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Intracanal microbial reduction after root canal preparation with different tapered instruments. An ex-vivo study

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Aim: The objective of this ex vivo study was to assess the dual impact of instruments taper and irrigation activation methods on bacterial reduction in infected root canals.

Materials and methods:42 extracted human molar teeth were inoculated with Enterococcus faecalis for 2 weeks to develop a mature biofilm. The samples were divided into four experimental groups and two control groups. The experimental groups underwent root canal preparation using two different instruments: XP Shaper (taper 0.04) and Reciproc Blue R25 (taper 0.08). Additionally, two different activation methods were tested: mechanical (XP Finisher) and sonic (EDDY). Bacterial reduction was assessed using Colony Forming Unit (CFU) analysis and Confocal Laser Scanning Microscopy (CLSM) to evaluate the efficacy of the treatments.

Results: Results showed significant microbial reduction in all groups after root canal preparation. Root canal preparation with the Reciproc Blue R25 (0.08 taper) achieved a significantly higher bacterial reduction compared to the XP-Endo Shaper (0.04 taper). Additionally, EDDY-activated irrigation resulted in a greater bacterial reduction than the XP Finisher. The interaction between instrument taper and activation method was statistically significant ($p<0.001$), indicating that both factors contribute to the efficacy of microbial reduction in root canals.

Conclusion: Both the instrument taper and the method of irrigant activation significantly affected the reduction of intracanal bacterial load. The use of larger tapers and sonic activation provides microbial reductions that may improve clinical outcomes in endodontic treatments.

Keywords: CLSM, XP Shaper, Reciproc R25, EDDY, XP Finisher, Enterococcus faecalis, Biofilm, Preparation taper, Root canal disinfection, Sonic irrigation, Microbial reduction.

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Introduction

The main objective of endodontic treatment in infected root canals is to reduce the microbial load within the root canal system, thereby creating an environment conducive for healing. The eradication of bacteria is fundamental for the longstanding success of endodontic treatment, as the presence of residual bacteria is the leading cause of treatment failure.[1]

Enterococcus faecalis, a facultative gram-positive coccus, emerges as the predominant species in secondary apical periodontitis, distinguishing itself from the typically anaerobic-dominated bacterial flora observed in primary apical periodontitis. [2] E. faecalis ability to invade and survive in dentinal tubules contributes to refractory infections and post-treatment apical periodontitis.[3,4]

The chemo-mechanical preparation of root canals combines the mechanical processes of instrumentation and irrigation with the chemical properties of irrigating solutions to thoroughly clean, shape, and disinfect the root canal system. While mechanical preparation combined with non-antimicrobial irrigants can decrease intracanal bacterial populations, the inclusion of antimicrobial agents is crucial for optimal disinfection.[5]

The reduction of bacterial counts with large instruments presents a logical and compelling avenue for further investigation. Achieving larger finishing sizes, specifically size 35 taper 4, with minimal procedural complications can be effectively facilitated by utilizing flexible NiTi engine-driven instruments. It is important to note that opting for finishing sizes smaller than size 35 may negatively impact periapical healing outcomes. [6] Nevertheless, it is essential to ponder the possible clinical implications of large instrumentation, for instance compromised restorability, increased fracture susceptibility, and alterations to the canal path. [7]

The mechanical preparation of root canals is widely acknowledged as a critical phase in root canal treatment. The apical width of the preparation is a crucial factor in the effective treatment of infected root canals. Clinical studies have demonstrated that increasing the apical preparation size significantly enhances disinfection efficacy, independent of the irrigant used. [8,9] Despite numerous advancements in file designs over the past decades, numerous studies have consistently shown that there is no technique to eliminate bacteria entirely from the root canals, principally due to the existing anatomic complexties.[10]

In recent years, various single-file systems for root canal instrumentation have been introduced. One system is the Reciproc Blue R25 (VDW, Munich, Germany), which shapes the root canal with a 0.25 mm apical diameter and a taper of 0.08 in the initial apical millimeters.[11-12] Similarly, the XP-Endo Shaper (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) is a single-file instrument that employs rotary motion for canal cleaning and shaping. The XP-Endo Shaper has a minimal taper, with an apical diameter of 0.30 mm and an initial taper of 1%. Utilizing MaxWire alloy technology (FKG Dentaire SA), the XPS transforms into an austenite phase at body temperature, taking on a snake-like shape that can expand up to a 4% taper.[13]

In this context, the implementation of irrigation techniques and delivery systems was initiated with the aim of enhancing the disinfection process of root canals. A notable example of recently developed activation techniques is sonic activation, such as the EDDY system by VDW in Munich, Germany.[14] The EDDY device is a high-power sonic activation tool featuring a smooth, flat polymer tip. It operates at 6 kHz and is driven by a sonic air-powered

handpiece. Furthermore, the XP Endo Finisher (FKG Dentaire, Switzerland) is a recently introduced file designed for final disinfection, aiming to disrupt bacterial biofilms. According to the manufacturer, this instrument offers effective cleaning of the root canal system while safeguarding the integrity of dentin.[15]

Recent studies have employed confocal laser scanning microscopy (CLSM) to visualize bacteria within dentinal tubules, allowing for three-dimensional imaging and differentiation of live and dead bacteria in infected dentin. CLSM has also been proven to be a valuable quantitative assay for distinguishing between live and dead bacteria.[16-19] Currently, consensus is lacking regarding the optimal master apical file size and taper, as well as the most effective irrigation activation technique to achieve bacterial reduction within root canals and promote healing of periapical tissues.

This ex vivo study aims to assess the influence of apical preparation taper as well as the irrigation methods on the reduction of bacteria in infected root canals. The null hypothesis tested is that neither the apical preparation taper nor the irrigant activation method impact intracanal bacterial reduction.

Materials and Methods

Ethical committee approval

This study has ethical clearance from the research ethics committee at the Faculty of Dentistry, the British University in Egypt, with the approval number (FD BUE REC 22-002). The Ethics Committee decision, made on 23/1/2022, stated that this research is exempted from approval of the research ethics committee as it does not involve human subjects.

Sample size calculation

A power analysis was conducted to ensure sufficient power for testing the null hypothesis, which conceives no significant difference between the groups in terms of intracanal bacterial reduction. The analysis used an alpha level of 0.05, a beta of 0.05 (power = 95%), and an effect size (f) of 0.637. The calculated sample size required 42 samples.

Sample Selection

Teeth that were extracted for periodontal reasons were used in this study. Each tooth had a mature apex and was free of cracks, internal or external resorption, and had no history of previous endodontic treatment. All teeth were scrubbed under tap water for 30 minutes to remove adherent soft tissues. They were then disinfected by immersion in a 5.25% NaOCl solution for 1 hour. Preoperative radiographs confirmed the absence of root caries, fractures, multiple canals, calcifications, or significant curvatures. Specimens were selected based on relative dimensions and root morphological similarity. Subsequently, the teeth were stored in 0.5% thymol and used within one month of storage.

Sample Preparation

Coronal access cavities were prepared on all teeth utilizing a Round Carbide Bur #4 and an Endo Z Bur, employing a high-speed handpiece. Canal patency was confirmed using Edge K-Files #8 and #10. A glide path was established to the working length (WL) with #15 and #20 K-files. Edge rotary files #20 and #25 were operated by the Eighteeth E-connect cordless Endo Motor. Longitudinal grooves were made along the long axis of the mesial roots on the buccal and palatal/lingual surfaces without breaching the root canal system. Finally, all samples received a final irrigation sequence using a 30-gauge side-vented needle, consisting of 5 ml of 17% EDTA (Meta Biomed, Cheongju-si, Korea), followed by 5 ml of 2.5% NaOCl and 5 ml of distilled water to remove the

smear layer. Teeth were air-dried and steam autoclaved at 121°C for 30 minutes after coating the root surface with two layers of nail polish (Max factor, cosmetics, and fragrances, London, UK).[20,21]

Samples Grouping

The four experimental groups represented the instrumentation and active irrigation technique used. The two control groups consisted of a positive control group that was infected but not prepared to represent the gross microbial load, and a negative control group that was neither infected nor prepared, and later cultured to verify the sterility of the procedures.

Positive Control Group (n=7): inoculated with E. faecalis, incubated only, and analyzed under similar conditions as the experimental groups.

Negative Control Group (n=7): These samples were immersed in vials filled with sterile BHI broth and replenished with sterile saline every 72 hours.

The study comprised four experimental groups, each employing a distinct instrumentation and irrigation combination:

Group 1 (n=7): instrumented using the XP shaper taper 0.04 followed by active irrigation using the XP finisher.

Group 2 (n=7): instrumented using XP shaper taper 0.04 followed by active irrigation using EDDY.

Group 3 (n=7): instrumented using Reciproc R25 taper 0.08 followed by active irrigation using the XP finisher.

Group 4 (n=7): instrumented using Reciproc R25 taper 0.08 followed by active irrigation using EDDY.

Bacterial biofilm development

A 24-hour culture of bacteria was cultivated in brain heart infusion (BHI; Difco). A standardized suspension of E. faecalis (1 x 10^8 cells/mL) was created. Using sterile 1-mL insulin syringes equipped with a 30-gauge needle, each canal was filled to the level of the orifice with the E. faecalis suspension. This process was repeated every 72 hours using a 24-hour pure culture, which was adjusted to match the No. 1 MacFarland turbidity standard. The teeth were kept in a humid environment at 37°C for 14 days at 100% humidity to facilitate the recolonization of bacteria in the canal walls and dentinal tubules.[21]

Teeth preparation

For all groups, the glide path was re-established using a K-file #15 followed by a #20 K-file. For Groups 1 and 2 mechanical preparation was performed with the XP-Endo Shaper (FKG Dentaire, La Chaux-deFonds, Switzerland) activated in an E-connect Endo Motor at 800 rpm and 1 Ncm until the working length was reached.

For Group 1, irrigation was carried out with 10 ml of 5.25% sodium hypochlorite (NaOCl) using a 30-gauge side-vented needle throughout the root canal instrumentation, between each successive instrument, and as a final flush, followed by irrigant activation with an XP Finisher (FKG Dentaire, La Chaux-deFonds, Switzerland) for approximately one minute with slow and gentle lengthwise movements.

In Group 2, 10 ml of 5.25% NaOCl was used similarly for irrigation, and irrigant activation was performed using the EDDY (VDW GbmH, Munich, Germany) driven by an air handpiece according to the manufacturer's recommendations for 30 seconds, repeated three times with irrigation in between.

For Groups 3 and 4, mechanical preparation in these groups was done using a Reciproc R25 VDW (GbmH, Munich, Germany) with a tip size of 25 and 0.08 taper, activated at 300 rpm and 2 Ncm.

In Group 3, 10 ml of 5.25% NaOCl was used throughout the root canal instrumentation, and irrigant activation was

performed with an XP Finisher for approximately one minute with slow and gentle lengthwise movements. For Group 4, the same irrigation protocol was followed, but irrigant activation was performed using the EDDY driven by an air handpiece according to the manufacturer's recommendations for 30 seconds, repeated three times with irrigation in between.

Evaluation methods

Colony Forming Unit (CFU) Analysis

Bacterial samples were collected following a standard procedure. Sterile saline was used to fill the root canals as the transport medium. A #15 K-file was inserted into the canal up to 1 mm from the working length and moved circumferentially for 10 seconds. Sterile absorbent paper points were then used to absorb the transport fluid, which was transferred into a test tube containing 1.0 ml of saline. The samples were vortexed for 20 seconds, and 10-fold dilutions were prepared using saline. Aliquots of 0.1 ml were spread onto BHI agar plates and incubated at 37°C for 48 hours, after which colony-forming units (CFU) per 1 mL were counted.[21]

Confocal Laser Scanning Microscope (CLSM)

All samples were visually inspected to select three samples from each group with comparable canal dimensions for confocal laser scanning microscopy analysis. Comparable specimens from each group were selected for analysis. Selected specimens were split with a hammer and chisel into two halves. The mesial roots were stained with LIVE/DEAD Bacterial Viability stain and prepared as followed: each specimen was placed in 1 ml of deionized water, then 10 µL of Acridine Orange (100 µg/mL) and 10 µL of Propidium Iodine (100 µg/mL) were added.[17-18] The solution was mixed for 30 seconds with a Neuation i Swix Jr VT Vortex Mixer, left for 15 minutes at room temperature in darkness, and washed three times with 1 ml of deionized water. Specimens were then placed on glass slides and scanned using the Leica DMi8 with a 40X oil immersion lens at excitation/emission wavelengths of 458 nm and 514 nm for Propidium Iodide and Acridine Orange, respectively. Three random locations at each root level were scanned, and images were acquired in TIFF format at a resolution of 512 × 512 pixels using Leica Application Suite-Advanced Fluorescence software (LAS AF). Images were processed for background noise reduction and analyzed with Image J software using the Automatic Live/Dead Quantification Macro plugin to quantify viable (green) and nonviable (red) bacteria.[22] Percentages of live and dead cells were calculated at the coronal, middle, and apical levels for selected samples. The percentage of bacterial viability was determined using the formula: [23]

$$Percent = \frac{Live\ bacteria}{Dead\ bacteria + Gree\ \ bacteria} x\ 100$$

Data collection, management, and analysis

Numerical data were expressed as means and standard deviation (SD) values. The normality of data distribution was assessed using the Shapiro-Wilk test and confirmed a normal distribution. A two-way ANOVA was employed for the analysis, with simple effects comparisons carried out using the ANOVA error term. P-values were adjusted via the False Discovery Rate (FDR) method. The significance level was established at p. Statistical analyses were conducted using R software, version 4.4.1 for Windows.[24]

Results

CFU analysis

The percentage reduction in bacterial counts for different tapers and activation methods is presented in Table (1). Statistical analysis of the results showed significant bacterial

reduction after root canal preparation with the 0.08 taper instruments and after EDDY activation ($p<0.001$).

Table 1: Comparisons and summary statistics of bacterial reduction (%) for different Tapers and activation methods.

Taper / Activation	Bacterial reduction (%) (Mean±SD)		p-value
	Taper 0.04 XP Shaper	Taper 0.08 Reciproc R25	
XP finisher	95.51±1.30	99.56±0.13	**<0.001***
EDDY	99.13±0.21	99.92±0.03	**0.034***
p-value	**<0.001***	**0.320ns**	

*** Significant, ns not significant**

Statistical analysis of the interaction between the variables (Table 2) revealed that instrument taper exhibited a more pronounced effect on reducing bacterial counts compared to the activation method alone, or the combined effect of both ($p<0.001$).

Table 2: Effect of different variables and their interactions on bacterial reduction (%).

Source	Sum of Squares (II)	df	Mean Square	f-value	p-value
Taper	41.14	1	41.14	**94.35**	**<0.001***
Activation method	27.71	1	27.71	**63.55**	**<0.001***
Taper * Activation method	18.63	1	18.63	**42.72**	**<0.001***

df degree of freedom, * significant ($p<0.05$).

CLSM examination

CLSM was used to differentiate between live and dead bacteria in the dentinal tubules following staining. As illustrated in **Figure 1**, representative images display live (L), dead (D), and consolidated (C) bacteria across various groups within the dentinal tubules of treated roots from different segments (coronal, middle, and apical).

The percentage reduction in the count of live bacterial cells at different canal thirds after root canal preparation with different tapers and activation methods is presented in Table (3). Statistical analysis of the results showed that significant reduction was achieved at the coronal third after using 0.08 taper instruments and EDDY activation ($p<0.001$).

Table 3: Comparisons and summary statistics of reduction (%) in the count of live bacterial cells at different canal thirds after root canal preparation with different tapers and activation methods

Root section	Taper / Activation	Bacterial cells' reduction (%) (Mean±SD)		p-value
		Taper 0.4 XP Shaper	Taper 0.8 Reciproc R25	
Coronal	XP finisher	40.86±0.96	68.49±1.05	**<0.001***
	EDDY	45.49±0.44	85.84±0.98	**<0.001***
	p-value	**<0.001***	**<0.001***	
Middle	XP finisher	39.51±0.86	70.32±1.05	**<0.001***
	EDDY	55.59±0.64	76.69±0.53	**<0.001***
	p-value	**<0.001***	**<0.001***	
Apical	XP finisher	37.31±1.41	58.15±0.33	**<0.001***
	EDDY	54.26±0.43	79.32±0.44	**<0.001***
	p-value	**<0.001***	**<0.001***	

*** Significant.**

Statistical analysis revealed a significant interaction between the variables (Table 4) in which instrument taper exhibited the most substantial effect on reducing bacterial counts compared to the other variables alone, or in combination ($p<0.001$).

Table 4: Effect of different variables and their interactions on bacterial cells' reduction (%).

Source	Sum of Squares (II)	df	Mean Square	f-value	p-value
Taper	6870.67	1	6870.67	**4199.35**	**<0.001***
Activation method	1703.73	1	1703.73	**1041.32**	**<0.001***
Root section	77.10	1	66.47	**187.09**	**<0.001***
Taper* Activation method	13.08	1	13.08	**8.00**	**0.022***
Taper* root section	195.49	1	168.52	**474.34**	**<0.001***
Activation method* section	126.66	1	109.19	**307.35**	**<0.001***
Taper* Activation method* section	192.31	1	165.78	**466.64**	**<0.001***

df degree of freedom, * significant ($p<0.05$).

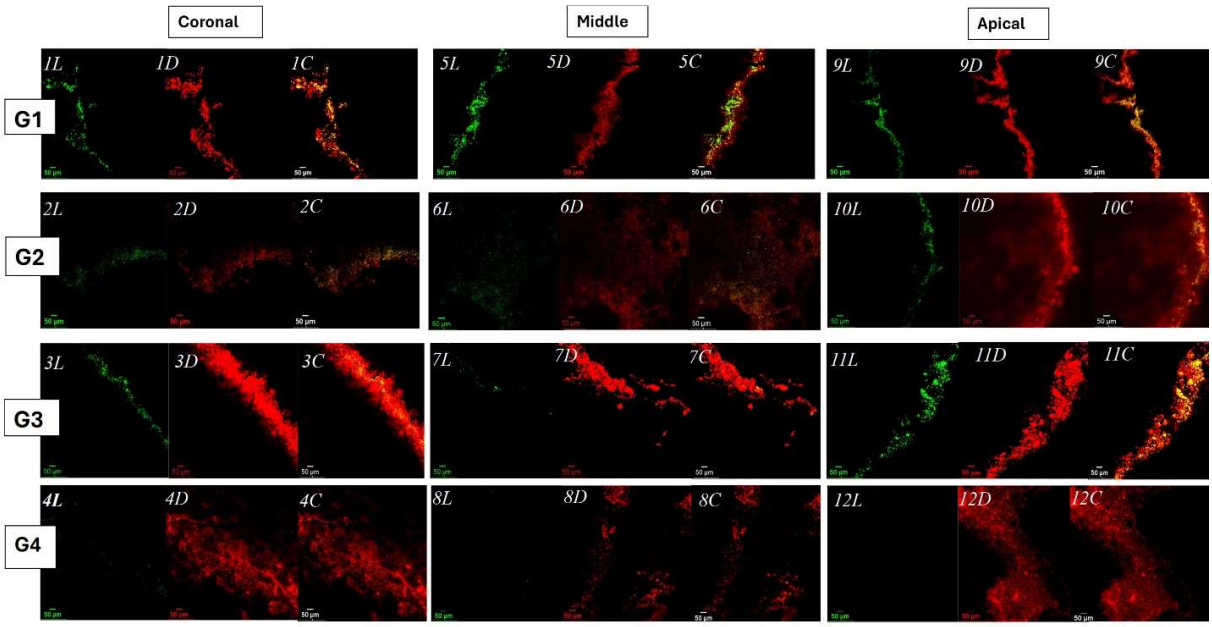

Figure 1: At the coronal third level: 1L: G1 (L), 1D: G1 (D), 1C: G1 (C), 2L: G2 (L), 2D: G2 (D), 2C: G2 (C), 3L: G3 (L), 3D: G3 (D), 3C: G3 (C), 4L: G4 (L), 4D: G4 (D), 4C: G4 (C).

At the middle third level: 5L: G1 (L), 5D: G1 (D), 5C: G1 (C), 6L: G2 (L), 6D: G2 (D), 6C: G2 (C), 7L: G3 (L), 7D: G3 (D), 7C: G3 (C), 8L: G4 (L), 8D: G4 (D), 8C: G4 (C).

At the apical third level: 9L: G1 (L), 9D: G1 (D), 9C: G1 (C), 10L: G2 (L), 10D: G2 (D), 10C: G2 (C), 11L: G3 (L), 11D: G3 (D), 11C: G3 (C), 12L: G4 (L), 12D: G4 (D), 12C: G4 (C).

Discussion

The primary goal of endodontic treatment is to eliminate the infection and prevent microbial re-invasion. In this study, we studied the impact of apical preparation taper and irrigation activation method on bacterial reduction in infected root canals, selecting Enterococcus faecalis as the biological marker, due to its resilience and association with endodontic treatment failures. [3-4,25] The methodology was meticulously designed to ensure accurate simulation of clinical conditions and reproducibility.

Mesial canals of mandibular and maxillary molars were chosen for their anatomical complexity, which presents significant challenges in endodontic treatment.[26-27] This complexity is further compounded by the presence of isthmuses in 54–64% of the cases, which are areas that are not typically reached by mechanical instrumentation and therefore depend heavily on the efficacy of irrigation solutions for proper disinfection.[28] The decision to use different tapers (0.04 for XP Shaper and 0.08 for Reciproc R25) and irrigation activation methods (XP Finisher and Eddy) was based on the hypothesis that these variables significantly influence bacterial reduction.

The evaluation of antibacterial efficiency in this study utilized two methods: Colony Forming Unit (CFU) counting and Confocal Laser Scanning Microscopy

(CLSM). As opposed to other molecular techniques, which can detect uncultivable or difficult-to-grow bacteria, CFU is a useful primary investigation method to quickly quantify cultivable microorganisms. This was augmented by CLSM that offers both direct visualization of live and dead bacteria within the dentinal tubules, as well as a quantitative analysis of bacterial viability. Together, these methods offer a comprehensive assessment, combining numerical and visual insights into the effectiveness of antibacterial treatments.[29]

According to our results, the null hypothesis is rejected. Our findings confirmed that both taper and activation methods impacted bacterial reduction, with significant interaction between them. The Reciproc R25, with a larger 0.08 taper, consistently outperformed the XP Shaper (0.04 taper), demonstrating better bacterial reduction, particularly when paired with sonic activation.

These findings are consistent with studies by Rodrigues et al[8], Paraskevopoulou and Khabbaz [30], and Vossoghi et al[31], all of which emphasized the importance of larger tapers in improving bacterial reduction. This suggests that larger tapers allow for better irrigation dynamics and an active flow of the irrigating solution, which would hinder gas agglomeration apically, hence provide better mechanical cleaning, particularly in the apical third of the root canal, which is often the most challenging area to disinfect.

Nonetheless, the literature also presents contrasting views. For example, studies by Sandini et al [32], Alimadadi et al [33] and Barbosa et al [34] who reported no significant differences in bacterial reduction between different tapers, suggesting that other factors, such as the type of irrigant, its activation, and the specific anatomy of the root canal system, may play more crucial roles than taper size alone. These differences can be attributed to the use of unlike methodologies as Sandini et al [32] who used mechanical preparation without additional activation, while Alimadadi et al [33] used photodynamic therapy (PDT) as an adjunct, and Barbosa et al [34] used 0.03 and 0.05 tapers to prepare mandibular incisors, which have a relatively less complicated anatomy.

Our results also showed a superiority of sonic activation using Eddy over the XP Finisher. This agrees Guven et al [35] who reported the removal of intra canal medications can be more efficiently achieved with EDDY than with XP Finisher, passive ultrasonic irrigation, or needle irrigation, and Abdelkarim et al [36] who reported the lowest value of remaining calcium hydroxide following Eddy activation in comparison to ultrasonic activation and passive needle irrigation. Moreover, Chi et al [37] supported these observations by showing that EDDY significantly outperformed XP Finisher in clearing calcium hydroxide paste from the apical curve of S-shaped root canals. This can be attributed to its ability to generate cavitation and acoustic streaming, which enhance the penetration of the irrigant into the dentinal tubules and can disrupt biofilms more effectively.

In conclusion, our findings emphasize the importance of selecting appropriate taper and activation combinations to maximize bacterial reduction during root canal disinfection. The significant interaction between taper and activation methods suggests that these factors should not be considered in isolation but rather as complementary components of a comprehensive endodontic treatment strategy.

Further research is needed to explore the interplay between taper size, activation methods, irrigant type, in multi-species biofilm models, and in randomized clinical trials in order to develop more refined and effective endodontic treatment protocols across diverse clinical scenarios.

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Conflict of interest
The authors declare that they have no conflicts of interest related to this study. No financial support or other benefits have been received from any commercial entity that could be perceived to influence the outcomes of this research.

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Data Availability
upon request from the corresponding author.

Ethics approval and consent to participate
This study has ethical clearance from the research ethics committee at the Faculty of Dentistry, the British University in Egypt, with the approval number (FD BUE REC 22-002). The Ethics Committee decision, made on 23/1/2022, stated that this research is exempted from approval of the research ethics committee as it does not involve human subjects.

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