle irrigation (3). Irrigant agitation/activation techniques are recommended to improve the activity of irrigation solutions and increase the depth of penetration into The Root Canal System (1, 4).

Complete removal of this intracanal medicament is a desirable for filling the root canals, as remnant medicament interferes with sealer penetration into the dentinal tubules (5, 6). Although the

evidence-based implications of sealer penetration on clinical outcomes are weak, from a logical standpoint, sealer penetration may enhance the seal of root canal fillings (7). Sealer penetration into dentinal tubules might be associated with to the penetration of irrigating solutions into the dentinal tubules, and thus to its disinfection efficiency. There has been an increasing interest in bioactive sealers such as those based on hydraulic calcium silicates.

Guttaflow Bioseal is a new material containing gutta-percha and calcium silicate. The effects of GuttaFlow Bioseal on bond strength and dentinal tubule penetration (8), and the quality of root filling with this material has been previously evaluated (9). The impact of remaining intracanal medicaments on the adhesion of GuttaFlow Bioseal is still not well-known. Calcium hydroxide (CH) is frequently used in endodontics due to its antibacterial effects, organic tissue dissolution abilities and anti-inflammatory effects (10). However, because infection of the root canal system is considered to be polymicrobial, it is not possible to sterilize root canals in necrotic teeth using only CH, thus antibiotic combinations and chlorhexidine digluconate (CHX) have also been suggested for disinfecting root canals (11). Triple antibiotic paste removes necrotic pulp tissue, disinfects the root canal cavity and creates a suitable environment for regenerative treatment (12). The contents of the triple antibiotic paste (TAP) are minoxycline, ciprofloxacin and metronidazole. Since minocycline causes discolouration in the tooth, cefaclor was added instead and defined as a modified triple antibiotic paste (mTAP) (11). Most studies have focused on the removal of calcium hydroxide (10). Hence, this laboratory study was designed to investigate the dentinal tubule penetration and adhesion of Guttaflow Bioseal after the removal of two intracanal medicaments (mTAP and chlorhexidine) with three irrigation strategies (syringe-and-needle, sonic agitation and laser assisted irrigation). The null hypothesis was that neither the intracanal medicament nor the removal technique had a significant influence on the dentinal tubule penetration and bond strength of Guttaflow Bioseal.

MATERIALS AND METHODS

Single rooted mandibular premolars (n=96) with completely formed roots and closed apices were collected based on a protocol approved (03/08/2018-03) by the Research Ethics Board of University. Patients were informed in detail about the purpose and process of the study. Volunteer patients who signed the informed consent form approved by the ethics committee were included in the study. The exclusion criteria were caries, curved roots, open apices, cracks or previous root canal treatments. A radiograph was taken for each tooth sample to insure no intracanal abnormality (e.g., root resorption, sclerosed canal) was present. Roots with less than 10° curvature were also selected for standardization. The teeth were cleaned using curettes to remove the attached soft and hard tissue and stored in distilled water with 0.5% thymol until use. Following cleaning of the soft tissue debris and calculus from the surface of the teeth, the teeth were decoronated with a slow-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) under water cooling root lengths were standardized to 12 mm. A #15 K-file (VDW GmbH, Munich, Germany) was inserted into the root canal until the tip of the file was visible at the apical foramen, and the working length was determined after subtracting 1 mm from this length. The root canals were instrumented with ProTaper rotary files (Dentsply Maillefer, Ballaigues, Switzerland) up to file F3. At every instrument change, the root canals were irrigated with 2 mL of 5.25% sodium hypochlorite (NaOCl) solution. Final irrigation was performed using 5 mL of 17% EDTA for 2 min and 5 mL 5.25% NaOCl for 2 min (13).

The root canals were dried with paper points, and the teeth were randomly distributed into two groups (n=48) using a computer program (www.random.org), based on the intracanal medicaments used: Group 1, mTAP and Group 2, Chlorhexidine (CHX). mTAP was prepared by mixing equal proportions of Metronidazole (Nidazol 500 mg tablet, Ulagay, Istanbul, Turkey), Ciprofloxacin (Cipro 500 mg tablet, Biofarma, Istanbul, Turkey), and Cefaclor (Sanocef 750 mg tablet, Actavis, Istanbul, Turkey) with 1 mL sterile distilled water to form a paste to the mixture used clinically (14). It was entrenched into the root canals using a size #30 Lentulo spiral (Dentsply Maillefer) until visible at the apical foramen. In the second group, 2% CHX gel (Endogel, Itapetininga, SP, Brazil) was injected into the root canals using the special syringe provided by the manufacturer. After the medicaments was placed in the root canals, the roots of all samples were coated with 2 coats of nail varnish.

The orifice was sealed with Cavit (3M ESPE, Seefeld, Germany), and the roots were stored at 37°C with 100% humidity for 21 days. After the incubation period, the specimens were instrumented with ProTaper F3 and #30 Hedström files (Dentsply Maillefer) using 5 mL of 5.25% NaOCl as the irrigant until no medicament was noticeable on the files. Canals were then rinsed with 5 mL of 17% EDTA for 2 mins. Then, the specimens in both groups were divided into three subgroups according to the final irrigation technique used (n=16). Since the techniques vary in their usage parameters, the volume of irrigant was standardized, rather than the duration of use.

Subgroup A: Er,Cr:YSGG laser

The root canals were filled with 5.25% NaOCl and activated with Er, Cr: YSGG laser (Waterlase MD, Biolase Technology Inc., San Clemente, CA, USA) using the RFT2 tip (275 microns in diameter and 21mm length), placed 1 mm short of the working length. The parameters of laser used were output power of 2W energy, pulse frequency of 20 Hz (pulses per second), using 10% air and 10% water (2W- 23.15 J/cm^2). The canals were radiated from apical area to the coronal area in slow and helicoidal movements for 8s. This procedure was repeated until 2mL of NaOCl was used.

Subgroup B: Sonic agitation

The root canals were filled with 5.25% NaOCl and agitated using the red tip (25/.04) of EndoActivator (Dentsply). The tip was placed 2 mm short from the working length. The activation was performed in 1-minute cycles (10.000 cpm) until 2 mL of NaOCl was used.

Subgroup C (control): Syringe-and-needle irrigation

The root canals were irrigated with 5.25% NaOCl using a 27-gauge closed-end needle (Ayset, Adana, Turkey), placed 2 mm short of the working length. The needle was moved in

short vertical strokes of 2-3 mm amplitude at an approximate rate of 100 strokes/min (15). A total of 2 mL of NaOCl was used.

All canals were irrigated with 5 mL distilled water, dried with absorbent paper points and prepared for filling. Six specimens from each subgroup were allocated to the sealer penetration experiment, while 10 specimens were used for the push-out bond strength test. All procedures were performed by one experienced endodontist.

Sealer penetration into dentinal tubules

GuttaFlow® Bioseal (Coltène/Whaledent, Langenau, Germany) was mixed with 0.1% Rhodamine B dye (Sigma-Aldrich, St. Louis, MO, USA) (16, 20, 25). The stability of the dye mixed in the sealer was verified and confirmed in pilot studies. The canals were obturated with gutta-percha cones in combination with the sealer using a single cone technique (8). The root canal orifices were sealed with Cavit, and all samples were stored at 370 C and 100% humidity for 15 days.

The specimens (n=6) (16) were embedded into resin blocks and sectioned horizontally using an Isomet saw (Buehler, Lake Bluff, IL, USA) to obtain 1 mm thick sections from 2, 5 and 8-mm levels from the apex. The exact thickness of each slice was measured using a digital caliper to 0.04 mm accuracy (Mitutoyo, Tokyo, Japan). Specimens were visualized under confocal laser scanning microscope to measure sealer penetration depth into the dentinal tubules (17). representative images from each group at coronal and apical thirds are shown in Figure 1. The measurements were recorded using the digital ruler of the NIS-Elements Microscope Imaging software (Fig. 2). The data were averaged to carry a single value for each section. A single operator analyzed all the specimens to rule out any discrepancy.

Measurement of dislocation resistance

The canals were filled with the sealer, and matched gutta-percha cone (ProTaper Universal F3, Dentsply Maillefer) was then inserted to the working length. The orifices were sealed with Cavit, and the specimens were stored at 370 C and 100% humidity for 15 days. The dislocation resistance was measured using the push-out bond strength test. The specimens [(n=10) per each subgroup] were embedded into resin blocks and sectioned horizontally using an Isomet saw (Buehler, Lake Bluff, IL, USA) to obtain 1 mm thick sections from 2, 5 and 8 mm levels from the apex. The thickness of each slice was measured using a digital caliper to 0.04 mm accuracy (Mitutoyo, Tokyo, Japan). The specimens were subjected to compressive loading using stainless steel plungers of different diameters (0.3 mm for apical, 0.7 mm for middle and 1.10 mm for coronal), in a universal testing machine (Shimadzu Corporation, Kyoto, Japan) at a crosshead speed of 1 mm/min (18). The push-out force was applied in an apicocoronal direction until bond failure occurred, which was represented by a sudden drop in load deflection. This force was recorded in Newtons. It was then converted to MPa by applying the following formula.

Where Push-out bond strength (MPa)=N/A; N=maximum failure load, A=adhesion area (mm^2). The bonding surface area of each slice was calculated as: $[\pi (r1+r2)]x[(r1-r2)_2+h_2]_{1/2}$; where π is the constant 3.14, r1 and r2 are the smaller and larger radii, and h is the thickness of the section in mm (2, 19).

Statistical analysis

The data were first analyzed using the Shapiro–Wilk test to verify the assumption of normality. The data were analyzed using three-way analysis of variance (ANOVA) and Tukey post-hoc tests to detect the effects of the independent variables (intracanal medicaments, final irrigation techniques, and root canal thirds) on penetration depth of the sealer. Additionally, the data were analyzed using two-way analysis of variance (ANOVA) and Tukey post-hoc tests for push-out assessment, considering the intracanal medicaments and its removal techniques as independent variables. All statistical analyses were performed using IBMM SPSS Statistics V23 at a significance level of 0.05 and a confidence interval of 95%.

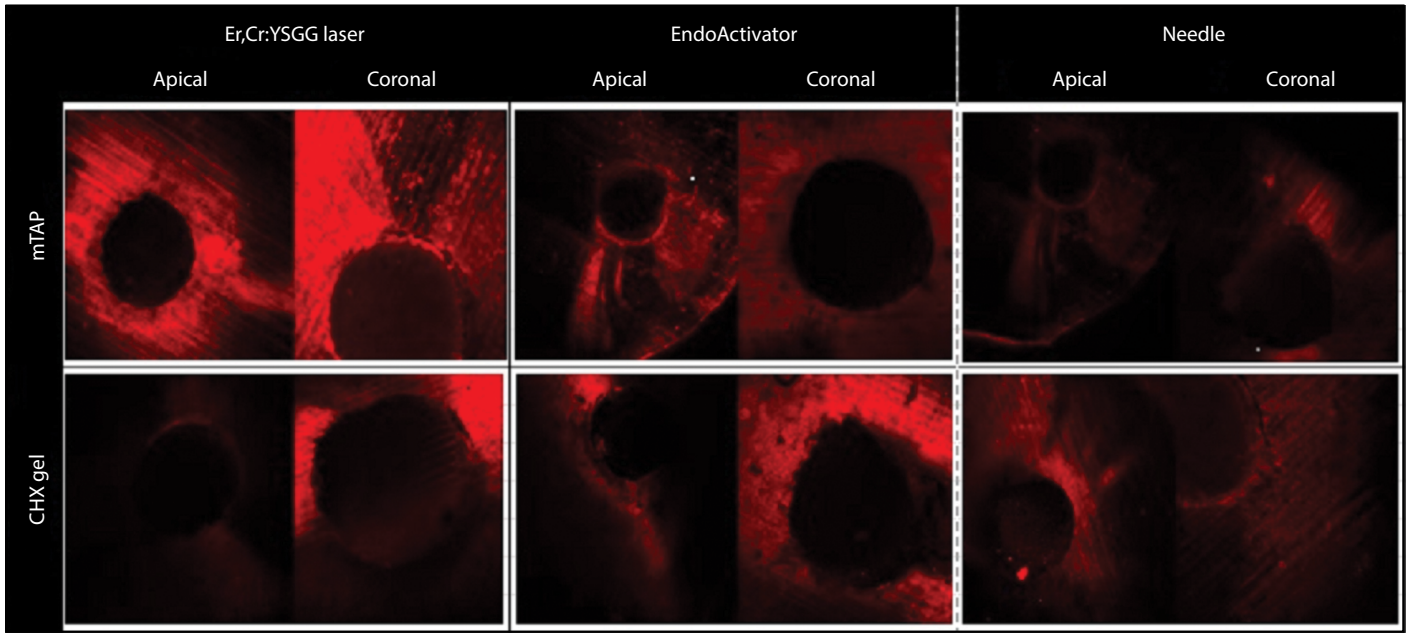

Figure 1. Representative confocal laser scanning microscope images from each group at the coronal and apical thirds

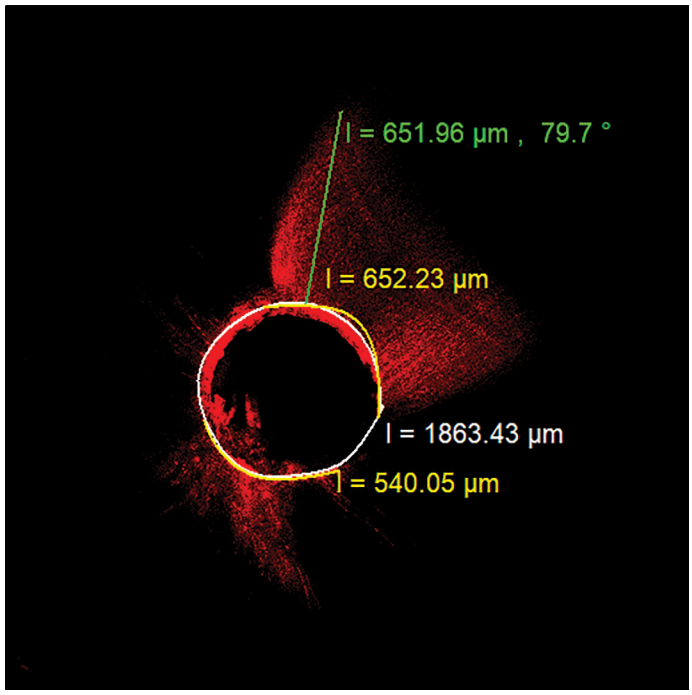

Figure 2. The measurements were recorded using the digital ruler of the NIS-Elements Microscope Imaging software

RESULTS

Sealer penetration into the dentinal tubules

The results of the three-way ANOVA test for sealer penetration depth are presented (Table 1). The interaction between the type of intracanal medicament and the sealer penetration values were not statistically significant (P=0.573). The final irrigation technique had a significant influence on sealer penetration (P<0.001). The average sealer penetration depths were recorded as 846.6 µm, 786.5 µm and 505 µm in the Er,Cr:YSGG laser, EndoActivator and control groups, respectively. The results showed that Er,Cr;YSGG laser resulted in a greater sealer penetration depth than the sonic agitation and syringe irrigation groups, although the difference between the laser group and the sonic group was not statistically significant (P>0.05). Syringe irrigation resulted in a significantly lesser sealer penetration than the other two groups (P<0.05). There were no significant differences in the penetration depth between the coronal, middle and apical thirds (P=0.057). Additionally, the interactions between the intracanal medicaments/the root-third, final irrigation technique/the root-thirds and the triple interaction (intracanal medicament, final irrigation technique, and root-thirds) were not statistically significant (P=0.97, 0.98 and 0.99, respectively).

The mean and standard deviations of sealer penetration depths according to the various intracanal medicaments, final irrigation techniques and the root canal thirds are presented in Table 2.

Bond strength to the root canals

The mean and standard deviation of the push-out strength values are presented in Table 3. The effect of the final irrigation technique on the bond strength was statistically significant (P<0.001) (Table 4). The syringe irrigation group demonstrated significantly less bond strength values than the other groups (P<0.05). The mean values were 9.08 in the Er,Cr:YSGG laser group, 8.44 in the EndoActivator group and 5.08 in the needle group. The mean bond strength obtained in group 3 (syringe-and-needle irrigation) was significantly less than the other groups (P<0.05).

DISCUSSION

This study investigated the effect of intracanal medicament type and technique of removal on the dentinal tubule penetration and dislocation resistance of a bioactive root canal sealer. The results showed that laser-assisted irrigation significantly increased sealer penetration and its dislocation resistance, immaterial of the intracanal medicament used. Hence, the null hypothesis is partially rejected.

Their direct effect on sealer penetration or dislocation resistance on the outcomes of root canal treatment are unclear. Considering the myriad variables that influence treatment outcomes, it may be unrealistic to correlate specific variables to success or failure of treatment. It has been reported that TAP penetrates deeper into dentinal tubules than calcium hydroxide (20). From a logical standpoint, complete removal of intracanal medicaments is recommended for better penetration of sealers into dentinal tubules as well its adaptation of sealers to dentine walls. This is specifically important for bioactive sealers that form a mineralized interface with biological substrates such as dentine (21). Theoretically, sealer penetration into the dentinal tubules could improve sealability by increasing the surface area contact of filling materials to dentinal walls (7). Furthermore, retention of root filling material might be developed by this mechanical locking. That theory also justifies the considerable research body studying the potential of dentinal

TABLE 1. Three-Way ANOVA for the intracanal Medicament, final irrigation technique, root canal third and the effect of their interactions on the maximum depth of penetration of the sealer

Source of variation	Type III Sum of squares	df	Mean square	F	p	Partial η^2
Medicament	23887.8	1.0	23887.8	0.3	0.573	0.004
Final irrigation technique	2393888.4	2.0	1196944.2	16.1	<0.001	0.263
Root canal third	441799.9	2.0	220899.9	3.0	0.057	0.062
Medicament *Final irrigation	4366.7	2.0	2183.4	0.0	0.971	0.001
Medicament *Root canal third	4564.9	2.0	2282.4	0.0	0.970	0.001
Final irrigation *Root canal third	27881.0	4.0	6970.3	0.1	0.984	0.004
Medicament *Final irrigation *Root canal third	7748.4	4.0	1937.1	0.0	0.999	0.001

df: Degree of freedom, F: Statistic table

TABLE 2. The mean and standard deviation of the maximum depth of penetration according to the various intracanal medicaments, final irrigation techniques and the root canal thirds

Final irrigation technique	Root canal third	Medicament		
		mTAP	CHX gel	Total
Er,Cr:YSGG laser	Apical	762.7±236.3	754.1±263.1	758.4±238.5
	Middle	879.8±214.1	884.9±207.7	882.4±201.1
	Coronal	916.0±290.7	882.1±217.0	899.1±245.2
	Total	852.8±243.5	840.4±225.5	846.6±231.4
EndoActivator	Apical	718.3±301.1	709.7±87.4	714.0±211.4
	Middle	807.2±228.9	757.5±372.3	782.3±295.8
	Coronal	898.0±219.5	828.2±415.0	863.1±318.6
	Total	807.8±248.9	765.1±310.1	786.5±278.0
Needle	Apical	450.0±210.2	392.4±196.7	421.2±196.4
	Middle	496.8±170.5	491.0±236.9	493.9±196.8
	Coronal	619.5±410.1	580.6±387.6	600.1±381.0
	Total	522.1±276.4	488.0±279.9	505.0±274.7
Total	Apical	643.6±276.2	618.7±247.9	631.2±259.0
	Middle	727.9±258.2	711.1±313.8	719.5±283.3
	Coronal	811.2±328.6	763.6±356.3	787.4±338.7
	Total	727.6±292.0	697.8±309.3	712.7±299.7

mTAP: Modified triple antibiotic paste, CHX gel: Chlorhexidine gel

TABLE 3. The mean and standard deviation of the push-out bond strength values of the experimental groups

Final irrigation technique	Medicament		
	mTAP	CHX gel	Total
Er,Cr:YSGG laser	9.19±5.32	8.98±4.34	9.08±4.84
EndoActivator	8.61±3.57	8.28±3.51	8.44±3.53
Needle	5.11±2.94	5.05±3.14	5.08±3.03
Total	7.63±4.43	7.44±4.06	7.53±4.25

mTAP: Modified triple antibiotic paste, CHX gel: Chlorhexidine gel

tubule penetration of filling materials. On the other hand, contrary to the common belief, the positive correlation between tubular dentine sealer penetration and the quality of the root filling has not been scientifically established.

Sen et al. (22) reported a lack of correlation between dentine sealer penetration and sealability. It was also shown that dentinal tubule penetration did not contribute to improved dislocation resistance for epoxy resin-based sealers (23). Thus, measuring sealer penetration as a sole outcome measure is insufficient. Therefore, this study used both sealer penetration and dislocation resistance as surrogate outcome measures to determine the efficacy of the irrigation techniques in removing the intracanal medicaments.

Sealer penetration into dentinal tubules has been evaluated using several techniques including scanning electron microscopy, stereomicroscopy, confocal laser scanning microscopy, microcomputed tomography and spiral computed tomography (24). This study used confocal microscopy as it is an easy and commonly used approach to visualize and quantify sealer penetration based on fluorescence. Although computed tomographic approaches are also non-invasive, confocal microscopic approaches are less demanding in terms of time required for imaging, reconstruction and analysis.

Sealer penetration was influenced by the irrigation technique, but not the intracanal medicament used and the root-third. While the root-third had no significant effect on the sealer penetration, the penetration depth in all the groups was higher in the coronal-third than in the middle and apical third. The finding that sealer penetration depth was higher in the coronal than in the middle and apical region is in accordance with previous studies (17, 25). Er,Cr:YSGG laser activation resulted in significantly higher sealer penetration depth than sonic agitation and syringe irrigation, immaterial of the root third and the medicament used. Despite the lack of ample studies on this aspect, one report demonstrated greater sealer penetration with Er, Cr: YSGG laser than with the EndoActivator (26). The reduced sealer penetration in the sonic agitation group may be attributed to the poor removal of intracanal medicaments from the dentinal walls. Lasers have been shown to be more

TABLE 4. Two-Way ANOVA for the intracanal medicament and final irrigation technique, and the effect of their interactions on the bond strength

Source of variation	Type III Sum of squares	df	Mean square	F	p	Partial η^2
Medicament	3.45	1.00	3.45	0.23	0.633	0.001
Final irrigation technique	1094.11	2.00	547.06	36.16	<0.001	0.172
Medicament *Final irrigation technique	1.07	2.00	0.53	0.04	0.965	0.000

df: Degree of freedom, F: Statistic table

effective in removing the smear layer (26, 27). Thus, it is likely that the hydrodynamics induced by the lasers resulted in better removal of the intracanal medicament and the smear layer from both the dentinal wall and from the dentinal tubules, resulting in a higher sealer penetration.

The difference between the EndoActivator group and syringe-and-needle group was not statistically significant. However, when the obtained values were examined, a higher penetration depth was obtained in the EndoActivator group than the syringe-and-needle group. There are both supporting and contradictory results in the literature on the cleaning efficiency of EndoActivator (28). In part, this is attributed to different parameters used in the studies, with regard to irrigation time, volume, concentration of irrigant and activation time. It may not be possible to standardize both time and volume of irrigants in studies using various agitation protocols. Furthermore, there is no clear consensus on the more important variable (time or volume or irrigant). In this study, the volume of irrigant was standardized. It is timely to review the literature by means of a systematic review to provide recommendations on the optimal time or volume of irrigation for different concentrations of irrigants and for different irrigant activation techniques.

The type of intracanal medicament did not demonstrate statistically significant effect on sealer penetration depth. However, when the results were evaluated, it was found that the root canal sealer penetrated deeper after mTAP removal in all 3 regions of the root (Table 2). There is a lack of studies evaluating the effect of these two intracanal medicaments on sealer penetration that preclude any comparison of our data. We speculate that the gel form of CHX may penetrate more into the dentinal tubules (29) compared to the mTAP slurry. However, this was not investigated in this study.

There are studies in the literature evaluating the effectiveness of NaOCl in removing triple antibiotic paste. They reported that NaOCl alone was not sufficient in removing triple antibiotic paste (30). In addition, Üstün et al. (31) reported that the use of NaOCl and EDTA together was effective in removing the triple antibiotic paste. For this reason, 5.25% NaOCl and 17% EDTA were used as standard in each group in this study. Also, Aydın et al. (32) reported that the use of EDTA in final irrigation increases the permeability of dentinal tubules and is effective on the penetration of the sealer. In this study, the authors believe that EDTA use has a positive effect on sealer penetration.

There are studies in the literature that contain contradictory results in which the effects of by-products formed by the interaction of NaOCl and CHX on sealer penetration are evaluated. Orhan et al. (33) reported that the brown solution formed by the interaction of NaOCl and CHX is a CHX solution and does not contain PCA. However, Bui et al. (34) reported that the by-product formed as a result of the interaction of NaOCl and CHX may affect sealer penetration. Wuerch et al. (13) used irrigation protocol with NaOCl without using saline to remove CHX gel and reported that it did not affect sealer penetration. In this study, Wuerch et al. (13) was taken as reference and saline was not used for the removal of mTAP and CHX gel. However, not using saline is one of the limitations of this study. Further *in-vitro* studies are needed to evaluate the effect of using NaOCl on sealer penetration in removing CHX gel.

One limitation of this study was that the amount of remaining intracanal medicament was not quantified; rather, the surrogate outcome measures (sealer penetration and dislocation resistance) were measured. Although analyzing the remaining medicament would have been interesting, it is more clinically relevant to demonstrate the effects of such remnants on the subsequent procedure in root canal treatment, i.e., root filling. Furthermore, using the same specimens for confocal microscopic analysis and push-out bond strength test would have enhanced the robustness of the data. This was not done as our pilot studies (data not shown) demonstrated that the bond strength of the sealers decreased when mixed with fluorescent dye.

CONCLUSION

The dentinal tubule penetration and dislocation resistance of GuttaFlow Bioseal were influenced by the final irrigation techniques used to remove intracanal medicaments, but not by the type of intracanal medicaments. Er,Cr;YSGG laser significantly increased the sealer penetration and dislocation resistance of the sealer compared to sonic agitation and syringe-and-needle irrigation.

Disclosures

Conflict of Interest: The authors has no conflict of interest.

Ethics Committee Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institu- tional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The use of extracted teeth was approved by the Institutional Review Board and Ethics committee of The University of Van Yüzüncü Yıl. (Approvel number: 03/08/2018-03)

Peer-review: Externally peer-reviewed.

Financial Disclosure: This Project was supported by Van Yüzüncü Yıl University Scientific Research Coordinator, Project No: TSA-2018-7442.

Authorship contributions: Concept – E.Ö., S.B., P.N.; Design – E.Ö., P.N., S.B.; Supervision – E.Ö.; Funding - E.Ö., E.A.; Materials - E.Ö., H.G., A.Y.U.; Data collection &/or processing – E.A., H.G., A.Y.U.; Analysis and/or interpretation – E.Ö., H.G., A.Y.U.; Literature search – E.Ö., H.G.; Writing – E.Ö.; Critical Review – S.B., P.N.

REFERENCES

1. Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. J Endod 2004; 30(8):559–67. [CrossRef]
2. Barbizam JV, Trope M, Teixeira EC, Tanomaru-Filho M, Teixeira FB. Effect of calcium hydroxide intracanal dressing on the bond strength of a resin-based endodontic sealer. Braz Dent J 2008; 19(3):224–7. [CrossRef]
3. Phillips M, McClanahan S, Bowles W. A titration model for evaluating calcium hydroxide removal techniques. J Appl Oral Sci 2015; 23(1):94–100.
4. Gu LS, Kim JR, Ling J, Choi KK, Pashley DH, Tay FR. Review of contemporary irrigant agitation techniques and devices. J Endod 2009; 35(6):791–804. [CrossRef]
5. Gomes-Filho JE, Duarte PC, de Oliveira CB, Watanabe S, Lodi CS, Cintra LT, et al. Tissue reaction to a triantibiotic paste used for endodontic tissue self-regeneration of nonvital immature permanent teeth. J Endod 2012; 38(1):91–4. [CrossRef]
6. Kamocki K, Nör JE, Bottino MC. Dental pulp stem cell responses to novel antibiotic-containing scaffolds for regenerative endodontics. Int Endod J 2015; 48(12):1147–56. [CrossRef]

7. Calt S, Serper A. Dentinal tubule penetration of root canal sealers after root canal dressing with calcium hydroxide. J Endod 1999; 25(6):431–3.
8. Akcay M, Arslan H, Topcuoglu HS, Tuncay O. Effect of calcium hydroxide and double and triple antibiotic pastes on the bond strength of epoxy resin-based sealer to root canal dentin. J Endod 2014; 40(10):1663–7.
9. Zhong X, Shen Y, Ma J, Chen WX, Haapasalo M. Quality of Root Filling after Obturation with Gutta-percha and 3 Different Sealers of Minimally Instrumented Root canals of the Maxillary First Molar. J Endod 2019; 45(8):1030–5. [CrossRef]
10. Gokturk H, Ozkocak I, Buyukgebiz F, Demir O. Effectiveness of various irrigation protocols for the removal of calcium hydroxide from artificial standardized grooves. J Appl Oral Sci 2017; 25(3):290–8. [CrossRef]
11. Devaraj S, Jagannathan N, Neelakantan P. Antibiofilm efficacy of photoactivated curcumin, triple and double antibiotic paste, 2% chlorhexidine and calcium hydroxide against Enterococcus fecalis in vitro. Sci Rep 2016; 6:24797. [CrossRef]
12. Mohammadi Z, Jafarzadeh H, Shalavi S, Yaripour S, Sharifi F, Kinoshita JI. A Review on Triple Antibiotic Paste as a Suitable Material Used in Regenerative Endodontics. Iran Endod J 2018; 13(1):1–6.
13. Wuerch RM, Apicella MJ, Mines P, Yancich PJ, Pashley DH. Effect of 2% chlorhexidine gel as an intracanal medication on the apical seal of the root-canal system. J Endod 2004; 30(11):788–91. [CrossRef]
14. Akman M, Akbulut MB, Aydınbelge HA, Belli S. Comparison of different irrigation activation regimens and conventional irrigation techniques for the removal of modified triple antibiotic paste from root canals. J Endod 2015; 41(5):720–4. [CrossRef]
15. Varela P, Souza E, de Deus G, Duran-Sindreu F, Mercadé M. Effectiveness of complementary irrigation routines in debriding pulp tissue from root canals instrumented with a single reciprocating file. Int Endod J 2019; 52(4):475–83. [CrossRef]
16. Generali L, Prati C, Pirani C, Cavani F, Gatto MR, Gandolfi MG. Double dye technique and fluid filtration test to evaluate early sealing ability of an endodontic sealer. Clin Oral Investig 2017; 21(4):1267–76. [CrossRef]
17. Cruz ATG, Grecca FS, Piasecki L, Wichnieski C, Westphalen VPD, Carneiro E, et al. Influence of the Calcium Hydroxide Intracanal Dressing on Dentinal Tubule Penetration of Two Root Canal Sealers. Eur Endod J 2017; 2(1):14–20. [CrossRef]
18. Ozlek E, Rath PP, Kishen A, Neelakantan P. A chitosan-based irrigant improves the dislocation resistance of a mineral trioxide aggregate-resin hybrid root canal sealer. Clin Oral Investig 2020; 24(1):151–6. [CrossRef]
19. Bitter K, Meyer-Lueckel H, Priehn K, Kanjuparambil JP, Neumann K, Kielbassa AM. Effects of luting agent and thermocycling on bond strengths to root canal dentine. Int Endod J 2006; 39(10):809–18. [CrossRef]
20. Deniz Sungur D, Aksel H, Purali N. Effect of a Low Surface Tension Vehicle on the Dentinal Tubule Penetration of Calcium Hydroxide and Triple Antibiotic Paste. J Endod 2017; 43(3):452–5. [CrossRef]
21. Saygili G, Saygili S, Tuglu I, Davut Capar I. In Vitro Cytotoxicity of GuttaFlow Bioseal, GuttaFlow 2, AH-Plus and MTA Fillapex. Iran Endod J 2017; 12(3):354–9.
22. Sen BH, Pişkin B, Baran N. The effect of tubular penetration of root canal sealers on dye microleakage. Int Endod J 1996; 29(1):23–8. [CrossRef]
23. Silva EJ, Perez R, Valentim RM, Belladonna FG, De-Deus GA, Lima IC, et al. Dissolution, dislocation and dimensional changes of endodontic sealers after a solubility challenge: a micro-CT approach. Int Endod J 2017; 50(4):407–14. [CrossRef]
24. Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Perdigão J, Lambrechts P, et al. Microscopy investigations. Techniques, results, limitations. Am J Dent 2000; 13(Spec No):3D–18D.
25. Uzunoglu-Özyürek E, Erdoğan Ö, Aktemur Türker S. Effect of Calcium Hydroxide Dressing on the Dentinal Tubule Penetration of 2 Different Root Canal Sealers: A Confocal Laser Scanning Microscopic Study. J Endod 2018; 44(6):1018–23. [CrossRef]
26. Chaudhry S, Yadav S, Talwar S, Verma M. Effect of EndoActivator and Er,Cr:YSGG laser activation of Qmix, as final endodontic irrigant, on sealer penetration: A Confocal microscopic study. J Clin Exp Dent 2017; 9(2):e218–22. [CrossRef]
27. Schoop U, Goharkhay K, Klimscha J, Zagler M, Wernisch J, Georgopoulos A, et al. The use of the erbium, chromium:yttrium-scandium-gallium-garnet laser in endodontic treatment: the results of an in vitro study. J Am Dent Assoc 2007; 138(7):949–55. [CrossRef]
28. Uroz-Torres D, González-Rodríguez MP, Ferrer-Luque CM. Effectiveness of the EndoActivator System in removing the smear layer after root canal instrumentation. J Endod 2010; 36(2):308–11. [CrossRef]
29. Ercan E, Erdemir A, Zorba YO, Eldeniz AU, Dalli M, Ince B, et al. Effect of different cavity disinfectants on shear bond strength of composite resin to dentin. J Adhes Dent 2009; 11(5):343–6.
30. Ok E, Altunsoy M, Nur BG, Kalkan A. Effectiveness of different irrigation solutions on triple antibiotic paste removal from simulated immature root canal. Scanning 2015; 37(6):409–13. [CrossRef]
31. Ustun Y, Düzgün S, Aslan T, Aktı A. The efficiency of different irrigation solutions and techniques for the removal of triple antibiotic paste from simulated immature root canals. Niger J Clin Pract 2018; 21(3):287–92.
32. Aydın ZU, Özyürek T, Keskin B, Baran T. Effect of chitosan nanoparticle, QMix, and EDTA on TotalFill BC sealers' dentinal tubule penetration: a confocal laser scanning microscopy study. Odontology 2019; 107(1):64–71. [CrossRef]
33. Orhan EO, Irmak Ö, Hür D, Yaman BC, Karabucak B. Does Para-chloroaniline Really Form after Mixing Sodium Hypochlorite and Chlorhexidine? J Endod 2016; 42(3):455–9. [CrossRef]
34. Bui TB, Baumgartner JC, Mitchell JC. Evaluation of the interaction between sodium hypochlorite and chlorhexidine gluconate and its effect on root dentin. J Endod 2008; 34(2):181–5. [CrossRef]