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ORIGINAL RESEARCH

A COMPARATIVE *IN VITRO* STUDY ON THE EFFECTIVENESS OF MALIC ACID, EDTA, HYALURONIC ACID, AND FUMARIC ACID IN SMEAR LAYER REMOVAL USING SCANNING ELECTRON MICROSCOPY EVALUATION

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Background: Effective smear layer removal is crucial for enhancing root canal disinfection and sealing. Traditional chelators like ethylenediaminetetraacetic acid (EDTA) are widely used; however, alternative agents such as fumaric acid, malic acid, and hyaluronic acid are being explored for their biocompatibility and efficacy.
Objectives: This *in vitro* study compared the smear layer removal capabilities of these agents using scanning electron microscopy (SEM).
Materials and Methods: Fifty extracted human single-rooted teeth with straight, fully formed canals were selected. Teeth with caries, fractures, or calcifications were excluded. Specimens were instrumented using the ProTaper Universal system and randomly divided into five groups (n = 10) based on the final irrigant used: 5% fumaric acid, 17% EDTA, 7% malic acid, 0.12 % hyaluronic acid, and distilled water (control). After irrigation, roots were longitudinally split, and SEM images were captured at coronal, middle, and apical thirds (×1000) and scored using Hülsmann's criteria by a blinded observer and AI-assisted analysis.
Results: Fumaric acid and EDTA demonstrated superior smear layer removal across all canal levels ($p < 0.001$), with no significant difference between them. Malic acid showed moderate efficacy, while hyaluronic acid and saline were largely ineffective. Cleaning efficacy consistently decreased from coronal to apical regions for all irrigants. Inter-observer reliability between human and AI scoring was fair to moderate ($\kappa = 0.385$, $p < 0.001$).
Conclusion: Fumaric acid (5%) and EDTA (17%) are the most effective agents for smear layer removal, outperforming malic acid and hyaluronic acid. Fumaric acid presents a promising, biocompatible alternative to EDTA for final root canal irrigation. Further clinical studies are recommended to validate these findings.

Keywords*:* Smear layer removal; Root canal irrigation; Fumaric acid; EDTA; Malic acid; Hyaluronic acid; Scanning electron microscopy (SEM); Chelating agents

INTRODUCTION

The presence of the smear layer may hinder the penetration of irrigants and sealers into dentinal tubules, compromising disinfection and sealing ability.[1,2] Effective root-canal therapy depends largely on thorough cleaning and debridement of the canal system.[3] Mechanical instrumentation alone cannot eliminate the smear layer, which consists of organic and inorganic debris produced during instrumentation and may compromise disinfection and subsequent obturation.[4] Chelating agents such as EDTA are widely used to remove the inorganic component of the smear layer by calcium chelation.[5,6]

More recently, organic acids such as maleic acid, hyaluronic acid, and fumaric acid have been investigated as possible alternatives.[7-10] Malic acid, a naturally occurring α-hydroxy acid, and fumaric acid, an unsaturated dicarboxylic acid, have shown chelating properties and low cytotoxicity.[11,12] Hyaluronic acid has been proposed for its biocompatibility and potential lubricating effects within dentin.[13]

Although fumaric, malic, and hyaluronic acids show promise in clearing smear layers without harming dentin structure[9,10,14], direct comparisons along the full root canal especially those combining expert assessment with AI-based imaging, are still limited. This lab study used SEM to evaluate how well 5% fumaric acid, 17% EDTA, 7% malic acid, 0.12% hyaluronic acid, and saline remove debris in coronal, middle, and apical zones. Instead of relying on one method, we combined visual grading with machine-assisted analysis. This study aimed to test whether fumaric acid and EDTA perform equally well, and better than others, in all sections. Because existing evidence lacks consistency, a side by side examination under controlled conditions seemed necessary. Thus, samples were randomly assigned to treatment groups to reduce bias. Each specimen underwent identical preparation steps before imaging.

Materials and Methods

Study design

This in vitro experimental study was conducted to evaluate the efficacy of removing the smear layer with four final irrigants in instrumented human root canals. A total of fifty freshly extracted human single-rooted teeth with fully formed apices and straight canals were selected via convenience sampling. Teeth exhibiting caries, fractures, resorption, or canal calcifications were excluded. Following extraction, samples were stored in normal saline to prevent dehydration and rinsed with distilled water before instrumentation. All teeth were decoronated to standardize root length using a water-cooled diamond disc, and a #10 K-file was inserted into each canal until it was visible at the apex; the working length was then set by subtracting 1 mm from this measurement.

Root canal preparation

Root canal preparation was performed using a crown-down technique with ProTaper Universal NiTi files (Dentsply Sirona) up to an F3 (30/.09) size as the final apical instrument. During chemomechanical preparation, canals were irrigated with 2 mL of 2.5% sodium hypochlorite after each instrument change. Upon completion, canals were flushed with 5 mL of distilled water to remove residual NaOCl and dried with absorbent paper points (Figure 1).

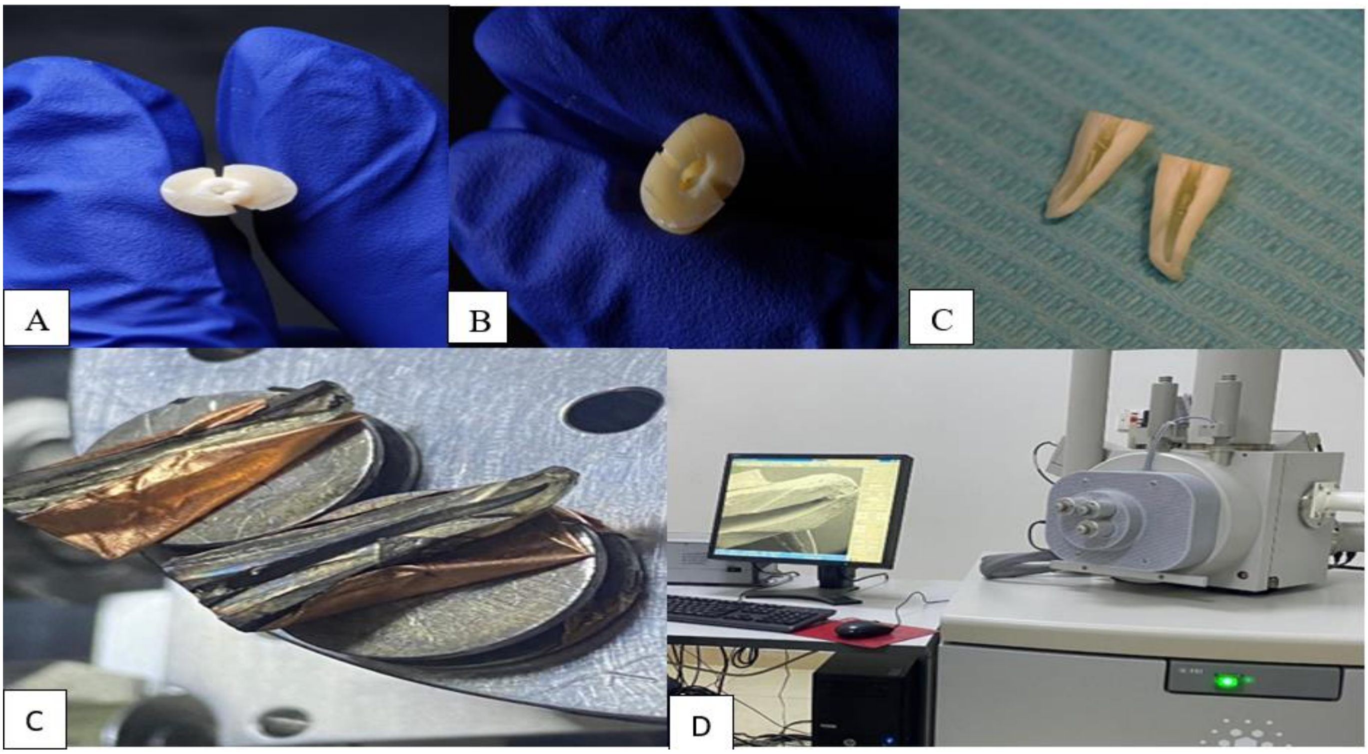

Figure 1. A: The preparation of the canal – B: After irrigation procedure of the canal – C: The splitting of the root – D: Preparation and coating of the sectioned tooth before SEM – D: The scanning electron microscope device.

Groups

The specimens were then randomly assigned into five groups (n = 10) based on the type of final irrigant used: 5% fumaric acid, 17% ethylenediaminetetraacetic acid (EDTA), 7% malic acid, 0.12 % hyaluronic acid, and distilled water (control). Each canal received 3 mL of the respective solution for 30 seconds, after which all canals were flushed with 5 mL of distilled water to neutralize the irrigant and dried again. The roots were longitudinally sectioned using a chisel and mallet after creating shallow external grooves without penetrating the canal lumen, and one half of each tooth was selected for scanning electron microscopy (SEM) analysis.

Scanning electron microscopy

Smear layer removal was independently assessed using Hülsmann's five-point scoring scale by a single calibrated and blinded human evaluator and a convolutional neural network (CNN) based image analysis system specifically trained to apply identical morphological criteria. Prior to implementation, the AI model underwent supervised training on a curated dataset of annotated scanning electron microscopy (SEM) images, achieving a validation concordance of κ = 0.82 against expert human scoring. For each specimen, the final smear layer score was calculated as the mean of the human and AI-assigned values (Figure 2).

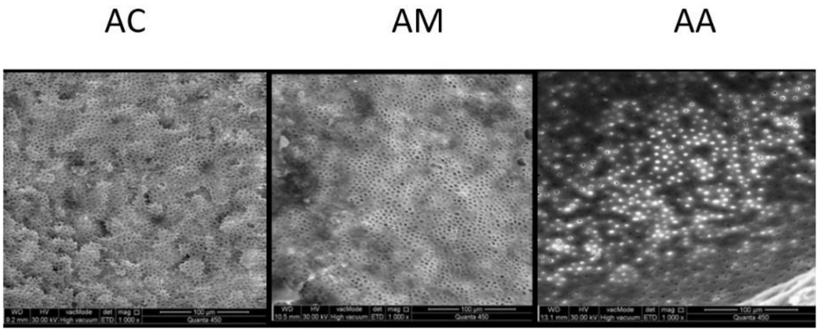

Figure 2. SEM of all groups in coronal, middle, and apical regions (A-E).MEAN

Note: (AC = Apical-Coronal segment [coronal third], AM = Apical-Middle segment [middle third], AA = Apical-Apical segment [apical third])

Data analysis

Data were analyzed using IBM SPSS version 26. As smear layer scores were ordinal, the Kruskal–Wallis test was used to compare differences among the five irrigant groups, while pairwise differences were assessed with the Mann–Whitney U test. The Friedman test with Bonferroni adjustment was used to evaluate regional (coronal, middle, apical) differences within each group. Inter-observer reliability between human and AI evaluations was assessed using weighted Cohen's Kappa, and statistical significance was set at $p < 0.05$.

RESULTS

Inter-Observer Reliability

Inter-observer agreement between the human evaluator and the AI-based scoring system was assessed using weighted Cohen's kappa (κ) across all 150 regions of interest (50 specimens × 3 canal levels). The overall κ value was 0.385 (95% CI: 0.312–0.458; $p < 0.001$), indicating fair-to-moderate but statistically significant concordance. Although the absolute agreement was modest, both evaluators exhibited consistent directional trends in the ranking of irrigants and regional smear layer removal, supporting the internal validity of the composite scoring framework.

Overall Smear Layer Removal Efficacy

A Kruskal–Wallis test demonstrated a statistically significant difference in overall smear layer removal efficacy among the five irrigant groups ($H(4) = 45.009$, $p < 0.001$). Post-hoc pairwise comparisons with Dunn–Bonferroni correction revealed that fumaric acid (mean rank = 9.6) and EDTA (mean rank = 11.4) both achieved significantly superior cleaning performance compared with malic acid (25.5), hyaluronic acid (35.5), and saline (45.5) (all adjusted $p \leq 0.002$).

No significant difference was detected between fumaric acid and EDTA ($p = 1.000$). Malic acid exhibited intermediate efficacy, performing significantly better than saline ($p = 0.021$) but not differing from hyaluronic acid ($p = 1.000$). Hyaluronic acid and saline were statistically indistinguishable ($p = 1.000$).

Collectively, these findings establish the following efficacy hierarchy: Fumaric acid ≈ EDTA > Malic acid > Hyaluronic acid ≈ Saline (Table 1 and table 2).

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Table 1. Mean Rank Scores and Interpretation for Smear Layer Removal Efficacy

Group	N	Mean Rank	Interpretation
Fumaric acid	10	9.6	Best smear layer removal
EDTA	10	11.4	Very effective
Malic acid	10	25.5	Moderate
Hyaluronic acid	10	35.5	Poor
Control (saline)	10	45.5	Very Poor

Table 2. Post-hoc Pairwise Comparisons (Adjusted p-values)

Comparison	Adjusted p-value	Significant
Fumaric vs EDTA	1.000	No
Fumaric vs Malic	0.022	Yes
Fumaric vs Hyaluronic	0.001	Yes
Fumaric vs Control	0.000	Yes
EDTA vs Malic	0.03	Yes
EDTA vs Hyaluronic	0.002	Yes
EDTA vs Control	0.000	Yes
Malic vs Hyaluronic	1.000	No
Malic vs Control	0.021	Yes
Hyaluronic vs Control	1.000	No

Regional Differences in Smear Layer Removal

Consistent with endodontic principles, smear layer removal efficacy declined progressively from the coronal toward the apical third across all irrigants (Friedman $\chi^2 \geq 8.882$, df = 2, $p \leq 0.012$ for each agent). The Kruskal–Wallis test confirmed significant inter-group variation at every canal level (coronal: H = 45.009; middle: H = 43.365; apical: H = 41.652; all $p < 0.001$) (Table 3).

Coronal third: Fumaric acid and EDTA achieved near-complete smear layer removal, significantly outperforming malic acid (p = 0.022 and 0.030, respectively), hyaluronic acid (both $p \leq 0.011$), and saline (both $p < 0.001$). Malic acid was also more effective than saline (p = 0.009) but not superior to hyaluronic acid (p = 0.880).

Middle third: The superiority of fumaric acid and EDTA persisted, with both agents performing significantly better than malic acid (p ≤ 0.027), hyaluronic acid (p ≤ 0.001), and saline (p < 0.001). Malic acid again exceeded saline efficacy (p = 0.034) but did not differ from hyaluronic acid (p = 0.793).

Apical third: Overall efficacy decreased substantially, yet fumaric acid and EDTA maintained significant advantages over hyaluronic acid (p = 0.009–0.012) and saline (p < 0.001). Although both agents showed numerically higher scores than malic acid (p = 0.059 and 0.070), these differences did not reach statistical significance after Bonferroni correction. Malic acid showed no significant difference relative to hyaluronic acid (p = 1.000) or saline (p = 0.104) (Figure 3).

Table 3. Smear-layer removal scores among irrigant groups at coronal, middle, and apical regions

Region	Kruskal–Wallis H (df = 4)	p-value	Pairwise Comparisons (Adjusted p-values)
Coronal	45.009	<0.001	Fumaric–EDTA = 1.000; Fumaric–Malic = 0.022; Fumaric–Hyaluronic = 0.002; Fumaric–Control = 0.000; EDTA–Malic = 0.03; EDTA–Hyaluronic = 0.011; EDTA–Control = 0.000; Malic–Hyaluronic = 1.000; Malic–Control = 0.009; Hyaluronic–Control = 0.880
Middle	43.365	<0.001	Fumaric–EDTA = 1.000; Fumaric–Malic = 0.0193; Fumaric–Hyaluronic = 0.001; Fumaric–Control = 0.000; EDTA–Malic = 0.0274; EDTA–Hyaluronic = 0.001; EDTA–Control = 0.000; Malic–Hyaluronic = 0.793; Malic–Control = 0.034; Hyaluronic–Control = 1.000
Apical	41.652	<0.001	EDTA–Fumaric = 1.000; EDTA–Malic = 0.070; EDTA–Hyaluronic = 0.012; EDTA–Control = 0.000; Fumaric–Malic = 0.059; Fumaric–Hyaluronic = 0.009; Fumaric–Control = 0.000; Malic–Hyaluronic = 1.000; Malic–Control = 0.104; Hyaluronic–Control = 0.432

Note: Bold p-values indicate statistically significant differences after Bonferroni adjustment (p < 0.05).

Friedman post-hoc analyses demonstrated that, for both fumaric acid and EDTA, the apical third was significantly less clean than the coronal (p = 0.020) and middle (p = 0.014–0.030) regions, while no difference was found between coronal and middle thirds (p =1.000). A comparable pattern was noted for malic acid (coronal vs. apical: p = 0.016). Hyaluronic acid, however, exhibited a significant decline specifically between the coronal and middle thirds (p = 0.016) (Table 4).

Table 4. Friedman Test Results for the Effectiveness of Different Solutions Across Root Canal Regions

Parameter / Region	Total N	Friedman Chi-Square	df	p (Asymp. Sig.)	Coronal–Middle (Adj. p)	Coronal–Apical (Adj. p)	Middle–Apical (Adj. p)
EDTA	10	8.882	2	0.012	1.000 (ns)	0.020(*)	0.030 (*)
Fumaric Acid	10	13.241	2	0.001	1.000 (ns)	0.020(*)	0.014 (*)
Malic Acid	10	17.590	2	0.000	0.172 (ns)	0.016 (*)	0.076 (ns)
Hyaluronic Acid	10	13.000	2	0.002	0.016 (*)	0.02(*)	1.000 (ns)

- ns = not significant; * = significant at α = 0.05

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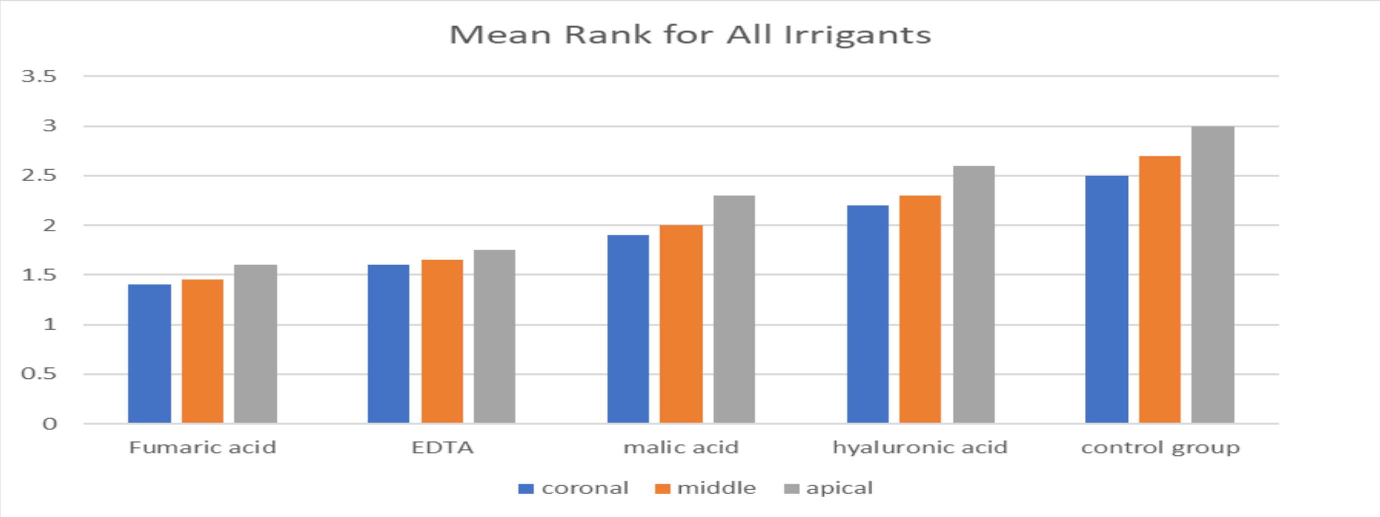

Figure 3. The relative effectiveness of four solutions and the control group—EDTA, Fumaric Acid, Malic Acid, and Hyaluronic Acid—across the coronal, middle, and apical regions of the root canal.

DISCUSSION

This *in vitro* study compared the smear-layer removal efficacy of four irrigants—fumaric acid (5%), EDTA (17%), malic acid (7%), and hyaluronic acid (0.1%)—using SEM analysis across the coronal, middle, and apical thirds of instrumented root canals.[3,4,11] Both fumaric acid and EDTA were highly effective across all regions, showing no statistically significant difference in performance, which corroborates previous studies by Jaiswal et al.[5] and Ballal et al.[12] that demonstrated comparable demineralizing capacities for these agents.[7,14] The superior efficacy of fumaric acid can be attributed to its unsaturated dicarboxylic structure, which enhances both chelating ability and dentinal tubule penetration.[15,16] In contrast, malic acid displayed moderate efficacy, likely due to its higher surface tension and limited calcium-chelating capacity, while hyaluronic acid and saline were largely ineffective, possibly due to their minimal demineralizing properties and, in the case of hyaluronic acid, high viscosity.[17]

A consistent decline in smear-layer removal from coronal to apical regions was observed across all groups, aligning with prior SEM-based research.[18-20] This pattern is commonly linked to challenges such as limited irrigant flow, vapor lock effects, and restricted access in the narrow apical third. These findings emphasize the necessity of advanced irrigation techniques, including ultrasonic or sonic activation, to enhance irrigant penetration and efficacy in clinical settings.[21,22] Notably, fumaric acid shows potential as a more biocompatible and environmentally friendly alternative to EDTA, with previous toxicological studies highlighting its safer profile for periapical tissues and reduced ecological impact.[23]

A novel aspect of this study was the use of an AI-based image analysis system for SEM evaluation, which demonstrated moderate agreement with human scoring. This suggests that AI-assisted assessment may offer reliable, reproducible, and objective analysis in future research, though further validation using larger datasets is needed to refine this approach.

Despite these promising findings, the study's limitations must be acknowledged. Because it was conducted *in vitro* using only straight, single-rooted teeth with fully formed apices and a relatively small sample size (n = 10 per group), the results may not fully reflect clinical conditions where root canal anatomy is more complex, often involving curvature, accessory canals, and biofilms. Future work should involve in vivo studies, the evaluation of irrigant combinations, and investigations in more anatomically complex models to better simulate clinical scenarios.

CONCLUSION

This study demonstrated that fumaric acid (5%) and EDTA (17%) were the most effective irrigants for smear-layer removal in instrumented root canals, exhibiting similar performance across the coronal, middle, and apical thirds, with fumaric acid offering the added advantages of biocompatibility and environmental safety. Malic acid (7%) showed moderate smear-layer removal, while hyaluronic acid and saline were ineffective. Smear-layer removal efficacy consistently decreased from coronal to apical regions for all irrigants, underscoring the clinical challenge of achieving adequate apical cleanliness. The integration of AI-based SEM analysis provided reproducible and objective scoring, suggesting its potential utility in future endodontic research. Overall, the findings support the use of fumaric acid as a viable alternative to EDTA for final root canal irrigation, combining efficient smear-layer removal with favorable biocompatibility and ecological profiles.

DECLARATIONS

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Availability of Data and Materials
The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests
The authors declare that they have no competing interests.

Ethical Considerations
This study was approved by the Ethical Review Committee of the Kurdistan Higher Council of Medical Specialties, Kurdistan Region, Iraq (1558-05/05/2025. The research was conducted in accordance with institutional guidelines and the ethical principles of the Declaration of Helsinki (2013 revision). The use of extracted human teeth for in vitro experimentation was authorized by the committee, and written informed consent was obtained from all donors or their legal guardians before sample collection.

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