Efficacy of a nickel-titanium ultrasonic instrument for biofilm removal in a simulated complex root canal

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Efficacy of a nickel-titanium ultrasonic instrument for biofilm removal in a simulated complex root canal

Directed by Professor Il Young Jung

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2023년 12월

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Disinfection of the root canal is a crucial process in endodontic treatment. Root canals have complex anatomical characteristics, and mechanical methods alone are insufficient to achieve the desired effect, which increases the importance of root canal irrigation. Sodium hypochlorite (NaOCl) is the most commonly used root canal irrigant,

with a wide antimicrobial spectrum, capable of dissolving necrotic and vital pulp tissue. However, if sodium hypochlorite is used for irrigation with a simple syringe, issues like stagnation or vapor lock may occur. To enhance the penetrative power of the irrigant and increase its irrigation efficacy, various activation methods have been discussed, including the use of sonic and ultrasonic devices. The energy produced in ultrasonic devices begins with the vibrating wire and is transmitted to the irrigant in the form of ultrasonic waves. This induces acoustic streaming and cavitation phenomena in the cleaning agent. On the other hand, sonic irrigation method involves generating mechanical vibrations at the tip of the device and features the use of flexible polymer tips to avoid mechanically shaping the root canal walls. The debate over which is more effective between ultrasonic and sonic devices is still ongoing. This study evaluated the effectiveness of nickel–titanium (NiTi) ultrasonic tips for *Enterococcus faecalis* biofilm removal in simulated complex root canals.

Sixty root canal models consisting of a 30-degree curved main canal and two lateral canals were constructed from polydimethylsiloxane and incubated with *E. faecalis*. In this study, a total of 5 different root canal cleaning methods were used, with a 3% sodium hypochlorite solution as the irrigant. Irrigants in root canals were activated using a manual syringe (SI-9), a stainless steel (SS-9) ultrasonic instrument, a NiTi ultrasonic instrument, or a sonic instrument (EA-2). Instruments of SI-9, SS-9, and NT-9 groups were placed 9 mm from the apex, whereas those in NT-2 and EA-2 groups were placed 2 mm from the apex. To evaluate the biofilms formed inside all root canals, observations were made using a fluorescence microscope. The efficacy of each method was determined as the ratio of fluorescence concentration before and after activation.

In the apical curved canal, the highest efficacy was found in the NT-2 group (99.40%), followed by SI-9 (84.25%), EA-2 (80.38%), SS-9 (76.93%), and NT-9 (67.29%) groups. In lateral canals 1 and 2, the efficacy was the highest in the NT-2 group and the lowest in the SI-9 group. The NiTi ultrasonic instrument could effectively remove biofilms in the curved canal and lateral canals. Furthermore, the SI-9 group showed a significantly lower irrigation effect compared to all other methods. The method with the highest irrigation effect in the lateral canal 2 was NT-2 (99.86%), while the SI-9 exhibited the lowest cleaning effect (76.19%). In the lateral canal 2, the SI-9 demonstrated a statistically significant lower irrigation effect compared to NT-2, NT-9, and stainless steel (SS-9) methods.

According to the linear mixed model analysis of irrigation efficacy based on the location of each root canal and the irrigation method used, there was an interaction between the irrigation method and the root canal location, indicating differences in irrigation efficacy based on the location (p=0.0195). Additionally, when the main efficacy was analyzed, with the irrigation method considered as a fixed effect, there were differences in irrigation efficacy based on the root canal location (p=0.0018, fixed effect: method). When the root canal location was considered as a fixed effect, there were also differences in cleaning effectiveness based on the cleaning method (p=0.0001, fixed effect: canal location).

In conclusion, in root canal systems featuring a curved main canal and two lateral canals, the use of ultrasonic activation with a NiTi instrument demonstrated enhanced efficacy in removing biofilms. when nickel-titanium files are operated using ultrasonic, they can effectively remove biofilms in the curved canals and lateral canals. This instrument should be introduced close to the working length. An up-and-down motion of the activation instrument is recommended.

Key words: Nickel titanium, Polydimethylsiloxane, Root canal, Ultrasonic, Biofilm

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I. Introduction

Disinfection of the root canal system is essential for successful endodontic treatment. However, disinfection is limited when using mechanical instrumentation alone because of anatomic complexities in root canal systems, such as curvatures, fins, grooves, isthmus, and lateral canals (Burleson et al. 2007, Gutarts et al. 2005). There is an increasing awareness of the importance of antimicrobial irrigants when cleaning these systems.

Sodium hypochlorite (NaOCl) is a commonly used irrigating solution with a broad spectrum of antimicrobial actions and has the ability to dissolve both necrotic and vital pulp tissues (Townsend, Maki 2009). A recent study reported that irrigants affect operative torque during root canal instrumentation. The operative torque means the amount of torque required by the instrument to reach the root apex (Mazzoni et al. 2020). NaOCl could reduce operative torque during intracanal instrumentation. However, a syringe irrigation method with NaOCl has some limitations, including the stagnation of the irrigant and vapor lock (Ram 1977, Tay et al. 2010). Various activation methods have been used to increase the penetration of irrigants and improve their efficacy. Sonic and ultrasonic devices are typically used (Bago et al. 2013). The energy of the ultrasonic device is transmitted from an oscillating file to the irrigant, which induce two physical phenomena: acoustic streaming and cavitation of the irrigant (Ahmad, Ford, Crum 1987, Ahmad et al. 1988, Mozo, Llena, Forner 2012, ROY*, AHMAD*, Crum 1994). Acoustic streaming is defined as a rapid movement of the fluid in a circular or vortex-like shape around the vibrating file. In contrast, cavitation is defined as the generation of steam bubbles (Mozo, Llena, Forner 2012). The acoustic streaming promoted by the ultrasonic device disrupts bacterial aggregation (Moreira et al. 2019). Passive ultrasonic irrigation produces higher frequency oscillation than sonic irrigation, ranging between 25 and 40 KHz (Merino et al. 2013). On the other hand, sonic activation generates mechanical oscillation at the irrigation tip. Sonic activation is performed with flexible polymer tips to prevent cutting the root canal wall (Jiang et al. 2010).

Many investigators have suggested that root canals are significantly cleaner after sonic or ultrasonic activation than after irrigation with a manual syringe (Burleson et al. 2007, Castelo-Baz et al. 2012, Conde et al. 2017, Duque et al. 2017). However, these activation methods also have some limitations. When treating the apical portion of curved main canals, unintended contact between the ultrasonic file and the root canal wall is inevitable because of the root canal system’s dimensions and complex geometry (Boutsioukis et al. 2013, Kato et al. 2016). This contact can reduce the ultrasonic energy and increase the risk of iatrogenic cutting of the root canal wall and instrument separation (Kato et al. 2016).

In the sonically activated tip, the apical root canal's small diameter can inhibit free oscillation of the tip, reducing the stream of irrigants into the canal, preventing cavitation either on the sonic tip or on the canal wall (Jiang et al. 2010). There is some controversy as to whether sonic or ultrasonic irrigation is more effective (Crozeta et al. 2020, de Gregorio et al. 2009, Merino et al. 2013).

Previous studies on the efficacy of root canal irrigation have used extracted teeth (single or multiple-rooted) or oversimplified root canal models (Boutsioukis et al. 2013, Crozeta et al. 2020, Mohmmed et al. 2016, Townsend, Maki 2009). The advantage of using extracted teeth is that it is easy to perform an experiment that reproduces a clinical situation. When evaluating the efficacy of various irrigation methods, the standardization of canals is of utmost importance. However, extracted teeth have very different morphological features, making it difficult to use them in in vitro studies under standardized conditions (Sun et al. 2018). To overcome these limitations, In many previous studies, artificial models have been created to simulate root canal anatomy, and materials such as plastic, resin, and polymer, among others, have been utilized for this purpose (Mohmmed et al. 2016, Park et al. 2023, Pereira et al. 2021). In one study, a 3D printing model was created using plastic, and when bacteria were matured, it was reported that bacteria did not colonized (Thakrar 2014). Another previous study (Mohmmed et al. 2018) attempted to make artificial root canal models using transparent resin material and three-dimensional (3D) printing to visualize the experimental disinfection of a root canal. In research that simulated a root canal model using polymer, it was reported that solidified Polydimethylsiloxane (PDMS) is widely used as a prototype for microfluidic chips (Pereira et al. 2021). In particular, PDMS is actively utilized not only in dental research on canal irrigation but also in medical research for experiments involving epithelial tissue replication (Pan et al. 2020). In another study, PDMS was used to investigate bacteria adhesion, and the results showed that soft PDMS, which is not stiff, enhanced bacterial adhesion and provided resistance to desorption against shear stress at the liquid interface (Valentin et al. 2019). The study evaluated whether several types of bacteria adhered to the surface of PDMS, and it reported that *Enterococcus faecalis* exhibited higher adhesion (Lou et al. 2020). Thus, we created a transparent, standardized, and anatomically complex root canal model that included the root canal curvature and lateral canals.

Enterococcus faecalis was the most frequently used test organism in endodontic biofilm model systems because it is frequently isolated from root canal-treated teeth with persistent apical pathosis (Swimberghe et al. 2019). *E. faecalis* a common resident of the oral cavity. *E. faecalis* employs various strategies to overcome the challenges of surviving within the root canal system (Stuart et al. 2006). *E. faecalis* was previously detected in approximately 18% of primary endodontic infections (Sundqvist et al. 1998). In approximately 67% of endodontic failure cases (Neelakantan et al. 2015), *E. faecalis* infection was considered to be the main cause of endodontic treatment failures (Siddiqui, Awan, Javed 2013). *E. faecalis* residing inside dentinal tubules has been reported to possess the ability to withstand calcium hydroxide-based dressings for more than 10 days and to survive in harsh environments(Haapasalo, Orstavik 1987, Orstavik, Haapasalo 1990). Furthermore, *E. faecalis* forms biofilms, which provide it with the ability to withstand the destructive effects of antimicrobials(Distel, Hatton, Gillespie 2002, Engstrom 1964). According to studies on the impact of Enterococci in root canals, it was reported that 12.1% of teeth were found to be affected when primary treatment was initiated in necrotic root canals. In the study by Molander et al., it was reported that *E. faecalis* was found in 68% of root canals when performing re-treatment of root canal therapy on teeth with apical lesions (Molander et al. 1998). A clinical research reported *E. faecalis* has been identified as a bacterium with strong resistance to sodium hypochlorite

(NaOCl) (Peciuliene et al. 2001). It was reported that, post-debridement, a relatively higher proportion of bacterial counts were observed compared to the initial counts.

A nickel-titanium (NiTi) ultrasonic (Endosonic Blue) file has been recently introduced into the market by Maruchi Co., Ltd. (Wonju, Korea). The NiTi file reportedly tends to straighten within a curved canal (Zupanc, Vahdat-Pajouh, Schäfer 2018), resulting in an unintentional cut in the canal wall. However, NiTi instruments in this study were in R-phase, which minimizes the risk of iatrogenic cutting of the canal wall and enhances fundamental flexibility.

According to recent studies, research on biofilm removal is being conducted using various mechanical-assisted irrigation methods (Jain et al. 2023, Park et al. 2023, Shin et al. 2023). Biofilm formation primarily involves the use of *E. faecalis* or *Campylobacter rectus*. Additionally, studies are being reported where research laboratories have independently developed equipment and compared them against existing methods. Common irrigation methods include syringe irrigation using sodium hypochlorite (NaOCl), sonic agitation, and ultrasonic activation. The positioning of the irrigation tip in each method is typically performed as per the manufacturer's instructions, often located approximately 1-2mm from the apical terminus during activation. The research on whether irrigation efficacy varies based on the device tip's position inside the root canal is currently limited. The aim of this study was to compare the efficacy of different activation methods for removing *E. faecalis* biofilm in a transparent root canal model. The irrigation efficacy based on location was also evaluated.

II. Materials and Methods

2.1. Fabrication of root canal models

Root canal models were simulated on microfluidic chips made with polydimethylsiloxane (PDMS) using a soft lithography technique (Qin, Xia, Whitesides 2010). A coat of negative photoresist (SU-8; MicroChem Inc., Westborough, MA, USA), which becomes insoluble when exposed to light, was applied to a silicon wafer and exposed to 365-nm ultraviolet light through a mask to make a mold for a channel. PDMS was then cast in the mold and bonded on a pre-fabricated flat PDMS block to make a microfluidic chip with an internal channel.

The microfluidic chip had a cuboid shape (20 mm × 40 mm × 5 mm). The simulated root canal consisted of a curved main canal and two lateral canals (Figure 1). The main canal consisted of a single curved canal with an apical size of #30 (0.3-mm diameter) and a 0.04-taper, which means that the diameter increases by 0.04 mm per 1 mm from the apex. A 30-degree curvature was made about 10 mm from the apex according to the Schneider method (Schneider 1971). Two lateral canals, each with a diameter of 100 μm, ramified from the main canal at 5 mm and 10 mm from the apex. The root canal model used in the study was a closed system.

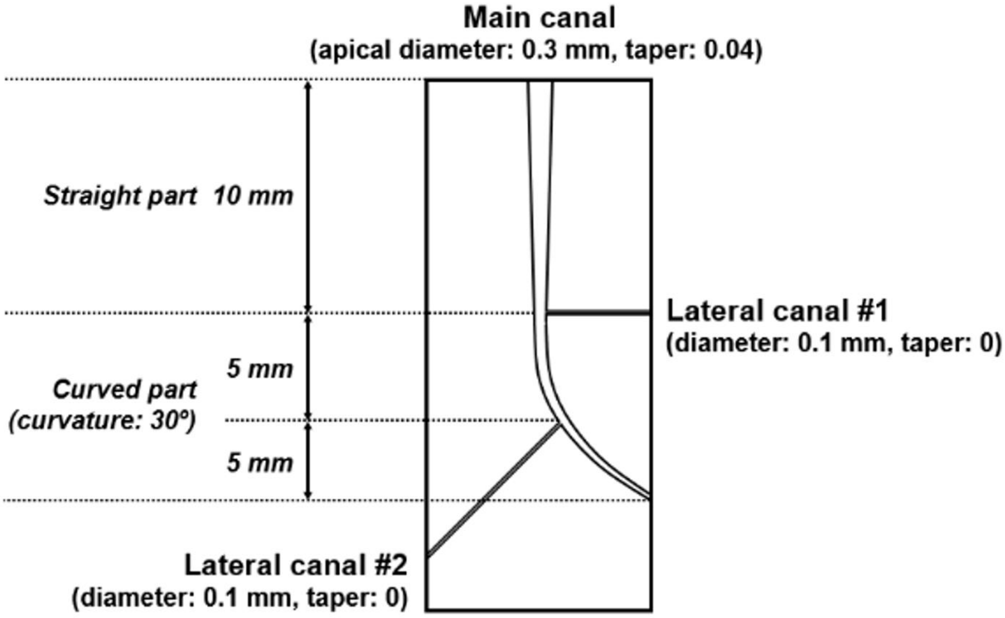

Figure 1. Schematic design of the simulated root canal model, including the lateral canal and the curved canal's apical portion. The lateral canals have a diameter of 0.1 mm. The curved canal's apical portion is created with an angle of 30 degrees from the straight main canal.

2.2. Formation of *E. faecalis* biofilm within the root canal model

E. faecalis (ATCC 19433) was inoculated into brain heart infusion (BHI) broth (Difco Laboratories Inc., Detroit, MI, USA) at a concentration of 1 × 10 CFU/mL and then incubated at 3℃ for 24 h with 80% N_2, 10% CO_2, and 10% H_2. Pure *E. faecalis* cultures were confirmed by colony morphology on BHI agar plates before the biofilm was generated. After 24 h of incubation, the root canal models were inoculated with 200 μL of *E. faecalis* for biofilm formation. The BHI broth was replenished daily and kept in an incubator for 10 days for the biofilm to develop.

The biofilm was visualized and quantified by a crystal violet binding assay, as previously described (Mohmmed et al. 2016). After 10 days of incubation with *E. faecalis*, loosely adherent bacteria inside the simulated root canal were rinsed out with 1 mL of sterile distilled water. The remaining biofilm was stained with 1 μL of crystal violet (212525; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for 1 min. Stained canals were subsequently washed with 3 mL of sterile distilled water for 1 min. The root canal model was placed on a microscopic slide table and observed under an Axio Imager M2 fluorescence microscope (Zeiss, Oberkochen, Germany). Images of two lateral canals and the apical portion of the main canal were captured (at 5× magnification) and analyzed with a ZEN pro software (Carl-Zeiss, Germany). The exit portals of the lateral and main canals were then blocked with sticky wax.

2.3. Irrigation procedures

Sixty root canal models were fabricated and randomly divided into five experimental groups (five different activation methods), as shown below. In all groups, a total of 9 mL of 3% NaOCl was delivered using a 10 mL syringe with a beveled 27-G needle. The needle was placed 9 mm from the apex into the canal. The syringe was attached to a programmed syringe pump to deliver the irrigant at a constant flow rate of 5 mL/min.

SI-9 group

In the syringe irrigation (SI-9) group, after the irrigation described above, the needle tip was placed 9 mm from the apex into the canal (Figure 2). The irrigant was left stagnant in the root canal model for 30 s. Then, all the debridement was washed out using 0.3 mL of 3% NaOCl.

SS-9 group

The agitation was carried out using a stiff stainless steel (SS) instrument with a 27G (DH tip; Epdent Co., Ltd., Seoul, Korea), which was mounted on a miniPiezon ultrasonic power unit (EMS Electro Medical Systems SA, Nyon, Switzerland). The instrument's tip was placed 9 mm from the apex into the canal, which was 1 mm short of its binding point in the curved canal (Figure 2). It was then activated at a power level of 4 with an ultrasonic power unit for 10 s, as directed by the manufacturer. Then, 0.1 mL of 3%

NaOCl was used for the washout debridement. The activation and debridement procedure described above was repeated two more times.

NT-9 group

The entire process was the same as that for the SS-9 group except a #15/.02 NiTi ultrasonic instrument (Endosonic Blue; Maruchi Co., Ltd, Wonju, Korea) was used instead of the stainless steel instrument. The ultrasonic instrument's tip was placed 9 mm from the apex into the canal (Figure 2).

NT-2 group

The same NiTi instrument was placed 2 mm from the apex into the curved canal (Figure 2). The entire process was the same as that used for the NT-9 group.

EA-2 group

Agitation was carried out using an Endoactivator (Dentsply Tulsa Dental, Tulsa, OK, USA) device, instead of an ultrasonic power unit, by placing the polymer tip with size #15/.02 2 mm from the apex into the curved canal (Figure 2). Then, activation was performed in a cyclic axial motion at the highest speed for 30 s, as suggested by the manufacturer. This was followed by debridement using 0.3 mL of 3% NaOCl.

After activation of the irrigant, crystal violet staining was repeated and the same area captured before irrigation was recaptured with the same microscope to measure the amount of remaining biofilm remaining.

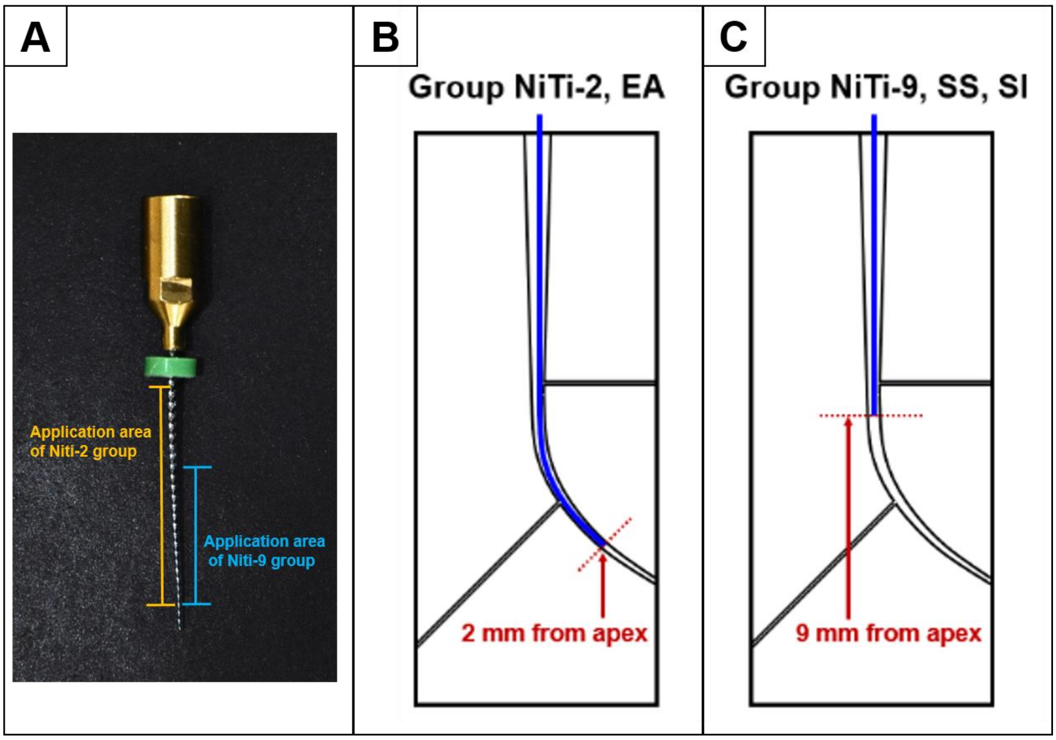

Figure 2. The left image (A) shows Ni-Ti ultrasonic instrument. NT-2 group means the file was placed 2mm from apex. NT-9 group means the file was placed 9mm from apex. The right image (B), (C) shows the five irrigation methods used in this study. Each method is indicated by a blue colored line in the designed root canal, representing the placement of irrigation instruments in this simulated anatomical structure.

2.4. Effects of irrigation on removal of *E. faecalis* biofilm

For each root canal model, images of two lateral canals close to the main canal and the area 2 mm from the apex were captured at 5x magnification with a fluorescence microscope before and after irrigation. All images were obtained from one side. The biofilm's fluorescence concentration was measured for each image (Figure 3) and analyzed using an Image-Pro Plus 6.0 software (Media Cybernetics, Inc., Washington, DC, USA). The efficacy of irrigation was determined as the ratio of fluorescence concentration before to after irrigation. To obtain images of the same location before and after irrigation, the same investigator marked a capturing point on the canal model's surface. The point where the lateral canal branched off from the main canal was used as the starting point of the image. The investigator marked the endpoint on the canal model to maintain the same capturing location of the lateral canal in all models. All images captured the same area three times with intervals, and their average values were used for analysis.

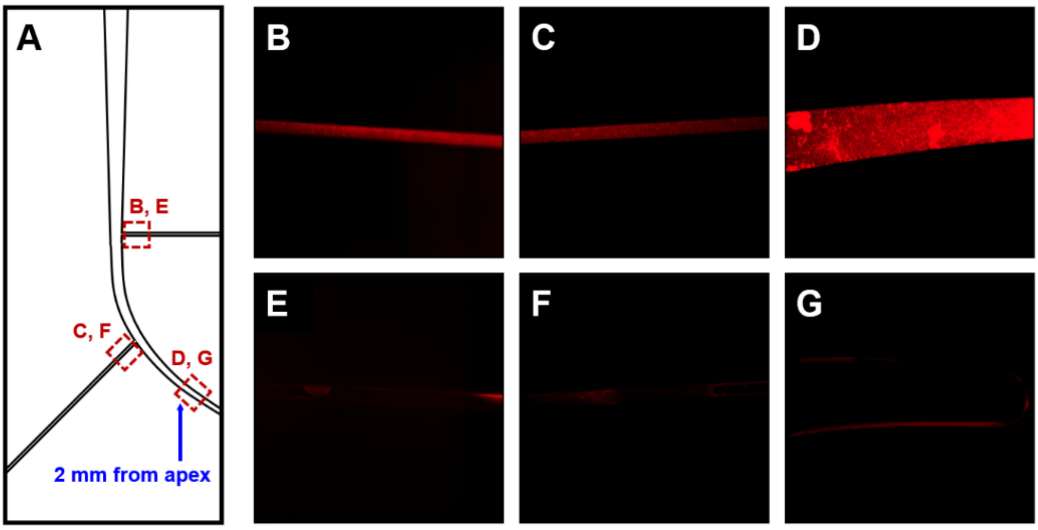

Figure 3. Representative fluorescence microscope images of *Enterococcus faecalis* biofilm at each root canal location (5x magnification) (**A**). The upper images show the growth of biofilm after incubation in lateral canal 1 (**B**), lateral canal 2 (**C**), and the apical portion of the curved canal (**D**). The lower images show each canal image after removal of biofilm from lateral canal 1 (**E**), lateral canal 2 (**F**), and the apical portion of the curved canal (**G**) after irrigation.

2.5. Statistical analysis

Normality of data was confirmed by the Shapiro-Wilk test. A linear mixed model was used to compare the efficacy of irrigation between the study groups. Differences in efficacy among the five irrigation methods depending on canal location were analyzed as method × location interactions. Tukey's post-hoc analyses were used to compare differences in the efficacy of irrigation between different methods and locations. All statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). A p-value < 0.05 was considered statistically significant.

III. Results

3.1 Difference in irrigation efficacy according to location or the method groups

Results for efficacy of irrigation according to each location in root canal model and irrigation methods are shown in Table 1-3. The linear mixed model analysis showed a significant difference in irrigation efficacy between methods according to canal location ($p=0.0195$, Table 1). When the root canal location had a fixed effect, there was a significant difference in the efficacy of irrigation according to the irrigation method (Table 2, $p = 0.0001$, fixed effect: canal location). When the irrigation method was fixed, the efficacy of irrigation differed significantly according to root canal location (Table 3, $p = 0.0018$, fixed effect: method).

Table 1. The percentage of irrigation efficacy values in terms of removing biofilm before and after irrigation using different irrigation methods.

Location	Mean (SE)					*p*-value
	NT-2	**NT-9**	**SS-9**	**EA-2**	**SI-9**	
Lateral canal #1	92.10 (6.80)	85.36 (7.40)	90.38 (7.08)	73.28 (7.40)	50.04 (6.80)	Method: 0.0001 Location: 0.0018 Method*Location: 0.0195
Lateral canal #2	99.86 (5.93)	95.14 (6.44)	99.14 (6.17)	84.95 (6.44)	76.19 (5.93)	
Main canal apex	99.40 (6.06)	67.29 (6.58)	76.93 (6.30)	80.38 (6.58)	84.25 (6.06)	

A linear mixed model analysis of the effect of location and method on irrigation efficacy in each root canal according to the type of irrigation method used. SE, standard error.

Table 2. Post hoc analysis of irrigation method by linear mixed model

Fixed variable	Method p-value									
	NT-2 Vs NT-9	NT-2 vs EA-2	NT-2 vs SS-9	NT-2 vs SI-9	NT-9 vs EA-2	NT-9 vs SS-9	NT-9 vs SI-9	EA-2 vs SS-9	EA-2 vs SI-9	SS-9 vs SI-9
Lateral canal #1	0.5048	0.0663	0.8612	<.0001	0.253	0.6257	0.0009	0.1005	0.0245	0.0001
Lateral canal #2	0.5918	0.0943	0.9328	0.0066	0.2686	0.6558	0.0348	0.1176	0.3215	0.0097
Main canal apex	0.0007	0.038	0.0129	0.0824	0.1652	0.2948	0.0632	0.7062	0.6673	0.406

Table 3. Post hoc analysis of root canal location by linear mixed model

Fixed variable	Location p-value				
	NT-2	NT-9	EA-2	SS-9	SI-9
Lateral canal #1 vs lateral canal #2	0.3382	0.2674	0.1866	0.2993	0.0019
Lateral canal #1 vs Main canal apex	0.4532	0.091	0.5015	0.1866	0.0008
Lateral canal #2 vs Main canal apex	0.9553	0.0026	0.6067	0.0111	0.3248

3.2 Irrigation efficacy of different activation methods at root canal location

Images of irrigation efficacy according to each location in root canal model and irrigation methods are shown in Figure 4 and 5. For the apical area of the main canal, the NT-2 group showed the highest irrigation efficacy (99.40%), followed by SI-9 (84.25%), EA-2 (80.38%), SS-9 (76.93%), and NT-9 (67.29%) groups. The irrigation efficacy was significantly higher in the NT-2 group than in the NT-9, SS-9, or EA-2 group. For lateral canal 1, the irrigation efficacy was the highest in the NT-2 group (92.10%) and the lowest in the SI-9 group (50.04%; Figure 5, Table 1). Irrigation efficacy was significantly lower in the SI-9 group than in any other study group. For lateral canal 2, the irrigation efficacy was highest in the NT-2 group (99.86%) and lowest in the SI-9 group (76.19%). The efficacy was significantly lower in the SI-9 group than in the NT-2, NT-9, or SS-9 group. All methods showed better efficacy for lateral canal 2 than lateral canal 1, although such findings were significant only for the SI-9 group.

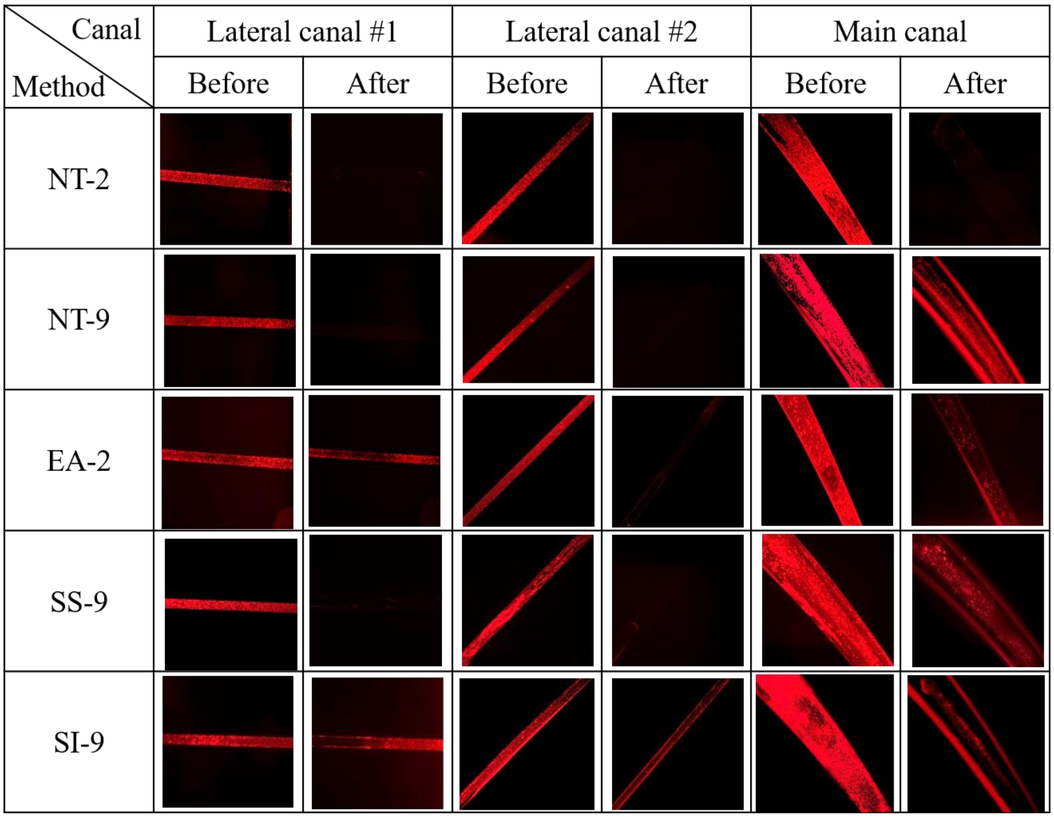

Figure 4. Representative Images before and after irrigation according to each canal locations and methods.

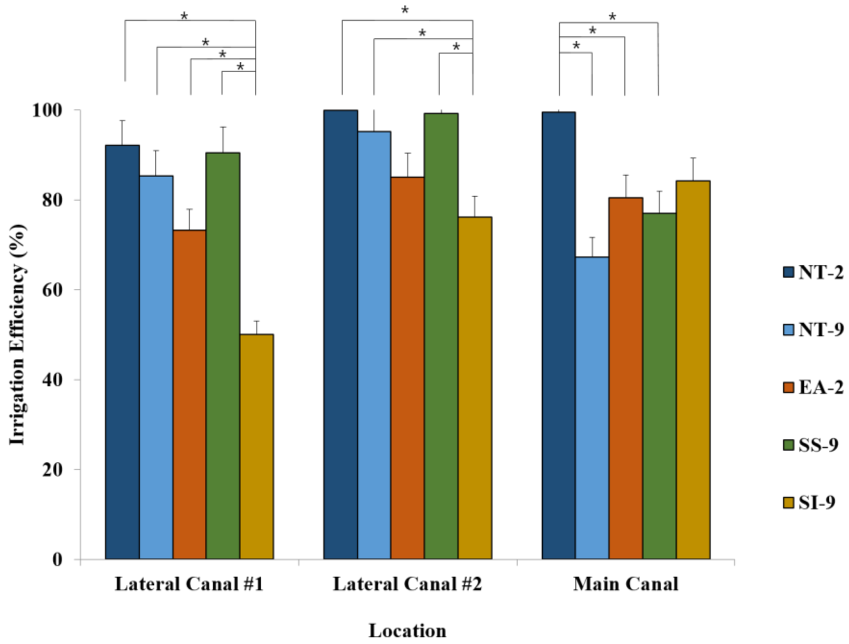

Figure 5. Irrigation efficacy of different activation methods at each location in the root canal. Post-hoc analysis found a significant difference between the different methods ($p < 0.05$), represented by an asterisk.

IV. Discussions

In this study, we evaluated NiTi ultrasonic tips' effectiveness in a simulated curved root canal model made with a PDMS microfluidic chip.

The NT-2 method showed the best performance for biofilm removal in the apical area of the canal. The efficacy of NT-2 was significantly better than that of other activation methods but not higher than SI-9. The biofilm removal was less effective in the EA-2 group than in the NT-2 group, meaning that the ultrasonic activation method was more effective than the sonic activation using an Endoactivator device; this finding is consistent with previous studies (Di Nardo et al. 2020, Jiang et al. 2010, Merino et al. 2013). The difference in efficacy might be due to differences in driving frequency, which was 30 kHz for the ultrasonic device and 160–190 Hz for the sonic device. The ultrasonic device’s higher frequency could induce a faster irrigant flow, resulting in effective acoustic streaming. Transient cavitation generated by ultrasonic devices might have also contributed to such a difference. By contrast, a sonic device generates mechanical oscillation at the irrigation tip without cavitation (Merino et al. 2013). In terms of increasing the irrigation efficacy, there is a limit to the application of only mechanical oscillations in a small curved canal.

When only ultrasonic devices were compared, there was no significant difference in efficacy between SS-9 and NT-9 groups, and the NT-2 group had better efficacy than SS-9 and NT-9 groups. These findings indicate that where the device is placed is more important than what it is made of. It can be inferred that the acoustic streaming and cavitation effect of the ultrasonic device are limited to the area close to the instrument tip. An SS-9 ultrasonic file can be used in a curved canal when the file is precurved. However, accurate bending is difficult in a clinical situation, which may cause severe file-wall contact, resulting in a poor streaming effect (Ahmad, Ford, Crum 1987, Boutsioukis et al. 2013). Thus, we chose the NiTi ultrasonic file that could be used all the way to the working length easily.

Ultrasonic instruments tend to fracture during use. The main cause of the fracture appears to be cyclic fatigue due to continuous oscillation (Ahmad, Roy 1994). Therefore, the instrument should show improved cyclic fatigue resistance. Several kinds of heat-treated NiTi alloys are available to manufacture endodontic instruments, including M-wire, R-phase (rhombohedrally distorted martensite phase), and CM wire (Ha et al. 2013, Lee et al. 2020, Zupanc, Vahdat-Pajouh, Schäfer 2018). Previous studies reported that the R-phase instrument revealed superior cyclic fatigue resistance and flexibility compared to conventional NiTi without heat treatment. The NiTi ultrasonic instrument used in this study is made by R-phase heat treatment technology and benefited from pre-bending. This pre-bending is due to the R-phase’s elastic modulus being lower than that of other alloys (Ha et al. 2013, Zupanc, Vahdat-Pajouh, Schäfer 2018). Therefore, the R-phase instrument allows a more effective canal preparation and irrigation procedure than do conventional systems. These properties of the NiTi instrument with the R-phase are appropriate for use in the irrigation procedure in curved root canals. Also, the size of the ultrasonic instrument used in the present study is #15/.02. If the diameter of the file is larger than the width of the root canal, a small diameter file is recommended because the contact with the canal wall will interfere with the creation of free oscillation (Amato et al. 2011).

On the other hand, the efficacy was lower in the SI-9 group than in the NT-2 group, but the difference between them was not significant. This means that 9 mL of irrigant with a 5 mL/min flow rate could also have adequate irrigation efficacy. It could be argued that the activation time and irrigant volume differed according to the method used in this study. Given that the optimal time and volume were not conclusive for each method, we did not consider that all the conditions should be the same for each method. Furthermore, this was not the aim of this study. Nonetheless, improved efficacy may be possible if a longer duration is used. However, increasing the treatment duration may be a disadvantage in clinical practice.

The results for lateral canals with a diameter of 100 μm were different from those for the main canal. All ultrasonic methods were significantly better than the SI-9 group for both lateral canals. Findings of the EA-2 group were better for the lateral canal 1 than in the SI-9 group. These results indicate that activation of the irrigant is important in removing biofilm in lateral canals with a diameter of 100 μm, although the activation methods might not be perfect. This result is consistent with that of a previous study (de Gregorio et al. 2010), confirming that it is difficult to remove biofilm formed in lateral canals with a small diameter, regardless of the irrigant used in traditional irrigation processes.

In all groups, more biofilm was removed from lateral canal 2 than from lateral canal 1, although the difference was only significant in the SI-9 group. These findings suggest that the irrigation efficacy differed according to the instrument's location, consistent with a previous study showing differences in cleaning efficiency (Ahmad, Pitt Ford, Crum 1987). The previous study suggested that the ultrasonic file's effect would be more intense at the apical section of the file because of acoustic streaming. Therefore, an up-and-down motion of the instrument or syringe is useful when cleaning a root canal system with lateral canals.

The PDMS is used for biomedical applications, such as microreactors, microchips for capillary gel electrophoresis, hydrophobic vent valves, and soft lithography (Fujii 2002, Whitesides et al. 2001). Bacterial invasion of dentinal tubules commonly occurs in endodontic infection (Siqueira Jr, Rôças, Lopes 2002). Although a porous structure similar to the dentin surface could not be reproduced using the PDMS material, a recent study has shown that PDMS-based root canal model is suitable for the imaging analysis of irrigation methods because it is transparent and has a contact angle with NaOCl similar to that of dentin (Layton et al. 2015). Song et al. reported that material properties affect bacteria-surface interaction and that the biofilm adheres to PDMS (Song, Koo, Ren 2015). A previous study using PMDS for biofilm removal has demonstrated bacterial growth and adhesion on this material's surface under conditions similar to those used for bacterial growth and adhesion to dentin (Mohmmed et al. 2017, Pereira et al. 2020). Experimental conditions using a porous structure of dentin are recommended for further in vivo studies.

The specifications of the PDMS microchip used in this study include an apical diameter of the main canal at 0.3mm with a taper of 0.04, and the lateral canal has a diameter of 0.1mm with taper of 0. Similarly, previous studies also created simulated root canal models with a length of 18 mm, an apical diameter ranging from 0.30 to 0.35 mm, and a taper of 0.06 (Mohmmed et al. 2016, Pereira et al. 2021). The most significant difference between previous research and our study showed the 0.04 taper size and the presence of a curved main canal in our research. Using PDMS, our study was able to create a more advanced form of a smaller taper, and it was also possible to mature *E. faecalis* biofilms in the curved canal. The mean primary curvatures of the mandibular first and second molars of the mesiobuccal canal have been reported to be 28.7 degrees (Cunningham, Senia 1992). Therefore, in this study, a simulated canal was fabricated with a curvature angle of 30 degrees from the end of the canal (Schneider method (Schneider 1971)), similar to a previous study (Townsend, Maki 2009), which was a strength of this investigation. Previous studies have evaluated the efficacy of irrigation to remove bacteria only in the main canal without considering the lateral canal (Mohmmed et al. 2016, Pedullà et al. 2019). Therefore, we developed a root canal model that included the lateral canals with a diameter of 100 μm and an anatomic structure similar to that of normal teeth (Al-Jadaa et al. 2009). To our knowledge, our study is the first to create a root canal model that includes both curvature and lateral root canals.

In recent research, isthmus was simulated as a canal model, and the efficacy of sonic and ultrasonic irrigation devices was compared (Park et al. 2023). A polycarbonate root canal model was produced, and experiments were conducted to simulate the removal of a hydrogel containing hydroxyapatite powder within isthmuses, which served as an environment mimicking biofilms. A previous results showed that ultrasonically activated irrigation exhibited a higher hydrogel removal effect compared to sonic activated irrigation. Additionally, in the isthmuses, ultrasonic activated irrigation showed significantly higher hydrogel removal in the apical part compared to the coronal part. This is attributed to the placement of the ultrasonic file tip 3mm from the apical portion of root canal, resulting in a more effective hydrogel removal in the apical portion compared to the coronal part. In contrast, our results confirmed the effective removal of biofilms in both two lateral canals and the curved main canal when the ultrasonic irrigation tip was positioned 2mm from the apex. The reason for these differences is speculated to be that in the previous research, an artificially created isthmus environment was simulated, and matured biofilms were not used in the that experiments conducted within the root canal. It is reasonable to assume that the hydrogel used in the previous research, which simulated the biofilm inside the canal, may not perfectly replicate the characteristics of real biofilms. Particularly, there might be differences, including variations in weight or mass, between the hydrogel and actual biofilms. The hydrogel, being a mixture of chemical components, is relatively heavier compared to biofilms. This is believed to have made it difficult to achieve hydrogel removal in the coronal portion when the ultrasonic file was positioned in the apical portion.

Compared to other studies, our research has the advantage of evaluating biofilm removal in very small sized lateral canals and a curved main canal. Furthermore, according to recent study that utilized a 3D root canal model inoculated with *Enterococcus faecalis, Streptococcus oralis, and Campylobacter rectus* to evaluate biofilm removal, a single root canal was used (Shin et al. 2023). The removal of biofilm was assessed based on the opening status of dentinal tubules, and additionally, the apical extrusion of NaOCl was evaluated. Ultimately, similar to our study, the previous study found that ultrasonic activation exhibited a high biofilm removal. A notable finding from the previous study was that ultrasonic activation resulted in less extrusion compared to sonic agitation. Based on these previous study results, our study also has its limitations. One of the limitations of our study is that we did not evaluate the formation of gas bubbles inside the root canal when using ultrasonically activated irrigation. In future research, it is necessary to use extracted teeth to form biofilms and capture the formation of gas bubbles inside the canal. Also, despite efforts to replicate complex curved canals and lateral canals in actual root canal models, different results may occur in the actual in vivo environment. Therefore, it is considered that a clinical study is needed. Furthermore, considering the current issues related to irrigation methods, conducting clinical studies on topics like irrigant extrusion or postoperative pain will be needed.

V. Conclusions

This study was compared the effects of the root canal activation method, and the conclusions are as follows.

1. In root canal systems designed with a curved main canal and two lateral canals, ultrasonic activation using a NiTi instrument showed improved efficacy in biofilm removal.
2. The effective removal of biofilm in curved main canals was observed when the instrument tip was in close proximity to the main canal. In the case of lateral canals, the efficacy of biofilm removal varied depending on the instrument tip position, suggesting that dynamic movements during instrumentation may contribute to enhanced biofilm removal.

Improved efficacy was found in the area around the instrument tip, clinicians should strive to make the tip come in contact with the root canal's entire length.

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ABSTRACT (IN KOREAN)

복잡한 근관 모형에서 세균막 제거를 위한 초음파 세정 효과

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근관계의 소독은 근관치료에 있어 매우 중요한 과정인데, 근관계의 복잡한 해부학적 특성으로 인해, 기계적인 방법으로는 충분한 효과를 얻을 수 없어, 근관 세정의 중요성이 커지고 있다.

차아염소산나트륨(Sodium hypochlorite, NaOCl)는 가장 많이 쓰이는 근관 세정제로 항균 범위가 넓고, 괴사 조직과 생활 치수 조직 모두를 용해시킬 수 있다. 그러나 차아염소산나트륨을 단순한 주사기 사용 방법으로 세정한다면 세

정제의 정체(stagnation)나 vapor lock 과 같은 현상이 나타날 수 있다. 이에 세정제의 침투력을 향상시켜 세정 효과를 높이기 위한 활성화 방법이 다양하게 논의되었고, 음파 및 초음파 기기들이 이용되었다.

초음파 기기에서 나온 에너지는 진동하는 와이어에서 시작되어 세정제로 초음파장 형태로 전달되는데, 이는 세정제의 음향류(acoustic streaming)와 공동화(cavitation) 현상을 유발하게 된다. 음향류는 진동하는 파일 주위에서 유체가 원형 또는 소용돌이 모양으로 빠르게 움직이는 것이고, 공동화는 증기 기포가 생기는 것을 의미한다. 초음파기기로부터 생성된 음향류는 박테리아 응집을 방해하게 된다. 또한 수동적 초음파 세정(Passive ultrasonic irrigation)은 25-40kHz 사이로 음파 세정에 비해 더 높은 주파수를 생성하게 되지만, 음파 세정은 세정기기 끝부분에서 기계적 진동을 발생시키고, 근관벽에 기계적 성형을 하지 않기 위해 유연한 폴리머 팁을 사용하는 특징을 갖는다.

다수의 선행 연구에서 세정제를 단순히 시린지 전달 방법으로 사용하는 것보다 음파 혹은 초음파 활성화 방법을 사용한 경우에 근관을 보다 우수하게 세정할 수 있었다고 보고하였다. 하지만 이러한 활성화 방법 또한 몇 가지 한계점을 갖고 있다. 만약 주 근관이 곡선형일 경우, 근관의 끝 부분을 치료할 때 근관의 너비 및 기하학적 복잡성으로 인해 초음파 파일과 근관 벽 사이에 의도치 않은 접촉이 발생할 수 있다. 이러한 접촉은 초음파 에너지를 감소시

키고, 근관 벽의 의인성 형성 및 기구 파절의 위험을 증가시킨다. 또한 음파로 활성화된 기구의 끝 부분은 치근단부의 작은 직경으로 인해 자유로운 진동이 어려워 근관내 세정제의 흐름은 물론 근관벽이나 기구의 끝 부분에서의 공동화 형성도 줄어든다. 근관 세정에서의 음파와 초음파 기기 중 어떤 것이 더 효율적인지에 대해서는 현재까지도 논쟁이 있는 실정이다.

한편 선행 연구에서 발치 치아들을 이용했을 때 근관의 형태가 다양하여 표준화된 조건을 조성하기 어렵다는 한계점이 있다고 보고하였다. 이에 따라 본 연구에서는 만곡된 근관과 측방근관을 지닌 해부학적으로 복잡성을 지닌 투명하고 표준화된 근관계를 고안하였다.

따라서 본 연구의 목적은 투명한 근관 모델에서 *E.faecalis* biofilm 제거를 위한 다양한 활성화 방법의 효능을 비교하였으며, 위치에 따른 세정 효율도를 평가하였다.

본 실험을 수행하기 위한 해부학적 근관 형태 재현을 위해 캐드 소프트웨어로 디자인하였고, 3D 프린터를 이용하여 근관 모델을 Polydimethylsiloxane(PDMS)로 총 65개 제작하였다. 근관 모델은 30° 각도를 가진 1개의 곡선형 주 근관과 2개의 측방 근관으로 디자인하였다. 본 근관 모델 내부에 세균막을 형성하기 위하여 *E.faecalis*를 이용하였으며 Brain-heart infusion broth (BHI) (Difco Co., USA) 액체 배지에서 37 °C, 10% CO2 배양 조건

하에 성숙시켰다. 모든 근관 내부에 형성된 세균막을 평가하기 위해 형광현미경 (Axio imager M2, Zeiss, Oberkochen, Germany)으로 관찰하였다. 본 연구에서 사용한 근관 세정법은 총 5가지 방법을 이용하였고 세정제는 3% 차아염소산나트륨을 사용하였다. 근관 세정 전과 후의 이미지에서 세균막 제거 효과를 정량화 하기 위해 잔여 세균막의 형광 농도 차이를 평가하였다.

본 연구 결과, 근관 위치에 따른 근관 세정 방법 간의 차이를 비교하였을 때 근관의 근단부에서 세정 효과가 가장 높게 나온 세정법은 NT-2(99.40%)였다. 다음으로 SI-9(84.25%), EA-2(80.38%), SS-9(76.93%), NT-9(67.29%) 순서로 세정 효과가 나타났다. 이 때 NT-2가 NT-9, SS-9, EA-2 에 비해 통계적으로 유의미하게 높은 세정 효과를 나타냈다. 첫 번째 측방 근관에서 세정 효과가 가장 높게 나온 방법은 NT-2(92.10%)였으며, SI-9(50.04%)에서 가장 낮게 나타났다. 또한 단순한 주사기 사용법을 이용한 SI-9는 다른 모든 방법들에 비해 통계적으로 유의하게 낮은 세정 효과 양상을 나타났다. 두 번째 측방 근관에서 세정 효과가 가장 높게 나온 방법은 NT-2(99.86%)였으며 SI-9(76.19%)가 가장 낮은 세정 효과를 나타냈다. 두 번째 측방 근관에서 SI-9는 NT-2, NT-9, SS-9 방법들에 비해 통계적으로 유의미하게 낮은 세정 효과가 나타났다.

각 근관의 위치와 세정법에 따른 세정 효과를 Linear mixed model 분석결과, 세정법와 근관 위치 간의 교호 작용이 있었으므로 위치에 따른 방법 간의 세

정 효과의 차이가 나타났다 (p=0.0195, table 1). 또한 주 효과 검정 결과 세정방법을 고정 (fixed effect)로 하였을 때, 근관의 위치에 따라 세정 효과의 차이가 있었으며 (p=0.0018, fixed effect: method), 근관위치를 고정(fixed effect)로 하였을 때, 세정방법에 따라 세정 효과의 차이가 있었다 (p=0.0001, fixed effect: canal location).

결론적으로 니켈-티타늄 파일을 초음파로 작동하면, 곡선 근관과 측면 근관의 세균막을 효과적으로 제거할 수 있었다. 효과적인 세균막 제거를 위해 초음파 기구를 작업 길이(working length)에 근접하게 위치시켜 상하 움직임으로 기구를 동작하는 것을 권장한다.

핵심되는 말: 니켈-티타늄, 폴리디메틸실록산, 근관, 초음파, 세균막.