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Evaluation of the effectiveness of different irrigation activation methods in removing calcium hydroxide from root canals

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Purpose: The aim was to evaluate the effectiveness of different irrigation activation methods in removing calcium hydroxide ($Ca(OH)_2$) from two different root canal isthmuses (apical and middle levels) created using three-dimensional (3D) modelling.

Methods: In this in vitro study, 150 acrylic blocks were divided into 10 groups (n=15). Models fabricated and filled with pure injectable $Ca(OH)_2$ paste (UltraCal XS, Ultradent, South Jordan, UT). Five irrigation activation groups were established (conventional needle irrigation (CNI), EDDY activation, passive ultrasonic activation, XP Endo Finisher activation and Er,Cr:YSGG laser activation). The amount of residual $Ca(OH)_2$ in the root canal isthmus was evaluated with a stereomicroscope at ×10 magnification. All procedures were performed by a single operator. The area containing $Ca(OH)_2$ was subtracted from the total area measurement and the cleaning percentage and area calculation were made using the Image J program. The obtained data were recorded in the Microsoft Excel program, and IBM SPSS v29 package program was used for analysis and visualization of the data.

Results: Er,Cr:YSGG laser showed significantly lower amount of $Ca(OH)_2$ remnants compared to other methods ($p<0.001$). In the apical isthmus region, EDDY was the least effective method, while in the middle isthmus region, the CNI method was the least effective.

Conclusion: Er,Cr:YSGG laser-activated irrigation technique demonstrated superior efficacy in the removal of $Ca(OH)_2$ from root canal isthmus regions than other activation methods. 3D modelling enabled the comparison of irrigation activation methods in a standardized manner.

Keywords: Calcium hydroxide removal; EDDY; Er, Cr: YSGG; irrigation activation; isthmus; passive ultrasonic activation; XP finisher.

Introduction

Interim intracanal medication commonly relies on calcium hydroxide ($Ca(OH)_2$) for antimicrobial control. If residues remain, they may hinder sealer bonding and lead to greater apical leakage—factors associated with endodontic treatment failure (1). Numerous studies have explored different irrigants and activation techniques to improve $Ca(OH)_2$ removal (2-4). The most common approach involves shaping the root canal with a master apical file, followed by continuous irrigation activation with sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA). Despite this, there is no consensus on the most effective method for $Ca(OH)_2$ removal (5).

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The isthmus, defined as a slender corridor connecting adjacent canals, is a common and complex morphology in maxillary premolars and mandibular first molars (6). Traditional methods are often inadequate due to intracanal irregularities such as isthmuses. The locations of the isthmuses are important in the effectiveness of the irrigation activation method. Studies have demonstrated that isthmuses in the coronal and middle thirds of the root canal are generally easier to clean, whereas cleaning efficacy decreases in the apical region. This observation has been consistent regardless of the irrigation method employed (7). Additionally, the width and length of the isthmus influence its cleanability; short and wide isthmuses are the easiest to clean, whereas narrow isthmuses are the most challenging. It is emphasized that width is a more critical anatomical parameter compared to length (7). For this reason, both the location and the morphological features of the isthmus directly affect the success of cleaning in root canal treatment (7,8).

Although numerous studies have compared irrigation activation methods for cleaning isthmuses, there is still no definitive consensus on which technique is most effective (7). High-frequency sonic irrigation (EDDY) and laser-activated irrigation (LAI) consistently demonstrate superior debris and biofilm removal in isthmus models, often outperforming passive ultrasonic irrigation (PUI), sonic activation, and conventional needle irrigation (CNI), especially in the coronal and middle thirds of the canal (9). However, all methods show reduced efficacy in the apical third, and none can guarantee complete cleaning in every anatomical scenario (10). Thus, while activation methods enhance isthmus cleaning compared to CNI, the search for an optimal, consensus-based protocol continues. Therefore, this in-vitro study aims to evaluate the effectiveness of five different activations methods including CNI, sonic activation, PUI, LAI, and XP-Endo Finisher (XP, FKG Dentaire, La Chaux-de-Fonds, Switzerland) in removing $Ca(OH)_2$ from root canals with standardized artificial isthmuses at two different levels. The first hypothesis is that laser activation will be more effective than other activation methods. The second hypothesis is that middle isthmuses can be cleaned more effectively than apical isthmuses.

Materials and Methods

In order to investigate the effects of acrylic blocks (located in the middle and apical thirds of the isthmus) and activation methods (CNI, laser activation, ultrasonic activation, and activation with EDDY and XP Finisher), a two-way factorial ANOVA was employed. Based on calculations using G*Power version 3.1.9.4, with a medium to large effect size (f=0.40), an alpha level of 0.05, and a power of 0.95, the minimum required sample size per group was 14 (11,12). Given that there are ten groups, the total sample size required was 140. This study utilized a total of 150 acrylic root canal models (75 middle isthmus, 75 apical isthmus). Models were designed using Chitubox V1.9.5 software and produced with a 3D printer (Sonic Mega 8K) (Fig. 1).

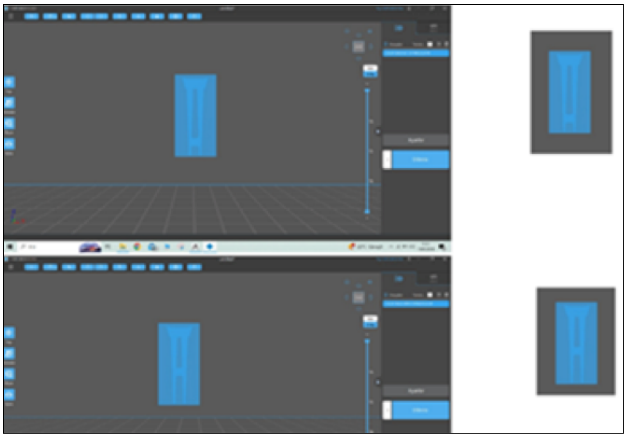

Fig. 1. Visualization of 3D printed root canals isthmuses using Chitubox V1.9.5.

The acrylic block models used in this study were designed to simulate root canal isthmus morphology in two distinct configurations. In the models with a middle-level isthmus, the isthmus area was designed with dimensions of 4 mm in height, 5 mm in length, and 0.4 mm in width. In the models with an apical isthmus, the corresponding dimensions were 2 mm in height, 3 mm in length, and 0.15 mm in width. All models were standardized with a root canal length of 16 mm, a taper of 0.06, and an apical diameter of 0.30 mm (11).

Injectable calcium hydroxide was applied to acrylic models with apical and middle isthmuses (Fig. 2A, 2B). After homogeneity was verified under dental operation microscope, canal orifices were sealed with teflon tape and temporary restoration material (Cavit-W, 3M Espe, Seefeld, Germany) and stored at 37°C in 100% humidity for 1 week. Samples were divided into 5 groups according to the irrigation activation method:

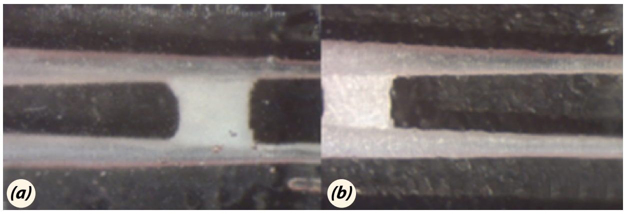

Fig. 2. Representative images of intra-isthmuses material removel. **a)** Apical isthmus filled $CaOH_2$; **b)** Middle isthmus filled $Ca(OH)_2$.

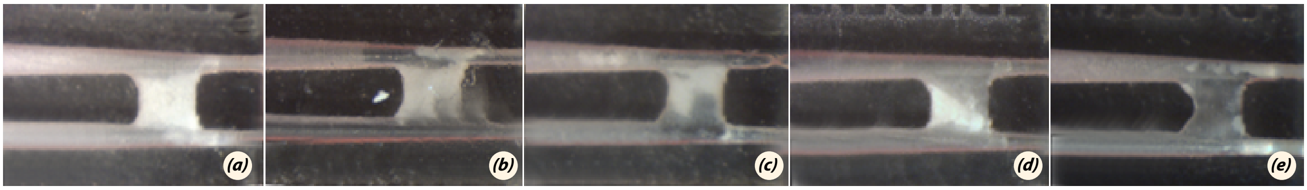

Fig. 3. Apical isthmuses after irrigation activation methods. **a)** CNI activation; **b)** EDDY activation; **c)** PUI activation; **d)** XP Endo Finisher activation; **e)** Laser activation.

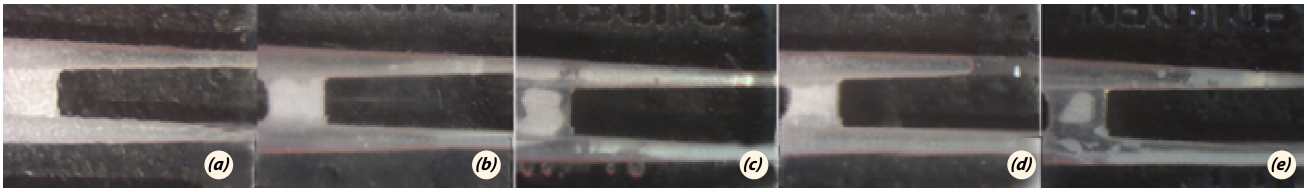

Fig. 4. Middle isthmuses after irrigation activation methods. **a)** CNI activation; **b)** EDDY activation; **c)** PUI activation; **d)** XP Endo Finisher activation; **e)** Laser activation.

Group 1: CNI (n=30) (Fig. 3A; Fig. 4A)

Group 2: Activation with EDDY (n=30) (Fig. 3B; Fig. 4B)

Group 3: Passive ultrasonic activation (n=30) (Fig. 3C; Fig. 4C)

Group 4: Activation with XP Endo Finisher (n=30) (Fig. 3D; Fig. 4D)

Group 5: Activation with Er,Cr:YSGG laser (n=30) (Fig. 3E, Fig. 4E)

A standard irrigation protocol was applied to all groups: Irrigation was performed for 120 seconds with 6 mL 5% NaOCl, 2 mL distilled water, 6 mL 17% EDTA, and finally 10 mL distilled water (6). The relevant activation method in each group was used according to the manufacturer's instructions. All procedures were performed by a single operator. All samples were dried with paper points to working length. Images were taken at x10 magnification using an AxioCamERc5 camera (Zeiss) attached to a stereomicroscope (Stemi 2000-C; Zeiss, Gottingen, Germany) (Fig. 3; Fig. 4). The obtained images were processed using Image J software (Image J, US National Institutes of Health, Bethesda, MD, USA) to mark the total size of the isthmus and Ca(OH)2 residues (Fig. 5). The area of each was calculated in pixels using the computer program. During the assessment of residual Ca(OH)2, the experimental groups were kept blinded from the evaluator.

The formula for calculating the percentage of $Ca(OH)_2$ removal from artificial isthmuses is: Removal Percentage (%) = [(Initial amount of $Ca(OH)_2$- Remaining amount after procedure) / Initial amount of Ca(OH)2] × 100. This formula is widely used in studies evaluating the effectiveness of different irrigation techniques for removing $Ca(OH)_2$ from root canal isthmuses (11).

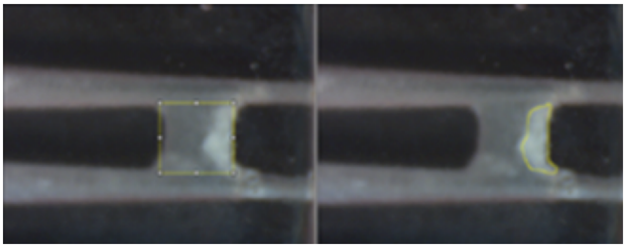

Fig. 5. Image calculation of residual $Ca(OH)_2$ using Image J software.

The measurements were performed blindly by two independent operators. The recorded values were saved in an Excel program. In this study, the percentages of residual $Ca(OH)_2$ in the acrylic models' isthmuses were compared based on the scoring system (9):

Score 1: <20%, Score 2: Between 20% and 60%, Score 3: >60%.

Statistical Analyses

Statistical analyses were performed using IBM SPSS v29 (IBM Corporation, Armonk, New York, USA) package program. Chi-Square Test was used for comparison of categorical variables, Mann-Whitney U Test for two-group comparisons, and Kruskal Wallis Test for comparisons of more than two groups. The level of statistical significance was accepted as $p<0.05$.

Results

The quantitative and qualitative outcomes of the $Ca(OH)_2$ removal process are summarized in Tables 1–3. Table 1 presents the initial pixel values representing the total volume of isthmuses filled with $Ca(OH)_2$, the post-activation residual pixel measurements, and the cor-

Table 1. Initial and post-activation remaining calcium hydroxide amounts according to irrigation activation methods (pixels)

Irrigation activation method	Initial value Median (25th–75th percentile)	Post-activation value Median (25th–75th percentile)	Reduction percentage (%) Median (25th–75th percentile)
Conventional (n=31)	97.104 (94.484–101.370)	52.682 (41.510–59.315)	48.2 (40.6–57.0) A
EDDY (n=30)	100.864 (97.792–107.844)	42.575 (27.936–55.685)	57.0 (45.3–73.3) B
XP Finisher (n=31)	94.392 (90.262–98.698)	34.010 (28.033–37.803)	64.1 (60.4–70.7) C
Ultrasonic (n=30)	93.500 (91.680–97.520)	32.859 (29.611–42.348)	63.9 (57.8–70.8) D
Er,Cr:YSGG (n=30)	94.270 (89.680–97.920)	5.645 (2.104–16.177)	93.7 (82.8–97.9) E

EDDY: High-frequency sonic irrigation. Kruskal–Wallis Test: Initial value: p>0.05; Post-activation value: p<0.001; Reduction percentage: p<0.001. Multiple comparisons (letters): E > A, B, C, D; A < C, D, E.

Table 2. Initial and post-activation remaining calcium hydroxide amounts according to isthmus location (pixels)

Irrigation activation method	Measurement Median (25th–75th percentile)	Apical isthmus Median (25th–75th percentile)	Middle isthmus	p
Conventional (n=31)A	Initial value	94.672 (92.252–100.966)	99.960 (96.432–101.370)	p>0.05
Conventional (n=31)A	Post-activation	50.480 (41.480–57.115)	52.941 (41.772–61.640)	
Conventional (n=31)A	Reduction percentage (%)	46,6 (55,0–43,4)	44,7 (56,6–39,1)	
EDDY (n=30)B	Initial value	100.640 (96.348–107.516)	101.088 (99.926–108.532)	
p<0.001				
EDDY (n=30)B	Post-activation	55.685 (51.321–58.867)	27.936 (19.210–35.735)	
EDDY (n=30)B	Reduction percentage (%)	45,3 (46,7–45,2)	72,3 (80,7–67,0)	
XP Finisher (n=31)C	Initial value	90.240 (88.160–91.512)	98.648 (96.672–100.528)	p>0.05
XP Finisher (n=31)C	Post-activation	33.939 (25.777–35.360)	34.010 (32.262–38.747)	
XP Finisher (n=31)C	Reduction percentage (%)	62,3 (70,7–61,3)	65,5 (66,6–61,4)	
Ultrasonic (n=30)D	Initial value	93.696 (92.580–96.372)	93.304 (90.288–97.712)	
p<0.001				
Ultrasonic (n=30)D	Post-activation	42.348 (32.883–46.859)	29.708 (24.835–32.858)	
Ultrasonic (n=30)D	Reduction percentage (%)	54,8 (64,4–51,3)	68,1 (72,4–66,3)	
Er,Cr:YSGG (n=30)E	Initial value	89.680 (85.616–91.232)	97.812 (95.510–99.762)	p>0.05
Er,Cr:YSGG (n=30)E	Post-activation	5.473 (707–14.971)	10.219 (3.236–18.208)	
Er,Cr:YSGG (n=30)E	Reduction percentage (%)	93,8 (99,1–83,5)	89,4 (96,6–81,7)	

EDDY: High-frequency sonic irrigation. Kruskal–Wallis Test: p<0.001; E > A, B, D. Kruskal–Wallis Test: p<0.001; B, E > A.

responding percentage reductions for each group. There was no significant difference between groups in terms of initial $Ca(OH)_2$ amount (p>0.05). When examining the remaining $Ca(OH)_2$ amount after irrigation activation, the lowest median value was observed in the Er,Cr:YSGG laser group, while the highest value was observed in the CNI group (5,645; 52,682; respectively). The difference between groups was found to be statistically significant (p<0.001) (Table 1).

Table 1 presents the percentage reduction of $Ca(OH)_2$ in isthmuses according to the irrigation activation method used. The highest percentage of reduction was observed with the Er,Cr:YSGG laser method, while the lowest was with the CNI method (93.7%; 48.2%; respectively). The difference in reduction percentages between these methods was statistically significant (Mann Whitney U Test; p<0.001) (Table 1).

According to the results of pairwise comparisons performed with Bonferroni correction, the residual $Ca(OH)_2$ value in the Er,Cr:YSGG method was found to be lower than that of the other methods, and this difference was statistically significant (p<0.001) (Table 1).

Table 2 provides a location-specific comparison of the initial and post-irrigation pixel values of $Ca(OH)_2$ in the apical and middle isthmuses, along with the corresponding reduction percentages. The highest median reduction after irrigation activation was observed in both the apical and middle isthmuses with the Er,Cr:YSGG method (93.8% and 89.4%, respectively), while the lowest median reduction was observed in the apical isthmus with the EDDY

Table 3. Scoring of remaining calcium hydroxide amounts

Irrigation activation method	All models Score 1 (<20%)	All models Score 2 (20–60%)	All models Score 3 (>60%)	Apical isthmus Score 1 (20–60%)	Apical isthmus Score 2 (20–60%)	Apical isthmus Score 3 (>60%)	Middle isthmus Score 1 (<20%)	Middle isthmus Score 2 (20–60%)	Middle isthmus Score 3 (>60%)
Conventional (n=31)	0	24 (77.4%)	7 (22.6%)	0	13 (81.3%)	3 (18.8%)	0	11 (73.3)	4 (26.7)
EDDY (n=30)	4 (13.3%)	25 (83.3%)	1 (3.3%)	0	14 (93.3%)	1 (6.7%)	4 (26.7)	11 (73.3)	0
XP Finisher (n=31)	0	30 (96.8%)	1 (3.2%)	0	14(100.0%)	0	0	16 (94.1)	1
Ultrasonic (n=30)	2 (6.7%)	28 (93.3%)	0	0	15 (100.0%)	0	2 (13.3)	13 (86.7)	0
Er,Cr:YSGG (n=30)	24 (80%)	6 (20%)	0	13 (86.7%)	2 (13.3%)	0	11 (73.3)	4 (26.7)	0

EDDY: High-frequency sonic irrigation. Chi-Square Test: $p<0.001$ (All models). Chi-Square Test: $p<0.001$ (Apical isthmus). Chi-Square Test: $p<0.001$ (Middle isthmus)
Three scores were given according to the percentage of remaining calcium hydroxide in acrylic models:. Score 1: Isthmus with less than 20% remaining calcium hydroxide. Score 2: Isthmus with 20–60% remaining calcium hydroxide. Score 3: Isthmus with more than 60% remaining calcium hydroxide.

method and in the middle isthmus with the CNI method (45.3% and 44.7%, respectively). The Kruskal-Wallis test revealed that this difference was statistically significant for both isthmuses ($p<0.001$) (Table 2).

Table 3 presents the categorical score-based assessment of the residual $Ca(OH)_2$ levels (score 1: <20%, score 2: 20–60%, score 3: >60%). In the samples treated with the Er,Cr:YSGG method, 80% of the specimens exhibited less than 20% residual $Ca(OH)_2$ after the procedure. Conversely, none of the samples subjected to the CNI or XP Endo Finisher methods showed residual $Ca(OH)_2$ levels below 20%. The Chi-square test indicated a statistically significant difference ($p<0.001$) (Table 3).

When evaluating the efficacy of the irrigation activation methods based on isthmus location, it was observed that, in the apical isthmus, residual $Ca(OH)_2$ levels did not fall below 20% in any method except for the Er,Cr:YSGG group, which showed a 73.3% success rate. In the middle isthmus, 26.7% of acrylic models treated with the EDDY method, 13.3% with PUI, and 73.3% with the Er,Cr.YSGG method achieved residual $Ca(OH)_2$ levels below 20%. Both locations demonstrated statistically significant differences among the groups according to the Chi-square test ($p<0.001$) (Table 3).

Discussion

In the present in vitro study, the efficacy of five different irrigation activation techniques in removing $Ca(OH)_2$ from root canal isthmuses was evaluated using standardized 3D printed models. The investigation focused on two distinct anatomical levels—apical and middle isthmuses—representing regions where isthmus prevalence is reported to be highest. Previous studies using micro-computed tomography and stereomicroscopy have shown that isthmuses, particularly in the mesial roots of mandibular and maxillary first molars, most commonly occur within 3–5 mm from the apex, with both complete and incomplete forms frequently observed, especially in teeth with two canals (13,14).

The morphological characteristics of isthmuses, including their width and length, have been shown to significantly influence the effectiveness of irrigant activation. Robberecht et al. (15) demonstrated that short and wide isthmuses are more amenable to debridement, regardless of the activation technique employed. In the present study, the middle and apical isthmus regions were designed based on those morphological principles, with dimensions of 4 mm × 5 mm × 0.4 mm and 2 mm × 3 mm × 0.15 mm, respectively, to replicate clinically relevant anatomical variations.

This study's findings revealed that CNI was the least effective method overall, consistent with the existing literature (16,17). However, a noteworthy exception was observed in the middle isthmus region, where CNI demonstrated greater efficacy than the EDDY system—a result that diverges from previous reports. This discrepancy may stem from limitations inherent in the present methodology, particularly the use of two-dimensional digital image analysis (ImageJ) to assess residual $Ca(OH)_2$, which may have failed to capture the full volumetric extent of remnants in complex isthmus anatomies (18).

Previous studies have demonstrated that activation techniques such as XP Endo Finisher, PUI, and EDDY are significantly more effective than CNI in the removal of $Ca(OH)_2$ from root canal systems. Isthmus morphology and the duration of activation further modulate the effectiveness of these techniques; nonetheless, traditional needle irrigation remains consistently less effective across studies (19,20). EDDY, which utilizes a flexible polyamide tip driven by an air scaler, produces substantial hydrodynamic agitation, thereby enhancing irrigant flow and debris elimination, particularly in anatomically complex regions such as isthmuses (21). The XP Endo Finisher, owing to its adaptive alloy structure, can physically engage canal walls and disrupt $Ca(OH)_2$ residues even in highly irregular spaces. PUI employs ultrasonic energy to induce acoustic streaming and cavitation effects, thus facilitating deeper irrigant penetration and improved debridement efficacy (19). Certain studies suggest that EDDY and the XP Endo Finisher may outperform PUI in isthmus regions due to their superior adaptability to canal irregularities and more robust mechanical agitation (19,20). However, PUI continues to be regarded as a highly effective and widely adopted technique, and the differences in performance among these methods are not consistently found to be statistically significant (21). In the present in vitro study, no statistically significant difference was observed between the efficacy of PUI and XP Endo Finisher in $Ca(OH)_2$ removal, a finding that aligns with existing literature (22,23).

In the present study, irrigation activation using the Er,Cr:YSGG laser was found to be significantly more effective in the removal of $Ca(OH)_2$ from both the apical and middle isthmus regions, with statistically significant results. Notably, no $Ca(OH)_2$ residues were observed in any of the three acrylic models following laser activation. Previous studies employing standardized canal grooves to simulate isthmuses have reported that the Er,Cr:YSGG laser, particularly when used with a radial firing tip, achieves superior $Ca(OH)_2$ removal compared to other techniques. However, residual material was still detected across all experimental groups (24). Moreover, the existing literature consistently indicates that no activation method, including the Er,Cr:YSGG laser, is capable of achieving complete elimination of $Ca(OH)_2$ from all canal irregularities (25). In contrast to these findings, the present study demonstrated complete removal in the three acrylic models. This outcome is likely attributable to the injectable formulation of the $Ca(OH)_2$ employed and the relatively wide isthmus dimensions of the acrylic models used in the experimental setup.

Robberecht et al. (15) reported that laser activation was more effective than both EDDY and CNI methods in the removal of intracanal medicaments. Similarly, Swimberghe et al. (26) compared various activation techniques including EDDY, PUI, and laser, and found that laser activation demonstrated significantly superior efficacy. These findings support the acceptance of the first null hypothesis of the present study.

In this in vitro investigation, a higher amount of residual $Ca(OH)_2$ was observed in the middle isthmus region. Accordingly, the second null hypothesis was rejected. In contrast, Widbiller et al. (11) reported that greater debris accumulation tends to occur in the apical third of the root canal. It has been suggested that short and wide isthmuses are more amenable to effective cleaning than narrower ones, irrespective of the activation technique employed. Among the morphological parameters, isthmus width appears to be a more critical determinant of cleaning efficacy than length (11). The observed difference in this study is likely due to the relatively wider dimensions of the middle isthmus in the experimental models.

The present study has several limitations that should be acknowledged. First, dentinal surface cleanliness could not be assessed due to the methodological constraint of using a two-dimensional imaging system, which limited the evaluation to specific areas and excluded portions of the isthmus walls from analysis. As such, a comprehensive three-dimensional understanding of residual material distribution was not achieved. Second, the dimensional differences between the middle and apical isthmuses may have influenced the outcomes, particularly in terms of $Ca(OH)_2$ retention, and this morphological variability was not standardized across samples. Third, only an injectable form of $Ca(OH)_2$ was used in the experimental design. As a result, the findings cannot be generalized to other formulations such as hard-set or paste-based $Ca(OH)_2$, and the impact of material consistency on removability from isthmuses could not be assessed. These limitations should be considered when interpreting the results and their applicability to clinical scenarios.

Conclusion

Within the limitations of this in vitro study, the Er,Cr:YSGG laser-activated irrigation technique demonstrated superior efficacy in the removal of $Ca(OH)_2$ from root canal isthmus regions compared to other activation methods. The use of standardized 3D printed acrylic blocks with two distinct isthmus configurations enabled a controlled comparison free from anatomical variability. All activation techniques enhanced removal efficiency relative to CNI. These findings underscore the continued challenges associated with effective cleaning of anatomically complex areas and highlight the necessity of employing optimized irrigation protocols to address these difficulties.

Authorship Contributions: Concept: N.S.E.; Design: N.S.E.; Supervision: D.T.; Materials: N.S.E.; Data: N.S.E.; Analysis: N.S.E.; Literature Search: N.S.E.; Writing: N.S.E.; Critical Revision: D.T.

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Informed consent: Not applicable.

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