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Efficacy of EDTA, Glycolic Acid, and Ethanol in Removing Calcium Hydroxide from the Apical Third: A SEM Study

SUMMARY

Objective: *This study aimed to evaluate the effectiveness of 17% ethylenediaminetetraacetic acid (EDTA), 10% glycolic acid (GA), and 70% ethanol as final irrigation solutions for removing calcium hydroxide [$Ca(OH)_2$] from the apical third of root canals.* ***Materials and Methods:*** *Seventy-six extracted single-rooted human mandibular premolars were instrumented and irrigated with 10 mL of 2.5% sodium hypochlorite followed by 2 mL of 17% EDTA. $Ca(OH)_2$ paste was placed in the canals, and specimens were stored at 37°C and 100% humidity for seven days. Samples were randomly assigned to three experimental groups (n=22 each) and two control groups (n=5 each). Final irrigation was performed using 6 mL of either 70% ethanol (Group 1), 17% EDTA (Group 2), or 10% GA (Group 3), with all groups receiving sonic activation. Control groups were irrigated with distilled water. Roots were sectioned buccolingually, and the apical third was evaluated using scanning electron microscopy (SEM) at 2000× magnification. Residual $Ca(OH)_2$ was assessed using Salgado's five-grade scale.* ***Results***: *Interobserver reliability was high (κ = 0.737–0.835). EDTA and GA groups showed no significant differences in scores 1, 4, and 5 ($p > 0.05$). The ethanol group was significantly less effective than EDTA and GA ($p < 0.001$). No significant differences were observed among groups for score 2, and GA and ethanol were comparable for score 3 ($p > 0.05$).* ***Conclusion***: *EDTA and GA demonstrated similar and superior efficacy in removing $Ca(OH)_2$ from the apical third. Ethanol was significantly less effective. Complete removal was not achieved with any solution.*

Keywords: calcium hydroxide, EDTA, ethanol, glycolic acid

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Introduction

Successful root canal treatment largely depends on the effective elimination of microorganisms from the root canal system. It is widely accepted that, in addition to mechanical instrumentation and irrigation, the use of intracanal medicaments is necessary to eradicate persistent infections, a process referred to as biomechanical preparation. However, complete disinfection may still be unattainable due to the complex anatomy of infected root canals[1].

To achieve optimal treatment outcomes in infected cases, multiple-visit endodontic procedures involving intracanal medicament placement are often preferred[2]. Calcium hydroxide ($Ca(OH)_2$) is frequently employed between treatment sessions due to its antimicrobial properties and its capacity to promote apical healing. Its effectiveness stems from its high pH, which can degrade lipopolysaccharides, dissolve tissue, inhibit osteoclastic activity, and readily dissociate into hydroxyl and calcium ions[2,3].

Complete removal of $Ca(OH)_2$ prior to obturation has been recommended[4], as its remnants may interfere with sealer penetration into dentinal tubules, compromise the bond between sealer and dentin, and impair the hermetic seal of the root filling[5]. Residual $Ca(OH)_2$ has also been shown to alter the physical properties of sealers and reduce their penetration depth[4].

Various irrigants, including saline, sodium hypochlorite (NaOCl), and chelating agents such as EDTA, have been used to remove $Ca(OH)_2$. Among these, chelators like maleic acid, citric acid, and EDTA have demonstrated superior efficacy[6]. However, due to EDTA's demineralizing effect on root dentin over prolonged use, alternative agents are being explored.

Glycolic acid (GA), a biodegradable α-hydroxy acid, has shown better antibacterial activity against Enterococcus faecalis than EDTA and is used at concentrations between 5–17% in endodontics[7-10]. While there are studies evaluating GA's effect on smear layer removal and dentin erosion, data regarding its effectiveness in removing $Ca(OH)_2$ is scarce [11,12].

Ethanol, another agent with limited use in endodontics, has been reported to enhance sealer penetration and reduce surface tension when used as a final rinse[13,14]. However, only one study to date has investigated its efficacy in removing $Ca(OH)_2$ from root canals[13].

Although various techniques—including sonic and ultrasonic devices, rotary systems, and brushes—have been proposed for $Ca(OH)_2$ removal, none have been shown to completely eliminate the medicament, particularly from the apical third of the canal [4,15,16].

Therefore, this study aimed to compare the effectiveness of EDTA, GA, and ethanol solutions, activated by a sonic system, in removing $Ca(OH)_2$ from root canals using scanning electron microscopy (SEM). The null hypothesis (H_0) posits that there is no significant difference among the irrigants in their ability to remove $Ca(OH)_2$.

Materials and Methods

This study aimed to evaluate the efficacy of 17% EDTA, 10% glycolic acid (GA), and 70% ethanol solutions in removing calcium hydroxide ($Ca(OH)_2$) from root canals. The study protocol was approved by the Institutional Review Board (Certificate No. 2022/15, dated 30.03.2022).

Tooth Selection

To determine the required sample size, a one-way ANOVA power analysis was performed using the G*Power software. With a confidence level of 95% (1-α), statistical power of 95% (1-β), and an effect size of f = 0.502, it was determined that 22 samples per group were needed, totaling 66 teeth for the three experimental groups.

Seventy-six extracted single-rooted human mandibular premolars with fully developed apices, no caries, and no prior endodontic treatment, extracted for orthodontic or periodontal reasons, were stored in 0.9% saline at room temperature until use. Periapical radiographs and stereomicroscopic examination were used to exclude teeth with calcified canals, internal or external resorption, cracks, or fractures.

Sample Preparation

To standardize root length, crowns were removed at the cementoenamel junction using a water-cooled diamond disc, resulting in a 15 mm root length (Figure 1a). Working length was established by inserting a #15 K-file into the canal until visible at the apical foramen and subtracting 1 mm. Teeth in which apical enlargement exceeded a #20 K-file were excluded.

To simulate clinical conditions, each root was embedded in a silicone impression material (Zetaplus, Zhermack Spa, Italy), leaving 2–3 mm of the coronal portion exposed for ease of access during instrumentation and irrigation (Figure 1b).

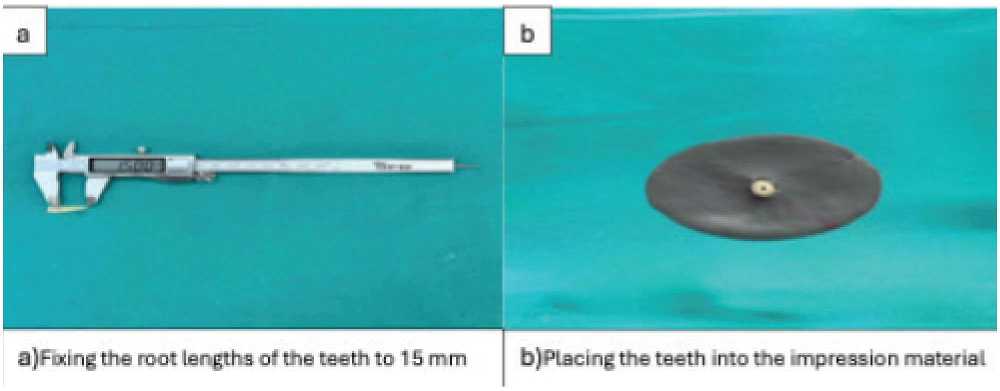


Figure 1. Preparation of the Teeth.

Root canal preparation was performed by a single operator using an E Connect S endodontic motor (Eighteeth, Changzhou, China) with Reciproc R40 NiTi files (VDW, Munich, Germany), employing a single file per canal. Apical patency was maintained with a #10 K-file. During instrumentation, canals were irrigated with 10 mL of 2.5% NaOCl using a 30-gauge side-vented irrigation needle positioned 1 mm short of the working length.

To remove the smear layer, canals were irrigated with 2 mL of 17% EDTA (Wizard, Rehber Kimya, Istanbul, Turkey) and left for 1 minute, followed by neutralization with distilled water. The canals were dried with paper points (Dentsply Maillefer, Ballaigues, Switzerland).

Placement of Calcium Hydroxide

A total of 76 teeth were allocated into three experimental groups (n=22 each) and two control groups (n=5 each). Injectable $Ca(OH)_2$ paste (Dentsply Maillefer, Switzerland) was placed homogeneously into all canals using disposable plastic tips, in accordance with the manufacturer's instructions. Periapical radiographs were taken to confirm complete canal filling.

Access cavities were sealed with sterile Teflon tape and 3 mm of temporary restorative material (Cavit-G, 3M ESPE, USA). Each sample was stored in an Eppendorf tube and incubated at 37°C in 100% humidity for 7 days.

Experimental Groups and Final Irrigation

After incubation, temporary materials were removed. Grooves were prepared along the buccolingual axis under water cooling using a diamond disc (Figure 2), enabling the samples to be split using a thin spatula.

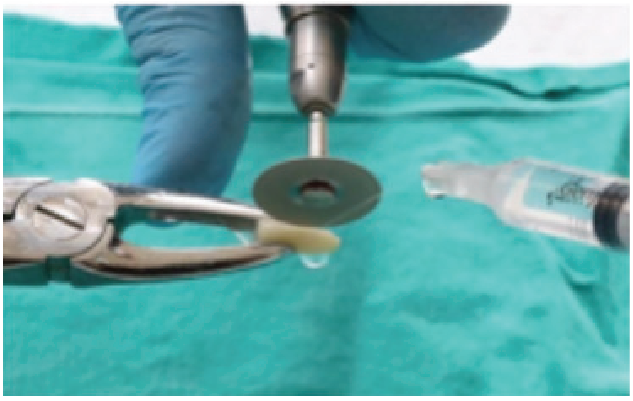


Figure 2. Tooth Separation Performed Under Continuous Water Cooling.

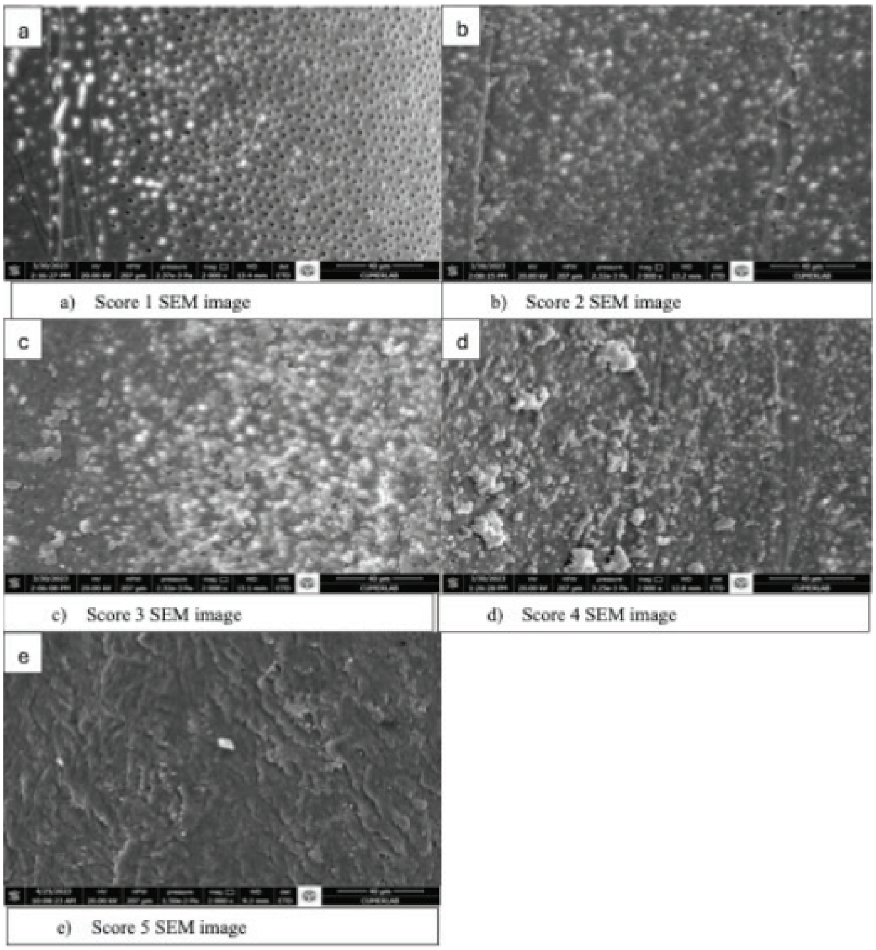


Figure 3. SEM Images of the EDTA Group

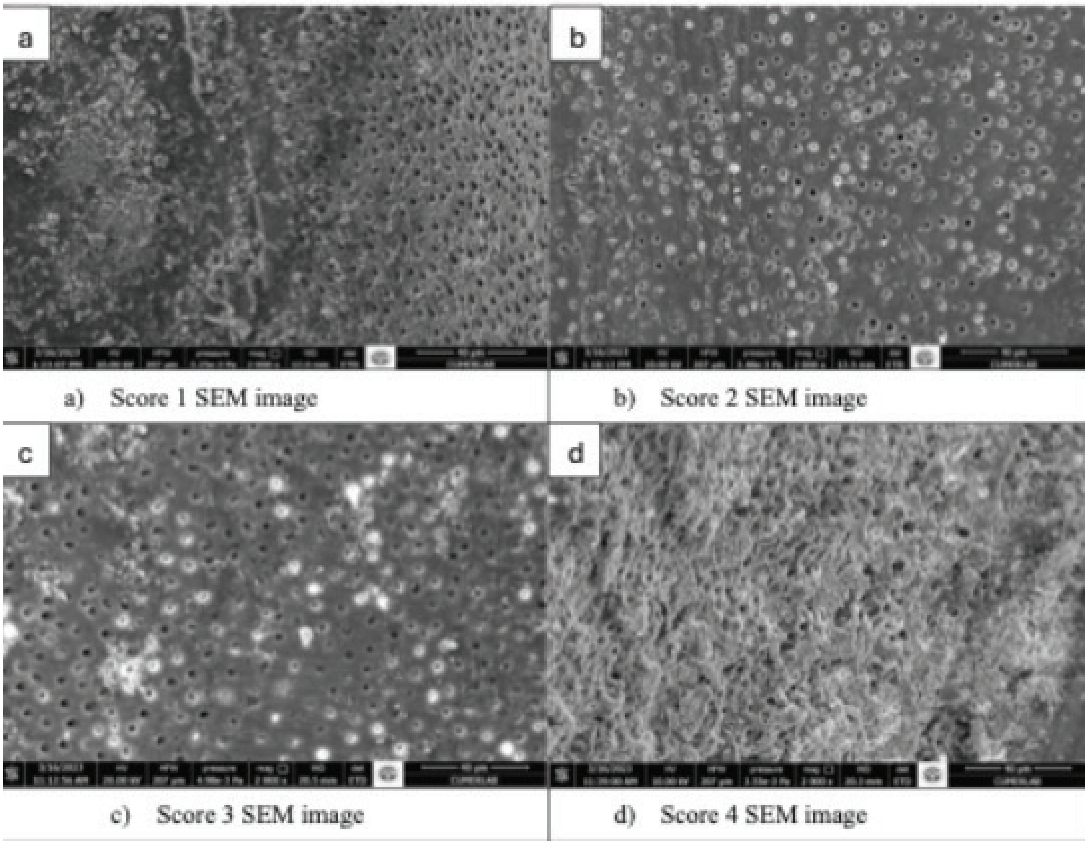


Figure 4. SEM Images of the GA Group

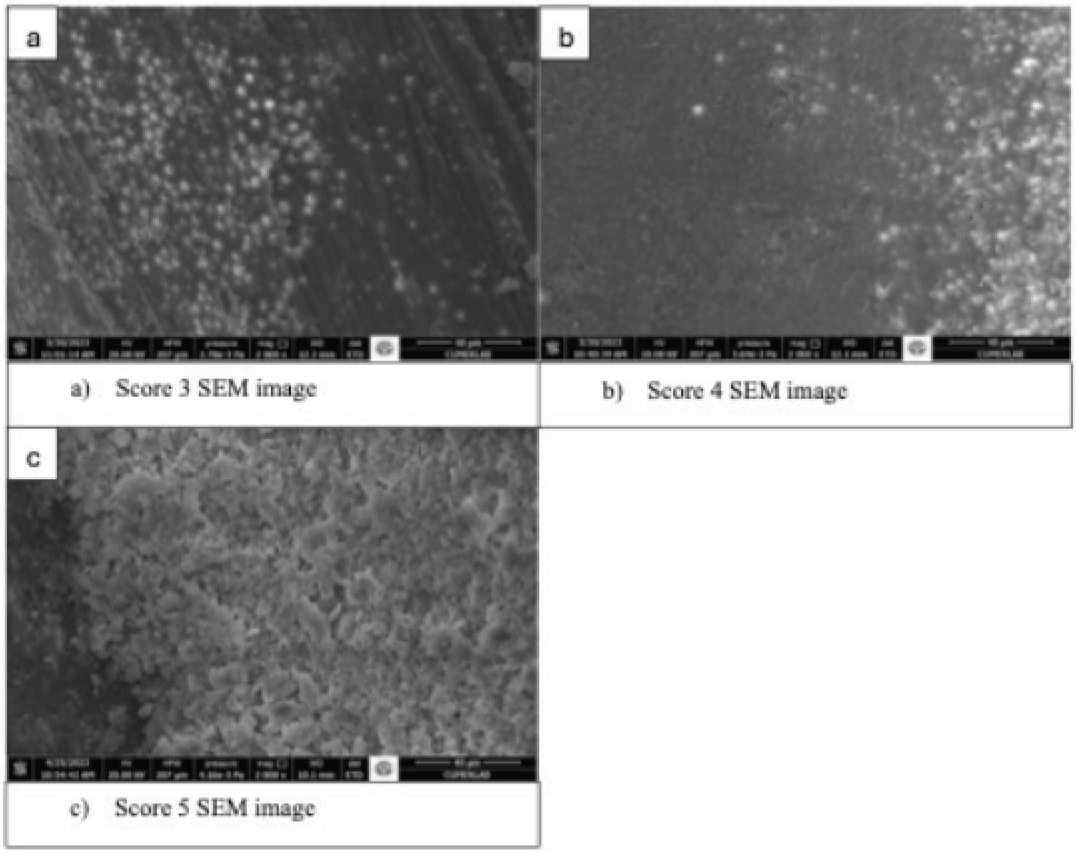


Figure 5. SEM Images of the Ethanol Group

The groups were as follows:
Group 1: 6 mL of 17% EDTA + Sonic Irrigation (SI) (n=22)
Group 2: 6 mL of 10% Glycolic Acid + SI (n=22)
Group 3: 6 mL of 70% Ethanol + SI (n=22)
Positive Control Group: 5 mL of distilled water (n=5)
Negative Control Group: 5 mL of distilled water (n=5)

Before final irrigation, canals were flushed with 10 mL of saline, and the master apical file was used to verify patency. Irrigants were then introduced and activated for 1 minute using the VDW EDDY sonic tip (VDW GmbH, Munich, Germany) connected to a W&H Synea ZA55 air scaler (W&H, Bürmoos, Austria). Irrigation tips were positioned 1 mm short of the working length. After activation, canals were flushed with 5 mL of distilled water and dried with paper points.

SEM Evaluation

Each root was split into two halves using a mouth spatula and placed in the SEM device. SEM imaging was performed at 2000× magnification and 20.0 kV, capturing 76 images from the apical third of each canal.

Residual $Ca(OH)_2$ was assessed using the five-grade scoring system proposed by Salgado:

Score 1: 80–100% removal (completely clean)
Score 2: 60–80% removal (very good cleanliness)
Score 3: 40–60% removal (partial cleanliness)
Score 4: 20–40% removal (limited cleanliness)
Score 5: 0–20% removal (uncleaned)

Two independent observers scored the samples based on SEM images. Representative images for each score are provided in Figures 3–5.

Results

Statistical Analysis

All statistical analyses were performed using IBM SPSS Version 23 (IBM Inc., Chicago, USA).

Interobserver agreement was assessed using the Kappa statistic. The distribution of calcium hydroxide ($Ca(OH)_2$) removal scores across the groups was evaluated using the Pearson Chi-square test. Multiple comparisons were conducted using the Bonferroni correction. Results were presented as frequencies (percentages), with statistical significance set at $p< 0.05$.

Interobserver Agreement

EDTA Group: Substantial agreement was observed between observers ($\kappa = 0.749$; $p < 0.001$). For scores 1 through 5, the agreement percentages were 100%, 100%, 70%, 100%, and 50%, respectively (Table 1).

GA Group: Substantial agreement was again confirmed ($\kappa = 0.737$; $p < 0.001$). Agreement percentages were 90%, 60%, 75%, and 100% for scores 1 through 4, respectively (Table 2).

Ethanol Group: Interobserver agreement was excellent ($\kappa = 0.835$; $p < 0.001$), with agreement percentages of 100%, 91.7%, and 88.9% for scores 3 through 5, respectively (Table 3).

Group Comparison by Score Distribution

The distribution of $Ca(OH)_2$ removal scores differed significantly among the three experimental groups ($p < 0.001$). Notable findings include (Table 4):

Table 1. Analysis of Inter-Observer Agreement in the EDTA Group

		Observer 1					Kappa	p
		1	2	3	4	5		
Observer 2	1	6 (100)	0 (0)	0 (0)	0 (0)	0 (0)		
	2	0 (0)	3 (100)	1 (10)	0 (0)	0 (0)		
	3	0 (0)	0 (0)	7 (70)	0 (0)	1 (50)	0,749	**<0,001**
	4	0 (0)	0 (0)	2 (20)	1 (100)	0 (0)		
	5	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)		

Frequency (percentage)

Table 2. Analysis of Inter-Observer Agreement in the GA Group

		Observer 1				Kappa	p
		1	2	3	4		
Observer 2	1	9 (90)	1 (20)	0 (0)	0 (0)		
	2	1 (10)	3 (60)	1 (25)	0 (0)	0,737	**<0,001**
	3	0 (0)	1 (20)	3 (75)	0 (0)		
	4	0 (0)	0 (0)	0 (0)	3 (100)		

Frequency (percentage)

Table 3. Analysis of Inter-Observer Agreement in the Ethanol Group

		Observer 1			Kappa	p
		3	4	5		
Observer 2	3	1 (100)	1 (8,3)	0 (0)	0,835	**<0,001**
	4	0 (0)	11 (91,7)	1 (11,1)		
	5	0 (0)	0 (0)	8 (88,9)		

Frequency (percentage)

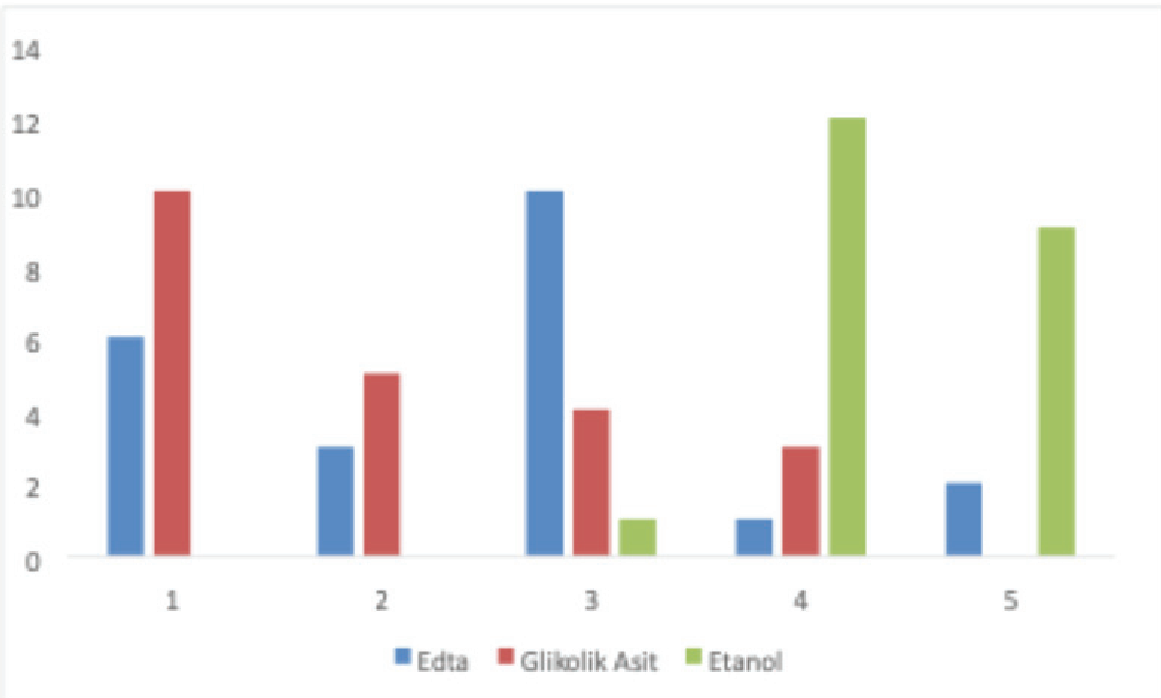
Table 4. Distribution of Scores by Groups

Score	EDTA	Glycolic Acid	Ethanol	Test Stat.	p
1	6 (27.3)a	10 (45.5)a	0 (0)b		
2	3 (13.6)	10 (45.5)a	5 (22.7)		
3	4 (18.2)ab	0 (0)	1 (4.5)b	47.707	<0.001
4	1 (4.5)a	3 (13.6)a	12 (54.5)b		
5	2 (9.1)a	0 (0)a	9 (40.9)b		

Pearson Chi-Square Test; frequency (percentage);

a-b: No significant difference between groups sharing the same superscript letter.

Table 5. Score distributions by group.



Score 1: GA group had the highest percentage (45.5%), followed by EDTA (27.3%). Ethanol group showed no samples with Score 1.

Score 2: Highest in GA (22.7%), followed by EDTA (13.6%), and none in ethanol group.

Score 3: EDTA showed the highest frequency (45.5%), followed by GA (18.2%) and ethanol (4.5%).

Score 4: Ethanol group had the highest (54.5%), GA had 13.6%, and EDTA only 4.5%.

Score 5: Ethanol group again had the highest (40.9%), while GA had none, and EDTA had 9.1%.

According to the graphical analysis:

The GA group exhibited the highest frequency of Score 1, while the ethanol group did not show any instances of this score. Similarly, Score 2 was most frequently observed in the GA group. For Score 3, EDTA showed the highest frequency, whereas ethanol had the lowest. In contrast, Scores 4 and 5 were most frequently associated with the ethanol group, indicating its inferior performance in calcium hydroxide removal (Table 5).

Discussion

One of the primary objectives of root canal therapy is the elimination of bacteria and their byproducts from the root canal system. However, due to the complex anatomy of root canals, including irregularities, isthmuses, and lateral canals, certain areas remain inaccessible to biomechanical procedures, providing shelter for microorganisms. To effectively disinfect these regions, the use of intracanal medicaments is essential. Calcium hydroxide ($Ca(OH)_2$), the most frequently used medicament for this purpose, possesses key properties such as broad-spectrum antibacterial activity against endodontic pathogens, inhibition of osteoclastic activity, and promotion of hard tissue repair [17].

Studies have shown that placing $Ca(OH)_2$ in the root canal for at least one week significantly enhances bacterial elimination, particularly in teeth with necrotic pulp [17,18]. Sjögren et al. reported that $Ca(OH)_2$ application effectively eliminated bacteria in 100% of cases within one week [19].

Prior to the final restoration phase of endodontic treatment, complete removal of $Ca(OH)_2$ is crucial, as its remnants can compromise the seal of the root canal filling[17,20]. Residual $Ca(OH)_2$ can block dentinal tubules, hinder sealer penetration, cause apical leakage, and alter the chemical properties of sealers[17,21,22]. Nonetheless, conventional irrigation methods alone are insufficient for complete removal, especially in the apical third [17,23]. Therefore, activation of irrigants is necessary to improve their efficacy.

Contemporary endodontic practice employs various irrigation activation techniques, including ultrasonic, sonic, multisonic, and laser systems. EDTA has been shown to be particularly effective in removing $Ca(OH)_2$, especially when used in combination with other chelators like GA, citric acid, and maleic acid [6,24,25]. The volume and chemical composition of the irrigant also significantly influence its efficacy. Additionally, studies have demonstrated enhanced $Ca(OH)_2$ removal when EDTA is used in combination with NaOCl [17,26].

Initially introduced in restorative dentistry for surface conditioning, GA has recently been shown to effectively remove the smear layer in endodontics[12]. In vitro and in vivo studies report that GA promotes collagen synthesis and fibroblast proliferation [27,28,29]. Its low pKa and molecular weight make GA particularly suitable for use on highly mineralized surfaces like dentin [10].

GA has surface tension properties similar to EDTA and citric acid but is more biodegradable and less cytotoxic. It also shows stronger antibacterial activity against Enterococcus faecalis [7,9,30].

In a study by Keskin et al., SEM analysis revealed that 10% GA provided the most effective removal of $Ca(OH)_2$ in artificially created resorption cavities in the apical third [19]. These findings support the current study's results, where the GA group showed the highest proportion of Score 1 outcomes, indicating superior cleanliness.

Cunningham et al. first investigated the effects of ethanol on NaOCl surface tension in 1982. They found that ethanol significantly reduced surface tension and improved irrigant penetration in vitro[31]. However, subsequent studies have reported mixed results regarding ethanol's efficacy as an irrigant. Some have noted that ethanol is ineffective at smear layer removal and can result in debris-covered surfaces when used to dissolve filling materials[32]. Others, such as De Lima et al., reported that ethanol achieved superior $Ca(OH)_2$ removal compared to other agents[13], though application times were not always specified.

Variability in results may stem from differences in application duration, as noted by Dainezi et al., who found that prolonged ethanol exposure caused protein denaturation and coagulation. Another consideration is the imaging technique; ethanol may quench the fluorescence of rhodamine-labeled $Ca(OH)_2$, potentially obscuring residual material during CLSM evaluation [32,33]. However, De Lima argued that even at high concentrations, alcohols have limited potential to interfere with CLSM imaging [13].

Another factor that may have contributed to the discrepancy is that ethanol might have neutralized the fluorescence of rhodamine used in confocal laser scanning microscopy (CLSM), potentially leading to residual $Ca(OH)_2$ remaining in some areas. These rhodamine-stained $Ca(OH)_2$ residues may have lost their fluorescent properties, which could have compromised the accuracy of the imaging results [34]. In contrast, De Lima cited literature suggesting that alcoholic solutions, even at high concentrations, have limited potential to interfere with rhodamine performance or affect the effectiveness of CLSM imaging [13].

The type of $Ca(OH)_2$ carrier used also affects removal efficacy. Studies have shown that oily vehicles like silicone oil-iodoform mixtures leave more residue than aqueous vehicles like distilled water or propylene glycol, due to their higher viscosity and resistance to dissolution [35,36]. In our study, AH Temp, a widely used commercially available pre-mixed $Ca(OH)_2$ product, was employed. Although its manufacturer claims it is easy to remove, additives in such products may enhance retention.

Interobserver agreement for scoring was substantial (kappa values: 0.749, 0.737, 0.835). Statistical comparisons showed no significant difference between the EDTA and GA groups for Scores 1, 4, and 5. However, ethanol was significantly less effective than both. No significant differences were observed among groups for Score 2. For Score 3, no differences were found between EDTA-GA or GA-ethanol comparisons. Thus, the null hypothesis was rejected.

Our results indicated that the GA group had the highest percentage of Score 1 outcomes (45.5%), followed by EDTA (27.3%), while ethanol showed none. This suggests that GA is at least as effective as EDTA in removing $Ca(OH)_2$ and may serve as a favorable alternative due to its low cytotoxicity and biocompatibility.

In contrast, ethanol had the highest proportion of low-cleanliness scores. For Score 4, EDTA, GA, and ethanol groups showed rates of 4.5%, 13.6%, and 54.5%, respectively. For Score 5, the values were 9.1% for EDTA, 0% for GA, and 40.9% for ethanol. These results indicate that ethanol was the least effective among the tested irrigants.

Considering the inconsistencies in previous literature, including variations in ethanol application time, vehicle formulation, and imaging techniques, further research is warranted before ethanol can be recommended as a final irrigant in clinical practice.

Conclusions

In this study, the efficacy of 17% EDTA, 10% glycolic acid (GA), and 70% ethanol—activated using a sonic irrigation device—for the removal of calcium hydroxide ($Ca(OH)_2$) from the apical third of root canals was compared. Although overall scores indicated that the EDTA and GA groups demonstrated similar levels of effectiveness, the highest rates of optimal cleanliness (Scores 1 and 2) were observed in the GA group. Conversely, the poorest cleanliness scores (Scores 4 and 5) were most frequently found in the ethanol group. None of the tested irrigants were able to completely remove $Ca(OH)_2$ from the apical region. The comparable efficacy of GA to EDTA highlights its potential as a viable and effective alternative irrigation solution in endodontic therapy. On the other hand, due to its limited cleaning performance, ethanol does not appear to be a suitable irrigant. Further research is warranted to better understand the effects of ethanol on dentin structure and its ability to remove $Ca(OH)_2$.

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