Acta Odontologica Turcica
The official journal of Gazi University Faculty of Dentistry

DOI: http://doi.org/10.17214/gaziaot.1659744

Original research article

The effectiveness of EDDY in removing calcium hydroxide, n-acetylcysteine, and diclofenac sodium medicaments from root canals

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Abstract

Objective: To compare the removability of calcium hydroxide ($Ca(OH)_2$), n-acetylcysteine (NAC), and diclofenac sodium (DCF) from root canals and to assess the effectiveness of the EDDY sonic activation tip in facilitating their removal.

Materials and Methods: Sixty-six upper central incisors were instrumented using T-Endo Must files (#40.04) and divided into six groups (n = 11) on the basis of the root canal medicament ($Ca(OH)_2$, NAC or DCF) and medicament removal protocol (needle or EDDY). The residual medicament was evaluated under ×20 magnification using a stereomicroscope. The Kruskal–Wallis test was used to compare the different medicament groups, whereas the Mann–Whitney U-test was employed to compare the needle irrigation and EDDY groups. The statistical significance level was set at 0.05.

Results: The highest residual medicament was observed in the $Ca(OH)_2$ group ($p < 0.05$), with no significant difference between the NAC and DCF groups in the needle irrigation group ($p > 0.05$). In the EDDY activation group, the DCF group presented the lowest residual medicament ($p < 0.05$), whereas no significant difference was observed between the Ca(OH)2 and NAC groups ($p > 0.05$). Compared with needle irrigation, EDDY activation significantly reduced the residual medicament for all the root canal medicaments ($p < 0.05$).

Conclusion: EDDY activation significantly reduces residual medicament. DCF resulted in less residual medicament remaining in both the needle and EDDY groups.

Keywords: Calcium hydroxide; diclofenac sodium; n-acetylcysteine; root canal medicaments

Citation: Sarı M, Tüfenkçi P, Ayhan AA, Peker Göülal Ö, Çelikten B. The Effectiveness of EDDY in Removing Calcium Hydroxide, N-Acetylcysteine, and Diclofenac Sodium Medicaments from Root Canals Acta Odontol Turc 2026;43(2):99-104

Editor: Bağdagül Helvacıoğlu Kıvanç, Gazi Universitey, Ankara, Türkiye

Conflict of Interest: The authors declare no conflict of interest related to this study.

[Abstract in Turkish is at the end of the manuscript]

Introduction

Calcium hydroxide ($Ca(OH)_2$) is one of the most frequently used root canal medicament (RCM) for achieving effective disinfection in mechanically difficult-to-reach areas of the root canal system because of its antimicrobial properties. $Ca(OH)_2$ dissociates into Ca^{2+} and OH^- ions, creating an alkaline environment; this high pH (12.5) damages the bacterial cell membrane and protein structure[1]. Another benefit of $Ca(OH)_2$ is its ability to neutralize bacterial lipopolysaccharides (LPS), a key virulence factor in the development of apical periodontitis[2]; however, there are concerns about its inability to eliminate LPS completely from the root canal[3]. Additionally, the buffering effect of dentin may prevent high pH levels from being fully reached in the dentinal tubules[4] and bacteria frequently encountered in endodontic infections, such as Enterococcus faecalis are resistant to $Ca(OH)_2$[5]. Therefore, the search for alternative RCMs continues.

Received: March 17, 2025; Accepted: May 30, 2025
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N-Acetylcysteine (NAC) is an amino acid derivative with various therapeutic properties. It exhibits antioxidant activity through its thiol group, which allows it to induce glutathione synthesis and directly neutralize reactive oxygen species[6]. Additionally, NAC helps control inflammation by reducing the expression of the inflammation-inducing nuclear factor kappa B gene and decreasing the levels of proinflammatory cytokines such as interleukin-1ß, interleukin-8, and tumor necrosis factor[7].

Diclofenac sodium (DCF), one of the most effective inhibitors of prostaglandin production via the cyclooxygenase pathway, is frequently used as a nonsteroidal anti-inflammatory drug for the treatment of acute pain and inflammation[8]. The antimicrobial and antibiofilm activity of DCF[9] has led to its potential use as an intracanal medication.

One of the major disadvantages of $Ca(OH)_2$ is the difficulty in removing it from root canals. Residual $Ca(OH)_2$ can negatively impact the physical properties and sealing ability of root canal sealers to dentinal walls. Over time, any remaining $Ca(OH)_2$ in the root canals may dissolve, compromising the seal of the root canal filling and leading to bacterial infiltration[10, 11]. The cleaning of $Ca(OH)_2$ from root canals remains a challenging aspect of endodontic treatment. However, the removal efficacy of promising RCMs such as NAC and DCF has not yet been examined. This study aimed to compare the removability of $Ca(OH)_2$, NAC, and DCF from root canals and to assess the effectiveness of a sonic activation tip, EDDY (VDW, Munich, Germany), in facilitating their removal. The null hypothesis was that no significant differences will be observed among the groups.

Materials and Methods

Prior Sample Size Calculation

Six different artificial tooth groups were created using various root canal medicaments and activation techniques. To test whether there were differences in the residual medicament amounts among the groups, a priori sample size calculation was performed using two-way analysis of variance with G*Power 3.1.9.4 software (Heinrich Heine University, Dusseldorf, Germany). Based on the calculation, a total sample size of 64 was determined, considering an effect size of 0.40 (Cohen's f = 0.40), % α error (α = 0.05), and 80% power (1-β = 0.80). To ensure an equal number of samples in each group (n = 11), a total of 66 teeth were included in the study.

The study design was approved by the Hatay Mustafa Kemal University Non-Interventional Clinical Research Ethics Committee with Protocol Number: 30/10/2024-26.

Sample Selection

Sixty-six single-rooted, single-canal upper central incisors were selected. Teeth presenting an open root apex, internal or external root resorption, fractures, cracks, or previously filled root canals were excluded. Dental calculus and organic debris were removed from the teeth, and the samples were stored in a 0.9% physiological saline solution until the experimental phase. All the crowns were shortened coronally to a standardized root length of 14 mm using a diamond disc under water cooling.

Chemomechanical Preparation

Access cavity preparation was performed using diamond fissure burs. The apical patency was checked using #10 and #15 K-files (Mani, Utsunomiya, Japan). Teeth with root canals larger than size #20 K-file were excluded from the study. A #15 K-file was visualized at the major apical foramen and then withdrawn by 1 mm to determine the working length (WL). Prior to root canal instrumentation, all the root apices were coated with wax. The root canals were shaped using T-Endo Must (Dentac, Istanbul, Turkey) Reciproc files (#40.04) with the crown-down preparation technique at the WL. The files were used with a pecking motion, limited to a maximum amplitude of 3 mm. After every 3 pecking motions, the file was withdrawn from the canal, and cleaned with damp gauze, and root canal irrigation was performed with 2.5% NaOCl. A 30-gauge Max-i-Probe (Dentsply Sirona, York, PA, USA) needle was used for irrigation and was placed 2 mm short of the WL. The total volume of NaOCl used during preparation was 10 ml, which was consistent for all the samples. After the preparation was complete, 5 mL of sterile saline (SS) solution was introduced into each canal. The canals were subsequently rinsed with 6 mL of 17% EDTA, 5 mL of SS, 6 mL of 2.5% NaOCl, and an additional 5 mL of SS. After the root canals were dried with absorbent paper points, the samples were randomly divided into three groups based on the RCMs: $Ca(OH)_2$ (Kalsin, Konya, Turkey), NAC (Aromel, Istanbul, Turkey), or DCF (Fagron, Rotterdam, Netherlands). Randomization was performed using block randomization with a block size of six to ensure equal distribution among the groups. An online software tool (www.randomizer.org) was used to implement the randomization process.

Placement of Root Canal Medicaments

All RCMs were freshly prepared by mixing with sterile saline at a 1:1 ratio. The root canals were filled to the WL with the prepared medicaments using a #30 Lentulo spiral. To confirm the complete placement of the RCMs within the canals, the wax seal was removed and the apices of the roots were examined. Once the RCMs were observed at the apical foramen, the wax was reapplied to proceed with the removal phase. The canal orifices were sealed with sterile cotton pellets, and the access cavity was temporarily sealed with Cavit-G (3M ESPE, Seefeld, Germany). The samples were then stored at 37°C in an environment with 100%

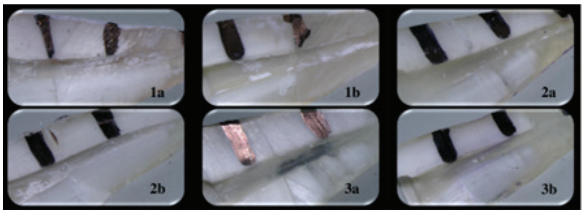

Figure 1: *Representative images of the experimental groups. 1: Calcium hydroxide, 2: N-Acetylcysteine, 3: Diclofenac sodium, a: Needle Irrigation, b: EDDY*

humidity for 1 week.

Removal of Root Canal Medicaments

Each medicament group was divided into two subgroups based on the medicament removal protocol. Randomization will be conducted using block randomization with a block size of two to ensure equal distribution among the subgroups. The RCMs were removed from the root canals by a T-Endo Must #40.04 file and rinsed with 5 mL NaOCl. Following this, 5 mL of SS was used in the root canals. In groups without activation the medicament was removed solely using needle irrigation. Six milliliters of 17% EDTA was placed in the canals for 1 minute. This was followed by a final rinse with 5 mL of SS, 6 mL of 2.5% NaOCl, and an additional 5 mL of SS. In the activation groups, the solutions were activated using an EDDY #25/.04 tip with a sonic device, the Micron TA-200 Air-Scaler (TA-200-S2H, Micron, Japan). The tip was placed 1 mm short of the WL and activated with a 3-4 mm up-down motion. The root canals were rinsed with 2 mL of 17% EDTA solution and activated for 20 s. This cycle was repeated two more times. To neutralize the effect of EDTA, 5 mL of SS was used. After irrigation with 2 mL of 2.5% NaOCl, the solution was activated for 20 s this procedure was repeated three times. A total volume of 6 mL of EDTA and 6 mL of NaOCl were used, the activation time for each solution was 60 s (3x20 s). Finally, 5 mL of SS was used in the root canals.

Evaluation of Residual Medicament

The roots were longitudinally divided into two halves using a precision cutting device. Only one half of each root was evaluated. Images of each half were captured under ×20 magnification using a stereomicroscope (Figure 1). The digital images were imported into the ImageJ software program (ImageJ 1.47V, National Institutes of Health, Bethesda, MD, USA). The boundaries of the root canal lumen were delineated, and the canal surface area was calculated. The areas with residual RCMs were also measured and expressed as a percentage of the total canal surface area[12].

Statistical Analysis

The data obtained from different RCMs and medicament removal protocols were analyzed using SPSS software (version 22, IBM Corp., Armonk, NY, USA). Differences among the RCM groups were assessed using the Kruskal-Wallis test, followed by post-hoc Dunn's test for pairwise comparisons. Comparisons between the needle irrigation and EDDY activation groups were performed using the Mann-Whitney U test. Cohen's kappa test was used to analyze interobserver reliability between two practitioners. A p-value less than 0.05 was considered statistically significant. Significance values were adjusted for multiple comparisons using Dunn's test with Bonferroni correction.

Results

The kappa value for interobserver reliability was 0.91. There were statistically significant differences among the RCMs and medicament removal protocols. Interaction between factors was not tested. With needle irrigation, the highest residual medicament percentage was observed in the $Ca(OH)_2$ group ($p < 0.05$), whereas the NAC and DCF groups presented similar results ($p > 0.05$). Under EDDY irrigation, the $Ca(OH)_2$ and NAC groups presented comparable results ($p > 0.05$); however, the DCF group presented the lowest percentage of residual medicament ($p < 0.05$) (Table 1). Compared with needle irrigation, EDDY activation significantly reduced the residual medicament percentages for all RCMs ($p < 0.05$) (Table 2).

Table 1: *Mean, standard deviation, median and IQR values of the residual medicament percentages.*

	Needle		EDDY	
Root Canal Medicaments	**Mean ± SD**	**Median-IQR**	**Mean ± SD**	**Median-IQR**
$Ca(OH)_2$	38.39 ^{A} ± 12.05	35.87 – 20.24	20.95 ^{A} ± 11.19	18.03 – 21.8
NAC	19.41 ^{B} ± 12.18	20.66 – 18.05	10.61 ^{A} ± 4.22	12.96 – 7.81
DCF	17.63 ^{B} ± 9.32	14.46 – 13.46	4.17 ^{B} ± 3.22	3.71 – 6.75
Pχ^2	0.000		0.000	
Chi-Square	13.598		18.442	
η^2	0.387		0.548	

Superscripts with different uppercase letters indicate statistically significant differences within the same column.
$p < 0.05$ indicates statistical significance. Significance values were adjusted for multiple comparisons using Dunn's test with Bonferroni correction.
χ^2: Kruskal Wallis test.

Table 2: *Mean rank values of the residual medicament percentages.*

	Needle	EDDY	P*	Z	r
Root Canal Medicaments	**Mean Rank**	**Mean Rank**			
$Ca(OH)_2$	15.18[a]	7.82[b]	0.008	-2.659	0.57
NAC	14.36[a]	8.64[b]	0.039	-2.069	0.44
DCF	16.36[a]	6.64[b]	0.000	-3.513	0.74

Superscripts with different lowercase letters indicate statistically significant differences within the same row.
$p < 0.05$ indicates statistical significance.
**:Mann-Whitney U test.*

Discussion

The intricate root canal system may not be accessible for conventional irrigation procedures, and $Ca(OH)_2$ may remain within the root canals[13]. No current strategy is able to completely clean $Ca(OH)_2$ medicament before root canal obturation. To achieve better results, a combination of chemical irrigation and mechanical activation is recommended for its removal[14, 15]. In this study, the effectiveness of conventional irrigation methods and the EDDY activation system in the removal of different RCMs was investigated, and the null hypothesis was rejected.

The smear layer impedes the penetration of RCMs into deeper regions of the root canal system, thereby diminishing their therapeutic efficacy[16]. Therefore, following canal preparation, we used NaOCl-EDTA-NaOCl sequentially to remove the smear layer.

Mobarakeh *et al.*[17] reported no difference in the efficacy of removing pure and injectable forms of $Ca(OH)_2$ from root canals. To follow a similar protocol, all RCMs were mixed with sterile saline, and the prepared medicaments were delivered into the root canals using a Lentulo spiral in this study.

In previous studies, the retention time of RCMs within root canals has frequently been reported to be one week[12, 14]. Keskin *et al.*[18] reported that the intracanal duration of triple antibiotic paste does not influence its removal. However, the impact of retention time on the removal of the RCMs used in this study remains unclear. A period of one week was chosen, considering the application of $Ca(OH)_2$ under clinical conditions[19]. We prepared RCMs by mixing powder with sterile saline at a 1:1 ratio to achieve a paste-like consistency suitable for placement into canals.

The minimum inhibitory concentration (MIC) of NAC against E. faecalis has been reported as 1.56 mg/mL[20] and 100 mg/mL NAC has been shown to completely disrupt mature multispecies endodontic biofilms[21]. The MIC of DCF against E. faecalis has been reported as 50 µg/mL[22], and DCSs at 5% and 2.5% have demonstrated greater antimicrobial effects than DCSs at 1.25%[23]. Further research is needed to investigate the effects of the concentration and type of transporter for NAC and DCF on the removal of these medicaments.

The most commonly used methods for cleaning $Ca(OH)_2$ involve mechanical shaping of the root canal at the WL using the master apical file, combined with extensive irrigation using NaOCl and EDTA[24, 25]. Despite the implementation of this approach, no sample was observed without residual medicament in this study.

Needle irrigation resulted in the highest residual medicament in the $Ca(OH)_2$ group, with no difference between NAC and DCF. Under EDDY irrigation, $Ca(OH)_2$ and NAC were similar, but DCF had the lowest residue. It is not possible to directly compare our study results, as the removal of NAC or DCF as root canal medicaments from root canals has not been previously investigated. However, the hydrophilic nature of NAC, resulting from its ability to form direct bonds with methyl groups, has been reported to facilitate its easy elimination from the body[7]. DCF is not biodegradable[26]; however, it is a water-soluble substance that easily accumulates in water[27], which may account for its low residue levels.

Studies have demonstrated that the antibacterial activity of NAC remains unaffected by the presence of dentin and is effective against both biofilm and planktonic forms of endodontic pathogens, including E. faecalis[20]. Jariyamana *et al.*[28] reported that NAC is effective in addressing inflammation associated with LPS. Furthermore, the combination of $Ca(OH)_2$ and NAC has been found to be more effective against Escherichia coli's LPS than either NAC or $Ca(OH)_2$ alone. The antibacterial efficacy of DCF against E. faecalis is superior to that of $Ca(OH)_2$[22], and the addition of DCF to $Ca(OH)_2$ enhances its antimicrobial effects against E. faecalis biofilms[29]. DCF exhibits bactericidal activity by disrupting bacterial DNA synthesis[30]. Additionally, it has been reported to effectively suppress peptidoglycan biosynthesis, biofilm formation, and the expression of genes and proteins associated with bacterial virulence[9,31,32]. Adl *et al.*[33] evaluated the antibacterial activity against E. faecalis and reported that DCF displayed similar effectiveness to that of $Ca(OH)_2$ and NAC at a depth of 100 µm. However, at a depth of 200 µm, DCF demonstrated superior efficacy to that of $Ca(OH)_2$ and NAC. The authors attributed the high efficacy of DCF to its resistance to dentin. We believe that the affinity of DCF for water may contribute to its enhanced effectiveness in deeper dentinal tubules.

Although NAC and DCF are easier to remove from root canals than $Ca(OH)_2$ is, their effects on the mechanical properties of dentin remain uninvestigated and require further examination.

Compared with needle irrigation, EDDY activation significantly decreased the residual percentage of all RCMs. The superiority of EDDY over needle irrigation in the removal of RCMs from simulated immature tooth models has been demonstrated[34]. Similarly, Güven *et al*.[35] reported that EDDY significantly improved the removal of $Ca(OH)_2$ from artificially created grooves in the apical region of root canals, whereas Silva *et al*.[36] highlights its significant effect in removing $Ca(OH)_2$ from simulated internal resorption cavities. The EDDY (VDW, Munich, Germany) sonic activation device is designed to increase the effectiveness of irrigation solutions. It features a flexible polyamide tip with a size of 25/.04 and operates at a frequency of 6000 Hz. This allows the irrigation solution to mechanically reach canal irregularities that are otherwise difficult to access[37].

The root canal configuration may affect the effectiveness of intracanal medicament removal[38]. Although root canals with a Type 1 Vertucci configuration were selected for this study, the lack of standardization in the canal morphology of the samples represents a significant limitation. Therefore, the removal of the RCMs was calculated proportionally for each sample. Different methods, such as scanning electron microscopy[36], or digital photography under a stereomicroscope[39], as used in this study—have been employed to evaluate RCMs within the root canal system. However, these techniques require longitudinal sectioning of the root to assess the internal surfaces. As a result, they only allow for the evaluation of the superficial layer of $Ca(OH)_2$ and make it difficult to accurately quantify the amount of medicament remaining within the canal system. In contrast, micro-computed tomography (micro-CT) enables quantitative analysis of the three-dimensional volume of $Ca(OH)_2$ remnants. Furthermore, the non-invasive nature of micro-CT allows the same specimens to be evaluated both before and after irrigation procedures, providing 3D superimposed images and more accurate quantitative measurements[40].

Conclusion

Compared with needle irrigation EDDY activation significantly reduces the residual percentage of all RCMs. While the $Ca(OH)_2$ group presented the highest residual medicament percentage with needle irrigation, the DCF group presented the lowest residual medicament percentage with EDDY activation.

Acknowledgments

We would like to express our gratitude to Sabit Coşar for his assistance in the precise sectioning of the samples.

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EDDY'nin kalsiyum hidroksit, n-asetilsistein ve diklofenak sodyum ilaçlarını kök kanallarından uzaklaştırmadaki etkinliği

Özet

Amaç: Bu çalışmanın amacı kalsiyum hidroksit ($Ca(OH)_2$), N-asetilsistein (NAC) ve diklofenak sodyumun (DCF) kök kanallarından uzaklaştırılabilirliğini karşılaştırmak ve bu süreçte EDDY sonik aktivasyon ucunun etkinliğini değerlendirmektir.

Gereç ve Yöntemler: Altmış altı üst santral kesici diş, T-Endo Must eğeleri (#40.04) kullanılarak şekillendirildi ve kök kanal medikamenti ($Ca(OH)_2$, NAC veya DCF) ile medikament uzaklaştırma protokolüne (iğne veya EDDY) göre altı gruba (n = 11) ayrıldı. Rezidüel medikament, stereomikroskop altında ×20 büyütme ile değerlendirildi. Farklı medikament gruplarını karşılaştırmak için Kruskal-Wallis testi, iğne irrigasyonu ve EDDY gruplarını karşılaştırmak için ise Mann-Whitney U testi kullanıldı. İstatistiksel anlamlılık düzeyi 0.05 olarak belirlendi. Bulgular: İğne irrigasyonu grubunda en fazla rezidüel medikament $Ca(OH)_2$ grubunda gözlendi ($p < 0.05$); NAC ve DCF grupları arasında anlamlı bir fark bulunmadı ($p > 0.05$). EDDY aktivasyon grubunda en düşük rezidüel medikament miktarı DCF grubunda gözlendi ($p < 0.05$), ancak $Ca(OH)_2$ ve NAC grupları arasında anlamlı bir fark yoktu ($p > 0.05$). İğne irrigasyonuna kıyasla, EDDY aktivasyonu tüm kök kanal medikamentleri için rezidüel madde miktarını anlamlı derecede azalttı ($p < 0.05$).

Sonuç: EDDY aktivasyonu rezidüel medikament miktarını önemli ölçüde azaltmaktadır. DCF, hem iğne hem de EDDY gruplarında daha az medikament kalıntısına neden olmuştur.

Anahtar Kelimeler: Diklofenak sodyum; kalsiyum hidroksid; kök kanalı ilaçları; n-asetilsistein.