

Penetration effect of sodium hypochlorite activated by the NSK Varios 370 ultrasonic system and the EQ-S sonic system in single-rooted premolars

Manuel Urrea¹, Julio García², Eduardo Campoy¹, Leticia Terán¹, Javier Domínguez²

1. Universidad de Sonora, Departamento de Medicina y Ciencias de la salud, Licenciatura de Odontología, Hermosillo, México

2. Universidad Autónoma de Baja California, Especialidad en Endodoncia, Facultad de Odontología Tijuana, México

ABSTRACT

The effectiveness of endodontic disinfection largely depends on the ability of sodium hypochlorite (NaOCl) to penetrate deeply into dentinal tubules, particularly in the apical third, where the anatomy is more complex and mechanical access is limited. Sonic and ultrasonic activation techniques have been proposed to enhance this penetration by generating dynamic fluid movement. **Aim:** To evaluate and compare the depth of penetration of 5.25% NaOCl into dentinal tubules using passive ultrasonic irrigation (PUI; NSK Varios 370) and sonic activation (SIM; EQ-S Metabiomed), in comparison to conventional needle irrigation. **Materials and Method:** thirty-six extracted human single-rooted premolars were instrumented to working length with ProTaper Gold F3. The teeth were randomly assigned to three groups (n = 12): PUI, SIM, and control. A standardized final irrigation protocol was performed, followed by the application of 1% methylene blue, activated or not, according to the group. Three transverse sections were obtained from each tooth (coronal, middle, and apical), and penetration depth was measured using ImageJ image analysis. Data were analyzed with non-parametric tests (Mann–Whitney U; $p < 0.05$). **Results:** Both PUI and SIM showed significantly greater penetration depths than the control group in all three thirds of the canal ($p < 0.05$). No significant difference was found between PUI and SIM ($p > 0.05$). The greatest penetration was observed in the coronal third, followed by the middle and apical thirds in all groups. **Conclusions:** Ultrasonic and sonic activation techniques produced greater marker penetration into dentinal tubules compared to conventional irrigation. Furthermore, the EQ-S system yielded similar results to those obtained with ultrasonic activation, with no statistically significant difference between the two systems.

Keywords: sodium hypochlorite - root canal irrigants - ultrasonic irrigation - sonic irrigation - dentin tubules

Efecto de penetración del hipoclorito de sodio bajo sistema de activación ultrasónico nsk varios 370 y sónico eq-s, en premolares unirradiculares

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Corresponding Author:

Manuel A Urrea Campoy
manuelalfredo.urrea@unison.mx

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RESUMEN

Introducción: La eficacia de la desinfección endodóntica depende en gran medida de la capacidad del hipoclorito de sodio (NaOCl) para penetrar profundamente en los túbulos dentinarios, especialmente en el tercio apical, donde la anatomía es más compleja y el acceso mecánico limitado. Las técnicas de activación sónicas y ultrasónicas han sido propuestas para mejorar dicha penetración mediante la generación de flujo dinámico. **Objetivo:** Evaluar y comparar la profundidad de penetración del NaOCl al 5.25% en los túbulos dentinarios utilizando activación ultrasónica pasiva (PUI; NSK Varios 370) y activación sónica (SIM; EQ-S Metabiomed), frente a irrigación convencional con aguja. **Materiales y métodos:** Se utilizaron 36 premolares unirradiculares humanos, instrumentados a longitud de trabajo con ProTaper Gold F3. Los dientes se dividieron aleatoriamente en tres grupos (n=12): PUI, SIM y control. Se realizó un protocolo estandarizado de irrigación final y posteriormente se aplicó azul de metileno al 1%, activado o no según el grupo. Se obtuvieron tres secciones transversales por diente (coronal, medio, apical) y se midió la profundidad de penetración mediante análisis de imagen con ImageJ. Los datos fueron analizados con pruebas no paramétricas (U de Mann-Whitney; $p < 0.05$). **Resultados:** Tanto PUI como SIM mostraron profundidades de penetración significativamente mayores que el grupo control en los tres tercios del conducto ($p < 0.05$). No se encontraron diferencias significativas entre PUI y SIM ($p > 0.05$). Las mayores penetraciones se observaron en el tercio coronal, seguidas del medio y apical en todos los grupos. **Conclusiones:** Las técnicas de activación ultrasónica y sónica mostraron una mayor penetración del marcador en los túbulos dentinarios en comparación con la irrigación convencional. Además, el sistema EQ-S arrojó resultados similares a los obtenidos con la activación ultrasónica, sin diferencias estadísticamente significativas entre ambos sistemas.

Palabras clave: hipoclorito de sodio - irrigantes - irrigación ultrasónica - irrigación sónica - túbulos dentinarios

INTRODUCTION

Endodontics focuses on the study of morphology, physiology, and pathology of the pulp cavity. Understanding the diagnosis and treatment of pulp and periapical diseases requires a broad knowledge of pulp biology. The goal of endodontic treatment is to eliminate infection from the root canal system and prevent or resolve apical periodontitis, while contributing to relieving dental pain associated with pulp and periapical inflammatory processes¹. These inflammatory processes and apical periodontitis are caused by bacteria that penetrate the root canal system¹⁻³.

Biofilm consists of individual cells or mixed multicellular bacterial colonies attached to a surface and embedded in a highly hydrated extracellular polymeric matrix (EPM). Bacteria form biofilms on nutrient-rich surfaces, and within root canals, these can organize into multiple cell layers. The EPM plays a fundamental role in the development, organization, and survival of the biofilm^{4,5}.

Once bacteria reach the root canals, lateral canals, or dentinal tubules, strategies must be applied to eliminate them⁵. Studies show that bacteria can penetrate 300–500 micrometers into the dentinal tubules⁶⁻⁸.

The main root canal is cleaned through chemo-mechanical action: manual or rotary instruments perform the physical removal of debris and shape the canal geometry to facilitate irrigant penetration. Irrigants play a crucial role in the chemical action by dissolving organic tissues, removing smear layer, reaching some lateral canals or apical deltas, and penetrating the dentinal tubules^{2,3,6}.

During canal shaping, 35–50% of root canal walls remain untouched by instruments due to anatomical irregularities, rendering those areas inaccessible to mechanical cleaning^{9,10}. Several authors have reported that the apical region is the most anatomically complex, making debridement more challenging⁷. Apical cleanliness depends on the apical preparation size and the irrigation protocol employed; the apical size determines how closely the needle can reach the working length, influencing irrigant flow and volume. Some studies associate larger apical preparations with improved apical disinfection⁷.

When irrigation is performed with a syringe and needle, greater canal enlargement becomes important. However, passive ultrasonic irrigation

may achieve cleanliness comparable to that in canals prepared to larger apical sizes, meaning that canals with smaller preparations can be just as clean as larger ones^{7,11}. Among irrigants, sodium hypochlorite (NaOCl) remains the gold standard due to its antimicrobial properties and ability to dissolve organic tissue^{6,7,11,12}.

Previous research has shown that 6% NaOCl can penetrate up to 300 µm into dentinal tubules^{7,8}.

Each irrigation technique influences how NaOCl penetrates the canal system and dentinal tubules. Another factor to consider is the air entrapment produced by a closed access cavity, due to the periodontal ligament and surrounding bone, which generates a vapor lock effect. This negatively affects irrigant flow in the apical region because an air bubble forms, preventing optimal irrigant penetration¹³.

Among the most commonly used activation techniques are: Dynamic Manual Irrigation using a conventional syringe; Passive Ultrasonic Irrigation (PUI), which uses a metallic file that vibrates at ultrasonic frequency; and Sonic Activation, which uses polymer tips vibrating at sonic frequency to enhance canal disinfection by removing debris and organic tissue. Sonic systems include EndoActivator (Dentsply Maillefer, Switzerland), Eddy tips (VDW, Germany), and EQ-S (Metabiomed, South Korea).

Given the availability of various systems that improve disinfection of the root canal complex, it is important to compare gold-standard systems with newer devices which generate apical-coronal motion in addition to sonic activation and lateral movement. The aim of this study was to evaluate the effect of sodium hypochlorite penetration using ultrasonic activation (NSK Varios 370, Dentsply Maillefer, Switzerland) and sonic activation (EQ-S, Metabiomed, South Korea) in single-rooted premolars.

MATERIALS AND METHOD

We used 36 recently extracted human single-rooted teeth with intact, straight roots and a single canal, obtained for orthodontic or periodontal reasons. These teeth were stored under controlled conditions in a sterile solution. Mesiodistal and buccolingual radiographs were taken to ensure the presence of a single canal and root lengths greater than 13 mm. Teeth with caries, root fractures,

open apices, calcified canals, previous root canal treatments, resorption defects, or posts were excluded.

Coronal access was prepared using a round diamond bur, after which the canal was located with a DG16 explorer (Hu-Friedy, Lyoner Frankfurt). Working length (WL) was determined using a size 15 K-file (Dentsply Sirona, Switzerland) inserted until the tip was visible at the apical foramen, and then reduced by 1 mm.

Teeth were divided into three activation groups:
Group 1: Ultrasonic activation (NSK Varios 370) Frequency: 28 – 32 KHz,

Group 2: Sonic activation (EQ-S Metabiomed) Frequency: 8 – 13 KHz,

Group 3: Control (methylene blue without activation).

Root canals were prepared to WL using ProTaper Gold F3 (size 30) (Dentsply Sirona, Switzerland). During instrumentation, canals were irrigated with 2 mL of 5.25% sodium hypochlorite (NaOCl) (Cloralex, Mexico) using a side-vented 30-gauge needle positioned 2 mm short of WL, applied between each instrument.

An apical barrier of LC Block-Out Resin (Ultradent Inc., USA) was placed to simulate a closed system, as in a non-extracted tooth. A layer of clear nail varnish was then applied to the external root surface to prevent external penetration of methylene blue.

Transverse grooves were made using a diamond disc (SYNDENT Tools Co., Ltd., China) at 2 mm (apical third), 5 mm (middle third), and 8 mm (coronal third) from the apex. The final irrigation protocol was standardized as follows:

1. NaOCl (5 mL, 1 min)
2. Distilled water (5 mL, 1 min)
3. 17% EDTA (5 mL, 1 min)
4. Distilled water (5 mL, 1 min)
5. NaOCl (5 mL, 1 min), activated for 30 s, followed by 30 s rest, and then 30 s additional activation.

To evaluate the penetration of 5.25% NaOCl into the dentinal tubules, the 36 teeth were randomly divided into three groups (n = 12) according to the activation system:

PUI Group: Passive Ultrasonic Irrigation (NSK Varios 370)

NaOCl (5 mL) was applied 2 mm from the working length (WL). A size 25/02 ultrasonic tip was

positioned 3 mm from the WL and activated for 30 seconds, in three activation cycles. The canal was then irrigated with 5 mL of saline solution, after which 1% methylene blue was introduced as a penetration marker 1 mm from the WL and ultrasonically activated for 30 seconds.

SIM Group: Sonic Activation (EQ-S Metabiomed – 10,000 cpm)

NaOCl (5 mL) was applied 2 mm from WL. The sonic tip size 25/02 was positioned 2 mm short of WL and activated for 30 seconds, in three activation cycles. The canal was then rinsed with 5 mL of saline, followed by placement of 1% methylene blue 1 mm from WL and sonic activation for 30 seconds.

Control Group: Conventional Needle Irrigation (No activation)

The irrigant (5 mL) was applied 2 mm from WL. NaOCl was then replaced with an additional 5 mL of fresh solution without activation, repeated three times. The NaOCl was flushed with 5 mL of saline, after which 1% methylene blue was placed and left undisturbed for 30 seconds.

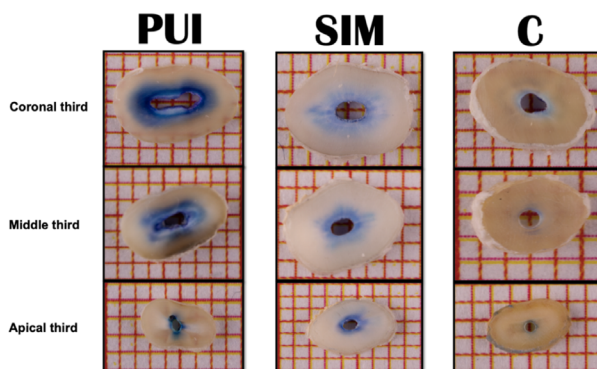


Fig. 1: Groups and their thirds.

Excess solution was removed using sterile paper points. The flowable resin was eliminated, the grooves were connected with a diamond disc, and any remaining irregularities were smoothed with a medium-grit polishing disc (3M Dental, USA). This procedure yielded one apical, one middle, and one coronal segment from each tooth, each 1 mm thick, providing a total 108 sections (Fig. 1).

The samples were examined under maximum magnification (Tokina AT-X 100 mm f/2.8 Pro D lens., Tokuyama Corporation, Japan) mounted on a camera (Nikon D3100, Nikon, Japan) to visualize the transverse plane of the canal walls. One image

was captured for each canal third—coronal, middle, and apical—together with a calibrated scale grid. All images were taken at the same focal distance of 0.3 meters, with fixed resolution and optimal focus, saved in TIFF format, and imported into ImageJ software (National Institutes of Health, Bethesda, Maryland, USA).

A calibrated straight line was drawn perpendicular from the internal canal wall to the deepest visible point of stain penetration. Twelve measurements were obtained from each image at equal angular intervals (clockwise direction). The mean NaOCl penetration depth for the coronal, middle, and apical segments of each group was calculated in micrometers (μm) (Fig. 2).

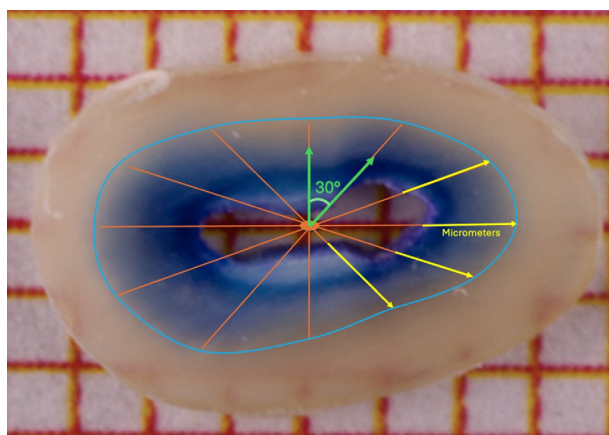


Fig. 2: Methodology for penetration depth. The blue area indicates methylene blue penetration.

The mean values and interquartile ranges (25–75%) were calculated for the full canal length within each group, as well as for the coronal, middle, and apical segments. A non-parametric statistical approach was employed due to the non-normal distribution of the data. Groups were compared using the Mann–Whitney U test, with significance level set at $p < 0.05$. All analyses were conducted using IBM SPSS Statistics, Version 27.0 (June 2020).

RESULTS

The penetration depth of sodium hypochlorite was evaluated in the apical, middle, and coronal thirds for each activation system (PUI and SIM). Both activation systems showed significantly greater penetration depths than the control group in all three

canal thirds ($p < 0.05$). No statistically significant differences were observed between PUI and SIM in any of the evaluated thirds ($p > 0.05$). Overall, penetration depth increased from the apical to the coronal third in all groups. Descriptive statistics, including median values, are presented in Table 1, and the distribution of penetration depths is shown in Figure 3.

Table 1. The penetration depth of sodium hypochlorite into the dentinal wall of the root canal

Techniques	Root Canal Level	Penetration Depth
SIM	Coronal	1112.2 $\pm$ 588.72
	Middle	799.39 $\pm$ 329.49
	Apical	596.76 $\pm$ 157.98
PUI	Coronal	1110.88 $\pm$ 507.07
	Middle	1019.34 $\pm$ 254.12
	Apical	346.13 $\pm$ 113.91
CONTROL	Coronal	549.62 $\pm$ 263.01
	Middle	407.01 $\pm$ 104.20
	Apical	173.65 $\pm$ 27.07

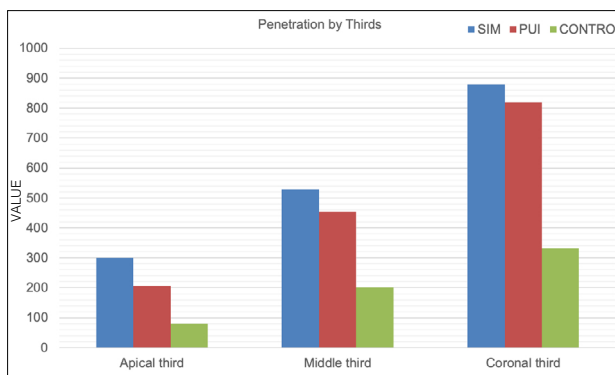


Fig. 3: Comparison of median values by thirds for each group.

Sodium Hypochlorite Penetration in the Apical Third

In the apical third, the minimum penetration depths, from lowest to highest, were 27.07 μm for the control group, 113.91 μm for PUI, and 157.98 μm for SIM (Fig. 4). Penetration depth was therefore markedly greater in both activated groups than in the control group. Statistical analysis confirmed a significant difference between the control group and both activation groups ($p < 0.05$), whereas no significant difference was observed between PUI and SIM ($p > 0.05$).

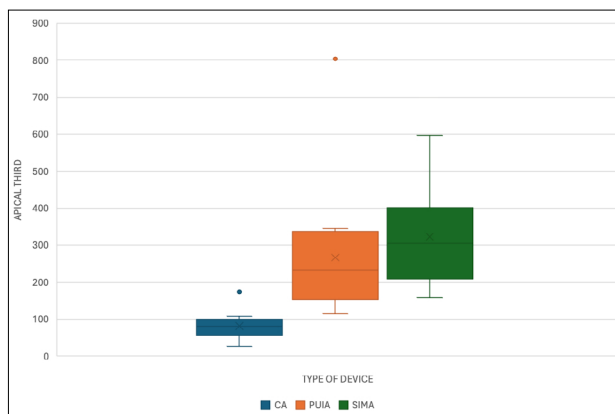


Fig. 4: Box-and-whisker plots of penetration depth for each activation device in the apical third.

Sodium Hypochlorite Penetration in the Middle Third

In the middle third, both PUI and SIM showed greater penetration depths than the control group (Fig. 5). Statistical analysis demonstrated significant differences between the control group and each activation system ($p < 0.05$), while no statistically significant difference was observed between PUI and SIM ($p > 0.05$).

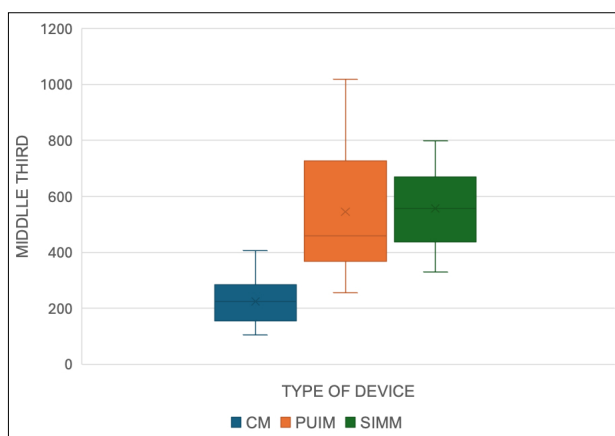


Fig. 5: Box-and-whisker plots of penetration depth for each activation device in the middle third.

Sodium Hypochlorite Penetration in the Coronal Third

In the coronal third, penetration depths were greater than those observed in the apical and middle thirds in all groups (Fig. 6). Both PUI and SIM showed significantly greater penetration depths than the control group ($p < 0.05$), with no statistically significant difference between the two activation systems ($p > 0.05$).

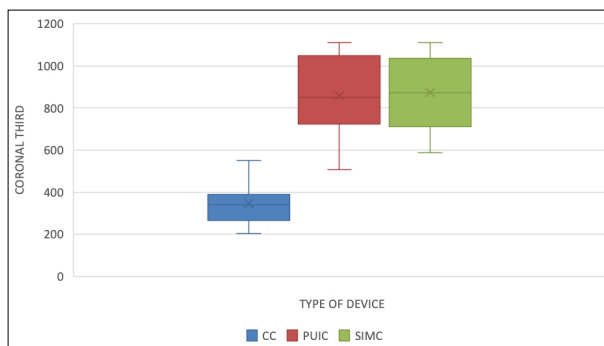


Fig. 6: Box-and-whisker plots of penetration depth for each activation device in the coronal third.

DISCUSSION

According to previous studies, sonic irrigation (EQ-S MetaBiomed) has shown a relevant role as a complementary method to improve root canal system cleaning. It enhances the penetration and efficacy of NaOCl by generating a dynamic flow (acoustic streaming) that facilitates irrigant access to irregularities, isthmuses, and flattened areas typical of oval canals. This effect promotes the removal of debris in areas unreachable by instruments, especially in the apical third. These findings are consistent with previous research indicating that sonic activation significantly reduces residual material in difficult-to-access regions¹⁴. Taken together, these results indicate that sonic activation can improve irrigant penetration and promote cleaning in hard-to-reach areas of the root canal. Furthermore, several studies have shown that the EQ-S system delivers results that are comparable or superior to conventional needle irrigation, especially in the apical third¹⁵.

In many cases, although EQ-S improved cleaning compared to conventional irrigation, it did not completely remove all material (e.g., gutta-percha/sealer, calcium hydroxide, or smear layer), highlighting that complete canal cleanliness remains challenging, especially in oval, curved, or complex anatomy. Therefore, various studies suggest combining activation techniques or irrigation sequences to optimize apical cleaning^{16, 17}.

Furthermore, the effectiveness of any irrigant activation or complementary disinfection technique largely depends on its ability to enhance the penetration of agents into dentinal tubules. In this regard, both sonic and ultrasonic activation have been shown to significantly improve the diffusion of methylene blue into the dentinal tubules¹⁸. This finding suggests that activating the irrigant promotes

its penetration into the dentin microstructures, which could improve the distribution of solutions such as sodium hypochlorite and chelating agents within the root canal system.

Therefore, these results support the idea that activation methods, particularly sonic, not only optimize direct cleaning of the main canal but may also enhance disinfection in difficult-to-access areas such as dentinal tubules, reinforcing their usefulness as adjuncts in contemporary endodontic therapy.

Moreover, conventional irrigation (solution with syringe alone) is often insufficient, frequently failing to remove smear layer adequately or achieve complete disinfection^{19,20}. These results reinforce the concept that sonic activation complements mechanical cleaning and enhances NaOCl infiltration into inaccessible areas, contributing to more thorough disinfection^{21,22}.

Several studies have also demonstrated that complete removal of calcium hydroxide in canals with complex anatomy remains a clinical challenge, even when using activated irrigation protocols. Sonic activation with the EQ-S system has been shown to significantly improve Ca(OH)_2 removal compared to conventional irrigation, particularly in moderately and severely curved canals. This superior performance is attributed to the greater oscillation amplitude of its flexible tips, which promotes a more dynamic irrigant flow and more efficient agitation in the apical region. However, even with this technology, residual medication persists in hard-to-reach areas, consistent with evidence that no method achieves fully predictable removal in anatomically challenging canals. These findings underscore the importance of complementing mechanical preparation with activated irrigation protocols, especially in cases of high morphological complexity, to optimize cleaning prior to obturation^{23,24}.

Sonic activation using the EQ-S system has also been shown to significantly improve debris removal in conservatively instrumented canals, especially where irrigant penetration and flow are limited. Its vibratory action promotes greater debris removal and better irrigant contact with canal walls, supporting its use as an adjunct in minimally invasive instrumentation protocols, where preservation of original anatomy may compromise cleaning

effectiveness without enhanced irrigation²⁵.

Additionally, EQ-S sonic activation facilitates smear layer removal, demonstrating adequate performance in anatomically complex areas and in the coronal and middle thirds of the canal. However, when compared to ultrasonic activation, the latter retains slight superiority in the apical region, where irrigant penetration and flow are more restricted. These results reinforce the idea that while ultrasonics remain more efficient in critical canal areas, EQ-S provides an effective, less aggressive alternative to optimize irrigation protocols in challenging anatomies^{26,27}. Although sonic activation offers clinically relevant benefits, ultrasonic activation maintains overall superiority, particularly in apical regions where hydrodynamic demands are higher. Nevertheless, the difference between the two techniques is not always pronounced, and sonic activation can be considered an effective and safe alternative, especially when preserving internal anatomy or when clinical conditions limit ultrasonic use. Overall, the evidence supports sonic activation as a valuable adjunct in final irrigation protocols, contributing significantly to intracanal cleaning and potentially improving endodontic treatment outcomes²⁸⁻³⁰.

CONCLUSION

Within the limitations of this study, efforts were made to standardize irrigation protocols and experimental conditions for each sample. Additionally, single-rooted teeth with wide canals and low anatomical complexity were selected. A limitation was the use of methylene blue as a penetration marker, since its physicochemical properties may differ from those of sodium hypochlorite; therefore, the results should be interpreted with caution. Based on the results obtained, the following conclusions are drawn:

Both the PUI and SIM techniques significantly increased irrigant penetration into the dentinal tubules compared to conventional needle irrigation. The greatest penetration was observed in the coronal third, followed by the middle and apical thirds in all groups evaluated.

No statistically significant difference was observed between the PUI and SIM systems in any of the analyzed thirds, indicating comparable behavior between both activation methods.

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CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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