

Comparative Evaluation of Irrigant Activation Systems on Sodium Hypochlorite Penetration into Dentinal Tubules in the Apical Third of Root Canals: A Confocal Laser Scanning Microscopy Study

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ABSTRACT

Background

Effective disinfection of the root canal system depends on adequate penetration and distribution of irrigants into areas inaccessible to mechanical instrumentation. Sodium hypochlorite (NaOCl) is widely used as an endodontic irrigant; however, its penetration into dentinal tubules, particularly in the apical third, is limited. Various irrigant activation techniques have been introduced to enhance irrigant penetration.

Aim

The aim of this study was to evaluate and compare the efficacy of conventional needle irrigation, sonic activation, ultrasonic activation, and diode laser activation on sodium hypochlorite penetration into dentinal tubules in the apical third of instrumented root canals using confocal laser scanning microscopy (CLSM).

Materials and Methods

Thirty-two extracted human mandibular premolars with single straight canals were selected and prepared using nickel–titanium rotary instruments up to size 25/.06 taper. The specimens were randomly divided into four groups ($n = 8$) according to the irrigation activation method: conventional needle irrigation, sonic activation, ultrasonic activation, and diode laser activation. Following final irrigation with 17% EDTA and 3% sodium hypochlorite, irrigant activation was performed according to the assigned protocol. The specimens were evaluated using CLSM, and the maximum penetration depth of sodium hypochlorite into dentinal tubules in the apical third was measured.

Results

A significant difference was observed among the four groups regarding sodium hypochlorite penetration depth ($P < 0.05$). The highest penetration depth was recorded in the diode laser activation group ($873.81 \pm 27.38 \mu\text{m}$), followed by the ultrasonic activation group ($789.71 \pm 30.25 \mu\text{m}$), sonic activation group ($556.80 \pm 37.91 \mu\text{m}$), and conventional needle irrigation group ($537.90 \pm 51.32 \mu\text{m}$). Post hoc analysis revealed significantly higher penetration with diode laser and ultrasonic activation compared with conventional needle irrigation, whereas no significant difference was observed between sonic activation and conventional needle irrigation.

Conclusion

The type of irrigant activation method significantly influences sodium hypochlorite penetration into dentinal tubules. Diode laser activation demonstrated the greatest penetration depth, followed by ultrasonic, sonic, and conventional needle irrigation. Laser-assisted irrigation may enhance irrigant penetration and contribute to improved disinfection of the apical third of root canals.

Keywords

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INTRODUCTION

Successful root canal treatment depends on the effective elimination of microorganisms, infected pulp tissue, and dentinal debris from the complex root canal system while preserving the structural integrity of the tooth. Owing to the intricate anatomy of root canals, including fins, isthmuses, lateral canals, and apical ramifications, mechanical instrumentation alone is unable to completely clean the canal walls. Therefore, chemical irrigation plays an indispensable role in achieving adequate canal disinfection.¹

Sodium hypochlorite (NaOCl) remains the irrigant of choice in endodontics because of its broad-spectrum antimicrobial activity, tissue-dissolving capability, and ability to neutralize bacterial endotoxins. However, its effectiveness depends on its ability to penetrate into dentinal tubules where residual microorganisms, particularly *Enterococcus faecalis*, may persist beyond the reach of mechanical instrumentation. Limited penetration of NaOCl into dentinal tubules has been attributed to its high surface tension and the anatomical complexities of the root canal system, especially in the apical third.²

The apical third of the root canal presents the greatest challenge for irrigation because of its narrow diameter, reduced dentinal tubule density, and limited irrigant exchange. Conventional needle irrigation (CNI), although widely practiced, relies mainly on positive-pressure delivery and has restricted irrigant replacement beyond the needle tip. Consequently, debris and microorganisms may remain in inaccessible areas, compromising treatment success.³

To overcome these limitations, several irrigant activation systems have been developed to improve irrigant penetration and fluid dynamics within the canal system. Sonic activation generates low-frequency oscillations that enhance irrigant movement through acoustic streaming. Ultrasonic activation operates at higher frequencies, producing vigorous acoustic streaming and cavitation, thereby improving smear layer removal, debris elimination, and antimicrobial efficacy. Numerous studies have demonstrated that passive ultrasonic irrigation significantly enhances the penetration of NaOCl into dentinal tubules compared with conventional syringe irrigation.⁴

Laser-activated irrigation has emerged as another promising technique for enhancing irrigant effectiveness. Laser energy produces photoacoustic streaming and cavitation, generating rapid fluid movement that facilitates deeper penetration of irrigants into dentinal tubules and anatomically complex regions. Previous investigations have reported superior irrigant penetration following

laser activation when compared with conventional irrigation and, in some cases, ultrasonic activation, although the available evidence remains limited and inconsistent.⁵

Confocal laser scanning microscopy (CLSM) has become a reliable and widely accepted method for evaluating irrigant penetration into dentinal tubules. The technique allows non-destructive visualization and precise measurement of fluorescent dye-labelled irrigants within dentin, providing accurate assessment of penetration depth while minimizing specimen preparation artifacts. Recent investigations have utilized CLSM to compare various irrigant activation techniques and have demonstrated improved penetration with activated irrigation systems compared with conventional needle irrigation.⁶

Although several studies have compared individual activation methods, direct comparison among conventional needle irrigation, sonic activation, ultrasonic activation, and diode laser activation using a standardized irrigation protocol in the apical third of mandibular premolars remains limited. Therefore, the present in vitro study aimed to evaluate and compare the efficacy of conventional needle irrigation, sonic activation, ultrasonic activation, and diode laser activation on the penetration of sodium hypochlorite into dentinal tubules in the apical third of instrumented root canals using confocal laser scanning microscopy.

MATERIALS AND METHODS

Sample Selection

Thirty-two extracted human mandibular premolars with single, straight root canals were selected for the study. Teeth extracted for periodontal or orthodontic reasons were included. Teeth exhibiting root fractures, dental caries, internal or external resorption, hypoplasia, calcified canals, or bifurcated/trifurcated canals were excluded. Following extraction, all specimens were cleaned of soft tissue remnants and calculus using an ultrasonic scaler and stored in distilled water until use.

Specimen Preparation

The crowns were removed at the cemento-enamel junction (CEJ) using a water-cooled diamond disc to obtain standardized root lengths of 15 mm. A #10 K-file (Mani Inc., Japan) was introduced into the canal until its tip became visible at the apical foramen. The working length was established by subtracting 1 mm from this measurement. Root canals were instrumented using NeoEndo S rotary nickel-titanium files (Neoendo, India) up to size 25/.06 taper according to the manufacturer's instructions. During instrumentation, canals were irrigated copiously with 3% sodium hypochlorite

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(NaOCl) between successive files.

Sample Grouping

The specimens were randomly allocated into four experimental groups ($n = 8$).

- Group I: Conventional Needle Irrigation (CNI)
- Group II: Sonic Activation
- Group III: Ultrasonic Activation
- Group IV: Laser Activation

Final Irrigation Protocol

The final irrigation protocol was standardized for all specimens. Each canal received 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) for 1 minute, followed by 5 mL of 3% sodium hypochlorite for 1 minute. After completion of the irrigation procedure, all canals were flushed with 2.5 mL of distilled water to eliminate residual irrigant.

Group I – Conventional Needle Irrigation

Irrigation was performed using a 30-gauge closed-end side-vented irrigation needle positioned 2 mm short of the working length. The needle was moved with gentle in-and-out (back-and-forth) motions during irrigation to facilitate irrigant exchange.

Group II – Sonic Activation

Following delivery of EDTA and NaOCl, irrigants were activated using a 25/04 polymer sonic tip positioned 2 mm short of the working length. Activation was performed at 10,000 rpm for 20 seconds, according to the manufacturer's recommendations.

Group III – Ultrasonic Activation

Irrigation was activated using the Ultra X Cordless Endodontic Ultrasonic Device (Eighteenth Medical, China) equipped with a #25 ultrasonic tip. The tip was positioned 2 mm short of the working length without contacting the canal walls. Activation was carried out for three cycles of 20 seconds each, using a total irrigant volume of 5 mL for each solution.

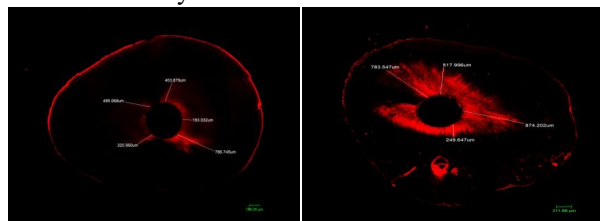
Group IV – Laser Activation

Laser activation was performed using a 980-nm diode laser with an output power of 1.5 W and a 200- μ m fiber tip. The fiber tip was introduced 2 mm short of the working length and moved in a slow helical motion from apical to coronal direction. Irradiation was performed for three cycles of 5 seconds, with intermittent pauses between cycles.

Confocal laser scanning microscopic evaluation

The sodium hypochlorite solution was labeled with Rhodamine B fluorescent dye before irrigation. Following completion of the irrigation protocol, the specimens were sectioned to obtain slices from the apical third of the root. The sections were examined using a confocal laser scanning microscope (LSM 800; Carl Zeiss, Jena, Germany) at $\times 5$ magnification using a 561-nm excitation laser. Digital images were obtained and analyzed using

the microscope software. The maximum penetration depth was defined as the linear distance from the canal wall to the deepest point of fluorescent dye penetration within the dentinal tubules. Measurements were recorded at four standardized locations around the canal circumference, and the mean value for each specimen was calculated for statistical analysis.



Statistical analysis

The sample size was determined using G*Power software version 3.1.9.4 (Franz Faul, Universität Kiel, Germany). Statistical analysis was performed using SPSS software (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean $\pm$ standard deviation. Data normality was assessed prior to inferential analysis. Intergroup comparisons were performed using one-way analysis of variance (ANOVA) for normally distributed data or the Kruskal–Wallis test for non-normally distributed data. Pairwise comparisons were performed using Dunn's post hoc test or the Mann–Whitney U test, where appropriate. Statistical significance was set at $P < 0.05$.

Result

The mean maximum penetration depth of sodium hypochlorite into dentinal tubules for each experimental group is presented in Table 1. Among the four irrigation activation systems, the laser activation group demonstrated the highest mean penetration depth ($873.81 \pm 27.38 \mu\text{m}$), followed by the ultrasonic activation group ($789.71 \pm 30.25 \mu\text{m}$), sonic activation group ($556.80 \pm 37.91 \mu\text{m}$), and the conventional needle irrigation group ($537.90 \pm 51.32 \mu\text{m}$).

Statistical analysis revealed a significant difference in the mean penetration depth among the four groups using one-way ANOVA ($F = 14.1529$, $P < 0.05$). Since the data also underwent non-parametric analysis, the Kruskal–Wallis test demonstrated a statistically significant difference among the study groups ($P < 0.05$), indicating that the method of irrigant activation significantly influenced sodium hypochlorite penetration into dentinal tubules. Post hoc pairwise comparisons using Dunn's test revealed that the laser activation group exhibited significantly greater penetration depth than the conventional needle irrigation, sonic activation, and ultrasonic activation groups ($P < 0.05$). Ultrasonic activation also demonstrated significantly greater penetration than both the conventional needle irrigation and sonic activation groups ($P < 0.05$). Although the sonic activation group showed greater mean penetration depth than the conventional needle irrigation group, this difference was not statistically significant ($P > 0.05$). Overall, the maximum penetration depth of sodium hypochlorite into dentinal tubules followed the order: Laser activation $>$ Ultrasonic activation $>$ Sonic activation $>$ Conventional needle irrigation. These findings indicate that activation of the irrigant significantly enhances sodium hypochlorite penetration into the apical dentinal tubules, with diode laser activation demonstrating the greatest efficacy among the evaluated irrigation techniques.

DISCUSSION

Successful endodontic therapy depends on effective elimination of microorganisms and organic tissue remnants from the root canal system. Although mechanical instrumentation contributes to canal shaping, it is unable to completely eliminate bacteria and debris from anatomical complexities such as fins, isthmuses, lateral canals, and dentinal tubules. Therefore, irrigation remains an essential component of root canal disinfection, and activation techniques have been developed to enhance irrigant penetration and improve cleaning efficacy.⁷

Sodium hypochlorite (NaOCl) is considered the primary irrigant in endodontics because of its antimicrobial activity and tissue-dissolving ability. However, its effectiveness depends on adequate contact with infected dentin and penetration into dentinal tubules. Zou et al. demonstrated that NaOCl penetration into dentin is influenced by factors such as concentration, exposure time, and dentinal characteristics, emphasizing the importance of improving irrigant delivery and activation.⁸

The present study evaluated and compared the effect of conventional needle irrigation, sonic activation, ultrasonic activation, and diode laser

activation on NaOCl penetration into dentinal tubules in the apical third using confocal laser scanning microscopy. The findings demonstrated significant differences among the groups, with the highest penetration depth observed in the laser activation group, followed by ultrasonic activation, sonic activation, and conventional needle irrigation.

The conventional needle irrigation group exhibited the lowest NaOCl penetration depth among all groups. This finding can be attributed to the limitations of positive-pressure irrigation, where irrigant replacement and fluid movement are restricted, particularly in the apical third. Without additional activation, conventional irrigation may result in stagnant irrigant zones, limiting penetration into dentinal tubules. Similar limitations of syringe irrigation have been described in previous investigations evaluating irrigant flow dynamics within root canals.

Sonic activation showed greater NaOCl penetration compared with conventional needle irrigation; however, the difference was not statistically significant. Sonic systems generate low-frequency oscillations that produce acoustic streaming and improve irrigant circulation within the canal. However, because sonic devices operate at lower frequencies compared with ultrasonic systems, the energy transferred to the irrigant is comparatively less. Previous studies have demonstrated that sonic activation improves canal cleanliness and irrigant distribution compared with syringe irrigation but may provide inferior results compared with ultrasonic activation.^{9,20}

Passive ultrasonic irrigation demonstrated significantly greater penetration depth than conventional needle irrigation and sonic activation. Ultrasonic activation produces acoustic streaming and cavitation through rapid oscillation of the ultrasonic tip, resulting in improved fluid dynamics and enhanced penetration of irrigants into inaccessible areas of the root canal system. Van der Sluis et al. reviewed the mechanism of passive ultrasonic irrigation and reported that acoustic streaming generated by freely oscillating ultrasonic instruments is responsible for improved irrigant replacement and cleaning efficiency.¹⁰

The increased penetration observed with ultrasonic activation in the present study is consistent with previous findings demonstrating improved NaOCl penetration following ultrasonic agitation. Ghorbanzadeh et al. compared conventional irrigation, passive ultrasonic agitation, and laser activation and reported enhanced penetration depth with activated irrigation methods compared with conventional irrigation.^{11,19}

Among all tested activation methods, diode laser activation produced the highest sodium hypochlorite penetration depth. The superior effect of laser activation may be explained by the photoacoustic and photomechanical effects generated by laser energy. Laser-induced vapor bubble formation and collapse create rapid fluid movement, improving irrigant exchange and facilitating deeper penetration into dentinal tubules.^{12,16} A systematic review comparing laser-activated irrigation and ultrasonic-activated irrigation reported that laser activation demonstrated promising results in improving debris removal, smear layer elimination, and antimicrobial efficacy, although variations exist among available studies.

The results of the present study agree with previous investigations reporting enhanced penetration after laser activation. Ghorbanzadeh et al. demonstrated that laser-activated irrigation resulted in greater NaOCl penetration depth compared with conventional irrigation and passive ultrasonic agitation. Similarly, recent systematic reviews have suggested that laser activation may provide superior irrigant activation effects compared with ultrasonic techniques because of enhanced fluid dynamics generated within the root canal system.

Confocal laser scanning microscopy was selected for evaluation because it enables three-dimensional visualization and quantitative measurement of irrigant penetration into dentinal tubules without extensive specimen preparation. This technique has been widely used in endodontic research for assessing penetration depth of irrigants and evaluating differences among activation protocols.^{13,14}

The present study has certain limitations. As an in vitro investigation, it does not completely reproduce clinical conditions such as complex canal anatomy, presence of biofilms, tissue remnants, and variations in dentinal structure. Additionally, only mandibular premolars with straight canals were evaluated, and the findings may not be directly applicable to teeth with complex anatomy. Further studies involving different tooth types, activation parameters, and microbiological evaluation are required to confirm the clinical relevance of these findings.

Within the limitations of this study, diode laser activation demonstrated the greatest sodium hypochlorite penetration into dentinal tubules, followed by ultrasonic activation, sonic activation, and conventional needle irrigation. These findings suggest that activation methods producing enhanced fluid dynamics can improve irrigant penetration and may contribute to more effective root canal disinfection.

CONCLUSION

Within the limitations of this study, irrigant activation significantly influenced sodium hypochlorite penetration into dentinal tubules. Diode laser activation showed the highest penetration depth, followed by ultrasonic activation, sonic activation, and conventional needle irrigation. Diode laser activation may enhance irrigant penetration and improve disinfection efficacy in the apical third of root canals.

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