Journal Endo Sapiens

Clinical Research

# Smear Layer Removal and Dentin Microhardness Following Use of an All-in-One Irrigant Versus Conventional NaOCl/EDTA: An In Vitro Study

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## Abstract

**Objective**

To compare Triton with a conventional sodium hypochlorite (NaOCl)/ethylenediaminetetraacetic acid (EDTA) final irrigation protocol regarding smear layer removal and effects on intracanal dentin microhardness.

**Methods**

Eighty single-rooted teeth with single canals were included. For smear layer analysis, 20 teeth were assigned to Triton or NaOCl/EDTA groups, instrumented to ProTaper Gold F4, and subjected to passive ultrasonic irrigation. Specimens were evaluated using scanning electron microscopy under 1000× magnification. For microhardness analysis, 60 teeth were assigned to saline, Triton, or NaOCl/EDTA groups. Knoop microhardness was measured in the coronal, middle, and apical thirds at 100 µm from the canal lumen using a 300-g load and 20-s dwell time.

**Results**

No significant difference was observed between Triton and NaOCl/EDTA in smear layer removal following passive ultrasonic irrigation. Mean Knoop hardness values were 36.1, 31.1, and 28.9 for saline, NaOCl/EDTA, and Triton groups, respectively. All groups differed significantly from one another ($p < 0.05$), with Triton demonstrating the greatest reduction in dentin microhardness.

**Conclusions**

Triton demonstrated smear layer removal comparable to NaOCl/EDTA when used with passive ultrasonic irrigation. However, the Triton irrigation protocol resulted in significantly lower dentin microhardness values than the conventional NaOCl/EDTA protocol and saline control under the conditions tested.

**Keywords:** microhardness; EDTA; Triton; smear layer; sodium hypochlorite

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Received for publication Jun 15, 2026; accepted for publication Jul 13, 2026.
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https://doi.org/10.71347/fg69yq12

# Introduction

Microorganisms within the root canal system play a central role in the initiation and persistence of apical periodontitis, making their elimination a primary objective of endodontic therapy (Kakehashi et al., 1965; Byström & Sundqvist, 1981). Chemo-mechanical preparation significantly reduces intracanal bacterial load; however, instrumentation alone cannot completely debride the complex anatomy of the root canal system, including isthmuses, fins, lateral canals, and dentinal tubules (Byström & Sundqvist, 1981; Peters et al., 2001). Instrumentation has been shown to leave portions of the canal wall untouched, while bacteria such as Enterococcus faecalis may penetrate deeply into dentinal tubules (Al-Nazhan et al., 2014; Carrigan et al., 1984). In addition, instrumentation creates a smear layer composed of organic and inorganic debris that may harbor microorganisms and interfere with penetration and adaptation of root canal sealers (McComb & Smith, 1975; Mader et al., 1984; Violich & Chandler, 2010; Torabinejad et al., 2002)

Irrigation therefore plays a critical role in root canal disinfection and smear layer removal. Sodium hypochlorite (NaOCl) remains the most widely used irrigant because of its antimicrobial properties and ability to dissolve organic tissue (Hand et al., 1978; Baumgartner & Ibay, 1987), while ethylenediaminetetraacetic acid (EDTA) is commonly used to remove the inorganic portion of the smear layer through chelation (Weinreb & Meier, 1965; Di Lenarda et al., 2000). The conventional irrigation protocol typically involves alternating NaOCl and EDTA, often with adjunctive activation methods such as passive ultrasonic irrigation (PUI) to improve irrigant penetration and smear layer removal, particularly in the apical third of the canal (Guerisoli et al., 2002; van der Sluis et al., 2007; Kuah et al., 2009; Caron et al., 2010; Haupt et al., 2020).

Although smear layer removal is generally considered beneficial for canal disinfection and sealer adaptation, chelating agents may adversely affect dentin properties. Previous studies have demonstrated that prolonged exposure to EDTA and other chelators can reduce dentin microhardness and contribute to dentinal erosion, with effects influenced by concentration and exposure time (Ari et al., 2004; De-Deus et al., 2006; Serper & Çalt, 2002; Uzunoglu et al., 2012; Pashley et al., 1985). Consequently, continued interest exists in developing irrigation systems capable of effective smear layer removal while minimizing detrimental effects on dentin.

Triton All-In-One Irrigation Solution (Brasseler USA) is a two-part irrigation system designed to provide simultaneous organic tissue dissolution and chelation through a proprietary combination of NaOCl, chelators, surfactants, and pH modifiers (Brasseler, USA, 2021). Recent studies evaluating Triton have demonstrated promising antimicrobial activity and smear layer removal; however, findings remain inconsistent, and data regarding its effects on dentin properties are still limited (Sheng et al., 2023; Aranda et al., 2026; Wakas Oraim & Al-Zaka, 2024). Additionally, its smear layer removal efficacy when used with passive ultrasonic irrigation, as well as its influence on dentin microhardness, has not been thoroughly investigated.

Therefore, the purpose of this study was to compare Triton with a traditional 5.25% NaOCl/17% EDTA irrigation protocol in smear layer removal following PUI using scanning electron microscopy (SEM), and to evaluate the effects of Triton, NaOCl/EDTA, and saline on dentin microhardness. The null hypothesis was that Triton would not differ significantly from the traditional NaOCl/EDTA protocol regarding smear layer removal or dentin microhardness.

# Materials and methods

**Specimen selection**

A total of 80 extracted human teeth were included in this study. Twenty teeth were used for smear layer evaluation and 60 teeth were used for dentin

microhardness testing (Fig. 1). Institutional review board approval for non-human subjects research was obtained (Pro00128260). Teeth were obtained from human cadavers through the university's anatomy laboratory, with all protected health information identifiers removed. Eligible specimens included single-rooted teeth with a single canal (maxillary central incisors, maxillary lateral incisors, maxillary canines, mandibular central incisors, mandibular lateral incisors, and mandibular premolars). Specimen selection was based on preoperative periapical radiographic evaluation. Preoperative periapical radiographs were used to confirm a single canal and evaluate canal curvature, open apices, previous root canal treatment, and resorptive defects. Teeth exhibiting an estimated canal curvature greater than 10° on radiographic examination, open apices, previous root canal treatment, or resorptive defects were excluded. Following extraction, adherent soft tissue and calculus were removed using hand scalers, and the teeth were stored in sterile saline until use.

Sample size estimation was performed a priori. Assuming a moderate-to-large effect size, $\alpha = 0.05$, and a statistical power of 0.80, a minimum sample size of 60 specimens was determined to be sufficient for the primary outcome of dentin microhardness.

**Smear layer evaluation**

Twenty teeth were decoronated to a standardized root length of 16 mm. Working length was established by inserting a size 10 K-file until visible at the apical foramen and subtracting 1 mm. Root apices were sealed with sticky wax. Teeth were assigned alternately to either a conventional irrigation group (5.25% NaOCl/17% EDTA) or a Triton group.

Specimens were initially instrumented to ProTaper Gold F3 while irrigating with 3–5 mL of the assigned irrigant. Teeth were then longitudinally sectioned using a diamond disc under water cooling and reapproximated with sticky wax to create a watertight seal. Following reassembly, canals were

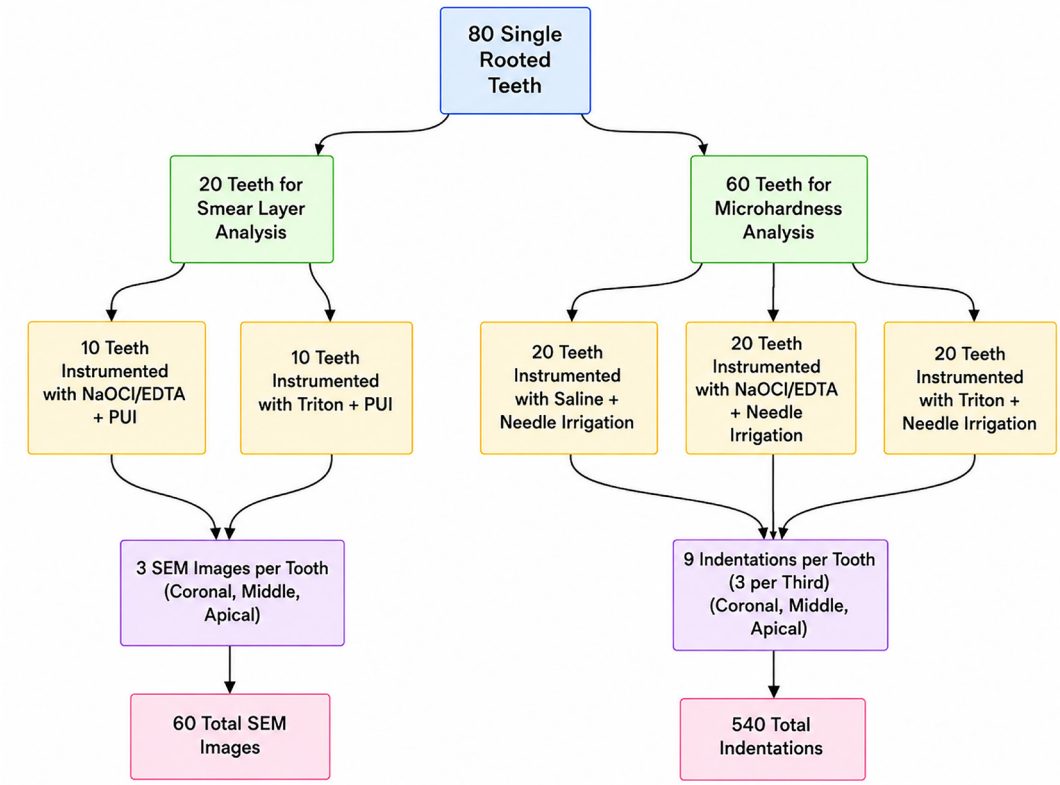



**Fig. 1. Flowchart illustrating specimen allocation and experimental design for smear layer evaluation and dentin microhardness analysis.**



**Fig. 2.** Shown is an image taken through the microscope of the Buehler Micromet 5114 Micro Hardness Testing Machine at 250x magnification. The vertical lines are adjustable to meet the edges of the diamond indentation created. After the measurement is determined by positioning the lines a HK score is calculated by the machine.

enlarged to ProTaper Gold F4.

In the NaOCl/EDTA group, canals were irrigated with 2 mL of 5.25% NaOCl followed by passive ultrasonic irrigation (PUI) for 20 s using ultrasonic U-files positioned 2 mm short of working length. This was followed by 1 mL of 17% EDTA for approximately 40 s with an additional 20 s of PUI, and a final rinse of 2 mL of 5.25% NaOCl with 20 s of PUI. PUI was performed using the Newtron P5 XS LED ultrasonic unit at 30 kHz.

In the Triton group, canals were irrigated with 2 mL of Triton followed by 20 s of PUI. This sequence was repeated three times for a total of 6 mL of Triton and 1 min of PUI. Specimens were then rinsed with saline according to manufacturer instructions.

Following instrumentation, specimens were dried in a vacuum chamber for 24 h and sputter-coated with gold prior to scanning electron microscopy (SEM) evaluation. SEM images were acquired at 1000× magnification using an accelerating voltage of 10.0 kV. Smear layer removal was assessed in the coronal, middle, and apical thirds using a previously published four-point scoring system: score 1, no smear layer with all dentinal tubules open; score 2, few areas covered by smear layer with most dentinal tubules open; score 3, smear layer covering most surfaces with few dentinal tubules open; and score 4, complete smear layer coverage. A single calibrated endodontic resident evaluated all SEM images.

**Microhardness evaluation**

Sixty teeth were decoronated to a standardized root length of 16 mm and instrumented to ProTaper Gold F4. Working length was established using a size 10 K-file positioned at the apical foramen and

subtracting 1 mm. Root apices were sealed with sticky wax. Specimens were divided into three groups (n = 20): saline control, NaOCl/EDTA, and Triton.

The saline group was irrigated with sterile 0.9% saline throughout instrumentation. The NaOCl/EDTA group received 5.25% NaOCl during instrumentation followed by a final irrigation protocol consisting of 3 mL 5.25% NaOCl, 1 mL 17% EDTA for 1 min, and a final rinse of 2 mL 5.25% NaOCl. The Triton group was irrigated with Triton throughout instrumentation followed by an additional 3 mL of Triton for 1 min and a final saline rinse according to manufacturer instructions. Irrigation was performed using needle irrigation within 2 mm of working length.

Teeth were longitudinally sectioned under water cooling, and one half of each specimen was mounted in acrylic resin and polished to 1600-grit silicon carbide paper. Microhardness testing was performed using a Knoop indenter under 250× magnification. Three measurements were obtained from the coronal, middle, and apical thirds for a total of nine measurements per specimen. Indentations were made 100 µm from the canal lumen using a 300-g load and 20-s dwell time (Fig. 2). Knoop hardness values (HK) were recorded for all specimens. Measurements were completed by two calibrated evaluators.

### Statistical analysis

Normality of the data was assessed using the Shapiro–Wilk test. As the data were not normally distributed, nonparametric statistical analyses were performed. Smear layer scores between groups were compared using the Wilcoxon rank-sum test. Comparisons among root thirds within groups were performed using the Kruskal–Wallis test. Post hoc pairwise comparisons were performed using the Dwass–Critchlow–Fligner procedure when significant omnibus differences were identified. Pairwise comparisons between corresponding root thirds were performed using the Wilcoxon rank-sum test. For dentin microhardness, overall group comparisons were performed using the Kruskal–Wallis test followed by the Dwass–Critchlow–Fligner procedure for pairwise comparisons. Statistical significance was set at $p < 0.05$.

## Results

### Smear layer analysis

For the smear layer removal portion, a total of 20 teeth were divided into the two experimental groups. A total of 60 SEM images were obtained at 1000× magnification and evaluated. Thirty images were evaluated per experimental group, with 10 images obtained from each regional third of the root canal. The mean smear layer removal score for the NaOCl/EDTA group was 2.1 overall, with regional mean scores of 1.8, 2.1, and 2.5 for the coronal, middle, and apical thirds, respectively. The Triton group demonstrated an overall mean smear layer removal score of 2.6, with regional mean scores of 2.2, 2.4, and 3.3 for the coronal, middle, and apical thirds, respectively. Representative SEM images are shown in Figure 3.

The Wilcoxon rank-sum test demonstrated no significant difference in smear layer removal between the Triton and NaOCl/EDTA groups ($Z = 1.98$, $p = 0.06$). Comparison of the coronal, middle, and apical thirds within each irrigation protocol using the Kruskal–Wallis test demonstrated a significant regional difference within the Triton group ($H = 6.1$, $p = 0.0478$). In contrast, no significant regional difference was observed within the NaOCl/EDTA group ($H = 3.4$, $p = 0.1835$). Additionally, subgroup comparisons between irrigant types within each canal third demonstrated no significant differences in the coronal ($Z = 1.2$, $p = 0.2333$), middle ($Z = 0.51$, $p = 0.6076$), or apical thirds ($Z = 1.9$, $p = 0.0608$).

### Microhardness analysis

For the microhardness portion, a total of 60 teeth

were divided into three groups consisting of one control group and two experimental groups. A total of 540 Knoop hardness (HK) measurements were recorded, including 180 measurements per group and 60 measurements per canal third. All measurements were performed by two calibrated evaluators who were not blinded to the study.

The mean HK values for the entire tooth were 36.1, 31.1, and 28.9 for the saline, NaOCl/EDTA, and Triton groups, respectively.

The omnibus Kruskal-Wallis test demonstrated a significant difference among the three groups ($H = 52.7$, $p < 0.0001$). Pairwise comparisons using the Dwass–Critchlow–Fligner correction demonstrated

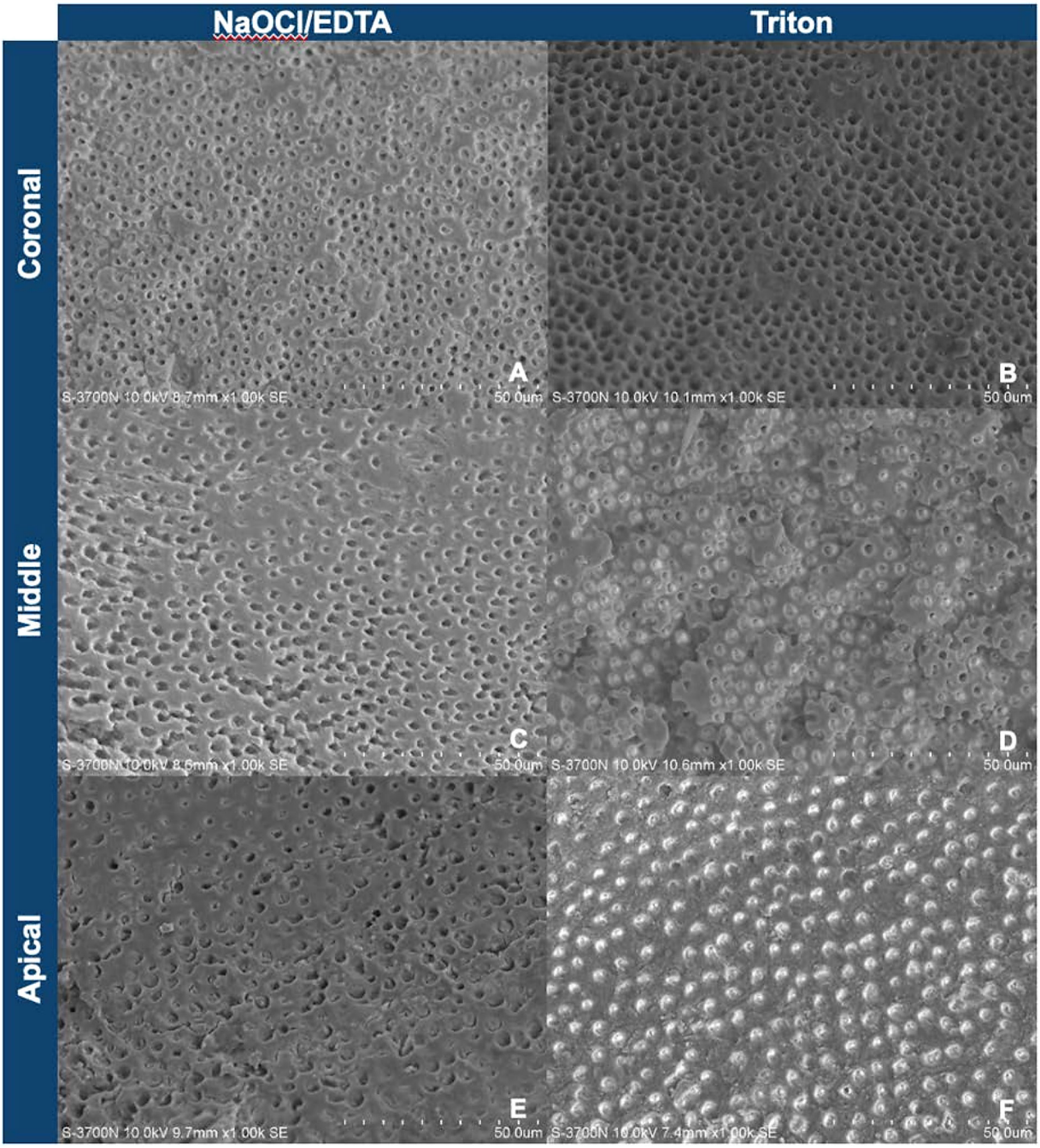



**Fig. 3.** Representative SEM images (1000× magnification) demonstrating smear layer removal following irrigation with NaOCl/EDTA + passive ultrasonic irrigation (PUI) and Triton + PUI. Images are shown from the coronal (A,B), middle (C,D), and apical (E,F) thirds of the root canal and represent increasing smear layer scores according to the four-point evaluation system.

that the NaOCl/EDTA group had significantly lower microhardness values than the saline control group ($p < 0.0001$). The Triton group demonstrated significantly lower microhardness values than both the saline control group ($p < 0.0001$) and the NaOCl/EDTA group ($p = 0.0093$).

Regional microhardness values are summarized in Figure 4. Mean HK values for the saline group were 34.2, 38.5, and 35.7 in the coronal, middle, and apical thirds, respectively. Corresponding values for the NaOCl/EDTA group were 30.0, 34.4, and 29.0, while the Triton group demonstrated values of 28.7, 31.3, and 26.8 in the coronal, middle, and apical thirds, respectively.

# Discussion

### Smear layer removal

The present study found no significant difference in smear layer removal between Triton and a conventional NaOCl/EDTA irrigation protocol when both groups utilized passive ultrasonic irrigation (PUI). These findings are generally consistent with previous studies evaluating Triton and smear layer removal (Sheng et al., 2023; Wakas Oraim & Al-Zaka, 2024; Castagnola et al., 2024).

Sheng et al. (2023) were among the first to evaluate Triton using SEM analysis and reported smear layer removal comparable to NaOCl/EDTA. However, their study utilized dentin discs immersed in irrigating solutions rather than a clinically relevant instrumentation protocol. Castagnola et al. (2024) later evaluated Triton during canal instrumentation and similarly found smear layer removal comparable to NaOCl/EDTA. In contrast, Wakas Oraim and Al-Zaka (2024) reported significantly less smear layer removal with Triton compared with EDTA and chitosan nanoparticles. Differences among studies may be related to variations in irrigation protocols, instrumentation methods, and exposure times.

To our knowledge, the effects of Triton used in combination with passive ultrasonic irrigation (PUI) have not been well described in the literature.

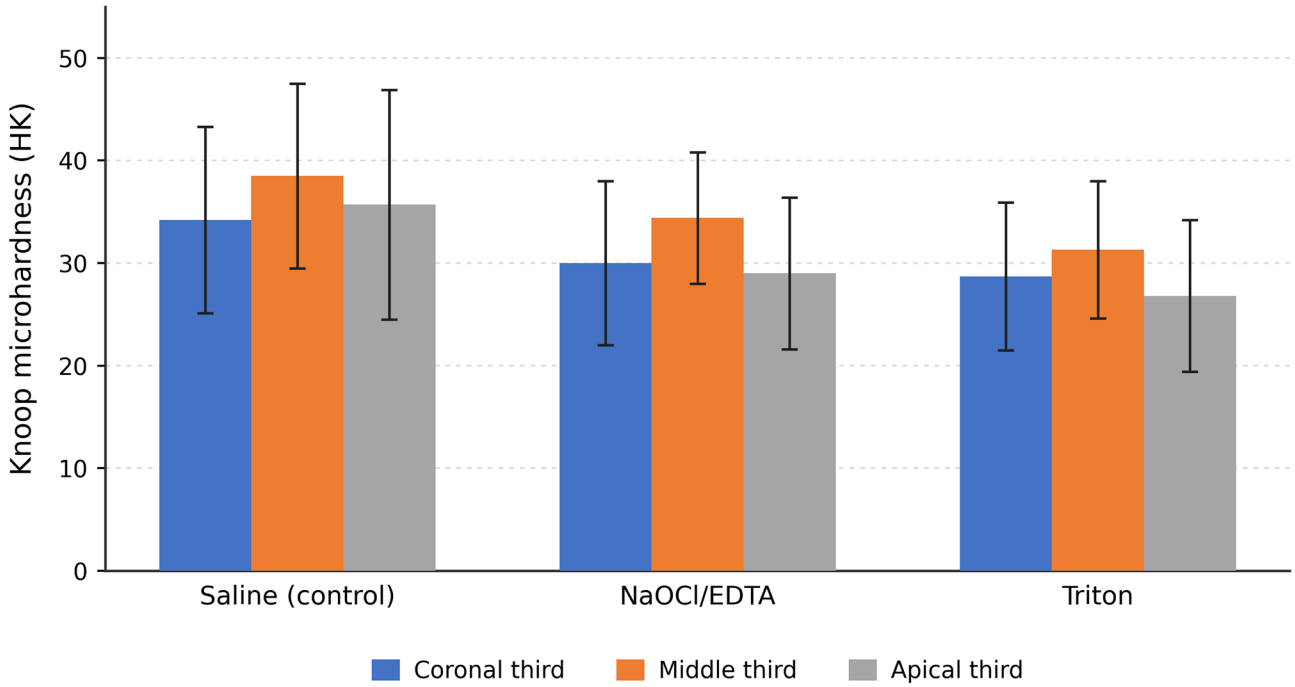



**Fig. 4.** Mean Knoop microhardness (HK) values by canal third for the saline control, NaOCl/EDTA, and Triton groups. Bars represent mean values, and error bars indicate standard deviations. Overall group comparisons demonstrated significantly lower microhardness values for both NaOCl/EDTA and Triton compared with the saline control, with Triton demonstrating significantly lower values than NaOCl/EDTA (see Results).

PUI has been shown to improve irrigant penetration and smear layer removal through acoustic streaming, particularly in anatomically complex regions of the canal system (van der Sluis et al., 2007; Kuah et al., 2009; Caron et al., 2010; Haupt et al., 2020). Our findings suggest that Triton performs similarly to conventional NaOCl/EDTA protocols when activated with PUI. Although Shekhar et al. (2022) demonstrated greater dentinal tubule penetration with citric acid compared with EDTA, their study utilized 10% citric acid, whereas Triton contains approximately 1% citric acid following mixture of both components. This lower concentration may partially explain the lack of significant differences observed in the present study.

**Microhardness evaluation**

Both the NaOCl/EDTA and Triton groups demonstrated significantly lower Knoop hardness values compared with the saline control group. This finding was expected because saline does not chemically alter dentin or produce a chelating effect (Ari et al., 2004; De-Deus et al., 2006). More importantly, Triton demonstrated significantly lower dentin microhardness values than NaOCl/EDTA.

One possible explanation is the prolonged exposure of dentin to chelating agents throughout instrumentation when using Triton, compared with the limited 1-min EDTA exposure in the conventional irrigation protocol. Previous studies have similarly demonstrated that prolonged exposure to citric acid and other chelators may increase dentin demineralization and reduce dentin hardness (Ari et al., 2004; De-Deus et al., 2006; Serper & Çalt, 2002; Uzunoglu et al., 2012; Sheng et al., 2023). Di Lenarda et al. (2000) reported that prolonged citric acid application effectively removed smear layer but also resulted in dentin over-etching. Machado-Silveiro et al. (2004) further demonstrated that 1% citric acid removed significantly more calcium from dentin than 17% EDTA over multiple time intervals. These findings may help explain the lower microhardness values observed in the Triton group. Therefore, the greater reduction in dentin microhardness observed with Triton should not necessarily be interpreted as an intrinsic property of the irrigant itself but may instead reflect differences in cumulative chelator exposure between the irrigation protocols.

In addition to differences in cumulative exposure time, the continuous availability of chelating components throughout instrumentation may have contributed to the greater reduction in dentin microhardness observed with the Triton irrigation protocol. Unlike the conventional protocol, in which EDTA is applied only as a short final rinse, Triton continuously exposes dentin to chelating agents during canal preparation. This prolonged interaction may promote gradual calcium ion removal and progressive demineralization of both peritubular and intertubular dentin. Although the proprietary formulation of Triton precludes a detailed understanding of its chelation kinetics, these differences in the timing and duration of chelator exposure may partially explain the reduction in dentin microhardness observed in the present study. Additional investigations evaluating calcium ion release and dentin mineral loss are warranted to better characterize these mechanisms.

Although statistically significant differences in dentin microhardness were identified, the clinical significance of these findings remains uncertain. Previous investigations evaluating fracture resistance following citric acid irrigation have reported no significant reduction in fracture resistance compared with controls (Arslan et al., 2014). Therefore, additional studies are needed to determine whether the observed reduction in dentin microhardness associated with Triton has meaningful clinical implications.

A secondary observation in the present study was a trend toward higher Knoop hardness values in the middle third compared with the coronal and apical

thirds. However, the study was not powered to evaluate regional differences within the root canal, and these findings should be interpreted cautiously.

**Limitations**

Several limitations should be considered. First, although the final irrigation protocols were standardized, cumulative irrigant exposure differed between groups because Triton was used throughout instrumentation whereas EDTA was applied only during the final irrigation step. Consequently, this study compared complete clinical irrigation protocols rather than isolating the effects of the individual irrigants. Future studies using equivalent irrigant exposure times are warranted to distinguish the effects of irrigant composition from exposure duration. Second, SEM evaluation assesses only a limited portion of the canal wall and may not fully represent the entire root canal system (Caron et al., 2010). Specimen sectioning before SEM analysis may have also introduced debris despite measures to minimize sectioning artifacts before final irrigation. Moreover, the absence of blinding during SEM image evaluation and microhardness measurements may have introduced observer bias. Regarding microhardness testing, the methodology did not allow each tooth to serve as its own control. Furthermore, dentin is a heterogeneous biological material, and variability in dentin structure may influence hardness measurements despite polishing procedures intended to standardize specimen surfaces (Pashley et al., 1985; De-Deus et al., 2011).

# Conclusion

Within the limitations of this study, Triton All-In-One Irrigation Solution demonstrated smear layer removal comparable to a conventional NaOCl/EDTA irrigation protocol when both groups utilized passive ultrasonic irrigation. However, the Triton irrigation protocol resulted in significantly lower dentin microhardness values than the conventional NaOCl/EDTA protocol and saline control under the conditions tested. These findings should be interpreted within the context of the irrigation protocols evaluated, including differences in cumulative irrigant exposure. Further studies are needed to determine whether these differences in dentin microhardness have clinically relevant effects on properties such as fracture resistance.

## Use of artificial intelligence (AI) in manuscript preparation

The authors used ChatGPT (OpenAI) to assist with language refinement and manuscript editing. The authors reviewed and edited all content and take full responsibility for the accuracy and integrity of the manuscript.

## Ethical Approval Statement

This study was approved by the Institutional Review Board
(Pro00128260).

## Acknowledgments

The authors would like to thank Dr. Jeremy L. Gilbert for providing access to his research facilities and equipment. We are grateful to Peter Kurtz and Dr. Girish for their invaluable assistance with sample analysis. This study was supported by the Medical University of South Carolina College of Dental Medicine Mentor Scholars Grant and the American Association of Endodontists Foundation for Endodontics.

This work was not funded by any research grant.

## Conflict of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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