ORIGINAL ARTICLE

OPTIMIZING ENDODONTIC DISINFECTION: SCANNING ELECTRON MICROSCOPY ANALYSIS OF APICAL PREPARATION SIZES AND PHOTODYNAMIC THERAPY IN MANDIBULAR FIRST MOLARS

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Background: Eliminating microorganisms from the root canal system is crucial for successful treatment. Rotary nickel-titanium systems have revolutionized endodontics, but their ability to reduce microbial counts within dentinal tubules remains under-researched. Proper apical size selection is crucial for cleaning without compromising radicular dentine. Removal of smear layer is crucial as it obstructs disinfectant penetration. To enhance traditional irrigation, photodynamic therapy utilizing photosensitizers and lasers, presents a novel antimicrobial approach. This research aims to explore the correlation between different final apical preparation sizes combined with photodynamic therapy in smear layer removal, utilizing SEM on extracted molars. **Methods:** Forty-two decontaminated human mandibular first molars were divided into four groups based on different apical size preparations. All groups were prepared with different apical preparation sizes. All groups underwent photo-activated disinfection along with standard irrigation. A diode laser, combined with a photosensitizer, was used for smear layer removal, followed by SEM assessment. SEM images were evaluated for smear layer removal in the apical third using established criteria. Data analysis employed One Way Anova. **Results:** Group-3 proved most effective in smear layer removal, while group 1 was the least effective. Both group 1 and Group-2 showed similar, minimal removal rates. The control group had a significant presence of smear layer. Statistical analysis revealed significant differences in smear layer removal efficacy across the groups, with a p-value<0.05. **Conclusion:** Photodynamic therapy effectively removes smear layer in apical third of the root when sufficiently prepared, serving as a valuable adjunct to conventional regimen.

Keywords: Photodynamic Therapy; Laser Induced Irrigation; Diode Laser; Apical Preparation Size

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INTRODUCTION

Persistence of vast microbial populations and intricate anatomical challenges within the root canal system poses a significant threat to the success of endodontic therapy.[1] Endodontics has been revolutionized with the introduction of rotary nickel-titanium (NiTi) systems.[2] Innovations in instrument design and variations in taper have been introduced to bolster safety and enhance the flare during preparations.[3] However, the effectiveness of these advancements in reducing root canal micro flora remains underexplored.[2–7] Canal preparation, be it manual or rotary creates a lot of shattered mineralized residue known as the smear layer.[8] Clearing the smear layer off the root canal walls encourage the creation of a good apical plug and maintains impervious seal after canal obturation.[7,9] This study underscores the critical role of the final apical size in facilitating the penetration of irrigants and optimizing root canal treatment outcomes.

While mechanical instrumentation aid disinfectants to access infected regions, it is found ineffective in apical third of the root due to apical ramifications leading to orthograde retreatments and apicectomies.[10] No single solution exhibits the ideal properties of an irrigant required for smear layer elimination. The pairing of NaOCl and EDTA is commonly employed as the standard irrigation method in clinical settings.[9] Emerging technologies, such as Laser assisted irrigation and Photoactivated Disinfection (PAD), outperform traditional irrigation in removing the smear layer.[11,12] Studies endorse the application of high power lasers to enhance the agitation of the irrigant within the canal but with potential risks, including tissue damage, ankylosis and resorption.[9,13] Laser technology can reach areas of the canal that are hard to access and

seal the openings of dentinal tubules particularly in the apical third of the root canal, where traditional irrigation methods often fall short in penetration.[9,14] Photoactivated Disinfection (PAD), is an emerging antimicrobial technique that uses non-toxic photosensitizers and a low-power laser to produce reactive oxygen species. This photochemical reaction disrupts biofilms and enhances antimicrobial action. However, the influence of apical preparation sizes on PDT's effectiveness remains unclear, necessitating further investigation.[15] According to Bao P *et.al*, Er:YAG laser-activated irrigation techniques, have demonstrated significant antibiofilm efficacy in apical artificial grooves and dentinal tubules. Keskin G in their study elaborated that PDT could be an effective supplemental treatment during endodontic therapy. Karoglu G also in their study reported that PDT protocols provide promising results in decreasing intra-canal antimicrobial loads. Although effective results of PDT as an adjunctive step during endodontic treatment has been explained, the existing literature provide limited insights into the interplay between final apical preparation sizes and the effectiveness of antimicrobial photodynamic therapy (PDT) in root canal disinfection. This study addresses this gap by investigating whether varying apical preparation sizes impact the efficacy of PDT in eliminating microbial biofilms and improving root canal disinfection outcomes. This study further aims to investigate the correlation between different final apical preparation sizes of root canals and the effectiveness of photodynamic therapy along with the standard protocol, in removing the smear layer at the apical third of mandibular molars using scanning electron microscopy (SEM).

MATERIAL AND METHODS

The study was an experimental study conducted at Hamdard University Dental Hospital following approval from the Institutional Review Board (IRB), with the assigned approval number 1132-03-24. A set of 42 recently extracted humans mandibular first molars were included in the study through convenient sampling, having undergone extraction due to periodontal disease. Written informed consent was obtained from all patients prior to tooth extraction, ensuring their voluntary participation in the study and explaining the purpose and use of their extracted teeth for research purposes. The teeth underwent a decontamination process involving immersion in a 5.25% sodium hypochlorite (NaOCl) solution for one hour which is cited in multiple studies for achieving optimal decontamination without compromising the structural integrity of teeth preserving a balance between efficacy and preservation of the specimen which is neutralized by rinsing with normal saline. Radiographic scrutiny was performed utilizing periapical radiographs. Digital radiographs were chosen for their superior image clarity, ease of manipulation (e.g., magnification and contrast adjustment), and ability to provide detailed visualization of the internal structures of the teeth was conducted to exclude teeth exhibiting cracks, fractures, resorption, calcification, or open apices. The radiographic evaluations were conducted by an experienced endodontist with more than 10 years of clinical expertise in radiographic interpretation. Then chamber opening was executed, establishing a straight-line access. Canal patency was verified using a size 10 K-file. (Dentsply). The working length was set, maintaining 1mm short from the estimated length using radiograph which was verified by apex locator. Teeth failing to meet specific criteria such as the apical foramen not being centrally located, an apical constriction diameter exceeding file size 15, or canals exhibiting more than a 25-degree curvature, were excluded from the study. Schneider's method was used to measure the angle formed between a line drawn along the long axis of the canal and a line drawn from the point of curvature to the apex. A template was used to standardize the angle and depth of cutting. A preoperative radiograph was used to calculate the angle. Root length standardization was achieved through recoronation using a diamond disc (SP 1600 Microtome, Leica, Nu Block, Germany) with water cooling where depth of cutting was determined using cement-enamel junction (CEJ) as anatomical landmark. Canal debridement was carried out with normal saline irrigation, facilitated by a 27-gauge needle.

Following tooth coding, the teeth were prepared using a size 15# k-file (Dentsply Maillefer, Switzerland). For the control group, teeth were chosen randomly without rotary instrumentation. Initial canal preparation was done using a size 15# K-file for glide path establishment. Manual instrumentation was performed using only hand files without rotary instrumentation. Progressively larger files were used to incrementally shape the canal, reducing the working length by 0.5 mm for each successive fill using step back technique. Irrigation was performed between each instrument to ensure debris removal and maintain canal cleanliness. Teeth were randomly assigned to a control group (no rotary instrumentation) or divided into three experimental groups (n=11each). Experimental groups underwent crown-down instrumentation with Pro Taper rotary files and an X-Smart motor controller, following manufacturer guidelines. Coronal pre-flaring was performed using an SX file (0.19). Instrumentation utilized the crown-

down technique with protaper rotary files (Dentsply Tulsa) and an X-Smart motor controller (Dentsply Maillefer, Switzerland), as per the manufacturer's guidelines. Pre-flaring of the coronal portion was conducted using SX (0.19) for all experimental groups.

Group-1:

The root canals received a 10 ml irrigation of 1% NaOCl using 28-gauge needles (Max I-Probe, USA) after each instrumentation for a duration of 1 minute to ensure sufficient time for the irrigant to act on the root canal walls, promoting effective disinfection and debris removal. This duration allows the irrigant to penetrate the intricate anatomy of the canal, dissolve organic tissues, and remove smear layers, enhancing the efficacy of subsequent instrumentation and reducing microbial load. Briefly explaining this provides clarity on how the chosen duration balances efficiency with practicality in a clinical or experimental setting. For the concluding irrigation step, 10 ml of 1% NaOCl combined with photoactivated disinfection was used. The procedure was finalized up to F1 (20/07).

Group-2:

The root canals received a 10 ml irrigation of 1% NaOCl using 28-gauge needles (Max I-Probe, USA) after each instrumentation for a duration of 1 minute. For the concluding irrigation step, 10 ml of 1% NaOCl combined with photoactivated disinfection was used. The procedure was finalized up to F2 (25/08).

Group-3:

The root canals received a 10 ml irrigation of 1% NaOCL using 28-gauge needles (Max I-probe, USA) after each instrumentation for a duration of 1 minute. For the final irrigation step, 10 ml of 1% NaOCL combined with photoactivated disinfection was utilized. The procedure was completed up to F3 (30/09).

Group-4 (Control):

Manual instrumentation (MI) was combined with photo activated disinfection.

Following the final irrigation, the canals were flushed with 5 ml of distilled water. Then a diode laser (Lasotronix) with an exogenous photo disinfectant laser fiber tip was used in a canal filled with a photosensitizer methylene blue (PAD SMART solution). For root canal disinfection, a 635nm wavelength, at 40mw continuous wave mode was employed. Following a two-minute period, laser irradiation was initiated. To maintain precision in measurement, a normal hand file stopper was utilized, with the laser fiber tip measured. The fiber tip, positioned 1 mm short of the working length, was carefully inserted into the canal with the laser off. Once inside, the laser was activated, and the fiber tip was moved outward from the apical to coronal ends in a circular motion at a speed of 2 mm per second. This constituted one cycle, which lasted for 60 seconds. The process was repeated four times, with a 20-second pause between each cycle. Following this, the canal was rinsed with distilled water to remove the photoactivated disinfectant.[15,16]

Following the cleaning and shaping process, paper points from Braseller (Savannah, USA) were used for drying the canals. Cotton pellets were used to seal the canal orifices, ensuring no debris entered during root sectioning. Longitudinal grooves were made on the buccal and lingual sides of the root using a diamond wire saw from MTI Corporation (Richmond, USA). These grooves did not penetrate the canal space. The roots were then bisected along their bucco-lingual axis using a bi-beveled chisel and mallet, resulting in two symmetrical halves without altering the inner surface.

All specimens underwent scanning electron microscopy (SEM) analysis. They were immersed in 2% glutaraldehyde for 24 hours. After a 1-hour osmium tetroxide treatment, samples were desiccated for 24 hours, mounted on metal stubs, and coated with a 20µ layer of gold. SEM photomicrographs were taken using backscatter mode on an XL30 microscope from Philips (Holland) and analyzed under 2500× magnification. Two blinded endodontic specialists assessed the debris and smear layer in the apical third of each root section, scoring them based on the criteria set by Schäfer and Schlingemann mentioned in Table 3.[17]

The data was organized and processed using SPSS software v 23, The one-way ANOVA test was employed to assess differences in score percentages across the apical thirds. Tukey multiple comparison test was used to analyze mean scores of different groups. A significance level of 0.05 was established for the test.

RESULTS

Two observers monitored the removal rate of the smear layer. The smear layer scores are detailed in Table 1, while Figure 1 displays the distribution of these scores. Figure 2 showcases SEM photomicrographs representing each group. According to the results deduced using one-way ANOVA, group-3 (F3+ CI+PAD) treated specimens displayed highest mean scores (3.64±0.50) of SL removal (score-1) from the canal. However, group-1 (F1+ CI+ PAD) exhibited the lowest mean scores (1.27±0.47) *i.e.* (score-3) among all the investigational groups. Intergroup comparison analysis revealed that group-1 (F1+CI+PAD) and group-2 (F2+CI+PAD) presented the lowest and comparable SL removal from the canal wall, i.e., (score1). It was also observed that group-3 demonstrated effective and comparable values of SL elimination from the canal. (p>0.05)

Photomicrographs from the control group (group-4) consistently showed a dense smear layer in the apical thirds. Notably, there were significant differences observed among the groups, with a *p*-value of <0.05.

Table-1: Mean Comparison of Scores using One Way ANOVA

Experimental groups	Mean ± SD	*p*-value ¥
Group-1: F1(20/07) + CI+ PAD	1.27±0.47 [b]	<0.05*
Group-2: F2(25/08) + CI+ PAD	2.73±1.10 [b]	
Group-3: F3 (30/09)+ CI+PAD	3.64±0.50 [a]	
Group-4: MI+ CI+PAD (Control)	2.91±0.94 [a]	

Conventional irrigation (CI), Photo-activated disinfection (PAD)
¥ Showing significant difference among study group (ANOVA)
***** Different superscript small alphabets denote statistically significant difference (Tukey multiple comparison test)

Table-2: The smear layer score frequency for different groups.

Groups	Score 1 Score 2 Score3 Score 4			
	n (%)			
Group-1: (F1+ CI+ PAD)	0	5	5	1
Group-2: (F2+ CI+ PAD)	3	7	1	0
Group-3: (F3+ CI+ PAD)	10	1	0	0
Group-4: (MI+CI)	0	0	2	9

Table-3: Smear layer removal by Schäfer and Schlingemann:

Score 0	No smear layer removed
Score 1	Minimal smear layer removed <25% dentinal tubules open
Score 2	Moderate l smear layer removed >50% dentinal tubules open.
Score 3	Maximum smear layer removed >75% dentinal tubules open

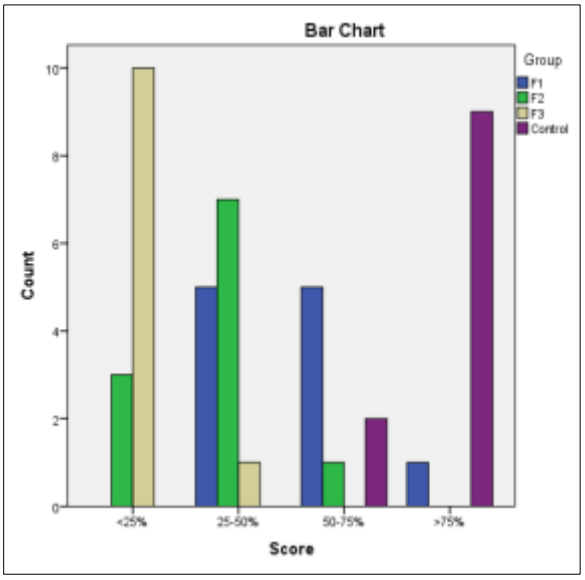

Figure 1: **Distribution of smear layer scores**

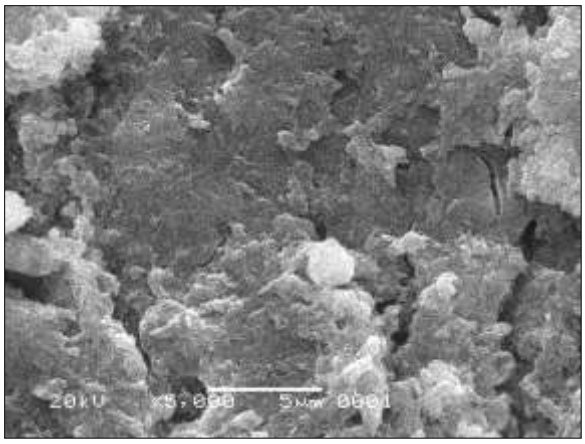

Figure 2: SEM photomicrographs scoring of each group (a) F1

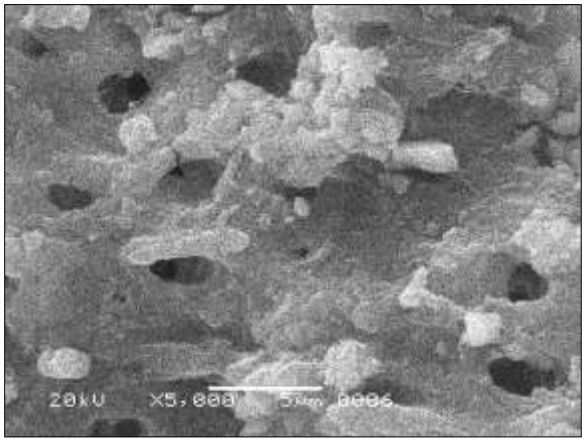

Figure 2: SEM photomicrographs scoring of each group (b) F2

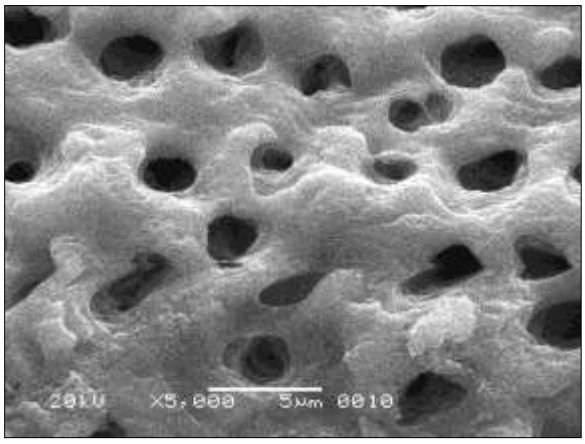

Figure 2: SEM photomicrographs scoring of each group (c) F3

DISCUSSION

In contemporary root canal disinfection, lasers are increasingly utilized alongside traditional chemo-mechanical methods. The versatility of lasers in

dentistry extends beyond root canal therapy to include tasks like caries detection, employing tools such as Diagnodent for diagnosing pulpal blood flow, treating dentinal hypersensitivity, performing pulp capping and pulpotomy procedures, removing smear layers, sterilizing root canals, preparing teeth for treatment, etching enamel, performing gingivectomy procedures, bleaching, disinfecting periodontal pockets, removing calculus, and sensitizing the root canal with lasers.[18] In 1986, Zakariasen and colleagues were the first to demonstrate the efficacy of lasers in endodontics, showing that lasers could effectively kill bacteria on root canal surfaces and penetrate deep into dentin layers.[19] While high power lasers are effective in bacterial elimination by generating heat in a dose-dependent manner they can also inadvertently inflict damage, including dentin charring, root ankylosis, cementum dissolution, root resorption, and periradicular necrosis.[19] Several factors can impact the effectiveness of the disinfection process, including canal preparation, canal morphology, disinfectant solutions, temperature, and laser settings like activation duration, pulse frequency, wavelength, power density, and energy.[20] The selected parameters—canal preparation, morphology, disinfectant solutions, temperature, and laser settings (activation duration, pulse frequency, wavelength, power density, and energy)—are critical for ensuring the effectiveness of the disinfection process. Each factor contributes to the proper delivery and performance of the disinfectant, while laser settings influence energy distribution and safety. These choices enhance the study's validity by controlling variables, simulating clinical conditions, reducing bias, and ensuring reliable, reproducible results. This study highlights the influence of photodynamic therapy with low power lasers when used as an adjunct to conventional irrigation in the apical third of mandibular first molars. Findings of this study suggest the correlation between the final apical preparation size and the removal of the smear layer in the apical third of mandibular first molars. The comparison was made between photodynamic therapy and different apical preparation sizes. The findings indicate that the application of Photodynamic Therapy (PDT) alongside preparing the apical third of the canal to size F3 results in superior removal of the smear layer and better outcomes compared to other approaches.

The findings suggested that low level laser therapy can also enhance disinfection in tooth root canals if prepared sufficiently at the root apex, even though the impact of such combinations hasn't been extensively explored across different parameters. The utilization of high power laser assisted irrigation for complete canal disinfection has sparked discussions due to the significant thermal effects linked to their use. Besides the detrimental effects of elevated temperatures on apical tissues, Matsouka *et al*. have reported the occurrence of cracks in dentin walls.[21] This study, however, examines a fresh approach involving Photodynamic Therapy (PDT) which utilizes low power lasers exhibit encouraging antimicrobial effects in laboratory experiments even in apical third of the root if prepared sufficiently.[22]

Unlike lasers, PDT avoids typical complications by using a light source of lower intensity and a cleaning process based on photochemical reactions. The settings for power, wavelength, and duration of the laser were determined based on prior research.[23] In this study, as in several prior studies, methylene blue (MB) served as the photosensitizer for PDT.[24] Previous researches have dismissed concerns about the cytotoxicity of this material. Methylene blue possess molecular characteristics that allow it to penetrate gram-negative bacteria via porin-protein channels in the outer membrane.[25]

In this research, SEM was employed to assess the outcomes. The detailed magnification capability of SEM facilitated precise examination of dentin tubules, making it a commonly utilized method in smear layer studies.[19] The study's results indicated that PDT effectively removed the smear layer while simultaneously cleansed the canal. Compared to the samples where traditional endodontic therapy was commenced with manual instrumentation, PDT proved to be notably more efficient in samples which were prepared through rotary instrumentation. The ability to eliminate the smear layer more predictably in the apical third of the root through rotary instrumentation enhances PDT's antimicrobial efficacy within the canal space. Earlier studies had advocated for PDT as a supplementary treatment method. Garcez *et al*. employed NaOCl and EDTA prior to PDT and found that this combined approach substantially enhanced the efficacy of PDT. This observation is in line with the conclusions of a systematic review by Cherpa *et al*.[19]

Previous research has shown the efficacy of various irrigants like NaOCl, EDTA, maleic acid, citric acid, and MTAD. In a study by Dalton *et al*., they observed that after irrigation with sterile saline, 72% of treated teeth retained a positive bacterial culture. Chemical irrigation in root canal treatment is more predictable in coronal and middle thirds.[26] Additionally, combinations involving lasers have also been demonstrated to be effective in coronal and middle thirds of the root.[27–29] EDTA and GA solutions were found to be more effective in removing the smear layer in the coronal and middle thirds of the canal as compared to the apical region.[30] As a universal protocol, many studies have employed 2.5% NaOCl

and 17% EDTA.[5,31] NaOCl and EDTA is seen to be routinely effective in removing dense smear layer in coronal and middle sections as confirmed by the previous studies but fails to treat apical third of the canal effectively where resides colonies of resistant bacterial species.[32] The apical third of the canal is often left untreated due to its complex anatomy, which makes it challenging to effectively clean and disinfect using traditional methods. Additionally, this region is often less accessible to instruments and irrigants, making complete removal of debris and microorganisms more difficult to achieve.

However, this study consumed EDTA and NaOCl along with PDT for the apical third of the root with efficient results. Use of PDT as an adjunct to conventional regimen was able to eliminate the smear layer from the canal in this section. The findings of this study align with those of Rathakrishnan *et al*., indicating that NaOCl and EDTA alone were not fully effective in removing the smear layer from the apical third of the root canal thus justifies the need of an adjunct treatment.[33] While the conventional approach effectively eliminates the smear layer from the coronal and middle portions of the root canal, it struggles to address the smear layer in the apical third due to the vapor lock phenomenon. This vapor lock arises from the canal's closed and narrower terminal end, inhibiting the proper circulation of irrigating solutions.[34] Gulabivala *et al*. suggested that the inadequate cleansing of the apical region might be attributed to the insufficient penetration of the needle tip and the formation of a stagnation plane beyond the tip. To address this issue, several studies have proposed different solutions. One effective method to enhance the efficacy of irrigants in the apical third involves the use of ultrasonic agitation.[35] To reach the apices of the root sufficient preparation is required for unimpeded access to the root end. Therefore, apical gauging holds utmost importance during cleaning and shaping to ensure superior disinfection throughout the entire root canal. The selection of the most suitable final apical size is important that thoroughly cleans the canal and at the same time leaves sufficient radicular dentine available without introducing erosion or vertical root fractures. In this study cleaning and shaping was ended upto apical size F1, F2 and F3 in experimental groups where F3 has shown better results when treated with PDT and conventional regimen.

CONCLUSION

This study underscores the significant potential of photodynamic therapy (PDT) as a treatment protocol for enhancing smear layer removal and optimizing root canal disinfection. Laser-treated samples with the widest apical preparation sizes demonstrated the most effective smear layer removal, highlighting the superior efficacy of combining PDT with an appropriately wide apical preparation. In contrast, laser-treated samples with the smallest apical preparation sizes exhibited limited smear layer removal, with intergroup analysis revealing comparable but less effective outcomes for these groups. Notably, samples with wider apical preparations consistently outperformed other groups in smear layer elimination, particularly in the apical third. Control group photomicrographs consistently displayed a dense smear layer, reinforcing the necessity for advanced treatment protocols such as PDT. Further research is essential to refine PDT parameters and establish their efficacy across a variety of clinical conditions, paving the way for improved outcomes in endodontic treatment.

Limitations of the study

The study's findings are limited to in vitro conditions, which may not fully replicate the complex and dynamic environment of the human root canal system in vivo. Also the samples used in the study may be limited, which could affect the generalizability and statistical power of the results. Variations in apical preparation sizes could influence SL removal and require further standardization. The study did not evaluate the long-term impact of smear layer removal on treatment success. Addressing these limitations in future studies will strengthen the evidence base and contribute to a more comprehensive understanding of the potential role and effectiveness of photodynamic therapy in endodontic treatment.

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Conflicts of interest

There are no conflicts of interest.

AUTHORS' CONTRIBUTION

HH: Conceptualization, literature search, write-up. FI: Data collection, literature search, write-up. HS: Write-up. AR: Data analysis, data interpretation. CK: Write-up. MH: Proof reading, write-up. SAA: Proof reading.

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