

**EDTA VS. TWIN KLEEN: A SITE-SPECIFIC STEREOMICROSCOPIC COMPARISON
OF THEIR EFFICACY IN ERADICATING INTRACANAL CALCIUM HYDROXIDE
USING PASSIVE ULTRASONIC AND SONIC ACTIVATION**

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ABSTRACT

Context: Calcium hydroxide is extensively utilized as an intracanal medicament due to its high pH and wide-ranging antimicrobial properties. However, its remnants on the canal walls can negatively impact dentin bond strength and obstruct sealer penetration into dentinal tubules. Hence, it is imperative to investigate techniques ensuring the complete elimination of this medicament from the canal walls. **Aim:** To assess the effectiveness of Twin Kleen solution and EDTA with two different irrigation activation techniques in removing calcium hydroxide from the root canal system. **Material and Method:** Sixty single-rooted anterior teeth were instrumented with Protaper rotary instruments and filled with oil-based calcium hydroxide with iodoform. There were four groups based on the irrigant used Group A: 17% liquid EDTA with sonic irrigation, Group B: Twin kleen solution solution with sonic irrigation, Group C: 17% EDTA with Passive Ultrasonic Irrigation (PUI) Group D: Twin kleen with PUI. Evaluation was done under stereomicroscope at three levels. **Statistical Analysis:** Mann Whitney U test, Wilcoxon signed rank test, Kruskal wallis test. **Result:** Liquid EDTA showed slightly better efficacy than Twin Kleen in removing calcium hydroxide from the root canal wall, but the differences were not statistically significant ($p > 0.05$). Sonic activation showed marginally better results than PUI in only apical third. **Conclusion:** Present study found no statistically significant differences in efficacy between sonic and ultrasonic irrigation methods using liquid EDTA or Twin kleen solutions at coronal, middle, or apical canal sites. ($p > 0.5$)

KEYWORDS: Calcium hydroxide, Passive ultrasonic irrigation, sonic irrigation, Twin Kleen.

INTRODUCTION

The primary objective of root canal therapy is to alleviate pain and eradicate infection through meticulous chemo-mechanical debridement, followed by the hermetic sealing of the root canal system. However, the intricate anatomical complexities of the root canal system, such as isthmuses, apical deltas, dentinal tubules, and ramifications pose significant challenges in the complete elimination of microorganisms solely through mechanical preparation.^[1] Consequently, the use of intracanal medicaments is crucial to ensure the eradication of residual bacteria from the root canals.^[1]

Calcium hydroxide ($\text{Ca}(\text{OH})_2$) is widely employed as an intracanal medicament due to its broad-spectrum antimicrobial activity. The bactericidal efficacy of calcium hydroxide is attributed to the generation of a

highly alkaline microenvironment within the root canal system.^[2] The vehicle used in the production of $\text{Ca}(\text{OH})_2$ affects its antibacterial efficacy.^[3] Prior to obturation, the complete elimination of calcium hydroxide from the canal walls is imperative, as residual $\text{Ca}(\text{OH})_2$ can impede sealer penetration into dentinal tubules, potentially compromising the long-term success of endodontic therapy.^[3]

Using a master apical file (MAF) and profuse irrigation with sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA), the most frequently employed strategy for calcium hydroxide removal is mechanically debriding the root canal.^[4]

A novel irrigant, Twin Kleen solution (Maarc Dental Innovations endo, India), has been introduced. This

solution comprises 9% 1-hydroxyethylidene 1, 1-bisphosphonate (HEDP), a mild calcium-chelating agent. For optimal efficacy, it must be freshly combined with 3% sodium hypochlorite (NaOCl) to create a single-step final irrigation protocol.

The incorporation of HEDP in endodontic irrigation has been advocated due to its favourable biocompatibility and compatibility with NaOCl. However, it is important to note that HEDP exhibits a lower chelating capacity compared to ethylenediaminetetraacetic acid (EDTA), a widely used chelator in endodontic therapy.^[5]

Traditional syringe-based irrigation protocols have demonstrated limited efficacy in the complete debridement of calcium hydroxide residues from the complex root canal system architecture, as demonstrated by numerous studies. One such method is sonic irrigation (SI) which generates a hydrodynamic effect in irrigants by vibrating a smooth, flexible polymer file at frequencies ranging from 1 to 10 kHz.^[6] Passive ultrasonic irrigation (PUI) has emerged as an extensively studied and benchmark methodology for irrigant activation in endodontics. While PUI has demonstrated efficacy in the elimination of residual calcium hydroxide from the coronal and middle segments of the root canal system, its performance in the critical apical third and anatomically complex areas remains suboptimal. Despite the implementation of this advanced irrigation protocol, a significant quantity of Ca(OH)₂ persists in these challenging regions, potentially compromising the overall success of endodontic therapy. This limitation underscores the need for further refinement of irrigation strategies to achieve comprehensive debridement of the entire root canal system, particularly in areas recalcitrant to conventional cleaning methods.^[7]

While extensive research has been conducted on the efficacy of ethylenediaminetetraacetic acid (EDTA) in the removal of calcium hydroxide residues from root canal systems, this investigation represents the first empirical evaluation of Twin Kleen, a novel irrigant comprising a mild calcium chelating agent in combination with sodium hypochlorite, for the elimination of calcium hydroxide remnants from the endodontic space.

The null hypothesis for this study posits that there is no statistically significant difference in the efficacy of 17% liquid EDTA and Twin Kleen irrigation solutions when utilized in conjunction with sonic agitation and passive ultrasonic irrigation (PUI) for the elimination of calcium hydroxide from the three different sites of the root canal system.

MATERIAL AND METHOD

Sample Size Calculation and Statistical Analysis

A priori power analyses were conducted to establish the smallest number of participants needed to achieve sufficient statistical power for their intended analyses.

For the Mann Whitney U test with two groups, a minimum sample size of 30 per group (total 60) was calculated to achieve a power of 0.8 for detecting a medium effect size (Cohen's $d = 0.5$) at a significance level of 0.05. The sample size was also evaluated to ensure sufficient power for the planned Wilcoxon signed rank test and Kruskal Wallis test using the same effect size and significance level. Statistical analyses, including Mann Whitney U test, Wilcoxon signed rank test, and Kruskal Wallis test, were conducted using SPSS Version 20.0.

Selection and Preparation of Samples

The study utilized 60 recently extracted single-rooted front teeth. Exclusion criteria included teeth with multiple canals, extensive decay, fractures, cracks, or any form of resorption. Post-extraction, the teeth underwent an 8-hour soak in 3% sodium hypochlorite to eliminate residual organic matter. Subsequently, all specimens were cleaned using ultrasonic scalers. The samples were then rinsed with distilled water and preserved in 10% formalin solution until needed for the study.

The roots were standardized to 12mm in length using continuous water cooling. Canal patency was confirmed with a size 10 K-file. Working length was established by inserting a size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) until visible at the apex, then subtracting 1mm. Root canal shaping was performed up to ProTaper Gold F3 rotary instruments. During preparation, canals were irrigated with 2ml of 3% NaOCl solution. Final irrigation consisted of 5ml of 3% NaOCl followed by 5ml of 17% EDTA.

After drying the canals with paper points, they were filled with an oil-based calcium hydroxide and iodoform paste (Metapex, Meta Biomed, Korea). The paste was applied using manufacturer-supplied disposable tips. The application involved slowly inserting the tip into the canal, gently pressing the syringe plunger, and gradually withdrawing the tip until the paste reached the canal orifice. To ensure the canal was fully filled, all samples were visualized under Radio Visiography (RVG) images. Root canals with voids were discarded from the study. Extra samples, which acted as a buffer, were included in the study to compensate for the loss of samples. Cavit (a temporary filling substance) and a cotton pellet were used to close the access cavities. The samples were stored in a closed sterile container filled with saline and kept in an incubator at 37°C and 100% humidity for 7 days.

Removal of Ca(OH)₂ from the Samples

After a 7-day period, the calcium hydroxide intracanal medicament was systematically removed from all experimental specimens. A size 30 K-file was employed using a circumferential filing technique. From the root canals of all groups, After 7 days, the calcium hydroxide dressing was removed from all samples. A 30-no. K file was used with a circumferential filing motion to remove

calcium hydroxide from the root canals of all groups, Following the manual procedure, an F3 Pro Taper Universal Rotary File was utilized. The samples were randomly divided into four groups (n=15) according to the Ca(OH)₂.

Removal Technique And Irrigating Solution Used

Group A: Samples were irrigated with 5ml of 17% EDTA liquid and activated using sonic irrigation with a blue 30/06 insert mounted on a sonic device (EndoActivator, Dentsply) at 10,000Hz per minute, 3 mm short of apex.

Group B: Samples were irrigated with 5ml Twin Kleen and activated using sonic irrigation as in Group A.

Group C: Samples were irrigated with 5ml of 17% EDTA liquid and activated using PUI with a no. 20 U file (MANI, INC) attached to an ultrasonic unit with Endo Mode power of 2, 3 mm short of apex.

Group D: Samples were irrigated with 5ml Twin Kleen and activated using PUI as in Group C.

Preparation of Samples for Evaluation

All samples had two longitudinal grooves created on their buccal and lingual surfaces using a diamond disc under water coolant following the final irrigation. The roots were split longitudinally into two halves from the created groove using a chisel. Both halves of each sample were viewed under a stereomicroscope (Luxio 4Z Steriozoom microscope) at 20X magnification, and images were captured with a digital camera. In present study, a stereomicroscope was chosen to evaluate residual calcium hydroxide on root canal walls instead of a scanning electron microscope (SEM). Although SEM provides higher magnification and resolution, its extensive sample preparation can introduce artifacts and alter structures, compromising accuracy. Stereomicroscopes enable non-destructive examination in normal conditions, preserving sample integrity for further analysis. Hence, the stereomicroscopic analysis was selected to ensure accurate and reliable results. Evaluation was done for the coronal, middle, and apical thirds by a single examiner.

Evaluation of Specimen Residues

A single endodontist, blinded to the experimental groups, conducted the assessment of all specimen images. The evaluator underwent calibration using the scoring system prior to the analysis. The quantification of residual material adhering to the canal walls was performed using a modified version of the scoring system developed by Kuga *et al.* (Brazilian Dental Journal, 2010; 21: 310-4). The scoring criteria were as follows:

- Score 0: No discernible residues.
- Score 1: Minimal residual coverage ($\leq 20\%$ of surface area).
- Score 2: Moderate residual coverage ($>20\%$ to $\leq 60\%$ of surface area).
- Score 3: Extensive residual coverage ($>60\%$ of surface area).

The evaluation was conducted on three distinct regions of each root canal: coronal, middle, and apical. These regions were demarcated by equally dividing the standardized 12 mm root length into three 4 mm segments. Both hemisected portions of each specimen were subjected to evaluation, with the higher score being recorded in cases of discrepancy. This methodology ensured a comprehensive assessment of residual material throughout the entire root canal system.

RESULT

Based on the data, the following observations can be made

1. For both Liquid EDTA and Twin kleen solutions, the mean scores for ultrasonic irrigation (liquid EDTA - 1.07,1.13,1.47; Twin kleen - 1.13, 1.20,1.53) were slightly lower than those for sonic irrigation (Liquid EDTA - 1.27,1.33,1.40; Twin Kleen - 1.33, 1.40, 1.47) at the coronal and middle thirds, suggesting that ultrasonic irrigation may be marginally more effective in these regions.

2. However, at the apical third, the mean scores for ultrasonic irrigation (Liquid EDTA - 1.47; Twin Kleen - 1.53) were slightly higher than those for sonic irrigation (Liquid EDTA - 1.40; Twin Kleen 1.47) for both solutions, indicating that sonic irrigation might be slightly more effective in this region.

3. The P values for all comparisons between ultrasonic and sonic irrigation at each site (coronal, middle, and apical) and for both solutions were greater than 0.05, indicating that there was no statistically significant difference between the two irrigation methods.

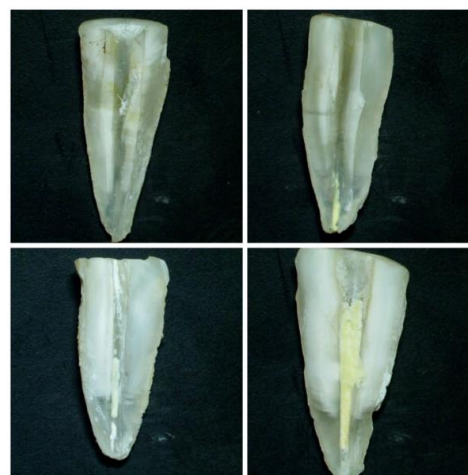


Figure 1: Stereomicroscopic images of the sample.

Table 1: Site wise mean residual Ca(OH)₂.

Irrigation method	Site	Group	Residual Ca(OH) ₂			p Value
			Number	Mean	SD	
Passive Ultrasonic irrigation	Coronal	Liquid EDTA	15	1.07	0.70	>0.05
		Twin Kleen	15	1.13	0.64	
	Middle	Liquid EDTA	15	1.13	0.49	
		Twin Kleen	15	1.20	0.77	
	Apical	Liquid EDTA	15	1.47	0.64	
		Twin Kleen	15	1.53	0.83	
Sonic Irrigation	Coronal	Liquid EDTA	15	1.27	0.59	>0.05
		Twin Kleen	15	1.33	0.49	
	Middle	Liquid EDTA	15	1.33	0.49	
		Twin Kleen	15	1.40	0.64	
	Apical	Liquid EDTA	15	1.40	0.74	
		Twin kleen	15	1.47	0.64	

DISCUSSION

The complete elimination of Ca(OH)₂ intracanal medicament prior to definitive obturation is crucial for maximizing the interface between root canal dentin and obturation materials.^[8] Multiple investigations have demonstrated that residual Ca(OH)₂ on canal walls can impede sealer penetration into dentinal tubules, diminish the bond strength with resin-based sealers, and compromise the sealing efficacy of silicone-based sealers.^[10,11] The complex anatomical variations in root canal morphology present significant challenges in achieving complete removal of the medicament.^[12]

This in vitro study utilized extracted single-rooted human teeth to evaluate the efficacy of two irrigating solutions: 17% liquid EDTA and Twin Kleen solution, in conjunction with PUI and sonic activation techniques. The study specifically focused on the removal of a calcium hydroxide-iodoform paste (Metapex, Meta Biomed, Korea) from the root canal system. The experimental design aimed to assess and compare the effectiveness of these irrigation protocols in overcoming the challenges posed by residual intracanal medicaments, with implications for optimizing root canal treatment outcomes. A stereomicroscope at ×20 magnification was used to evaluate the residual Ca(OH)₂ in coronal middle and apical third. The results showed that samples irrigated with 17 % liquid EDTA with PUI showed lesser remnant of Ca(OH)₂ in coronal, middle and apical third compared to samples in the other group.

EDTA based chelating solutions are routinely used in endodontics owing to their ability to react with calcium ions of dentine and forming soluble calcium chelates.^[13] EDTA functions as a chelating agent, sequestering calcium ions from the hydroxyapatite structure of dentin.^[14] Prolonged application of EDTA can result in excessive demineralization, leading to unintended erosion of both peritubular and inter-tubular dentin.^[9] 1-hydroxyethylidene 1,1-bisphosphonate (HEDP) is a newer chelating agent with good biocompatibility and availability with sodium hypochlorite. Twin kleen solution (Maarc Dental Innovations endo, India) which contains 9% HEDP is a weaker chelating agent when

compared with EDTA.^[15] Although, the results of the current study indicates that there is no statistically significant difference in the ability of the above two irrigating solutions in removing Ca(OH)₂ from the root canals.

Due to shortcoming of the conventional needle irrigation, many newer irrigation activation systems have been proposed. One such system is Passive Ultrasonic Irrigation (PUI) which employs non cutting file to activate the irrigating solution at ultrasonic frequency.^[17] Numerous studies have shown that PUI has improved efficacy to remove microorganisms and smear layer from root canals when compared to conventional needle irrigation^[18], it is observed that disinfecting efficacy of PUI seems to decrease in apical third of the root canals.^[18] However, ultrasonic irrigation has several disadvantages. For instance, when the oscillating tip contacts the root canal wall, it reduces energy and restricts file movement, with file-to-wall contact occurring about 20% of the time. The utilization of ultrasonic devices in endodontics, while effective, presents certain limitations. The metal alloy composition of ultrasonic files can lead to inadvertent dentin removal upon contact with canal walls, potentially altering canal morphology.^[17] In contrast, sonic devices offer several advantages: their oscillating tips, composed of polymer-based materials, maintain continuous movement upon wall contact and do not deform the canal structure, rendering them suitable for use in curved root canals.^[17] This inherent limitation of passive ultrasonic irrigation (PUI) is reflected in the study results, evidenced by increased Ca(OH)₂ residues in the apical third compared to the coronal and middle thirds.

The oscillating tips of sonic devices are fabricated from polymer-based materials, which allows for sustained oscillation even upon contact with canal walls. This property, coupled with their non-deforming nature, makes sonic devices particularly suitable for use in root canals with curvatures.^[17] The inherent limitations of passive ultrasonic irrigation (PUI) become apparent in the current study's findings. Analysis of the results reveals a higher concentration of Ca(OH)₂ residues in

the apical third of the root canal compared to the middle and coronal thirds.

Lambrianidis *et al.* reported that the calcium hydroxide concentration does not significantly influence its removal efficiency from root canal walls.^[19] However, numerous investigations have indicated that the vehicle used in Ca(OH)₂ formulations affects its retrievability, with oil-based vehicles presenting greater removal challenges.

While no single technique has been definitively established for complete Ca(OH)₂ removal from the root canal system, the present study demonstrates that 17% liquid ethylenediaminetetraacetic acid (EDTA) and Twin Kleen solution, when used in conjunction with PUI, are effective methods for medicament removal.

CONCLUSION

In conclusion, this study suggests that both sonic and ultrasonic irrigation methods are equally effective when used with either liquid EDTA or Twin kleen solutions across different canal sites. As Liquid EDTA has detrimental effects on the physical properties of the tooth the result of this study concludes that twin kleen combined with various irrigation activation method can be used as an effective alternative. To further elucidate the relative effectiveness of these irrigation protocols, subsequent investigations employing expanded sample populations and additional assessment parameters are warranted.

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