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Assessment of dentinal tubule penetration of AH plus, MTA Fillapex and Sealapex after various disinfection procedures: A confocal laser scanning microscopic study

Çeşitli dezenfeksiyon prosedürleri sonrası AH plus, MTA Fillapex Ve Sealapex'in dentin tübül penetrasyonunun değerlendirilmesi: Lazer taramalı konfokal mikroskop çalışması

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ABSTRACT

Objective: The aim of this study is to evaluate and compare dentinal tubule penetration of various root canal sealers obturated after the application of different irrigation activation procedures by a laser scanning confocal microscope.

Methods: A total of 150 extracted human permanent mandibular premolar single-rooted teeth were selected and randomly divided into 3 main groups according to the sealer type (n=50) as AH Plus, MTA Fillapex, and Sealapex. Each main group was randomly subdivided into 5 subgroups according to the irrigation activation protocols (n=10): Potassium–titanyl–phosphate laser irradiation, conventional needle, intra-kit, sonic irrigation, and ultrasonic irrigation procedures. After the activation procedures, the root canals were obturated with AH Plus, MTA Fillapex, and Sealapex mixed with 0.1% fluorescent rhodamine B isothiocyanate. Specimens were sectioned at 3, 6, and 9 mm from the apex. All sections were examined under a confocal laser scanning microscope to calculate the dentinal tubule penetration area.

Results: Data were analyzed using a 3-way analysis of variance and Tukey's post hoc tests ($P=.05$). Sealapex indicated a statistically lesser penetration than the other group ($P<.05$), MTA Fillapex ensured deeper penetration than AH Plus ($P<.05$). Ultrasonic irrigation provided significantly deeper penetration than other activation procedures ($P<.05$). The statistically highest percentage and the maximum depth of sealer penetration were shown in coronal third for all groups ($P<.05$).

Conclusion: The selection of root canal sealer, irrigation activation procedures, and root canal region plays a crucial role on the dentinal tubule penetration. AH Plus and MTA Fillapex applied with ultrasonic irrigation could achieve deeper sealer penetration in dentinal tubules.

Keywords: Dentin tubule penetration, confocal laser scanning microscopy, irrigation activation, root canal treatment

ÖZ

Amaç: Bu çalışmanın amacı, farklı dezenfeksiyon tekniklerinin uygulanmasından sonra uygulanan çeşitli kök kanal patlarının dentin tübüllerine penetrasyonunu bir konfokal lazer tarama mikroskobu kullanarak değerlendirmek ve karşılaştırmaktır.

Yöntemler: 150 adet çekilmiş insan daimi mandibular premolar tek köklü dişler seçildi ve rastgele olarak patların tipine göre AH Plus, MTA Fillapex ve Sealapex olarak 3 ana gruba ayrılmıştır (n=50). Her grup irigasyon aktivasyon prosedürüne göre rastgele olarak beş altgruba ayrılmıştır (n=10); KTP lazer ile ışınlama, geleneksel, intra-kit, sonik (Sİ) ve ultrasonik aktivasyon (Uİ) prosedüreleri.

Aktivasyon prosedürlerinin ardından kök kanalları % 0.1 floresan rodamin B izotiyosiyanat ile karıştırılmış AH Plus, MTA Fillapex ve Sealapex patlar ile dolduruldu. Örnekler apeksten itibaren 3, 6, ve 9 mm kesitler alındı. Bütün kesitler dentin tübül penetrasyonu alanını hesaplamak için bir lazer taramalı konfokal mikroskop altında incelendi. Veriler varyans üç yönlü analiz ve Tukey testleri kullanılarak analiz edildi ($P = ,05$).

Bulgular: Sealapex diğer gruplara göre anlamlı derecede düşük penetrasyon gösterdi ($P < ,05$). MTA Fillapex, AH Plus'a göre daha derin penetrasyon sağlamıştır ($P < ,05$), Uİ diğer dezenfeksiyon tekniklerine göre önemli ölçüde daha derin penetrasyon sağlamıştır ($P < ,05$). Tüm gruplarda koronal üçlüde, istatiksel olarak en yüksek oranda ve maksimum pat penetrasyon derinliği görülmüştür ($P < ,05$).

Sonuç: Kök kanal patı seçimi, irigasyon aktivasyon prosedürü ve kök kanal bölgesi dentin tübül penetrasyonu üzerine önemli bir rol oynamaktadır. Ultrasonik aktivasyonla birlikte uygulanan AH Plus ve MTA Fillapex ile dentn tübüllerinde daha derin pat penetrasyonu elde edilebilir.

Anahtar Kelimeler: Dentin tübül penetrasyon, lazer taramalı konfokal mikroskop, irigasyon aktivasyon, kök kanal tedavisi

INTRODUCTION

The main purpose of endodontic treatment is preventing and treating pathologies with pulpal or periradicular origin, thus maintaining the reducing microbial load in these areas. Thus, irrigation plays a key part in endodontic treatment. The aim of the irrigation process in endodontics is to remove microorganisms, necrotic or inflamed tissue, biofilm, and dentinal debris in the root canal system. Irrigation also claims better obturation via increasing the effectiveness of files.[1-3] At this point, canal irregularities, isthmuses and finns, lateral canals, and anatomical variations of the roots are challenging in the irrigation step.[1,3] Hence, traditional irrigation technique does not give the results aimed by professionals. To achieve better irrigation, the techniques have been developed and changed to a better version. Thus, different irrigation techniques are used for multiple aims to date.[3] Traditional irrigation technique is based on the agitation of the irrigant solution via small-size needles. The movement of the needle increases the effectiveness of the irrigant. It has been reported that this technique can be very effective and successful if made carefully. Also, it is cost effective and easy.[2,4] Additionally, NaOCl was used as an irrigant, and the results have shown that perforated irrigation needles provide better cleaning in root canals than conventional irrigation needles.[5] In this case, side-vented needles appear to be safer than open-ended needles because of the risk of leakage of the irrigant solution which can be very toxic to periradicular tissues. Potassium–titanyl–phosphate (KTP) laser systems have been used in dental practice mainly for bleaching and surgical purposes. These systems recently started to be used in endodontic practice besides other laser systems. Because of the photo-thermal properties of KTP lasers, it appears to be beneficial in irrigation procedures in endodontics. Romeo et al[6] conducted in their study that KTP laser was more effective in reducing primarily *Enterococcus faecalis* population than 980 nm diode laser. The traditional irrigation technique, also known as needle-delivered irrigation, is not sufficient enough in cleaning root canal system when compared to the sonic-activated irrigation technique. Sonic devices generally work at a frequency between 20 and 20 000 Hz.[3] It has been reported by Sabins et al[7] that sonically activated irrigation systems more effectively clean the root canal system than needle delivery systems. The thawing devices are the most preferred in sonic-activated irrigation techniques: EndoActivator, MicroMega, SonicAir1500, Rispi-Sonic, and Vibringe.[3] Ultrasonic activation was first used by Richman in 1957 with a Cavitron device and showed great results in the treatment. There are 2 types of ultrasonic irrigation (UI) systems which are passive UI and simultaneous UI.[3,8] A research that compared sonic irrigation (SI) and UI with traditional irrigation techniques, reported that SI and UI showed better results than needle delivery techniques alone .[9] Akcay et al[10] reported that UI is more advantageous in dentinal tubule penetration.

Obturation is another key part of endodontic therapy. The sealer are some biologic and biomechanical properties to have such as biocompatible, non-toxic, adhesive, dimensionally stable, flowable, and insoluble in tissue fluids.[11] Endodontic sealer materials should seal the root canal walls, laterally, middle, and apically, and should be well adapted to the root canal dentine. The penetration ability of the sealer into dentinal tubules is significantly important in the endodontic treatment process. It is also substantial in removing the residual bacteria and preventing reinfections.[10]

AH Plus is a resin-based sealer that is preferred frequently in endodontic therapy because of its beneficial standards such as sealing ability, dimensional stability, and high flow rate.[11,12] Another preferred sealer, MTA Fillapex, is an MTA (mineral trioxide aggregate)-based sealer material and beneficial due to the following properties: good sealing ability, biocompatible, non-cytotoxic, non-carcinogenic, and antibacterial.[13] Sealapex is a calcium hydroxide-based sealer known for its enhanced biocompatibility.[14]

The null hypothesis was created that there would be no difference among the sealer penetration degrees applied after various irrigation activation techniques into dentinal tubules. In this study, we aimed to evaluate and compare the effect of various irrigation activation techniques on dentinal tubule penetration of AH Plus, MTA Fillapex and Sealapex, KTP laser irradiation, conventional needle, intra-kit, SI, and UI procedures by using a laser scanning confocal microscope.

MATERIAL AND METHODS

Approval was obtained from the local human research ethics committee of Cumhuriyet University (ethics committee number: 2021-01/28). In the present study, 150 human mandibular premolar single-rooted teeth that were extracted , due to periodontal disease, orthodontic or prosthetic treatment planning, without root canal calcification and root surface fractures, cracks, and caries were used. The samples were divided into 3 main groups and according to the sealer type (n = 50) as AH Plus, MTA Fillapex, and Sealapex. Each group was randomly subdivided into 5 groups according to the irrigation activation protocol. All tissue and debris residues on the root surfaces of the teeth were removed and stored in physiological saline solution at +4°C until usage. Endodontic access cavities were prepared underwater cooling using diamond (Endo Access Bur; Dentsply Maillefer) bur with a high-speed handpiece. Size 10 K-file (Dentsply, Maillefer, Ballaigues, Switzerland) was advanced until it appeared apical foramen, and the working length was determined as 1 mm shorter than this length. To ensure standardization, the lengths of all teeth were standardized to 19 mm by flattening the crowns of the teeth with a diamond fissure, but root canals were shaped with ProTaper Next (Dentsply, Tulsa Endodontics, Oklahoma, USA), Nİ-Tİ rotary instruments, and endo motor system (Sendoline, Perfect Endo, Upplands Väsby, Sweden). Rotary instrument files X1 (size 17, 0.4 taper), X2 (size 26, 0.6 taper), X3 (size 30, 0.7 taper), and X4 (size 40, 0.6 taper) were, respectively, used at the Working Length (WL) for shaping the root canals. After each instrumentation, the root canals were irrigated with 2 mL of 5.25% NaOCl. About 17% Ethylenediamine Tetra Acetic Acid (EDTA), 5.25% NaOCl, and distilled water were applied for 5 minutes, respectively, to remove the smear layer that may affect the tubule penetration of the sealers to be used. Then, teeth were dried with paper points and sterilized using ethylene oxide. Teeth were randomly divided into a control group (without intracanal dressing) and 5 main groups according to the irrigation activation protocols (n=10) as KTP laser irradiation, conventional needle, intra-kit, SI, and UI activation procedures.

Main Groups

Conventional Needle Group: Conventional needle was inserted into the root canal 2 mm shorter than the working length and used in up and down motion in the canal, which facilitated the removal of debris (total time: 3 minutes).

Intra-Kit Group: NaviTip FX needle conventional needle was inserted into the root canal 2 mm shorter than the working length and used in up and down motion in the canal, which facilitated the removal of debris (total time: 3 minutes).

Sonic Activation Group: Around 10 mL of 2% NaOCl was delivered into the root canal. Then, it was activated by a sonic Vibringe system (Vibringe B.V. Corp, Amsterdam, Netherlands) without touching the canal walls (total time: 3 minutes).

Ultrasonic Activation Group: The ultrasonic activation was performed with a stainless steel #20/.00 file (IrriSafe; Satelec Acteon, Merignac, France) energized by a piezo electronic unit (Suprasson PMax; Satelec Acteon) at power setting "'blue" (total time: 3 minutes).

KTP Laser Irradiation Group: KTP laser irradiation was applied with 2.0 W (100 mj, 20 Hz) parameter and 532 nm wavelength (SMART LITE D, DEKA, Calenzano, Firenze, Italy) using with a fiber tip of 200 μm diameter. The fiber tip was positioned 2 mm shorter than the working length and then applied in 15-second recovery intervals for each irradiation, motions at a speed of 2 mm/s. This process was repeated 9 times (total time: 3 minutes).

The specimens were randomly divided into 3 groups for examining the sealing abilities of different sealer materials and their effects on dentinal tubule penetration following 5 different irrigation activation procedures. After that, all the root canal sealers were mixed in a 1:1 ratio according to the manufacturer's instructions with 10 μL 0.1% fluorescent rhodamine B isothiocyanate (Bereket Chemical Industry, Istanbul, Turkey) to give fluorescent images for Confocal Laser Scanning Microscopy (CLSM) analysis.

All root canal sealers that were constituent as the main groups of the research were placed into the canal 1 mm shorter than the WL using a size 40# lentulo spiral.

The Subgroups According to Sealers

AH Plus Sealer: A single gutta-percha cone (ProTaper Universal F4, Dentsply Maillefer) was then slightly coated with labeled epoxy resin-based sealer, AH Plus (Dentsply DeTrey, Konstanz, Germany), and placed in the root canal to the WL. After root filling, the coronal opening was filled with a temporary filling material (Cavit, 3M; ESPE, St. Paul, MN), and the specimens were stored at 100% humidity at 37°C for 1 week to completely set.

MTA Fillapex: Similar to the AH Plus group, the specimens were randomly divided into 3 groups, and the same procedure applied on the AH Plus group was performed but differently from the AH Plus group; MTA Fillapex were used as sealer material.

Sealapex: The specimens were divided into the same 3 groups, and the irrigation activation procedures were applied as in the AH Plus group. In this subgroup, Sealapex was applied as a sealer material.

Then, access cavities were sealed with Cavit W (3M ESPE, Seefeld, Germany), and teeth were incubated at 37°C for 24 hours before analysis.

Confocal Laser Scanning Microscope Analysis

After the sealers had been placed, each specimen was sectioned perpendicular to its long axis using a precision saw (IsoMet 1000; Buehler, Lake Bluff, Ill, USA) at a slow speed under water cooling. Following the medicaments had been placed in to the root canals, the teeth were sliced into 3 slices with approximately 1 ± 1 mm thickness and at depths of 2, 5, and 8 mm (apical, middle, and coronal). Similarly, a silicon carbide abrasive paper was used for polishing the sections. The samples were fixed by placing them on glass slides properly after processing, and it was examined with a Leica TCS-SPE confocal laser scanning microscope (Leica, Mannheim, Germany) set at 10× at wavelengths of 560-600 nm. If a single image of the root canal could not be obtained, different images of the canal were taken, and then these images were combined into a single image using Photoshop (Adobe Systems, Inc., San Jose, Calif, USA). Digital images obtained to measure and evaluate the total depth of dentin tubule penetration were transferred to the ImageJ program (ImageJ software, NIH, Bethesda, MD, USA). The maximum depth of sealer penetration was determined by measuring the distance from the canal wall to the deepest penetration point along the dentinal tubule. The sealer penetration area was calculated by subtracting the root canal area from the area measured by drawing around the dentin area in which the sealer penetrated. The percentage of sealer penetration was calculated from the ratio of the perimeter of the canal wall where the sealer had penetrated to the entire canal wall circumference. The dentinal tubule penetration area was measured as micrometers (μm) and converted to square millimeters (mm^2) for the statistical analysis. Data were analyzed using a 3-way analysis of variance (ANOVA) and Tukey's post hoc tests ($P=.05$). Confocal laser scanning microscopic images obtained from all groups of coronal parts are shown in Figure 1.

Statistical Analysis

The micro-crack incidence data were analyzed using the SPSS statistical software program version 14.0, (IBM Corp., SPSS Inc., Chicago, Ill, USA). A 3-way ANOVA was applied to compare the evaluated sealers penetration throughout the root canal. Analysis

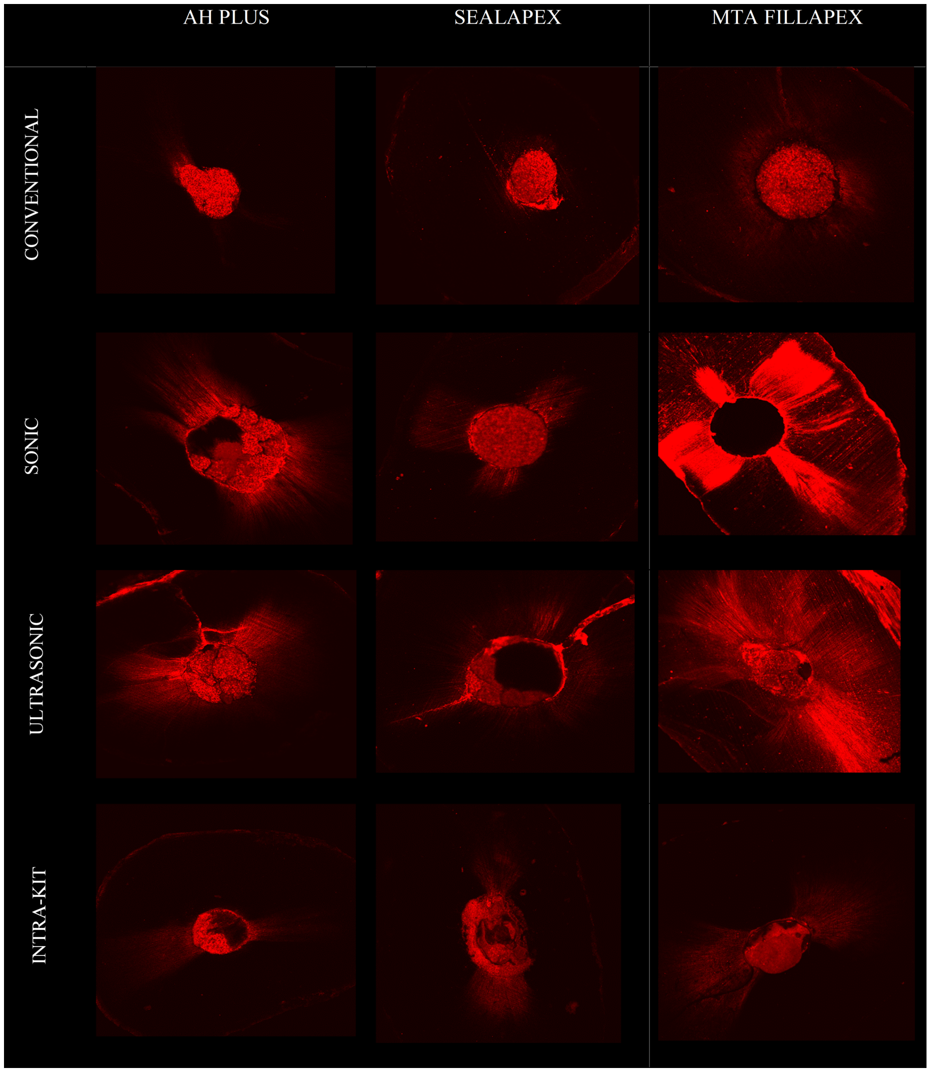

Figure 1. Confocal laser scanning microscopic images obtained from each group of coronal parts

of variance with post hoc Tukey's test was used for statistical analysis with a *P*-value at .05.

RESULTS

Mean and statistical data obtained from dentin tubule penetration area values by root canal sealer type, irrigation activation procedure, and root canal regions are shown in Table 1. Among all the sealers, although MTA Fillapex ensured the deepest penetration, Sealapex indicated a statistically least penetration than the other groups ($P < .05$). Ultrasonic irrigation provided a statistically significantly higher percentage and deeper dentinal tubules penetration than other activation procedures ($P < .05$). When the root canal regions were compared among themselves, the same irrigation activation techniques and sealers showed statistically higher tubule penetration in the coronal part compared to the middle third and the middle part compared to the apical third ($P < .05$).

DISCUSSION

Elimination of invasion of microorganisms and bacteria into dentinal tubules is a key part of endodontic infection control.[2] As in our knowledge, bacteria, most commonly *E. faecalis*, can invade deeply into the dentinal tubules as well as in root canals. Nevertheless, endodontic treatments consist of 3 main stages which are disinfecting, shaping, and filling the root canal systems, and all stages affect the other. Thus, the removal of these bacteria

Table 1. Mean and Statistical Data Obtained from Dentin Tubule Penetration Area Values by Root Canal Sealer Type, Irrigation Activation Procedure, and Root Canal Regions

	Sealapex			AH Plus			MTA Fillapex		
	Apical	Middle	Coronal	Apical	Middle	Coronal	Apical	Middle	Coronal
Conventional	$0.06 \pm 0.03^{aA\ddagger}$	$0.21 \pm 0.06^{bA\ddagger}$	$0.58 \pm 0.07^{cA\ddagger}$	$0.14 \pm 0.06^{aA\ddagger}$	$0.40 \pm 0.06^{bA\ddagger}$	$0.61 \pm 0.05^{cA\ddagger}$	$0.26 \pm 0.04^{aA\delta}$	$0.64 \pm 0.06^{bA\delta}$	$0.82 \pm 0.06^{cA\delta}$
Intra-kit	$0.14 \pm 0.06^{aA\ddagger}$	$0.35 \pm 0.07^{bA\ddagger}$	$0.71 \pm 0.06^{cA\ddagger}$	$0.22 \pm 0.06^{aA\ddagger}$	$0.56 \pm 0.07^{bA\ddagger}$	$0.77 \pm 0.05^{cA\ddagger}$	$0.34 \pm 0.06^{aA\delta}$	$0.74 \pm 0.06^{bA\delta}$	$0.96 \pm 0.06^{cA\delta}$
KTP laser	$0.23 \pm 0.05^{aA\ddagger}$	$0.51 \pm 0.07^{bA\ddagger}$	$0.79 \pm 0.05^{cA\ddagger}$	$0.30 \pm 0.08^{aA\ddagger}$	$0.68 \pm 0.06^{bA\ddagger}$	$0.86 \pm 0.07^{cA\ddagger}$	$0.46 \pm 0.04^{aA\delta}$	$0.85 \pm 0.07^{bA\delta}$	$1.12 \pm 0.07^{cA\delta}$
Sonic	$0.29 \pm 0.04^{aA\ddagger}$	$0.65 \pm 0.05^{bA\ddagger}$	$0.99 \pm 0.05^{cA\ddagger}$	$0.41 \pm 0.05^{aA\ddagger}$	$0.89 \pm 0.06^{bA\ddagger}$	$0.93 \pm 0.05^{cA\ddagger}$	$0.48 \pm 0.05^{aA\delta}$	$0.99 \pm 0.06^{bA\delta}$	$1.28 \pm 0.05^{cA\delta}$
Ultrasonic	$0.41 \pm 0.05^{aB\ddagger}$	$0.86 \pm 0.05^{bB\ddagger}$	$1.09 \pm 0.06^{cB\ddagger}$	$0.51 \pm 0.06^{aB\ddagger}$	$0.96 \pm 0.07^{bB\ddagger}$	$1.16 \pm 0.08^{cB\ddagger}$	$0.74 \pm 0.06^{aB\delta}$	$1.21 \pm 0.06^{bB\delta}$	$1.52 \pm 0.06^{cB\delta}$

Different superscript uppercase letters in the same column indicate a statistically significant difference ($P < .05$).
Different superscript lowercase letters in the same row (different thirds at the same sealers) indicate a statistically significant difference ($P < .05$).
Different superscript symbols ($^{\ddagger\delta}$) in the same row (same thirds at different sealers) indicate a statistically significant difference ($P < .05$).

from the root canal system is crucial in 3-dimensional obturation. The untouched and uncleaned areas in the root canal system constitute the focus of infection and menace the success of endodontic treatment consequently.[6] However, complete disinfection of the root canal system is not possible due to the complex anatomy of root canals and hard-to-reach areas. Moreover, there were disinfected areas after cleaning the root canals with NiTi systems, reported by Peters.[15] This makes the use of irrigant solutions essential in the endodontic treatment protocol.[16] The teeth that had endodontic treatment history are at a decreased risk of bacterial invasion compared to healthy teeth. Also, it is known that bacterial invasion of dentinal tubules are more prevalent in vital teeth than nonvital teeth because of the lack of odontoblastic activation and collagen fibers that organize the dentinal liquid transportation through dentinal tubules under a certain pressure.[17]

Thus, the present study data rejected the null hypothesis of there would be no difference among the sealer penetration degrees applied after various irrigation activation techniques into dentinal tubules.

Different irrigation techniques have been commonly used for infection control in endodontic practice. Moreover, removing the residual bacteria from dentinal tubules is significant in the cleaning procedure. Several facts affect the penetration depth of irrigation techniques such as root canal anatomy, the size and number of dentinal tubules, solubility, viscosity, the surface tension of the sealer, the particle size of the material, and the setting reaction of the material. The ideal irrigation solution should be non-toxic, non-allergic, antimicrobial, and dissolving on organic and inorganic dental tissues. Also, it should decrease the friction between dentin and endodontic instrument and so root canal preparation can be provided become more comfortable and less risky. [2] It was reported in a study that sealer penetration ratio was significantly higher in the apical third of the root among all thirds.[18] However, preventing fluid flow completely is not possible in endodontic treatments.[19]

Disinfecting and cleaning the root canal system can be made by a conventional needle delivery system. However, studies demonstrated that conventional needle delivery system is not as effective as sonic, ultrasonic, and laser systems.[3,8] Townsend and Maki[20] reported in their study that SI and UI is significantly more efficient than needle delivery system.

Over the past few decades, the endodontic practice has been developing itself, especially on irrigation protocols. There are several irrigation techniques, but any of them can completely clean the root canal system because of the unique anatomy and the variations of the canals. Thus, applying the most ideal irrigation technique is the key part of endodontic practice. Laser technology has been used in endodontics to improve the penetration depth and conduct an enhanced irrigation protocol.[6] Nevertheless, the penetration depth is one of the issues that endodontists face during irrigation protocols. To date, the most efficacious irrigation techniques are counted as laser irrigation techniques. The laser was found to be penetrating even on the inaccessible parts of the root canals and provides sufficient penetration and irrigation consequently.[6] According to studies on irrigation techniques in endodontics, the laser irrigation technique was found to be more effective on cleaning the dentinal debris, bacteria, and the smear layer in the coronal and apical parts of the root canals.[21–23] Moritz et al[24] conducted that it can be possible to eliminate a significant amount of bacteria from the root canals. Moreover, they achieved this result after 2 radiation treatments. They also pointed that exposure time and the management of the light fibers affect the sufficiency of irrigation. Kuştarci et al reported in their study that KTP laser irrigation is very antibacterial in infected root canals.[25] Similarly, it was reported in a study that the KTP laser was found to be more efficient than the 980 nm diode laser in reducing *the E. faecalis* population.[6]

Martin and Cunningham first detected that sonic and ultrasonic devices were successful in both mechanical and chemical debridement, disinfecting root canals and removing the smear layer. Also, they compared endosonic with the traditional techniques and concluded that endosonic was superior in previously counted manners.[26] Similarly, it was concluded that SI and UI were more efficient than conventional needle irrigation in a study.[7] Also, it was noted that sonic and ultrasonic systems can achieve the areas that cannot be achieved by traditional needle irrigation techniques.[7] Galler et al[27] reported that sonic, ultrasonic, and laser-induced activation showed enhanced penetration depth compared to manual activation. However, resulting of comparing the penetration ability of lasers and sonic agitation technique, lasers were found to be more effective and better in removing smear layer which can be ensure as a better sealer penetration .[28] In a recent study, it was reported that laser-activated irrigation was more effective in removing smear layer than antimicrobial photodynamic therapy.[29] In a study used with a confocal microscope, Nikhil and Singh put forth the better penetration in root canals of AH Plus sealer when applied with the ultrasonic file with 810 μm depth.[30] Nevertheless, Hachem et al[31] proved that the BC Sealer and NTS showed better tubule penetration than the AH Plus sealer. In another study, it has been reported that SI and UI are more effective in disinfecting the root canal system than needle irrigation. This result may be explained as less penetration ability of needle irrigation to the lateral canals.[9] All of these studies support the results of our study as UI is superior in the activation procedure than the conventional method. Nevertheless, to examine the effects of irrigation techniques, a confocal laser microscope is commonly used in studies because confocal microscopies provide a better, sharp image with less haze besides representing a thin cross-section opportunity.[32] Plus, creating a 3-dimensional image of a thin cross-section through a vertical axis is possible with confocal microscopes.[33] Thus, in this study, we preferred to use a confocal laser microscope.

The tubule penetration ability of AH Plus sealer was similar to the penetration ability of MTA Fillapex, reported by Amoroso-Silva et al.[34] In a study made with 96 human maxillary central incisors and divided into 3 experimental groups and used 3 sealers: AH Plus, BC Sealer, or NTS. The penetration depth of sealers was measured at 1 and 5 mm from the apex. The maximum penetration depth of all groups was significantly higher at 5 mm than 1 mm.

Additionally, Kok et al[35] demonstrated that AH Plus has a better tubule penetration than MTA Fillapex, but both sealers have no penetration ability after endodontic treatment.

Similar to our results, Sönmez et al[36] evaluated that MTA Fillapex showed significantly higher microleakage than AH Plus. Similarly, Silva et al[37] put forth that, MTA Fillapex was shown a higher flow rate than AH Plus according to ISO standards. Additionally, Shakya et al[38] compared several sealer materials such as AH Plus, MTA Fillapex, calcibiotic root canal sealer, and powdered gutta-percha (Gutta Flow 2). Thus, it can be concluded that AH Plus is a superior sealer than MTA Fillapex. They concluded that maximum inhibition of antibacterial property of *E. faecalis* was seen with AH Plus and followed by MTA Fillapex. Interestingly, Gutta Flow 2 did not inhibit *E. faecalis*.

Yücel et al[39] demonstrated the bacterial penetration rates in obturated root canals using different sealer materials. They used AH 26, AH Plus, Sealapex, and Ketac-Endo root canal sealer. Then they observed the bacterial penetration rates after 30 days of obturating. In conclusion, AH Plus showed the fastest bacterial penetration than other groups. This may be because of the protection ability of the sealer.

In conclusion, as many studies have evaluated, throughout the apical the tubule penetration depth is increasing comparing the coronal parts of the teeth because of the different intensities of tubules.Additionally, the apical part of the root canals contains fewer dentinal tubules that have smaller tubules diameters causes the more permeable than the middle and coronal parts.[40,41]

According to the result of our study, MTA Fillapex showed significantly better penetration than Sealapex and AH Plus sealers and UI provided a better tubule penetration depth than the SI and KTP laser irrigation techniques. Therefore, it has been determined that root canal irrigation methods have a significant effect on dentin tubule penetration of sealers, and there is also a significant difference between sealers.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Sivas Cumhuriyet University (Date: August 18, 2021, Number: 2021-01/28).

Informed Consent: Written informed consent was obtained from the all participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – R.Z.; Design – K.E.A.; Supervision – H.S.T., R.Z.; Resources – İ.H.; Materials – A.Ş.D.; Data Collection and/or Processing – R.Z., A.Ş.D.; Analysis and/or Interpretation – İ.H.; Literature Search – H.S.T.; Writing Manuscript – A.Ş.D.; Critical Review – R.Z.

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Hasta Onamı: Yazılı hasta onamı bu çalışmaya katılan tüm katılımcılardan alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir– R.Z.; Tasarım – K.E.A.; Denetleme –H.S.T.; Kaynaklar – İ.H.; Malzemeler – A.Ş.D.; Veri Toplanması ve/veya İşlemesi –R.Z., A.Ş.D.; Analiz ve/veya Yorum – İ.H.; Literatür Taraması – H.S.T.; Yazıyı Yazan – A.Ş.D.; Eleştirel İnceleme – R.Z.

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