

ORIGINAL ARTICLE

Efficacy of a novel remotely-generated ultrasonic root canal irrigation system for removing biofilm-mimicking hydrogel from a simulated isthmus model

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Abstract

Aim: To evaluate the efficacy of a novel ultrasonic irrigation device, remotely-generated irrigation with a non-invasive sound field enhancement (RINSE) system, in removing biofilm-mimicking hydrogel from a simulated isthmus model and compare it with sonically- and ultrasonically-activated irrigation systems.

Methodology: A polycarbonate root canal model containing two standardized root canals (apical diameter of 0.20 mm, 4% taper, 18 mm long with a coronal reservoir) connected by three isthmuses (0.40 mm deep, 2 mm high, 4 mm long) was used as the test model. The isthmuses were filled with a hydroxyapatite powder-containing hydrogel. The canals were filled with irrigant, and the models were randomly assigned to the following activation groups ($n = 15$): EndoActivator (EA), ultrasonically activated irrigation (UAI), and RINSE system (RS). Syringe irrigation (SI) with a 30G needle served as the control. Standardized images of the isthmuses were taken before and after irrigation, and the amount of hydrogel removed was determined using image analysis software and compared across groups using ANOVA ($p < .05$).

Results: Hydrogel removal was significantly higher with the RS (83.7%) than with UAI, EA, or SI ($p \leq .01$). UAI (69.2%) removed significantly more hydrogel than SI and EA ($p < .05$), while there was no significant difference between SI (24.3%) and EA (25.7%) ($p = .978$).

Conclusions: RINSE system resulted in the most hydrogel removal, performing better than UAI or EA. The effect of RS was also not reliant on the insert or tip entering the pulp chamber or root canal, making it particularly useful in conservative endodontics.

KEYWORDS

cavitation bubbles, gas bubbles, hydrogel biofilm model, irrigation technologies, minimally invasive approaches, vapor bubbles

INTRODUCTION

Apical periodontitis is a biofilm-induced disease, and root canal treatment (RCT) aims to eradicate bacterial biofilm from an infected canal (Siqueira & Rôças, 2022). Lateral canals, isthmuses, and apical ramifications have all been found to harbour bacterial cells, which are typically organized in biofilm-like structures (Ricucci & Siqueira, 2010). An isthmus is a thin, ribbon-shaped communication between two root canals (Teixeira et al., 2003), which is frequently clogged with hard tissue debris during instrumentation (Paqué et al., 2009), limiting accessibility to antibacterial solutions. Therefore, effective delivery of irrigants is critical for isthmus disinfection.

Although sodium hypochlorite presents excellent antibacterial properties and the ability to dissolve organic tissue, conventional syringe delivery of NaOCl does not consistently achieve complete disinfection of the root canal system (Siqueira et al., 1997). The main limiting factors in syringe irrigation are the complexity and variability of the root canal system and the characteristics of bacterial biofilms (Orstavik & Haapasalo, 1990; Siqueira & Rôças, 2022; Vertucci, 1984). Attempts to overcome these limitations have kindled interest in sonic and ultrasonic activation of irrigants within the root canal system. High-frequency vibration of a tip inserted into a root canal can create localized flow and cavitation bubbles along the tip, which has been suggested to contribute to the root canal cleanliness (Lumley et al., 1991; Macedo, Verhaagen, et al., 2014; Robinson et al., 2018; Roy et al., 1994) as well as physically disrupting biofilms (Vyas et al., 2019).

Minimally invasive approaches have been permeated in endodontics with the goal of tooth preservation (Neelakantan et al., 2022). They are primarily applied to access cavity preparation (Clark & Khademi, 2010) but have been extended to canal instrumentation (Gluskin et al., 2014; Krishan et al., 2014; Neelakantan et al., 2022). Conventional endodontics includes a traditional access cavity requiring straight line access (Silva et al., 2022) and conventional instrumentation with a minimum canal size of #25/0.06 or larger (Wang et al., 2018). In contrast, conservative endodontics uses a conservative access cavity, maintaining as much of the pulp chamber roof and the pericervical dentin as possible (Silva et al., 2022), with minimal instrumentation of an apical size less than #20–25 (Boutsioukis & Gutierrez Nova, 2021; Molina et al., 2015). Compared to conventional endodontic treatment, which creates enough space to deliver irrigants to the root canal system, conservative endodontics pursues preserving dentin as

much as possible without neglecting adequate canal cleaning (Neelakantan et al., 2022). The restricted space within such a minimally shaped root canal may compromise the efficacy of syringe irrigation (Boutsioukis & Gutierrez Nova, 2021) and sonic or ultrasonic activation of the irrigant (Jiang et al., 2010; Roy et al., 1994). Recently, laser-activated or multisonic irrigation have been introduced to improve the disinfection efficacy of minimally shaped canals (Molina et al., 2015; Ordinola-Zapata et al., 2014). Hence, in response to rising interest in modern irrigation strategies for enhancing canal cleanliness when canals are minimally prepared, a novel ultrasonic irrigation device, remotely-generated irrigation with a non-invasive sound field enhancement (RINSE) system, has been developed.

A recent study examined the cleaning effects of gas and vapour bubbles in ultrasonic cleaning and demonstrated that powerful ultrasonic waves could generate predominantly vapour bubbles in degassed liquids, which exist temporarily but exhibit powerful motion, thus improving the cleaning effect (Park et al., 2021). The RINSE system (RS) is designed to generate powerful acoustic waves to produce vapour bubbles within irrigants to remove all debris and, combined with sodium hypochlorite, eliminate microorganisms in the root canal system.

This study aimed to evaluate the efficacy of RS in removing biofilm-mimicking hydrogel from a simulated isthmus model and to compare it with conventional syringe irrigation and sonically and ultrasonically activated irrigation. The null hypothesis tested was that there would be no differences in the efficacy of these irrigation protocols for removing hydrogel from the isthmuses.

MATERIALS AND METHODS

This study was prepared in accordance with the PRILE 2021 Guidelines (Nagendrababu et al., 2021). Figure 1 shows the study design and its outcomes. The number of irrigation repetitions was estimated based on previous studies (Liu et al., 2022; Macedo, Robinson, et al., 2014; Macedo, Verhaagen, et al., 2014). Preliminary data were obtained from eight specimens and the effect size was established as Cohen's (1988) *d*. Using G*Power 3.1.9.7 software (Heinrich-Heine-Universität Düsseldorf) for one-way analysis of variance and the data from the pilot study, a minimal total sample of 48 specimens would support analysis with an effect size as 0.5, 80% power, and a 5% level of significance to statistically substantiate differences between experimental groups. A total of 60 samples were included in the final analysis, and each irrigation protocol was repeated 15 times ($n = 15$) per group.



FIGURE 1 PRILE 2021 flowchart.

Simulated isthmus model for hydrogel removal

A polycarbonate (PC) root canal model, specifically designed and manufactured by computer numerical control

(CNC) machining for this study was used, as shown in Figure 2. This model includes three separate parts with parting lines in the middle of the canals, and each part can be re-approximated with a bolt and nut. The PC model was modified from previous studies (Swimberghe

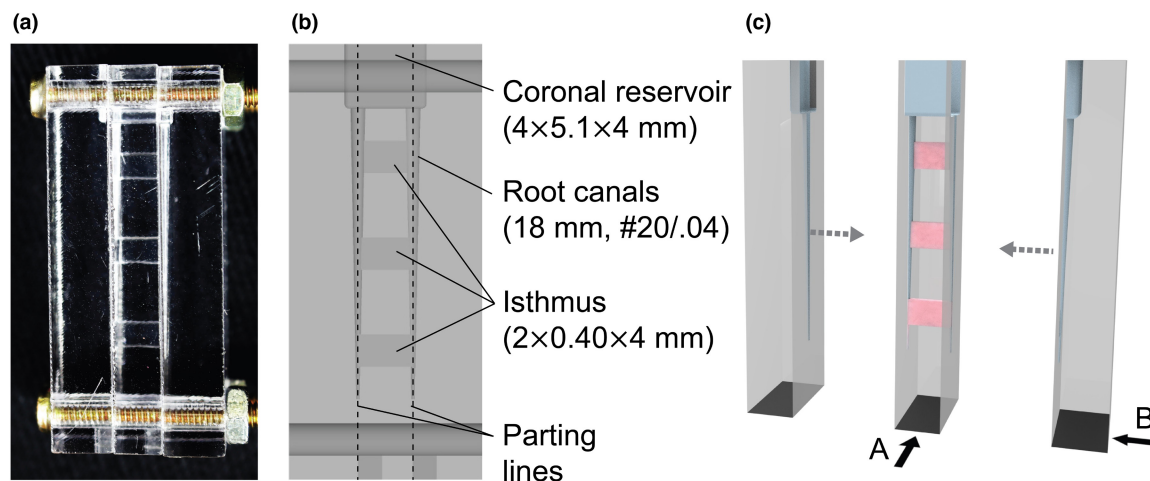


FIGURE 2 Polycarbonate (PC) root canal model with simulated isthmuses. (a) Clinical photo, (b) Schematic drawing, and (c) Three-dimensional model. Red indicates hydrogel filled in isthmuses.

et al., 2019) and simulated minimally shaped canals with multiple isthmuses (Liu et al., 2022). The model comprises a coronal reservoir (height 4 mm, length 5.1 mm, and width 4 mm) in which two root canals (length 18 mm, apical diameter 0.20 mm, and taper 0.04) end. The canals are connected by three isthmuses (height 2 mm, depth 0.40 mm, and length 4 mm), with the lowest one located 2 mm from the apical terminus. The other two were located every other 4 mm from the top of the lower one.

Biofilm-mimicking hydrogel with hydroxyapatite powders

Based on the biofilm-mimicking hydrogel model described by Macedo, Verhaagen, et al. (2014), the hydrogel was fabricated according to Swimberghe et al. (2019), but modified by adding 10- μ m hydroxyapatite powders (Sigma-Aldrich) in a 70/30 ratio (w/w), to resemble hard tissue debris during instrumentation. Before use, it was stored in an oven at 30°C. The hydrogel was placed in the isthmuses using a 30-G needle (NaviTip, Ultradent) and remained there for at least 1 min to cool and solidify at room temperature. After applying the hydrogel mixture in the isthmuses, the triple parts of the model were re-approximated and tightened using a bolt and nut. The canals and the coronal reservoir were then filled with distilled water (DW).

Degassed liquid preparation

We prepared degassed water for testing the RS. A vacuum pump (MVP 030-3DC, Pfeiffer Vacuum) created a flow of DW through a membrane (Liqui-Cel™ membrane contactor, 3M, Minneapolis, MN, USA), which removed a

significant amount of dissolved air in the water. The dissolved oxygen concentration was 7.2 mg/L and 1.2 mg/L before and after filtration using an oxygen meter (DO-31P, DKK-TOA).

Test groups

The hydrogel-containing models were randomly assigned to one of four irrigant activation groups, using computer software (www.random.org). DW was used as the irrigant and each irrigation condition was repeated 15 times.

In the EndoActivator (EA, Dentsply Sirona) group, which sonically activated an irrigant, a yellow Activator tip (#15/0.02) was equipped and operated at the highest speed. The tip was moved up and down manually over 4 mm, starting 1 mm from the apical terminus. In the ultrasonically activated irrigation (UAI) group, a non-cutting stainless steel #20/0.00 file (Irrisafe; Satelec Acteon) driven by an ultrasonic device (Suprasson P5 Booster; Satelec Acteon) at a power setting 6 was used in the canal as recommended by the manufacturer. The ultrasonic file was positioned centrally in the canal 3 mm from the apical terminus.

Activation in the EA and UAI groups was performed three times for 20 s per canal. Between each activation cycle, the canal was flushed with 3 mL irrigant for 10 s using a 27-G needle (Endo-Eze, Ultradent). Overall irrigation time was 180 s, including sonic or ultrasonic activation of 120 s. In the RS group, the 30-G nozzle of the handpiece was positioned above the coronal reservoir. Irrigation was performed for 180 s and approximately 20 mL of fluid was delivered per minute. The EA, UAI, or RS handpieces were fixed in a custom-made jig. In the control group, syringe irrigation (SI) was carried out using

a 3-mL syringe with a 30-G needle (NaviTip, Ultradent) at a flow rate of 0.15 mL/s. The syringe needle was moved up and down over 4 mm, starting from a binding point. After irrigation, the canals were dried with size 20 paper points (Dentsply).

Images and image analysis

A digital single-lens reflex camera (EOS 70D, Canon) with a 100-mm macro lens (RF 100mm f/2.8, Canon) was used to take standardized high-resolution images of the model before and after activation. A custom-made platform ensured that each sample was positioned identically. In each image, the hydrogel-covered area of the three isthmuses was determined and analysed using image analysis software (ImageJ, U.S. National Institutes of Health, Bethesda, MD). Representative images of RS and UAI were taken from the aspect indicated by A in Figure 2c at three sequential time points: before removal, midway through removal, and after complete removal.

Statistical analysis

The mean removed hydrogel proportions were calculated for each group. Statistical analysis was carried out using IBM SPSS Statistics version 26.0 (SPSS Inc.). Comparison of removed hydrogel proportions across groups was carried out using analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. Paired samples *t*-test was used to compare the amount of hydrogel before and after irrigant activation in each group. The significance level was set at .05.

Visualization of gas bubbles trapped in a root canal

We visualized gas bubbles trapped in a simulated root canal of the PC model after irrigation with RS and UAI for 2 min. In addition, we tested RS further with degassed or non-degassed water. As described previously, RS and UAI were operated in a PC model. Standardized high-resolution images were taken at the aspect pointed by B in Figure 2c, using a DSLR camera (EOS 70D, Canon) combined with a macro lens (RF 100 mm f/2.8, Canon).

RESULTS

The percentage of hydrogel removed was 24.3 (± 7.57) % for SI, 25.7 (± 9.65) % for EA, 69.2 (± 8.79) % for UAI,

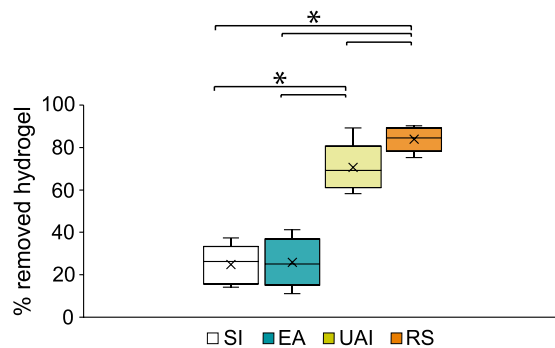


FIGURE 3 Ratio of hydrogel removed from isthmuses after irrigation per group (one-way ANOVA and Tukey's test, the bars and * above the box plots indicate significant differences; $n = 15$; $p < .05$).

and 83.7 (± 5.3) % for RS (Figure 3). Significant differences in the percentages of removed hydrogel between groups were found (ANOVA, $p < .05$). Hydrogel removal was significantly greater with RS than with SI, EA, or UAI ($p \leq .01$), and UAI removed significantly more hydrogel than SI and EA ($p < .05$). However, there was no significant difference in hydrogel removal between SI and EA ($p = .978$). As shown in Figure 4 (a, left), gas bubbles were rarely observed in the root canal during RS operation with degassed water. However, during RS operation with non-degassed water and UAI, many small gas bubbles were generated and merged into larger bubbles, and as a result, large gas bubbles trapped in the root canal were observed, as shown in Figure 4 (a, right) and (b).

DISCUSSION

This study aimed to evaluate the efficacy of RS in removing biofilm-mimicking hydrogel from a simulated isthmus model to compare it with SI and sonically activated (EA) and ultrasonically activated (UAI) irrigation. Under the experimental conditions of the current study, hydrogel removal differed significantly among the tested irrigation protocols; thus, the null hypothesis was rejected.

In conventional instrumentation, #30/0.06 is required for irrigant penetration into the apical third of the root canal (Khademi et al., 2006). In contrast, due to the confined space available in a minimally shaped root canal, the use of a needle in SI or an insert in EA or UAI may be ineffective for canal cleaning. In this study, 30-G needles (external and internal diameters of 308 μm and 196 μm , respectively) for SI, yellow tips (#15/0.02) for EA, and #20/0.00 Irrisafe files for UAI were used to get as close as 2 mm from the apical terminus without binding. A recent study reported that when 30-G needles are used in

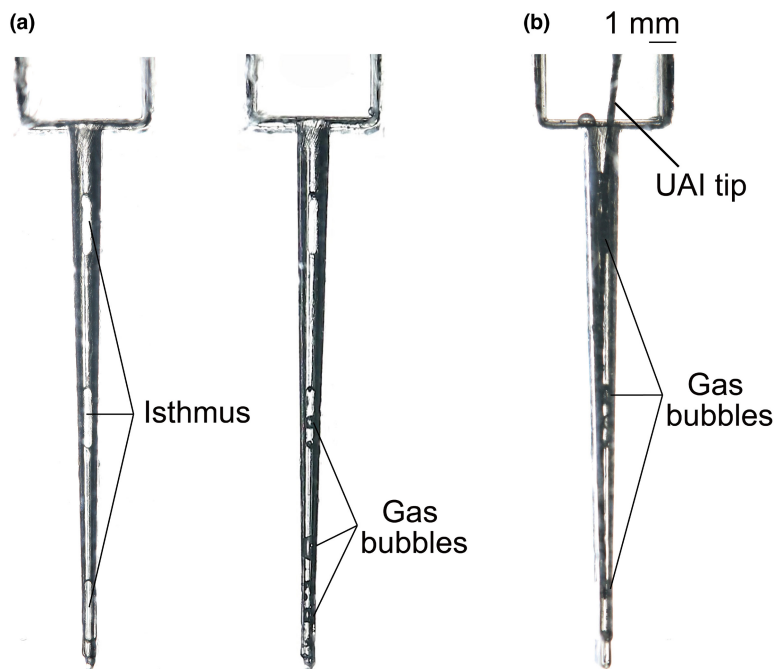


FIGURE 4 Representative images of gas bubbles trapped in a root canal. (a, left) RINSE system (RS) with degassing, (a, right) RS without degassing, and (b) ultrasonically activated irrigation (UAI). Images were taken from the aspect indicated by B in Figure 2c. Gas bubbles trapped in a root canal are shown for RS without degassing in (a, right) and for UAI in b.

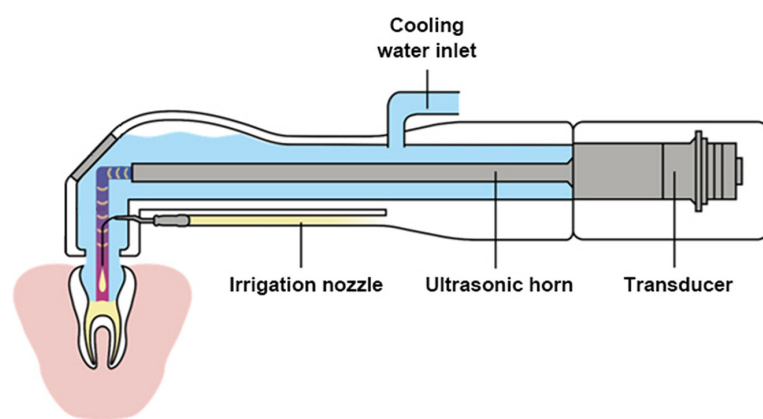


FIGURE 5 Schematic diagram of the RINSE system (RS).

minimally shaped root canals, irrigant penetration and shear stress in the apical third are compromised during SI (Boutsioukis & Gutierrez Nova, 2021). If the diameter of the apical canal is less than 0.5 mm, there is significant contact between the tip and the canal wall during sonically activated irrigation, which inhibits free oscillation of the sonic tip (Jiang et al., 2010; Macedo, Robinson, et al., 2014; Macedo, Verhaagen, et al., 2014). File-to-wall contact during UAI has been reported to lead to oscillation damping and, even worse, uncontrolled dentin removal (Boutsioukis et al., 2013; Boutsioukis & Tzimpoulas, 2016). These inadvertent effects may be aggravated in minimally shaped root canals; thus, conservative endodontics would benefit from the use of insert-free supplementary irrigation devices.

The RS has been developed to achieve irrigation without instruments insertion into the root canal system. For canal cleanliness, the RS employs large acoustic pressure

in degassed liquid to create vapour bubbles providing a better cleaning effect. Despite not being directly verified by the present study, its advantages were supported by an experimental model in our previous study (Park et al., 2021). The deliberate design of RS is shown in Figure 5. Compared to the ultrasonically oscillating tip used in UAI, a relatively large ultrasonic transducer was adopted in the RS to generate powerful acoustic pressure. While an irrigant stream is supplied through the nozzle of the handpiece, ultrasound waves emitted from the ultrasonic horn are guided into the root canal system. Consequently, acoustic waves can travel through the entire root canal system without requiring tip insertion into or the tight sealing of the pulp chamber.

The dynamic motion of cavitation bubbles has been reported to induce mechanical disruption of biofilms (Vyas et al., 2019). Cavitation bubbles can be classified as either gas or vapour bubbles depending on the relative

composition of the gas and vapour. Because vapour can rapidly turn into liquid, vapour bubbles exist only for short periods. In contrast, once gas bubbles are formed, gas cannot diffuse into the liquid on the time scale of bubble oscillation, so that gas bubbles survive much longer than vapour bubbles, often until they come out of the liquid (Park et al., 2021). Since we observed little trapped bubbles in the operation of RS, we assume that RS produced mainly vapour bubbles. Given that neither gas nor vapour cavitation bubbles were generated during sonically activated irrigation by EA (Jiang et al., 2010, Macedo, Robinson, et al., 2014, Macedo, Verhaagen, et al., 2014), one can conjecture that mixing irrigant with an acoustically agitating tip is the main mechanism of irrigation with EA. It has been reported that irrigation with UAI produces cavitation bubbles (Macedo, Robinson, et al., 2014; Macedo, Verhaagen, et al., 2014). Although cavitation can be important for root canal cleaning with UAI (Robinson et al., 2018), gas bubbles, typically exhibit weaker collapsing motions due to the cushioning effect of the gas, compared to the vapour bubbles employed in RS (Liu et al., 2014).

Figure 4 shows large gas bubbles trapped in a root canal, referred to as vapour lock. These large gas bubbles do not respond dynamically to ultrasound waves and block the transmission of ultrasound waves, thereby cleaning only in a limited region (Tay et al., 2010; Vera et al., 2012). Therefore, it is necessary to explore strategies to prevent the formation of gas bubbles, their accumulation, and consequent vapour lock. (Macedo, Robinson, et al., 2014; Macedo, Verhaagen, et al., 2014; Robinson et al., 2018). In this study, whether gas bubbles would be trapped in the canal, causing vapour lock, was compared in RS and UAI. In RS, we additionally used degassed or non-degassed

liquids, investigating how degassing affects vapour lock. As shown in Figure 4 (a, right), RS without degassing produced gas bubbles like UAI, but few gas bubbles were observed in RS with degassing. Despite degassing, tiny amounts of dissolved gas may remain in the degassed liquid; however, a continuous flow of irrigant through a nozzle can help remove occasional gas bubbles from the canal as well as detached biofilm and debris. Vapour lock formation and accumulation in UAI likely contributed to the limited hydrogel removal from isthmuses.

Representative images of RS and UAI removal of hydrogel from three simulated isthmuses are shown in Figure 6. RS cleaned the isthmuses from the coronal to apical aspects in a stepwise manner, whereas UAI cleaned them from the apical to coronal aspects. A similar pattern was found in a previous study of removal of calcium hydroxide from three isthmuses in simulated root canal models (Liu et al., 2022). Because the nozzle in RS is placed closer to the coronal than the apical side, nozzle-induced circulating flow may appear first on the coronal isthmus, resulting in cleaning. That contrasts with UAI, where the Irrisafe is held in place, and oscillation and fluid flow primarily occur in a single plane (Swimberghe et al., 2019). Moreover, the vapour lock created by gas bubbles in UAI may contribute to the limited cleaning effect in the coronal isthmus compared to the apical isthmus, where the file end with the largest oscillation amplitude was located. Regarding clinical application, RS employs a remote, simultaneous, and continuous activated irrigation. UAI necessitates canal insertion and activation of the tip near the isthmus, whereas the effect of RS with the nozzle above the access cavity is remote and not reliant on intracanal tip placement. Furthermore, UAI is activated in each canal individually with intermittent flushing. However, it is assumed that RS continuously replenishes

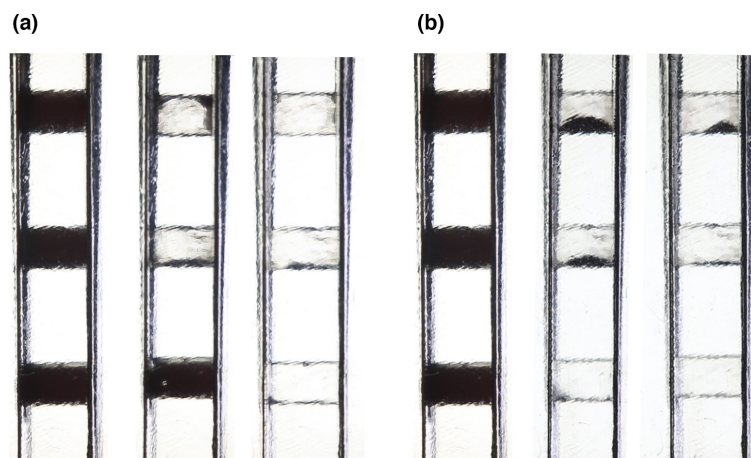


FIGURE 6 Representative images of intra-isthmus hydrogel removal. (a) RINSE system (RS) and (b) ultrasonically activated irrigation (UAI). Images were taken from the aspect indicated by A in Figure 2c. Three sequential images were taken before removal, midway through removal, and after complete removal from left to right. RS cleaned the isthmuses from the coronal to apical aspects in a stepwise manner, whereas UAI cleaned them from the apical to coronal aspects.

the irrigants in all canals simultaneously and circulates them, which may contribute to hydrogel removal, but further studies are warranted.

A biofilm is a structure composed of bacteria that are surrounded by a self-produced extracellular matrix (Koo et al., 2017). The matrix contributes to the viscoelastic properties of the biofilm, which protects the embedded bacteria from mechanical stress (Körstgens et al., 2001). The hydrogel presented in this study has been used as a substitute for a natural bacterial biofilm due to its viscoelastic properties (Fernandez-Rivas et al., 2012; Macedo, Robinson, et al., 2014). Moreover, HA powder was added to the hydrogel in this study to represent the considerable amount of hard tissue debris formed and packed into the isthmus area during canal instrumentation in clinical situations (Endal et al., 2011). This was consistent with other studies in which biofilm-mimicking hydrogel model was modified by adding hard tissue debris, resulting in a stiffer hydrogel that was less easily removed (Swimberghe De Clercq et al., 2019). The present study, however, employs HA powder particles of 10 microns, which appear significantly smaller compared to the debris produced by contemporary NiTi systems. Such minute debris may be comparatively easier to remove than the larger particles (150 microns) utilized in a previous study (Swimberghe De Clercq et al., 2019).

Nevertheless, a natural biofilm differs in composition and structure and can exhibit greater adherence to the substrate, whereas the hydrogel is attached with weaker adhesive force (Fernandez-Rivas et al., 2012; Swimberghe et al., 2019). Although a combination of antimicrobials and physical biofilm disruption via shear stress has been suggested as effective biofilm management (Koo et al., 2017), the present study purely assessed the mechanical effects of various activation systems. This study used distilled water as the irrigant because the chemical reaction between NaOCl and the hydrogel may generate stable bubbles that could impair the cleaning effect (Macedo, Robinson, et al., 2014; Macedo, Verhaagen, et al., 2014). Therefore, future studies are warranted to investigate tissue dissolution, the cleaning efficacy of *in vitro* microbial biofilms, and the removal of dentin debris in extracted teeth.

Lastly it should be noted that the image analysis program counts pixels on a single plane and does not consider volumetric changes of remaining or removed hydrogel. Therefore, the hydrogel removal results of this study could be underestimated. A future study could be done to evaluate the cleaning efficacy using three-dimensional quantification through microcomputed tomography. The current model has limitations regarding the difficulty of standardizing irrigant volume due to the different flow rates of each protocol. The amount of irrigant delivered during the 3 min experiment varied between groups depending on the flow rate and irrigant refreshment time, which may

have affected the results. Therefore, the results must be interpreted with caution. The experimental designs in this study aimed to conform to each system's recommended use rather than assessing the ultimate cleaning efficacy of different irrigation protocols under identical conditions, that is, the same active cleaning time per canal and flow rate.

Further limitations of the current model were that the PC isthmus model used was artificially designed, and the hydrogel was a mixture of chemical components; hence they may not reflect clinical situations *per se*. Nonetheless, this type of model is advantageous for comparative evaluation of the cleaning efficacy of various systems in terms of reproducibility, standardization, and visualization (Macedo, Robinson, et al., 2014; Macedo, Verhaagen, et al., 2014; Robinson et al., 2018; Swimberghe et al., 2019).

CONCLUSION

Within the limitations of this study, RS resulted in significantly greater removal of hydrogel from isthmuses in a simulated minimally shaped canal compared to the other test groups. Furthermore, the cleaning effect of RS was not reliant on the insert or tip entering the pulp chamber or root canals, which enables conservative access and minimal instrumentation.

AUTHOR CONTRIBUTIONS

The authors confirm contribution to the paper as follows: study conception and design: Won-Jun Shon; data collection: Eun Hyun Park, Ryeol Park, Jaedeok Seo; analysis and interpretation of results: Won-Jun Shon, Wonjung Kim, Ho-Young Kim; draft manuscript preparation: do Eun Hyun Park. All authors reviewed the results and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study does not require ethics committee approval.

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