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Comparison of Smear Layer Removal Rate by 5.25% Sodium Hypochlorite and Ozone Gel with Ultrasonic Activation

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Abstract

Background and objectives: Root canal irrigation removes tissues and debris that instrumentation cannot eliminate. The present study compared the amount of smear layer removal by sodium hypochlorite and ozone gel activated with ultrasound waves as an irrigation solution in root canal treatment.

Materials and methods: A total of 130 single-rooted mandibular teeth that met the study's inclusion criteria were selected. The tooth samples were prepared using the BioRaCe rotary system with the crown-down technique in five groups: group 1, irrigation with 5.25% sodium hypochlorite, followed by irrigation with 17% EDTA; group 2, irrigation with 5.25% sodium hypochlorite with ultrasonic activation, followed by irrigation with 17% EDTA; group 3, irrigation with ozone gel, followed by irrigation with 17% EDTA; group 4, irrigation with ozone gel with ultrasonic activation, followed by irrigation with 17% EDTA; and group 5, irrigation with 17% EDTA. Data were analyzed using the Kruskal-Wallis test and post-hoc comparisons via SPSS v.21, with significance set at $P < 0.05$.

Results: Groups 1 and 2 exhibited a significant increase in smear layer removal compared to the group 5 ($P < 0.001$). Groups 3 and 4 did not show a significant change in smear layer removal compared to the control group ($P > 0.05$). Groupn2had the highest smear layer removal among the studied groups, which was statistically significant.

Conclusion: Ozone gel and ultrasound-activated ozone gel cannot replace sodium hypochlorite as root canal irrigation solutions.

Keywords: Irrigation, Ozone, Smear layer, Ultrasonic activation.

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1. Introduction

The smear layer is a combination of inorganic microcrystals and organic debris particles. This layer consists of dentin and peritubular dentin, pulp residues, odontoblastic processes, remnants of irrigants, and finally, bacteria in infected root canals (1-3). The thickness of this layer ranges from 1 to 10 µm and is compressed within the dentinal tubules (3).

There is considerable debate in the literature regarding whether the smear layer should be removed or preserved during root canal treatment. Some researchers argue that the smear layer serves as a substrate for microorganism proliferation and minimizes the effectiveness of disinfectants, advocating for its removal (3). Conversely, some researchers believe that the smear layer aids in increasing the success rate of root canal treatment (4,5).

Root canal irrigation plays a central role in eliminating the smear layer. This procedure not only removes microorganisms, tissue remnants, and the smear layer from the root canal but also provides access to the most complex canal systems. Irrigation methods are categorized into active and passive types based on their activity. Passive irrigation involves the injection of a solution without additional

mechanical stimulation, while active irrigation uses systems like ultrasonics or specialized suction systems to enhance the penetration of the solution (6-8). Many mechanical methods are available for activation; however, most studies have focused on ultrasonic activation, which has been shown to lead to significant debridement compared to other methods (6).

Additionally, the solution used in root canal irrigation is vital in eliminating bacterial biofilms, inactivating endotoxins, and dissolving tissue remnants. Therefore, various materials have been proposed for root canal irrigation. Sodium hypochlorite is a cost-effective solution with antibacterial properties and strong solubility, commonly used in root canal irrigation. The efficacy of sodium hypochlorite during irrigation depends on its concentration and irrigation frequency (9). However, its substantial toxicity can lead to serious complications for patients (10). Chlorhexidine is used as a standard antibacterial solution for root canal irrigation in dentistry. The main disadvantage of this solution is its inability to dissolve pulp remnants (9). Ethylenediaminetetraacetic acid (EDTA) is another root canal irrigation solution with strong dissolving power, effectively capable of removing the smear layer (11-13); however, its antibacterial properties are inferior to those of sodium hypochlorite and chlorhexidine (14,15).

Recently, ozone has attracted attention in various medical fields due to its characteristics, including antimicrobial effects without developing drug resistance, the ability to stimulate angiogenesis, and high oxidation power (16). Ozone is beneficial for eliminating bacterial and fungal species like *Candida albicans* and can neutralize endotoxins (17,18). Ozone can be used as a solvent for debris within the canal and for disinfecting parts of the root canal that are mechanically inaccessible to instruments. It has been shown that ozone can effectively eliminate root canal-contaminating microorganisms (19). Furthermore, irrigation with 2% chlorhexidine and ozone gas for 24 seconds has been demonstrated to help treat infected root canals (20). Moreover, the gel formulation of ozone offers advantages of prolonged contact time, better handling in the canal system, and less risk of extrusion compared to gaseous ozone, which may make it more practical when combined with activation methods such as ultrasonic agitation

Based on our review, no study has investigated the possibility of smear layer removal using ozone gel and compared it with sodium hypochlorite. Therefore, considering the toxic effects of sodium hypochlorite and the antibacterial properties of ozone, the present study was designed to compare smear layer removal using electron microscopy after applying sodium hypochlorite and ozone gel activated with ultrasound as irrigation solutions in root canal treatment.

2. Materials and methods

In the present study, 130 single-rooted teeth with one root canal, extracted due to orthodontic treatment or periodontal issues, were selected. All the samples had a completely intact (closed) apical foramen and non-calcified root canals, verified through initial radiography. The degree of root curvature was determined from the periapical radiograph using the method described by Schneider et al. for preparation (21).

2.1. Sample Size

The sample size was calculated based on the results of a study by Shahriari et al., which reported the means and standard deviations of smear layer removal in the 5.25% NaOCl group and the control group at 1.93 ± 0.78 and 1.37 ± 0.49%, respectively (21). Considering a type I error of 5% and a power of 80%, a sample size of n = 23 was obtained for each group. The sample size was increased by 10% to improve the study's validity, resulting in 26 samples in each group, totaling 130 samples. Sample Size has been calculated using G*Power 9.1.3 software.

2.2. Inclusion and exclusion Criteria

Teeth with a single root and a single canal, straight roots without signs of calcification in the root canal and internal or external resorption, and a root canal length of 19–22 mm were included. Teeth with developmental anomalies, external resorption, vertical/horizontal root fractures, calcified root canals, endodontically treated roots, deep carious lesions, malformed teeth, and teeth with open apices were excluded.

2.3. Sample preparation

The teeth were immediately cleaned and debrided after extraction, and all soft and hard tissues attaching to them were gently removed using curettes. The teeth were immersed in 5.25% sodium hypochlorite (Chemident, Delhi, India) for 5 minutes to disinfect the surfaces. All the teeth were kept in 0.9% normal saline solution until preparation began (21). After cleaning the teeth, for the convenience of the study and standardizing the root lengths to 15 mm, the crowns were cut perpendicularly to the long axis below the CEJ, ensuring that the root length was 15 mm. Then, #10 and #15 K-files (Dentsply, Maillefer, Ballaigues, Switzerland) were placed into the canal to ensure there was only one root canal in the root and the root canal was open (21,22).

2.4. Root Canal Instrumentation

The apical parts of the roots were placed inside polyvinyl siloxane impression material during instrumentation (23). All the root canals were prepared using a similar mechanochemical preparation method to minimize confounding variables. First, coronal widening was performed using Gates-Glidden drills #2 and #3 (Dentsply, Maillefer, Ballaigues, Switzerland) without lateral pressure and with a passive up-and-down motion. The root canals were then cleaned to the working length with a #20 K-file, randomly divided into five groups of 26, numbered, and mounted in a laboratory putty (Figure 1A) (24).

2.5. Preparation of samples

Dental sample preparation was performed using the BioRaCe rotary system (FKG Dentaire, Swiss Dental Products, Switzerland) with the crown-down technique, following the manufacturer's instructions as follows:
BR0 (0.25, 0.08) for coronal preparation, followed by BR2 (0.25, 0.04), BR1 (0.15, 0.05), BR3 (0.25, 0.06), BR4 (0.35, 0.04), BR5 (0.40, 0.04), BR6 (0.50, 0.04), and finally BR7 (0.60, 0.02) for apical preparation. These instruments were used on an electric motor (TCM Motor 3000; Novage, Konstanz, Germany) with a torque of 1.5 Ncm and a rotation speed of 500 rpm.

2.6. Study groups

This study included five groups.
Group 1: During root canal debridement and between file and bur changes, the root canals were irrigated with 5 mL of physiologic serum (Chemident, Delhi, India). Finally, the internal surface of the root canals was conditioned with 5 mL of 5.25% NaOCl solution (Pakshoma, Tehran, Iran) for 5 minutes. Then, the root canal was irrigated with 17% EDTA (Merk, Darmstadt, Germany) before finally being rinsed with 5 mL of distilled water (Figure 1B).
Group 2: Similar to group 1, the root canals were irrigated with 5.25% NaOCl and then activated with an ultrasonic device X Blue Flexible Tip (Eighteenth, Changzhou, China), kept in the root canal for 1 minute passively. Ultrasonic activation was performed, followed by root canal irrigation with 17% EDTA. Finally, the root canals were rinsed with 5 mL of distilled water (Figure 1C).
Group 3: During root canal debridement and between file and bur changes, the root canals were irrigated with 5 mL of physiologic serum. Finally, the internal surfaces of the root canals were conditioned with 0.02% ozone gel (HealOzone; KaVo, Bibereach, Germany). The instruments were covered with ozone gel during instrumentation and used inside the root canals, followed by irrigation with 17% EDTA. Finally, the canals were rinsed with 5 mL of distilled water (Figure 1D).
Group 4: Similar to group 3, the root canals were irrigated with ozone gel, then activated with an ultrasonic device, and subsequently irrigated with 17% EDTA before finally rinsing with 5 ml of distilled water (Figure 1-E).
Group 5 (control): During file and bur changes, root canal debridement, and shaping, only 17% EDTA was used as the irrigation solution, followed by rinsing the root canals with 5 mL of distilled water (Figure 1F).
In all the groups, irrigation was uniformly divided between each instrument during and after instrumentation, and a #10 K file was placed 1 mm beyond the working length (WL) to maintain apical patency between instruments. The irrigating solutions were delivered through a sterile needle (RC Twents, Prime Dental Products Pvt. Ltd., Thane, India) with a gauge of 30, which penetrated 1–2 mm from the WL. All the root canals were then flushed with 5 mL of distilled water as a final rinse.
In the next step, the orifices of all the root canals in all the samples were sealed with small cotton pellets and dressing paste, and their apical ends were covered with wax and then stored in physiologic serum.

2.7. Root sectioning and SEM evaluation

Using a diamond disc (Bego, Bremen, Germany), two grooves were created on the roots, one on the buccal side and another on the lingual side. These grooves were made close to the canal without penetrating it. The roots were then split into two halves using a spatula with a wedging action at the created grooves (Figure 1G).
In the next phase, one of the halves was randomly selected for dehydration and SEM study. After dehydration, the samples were dried in a desiccator (Bayer Co, Berlin, Germany) and transferred to the SEM laboratory for gold coating in the presence of moisture-absorbing materials (Figure 1H).
In the laboratory, the middle segments of the samples were selected by applying aluminum foil on the coronal and apical areas (aluminum layers were used for electric connection) and then placed in a vacuum device (Baltech, Wetzlar, USA) for gold coating with a thickness of 10 nm (to create a conductive surface).
In the next stage, the samples were examined using a computer and an SEM (DSM 940 A; Vega Tescan, USA). The presence of the smear layer in the coronal, middle, and apical sections was scored based on the method reported by Hulsmann et al. (25). Sections of dentinal tubules inside the root canal were selected at a magnification of 1500×. Photomicrographs were taken and evaluated using the Y method (i.e., measuring the area covered by the smear layer compared to the total cross-sectional area by three independent researchers). In this method, the researchers scored the images based on a predefined scoring system for smear and debris removal. The scores ranged from 1 for the best condition to 8 for the worst Table 1 (26).

2.8. Statistical analysis

Median and Interquartile range were used to describe quantitative variables if the data were not normally distributed according to the Kolmogorov-Smirnov test ($r = 0.178$, $P < 0.001$), and frequencies and percentages were employed for qualitative variables. To compare the amount of removed layers between groups, the non-parametric Kruskal-Wallis test was used, and for pairwise comparisons between groups, the Kruskal-Wallis post-hoc test was applied. SPSS 21 was used for data analysis. The significance level was set at < 0.05 for all the tests.

3. Results

The measured average for smear layer removal in the various groups indicated that chemical agents alone or combined with the PUI technique performed better than the control group.

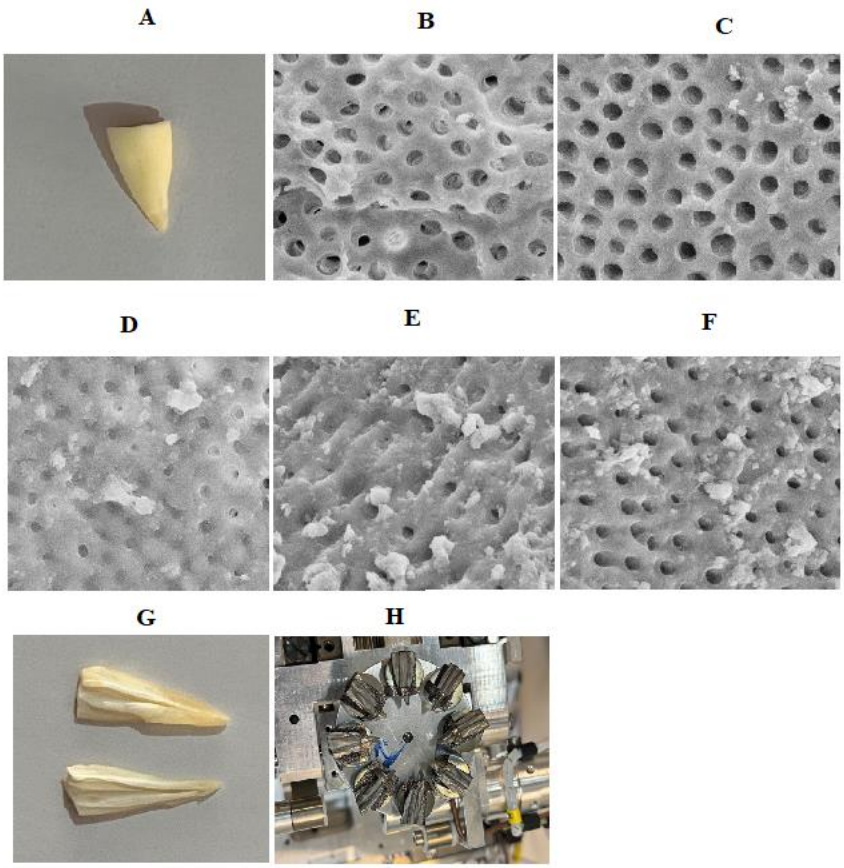

Figure 1. A) A tooth sample shortened to 15 mm from the apex. B) SEM image of the coronal area of a sample from the first group. C) SEM image of the coronal area of a sample from the second group. D) SEM image of the coronal area of a sample from the third group. E) SEM image of the coronal area of a sample from the fourth group. F) SEM image of the coronal area of a sample from the fifth group. G) A sample divided into two parts. H) A gold-coated sample. The findings indicated that the amount of smear layer removal in the middle and apical thirds of group C was significantly greater than in groups A, B, and D.

Table 1. Smear layer and debris removal scoring.

The surface is free of the smear layer and debris.	Score 1
The surface has no smear layer, but limited debris is visible.	Score 2
The surface is clean, but some sporadic smear layer and debris are visible.	Score 3
The surface has been cleaned, but the amount of the smear layer and debris is noticeable.	Score 4
The areas that have been cleaned are larger than the areas that are not free of the smear layer and debris.	Score 5
Almost half of the smear layer and debris have been removed.	Score 6
The majority of the smear layer and debris have remained.	Score 7
The surface is completely covered with the smear layer and debris.	Score 8

Statistical analyses showed that the differences between the groups regarding the amount of smear removal in all studied areas were significant (X2 = 35.91, DF = 4, $P < 0.05001$) (Table 2). The results of the pairwise comparison tests showed that in the coronal and middle areas, the use of group 2resulted in significantly greater smear layer removal compared to control group, ($P < 0.001$), followed bygroup 1, which also resulted in greater smear layer removal in the coronal regions (r = -65.239, $P < 0.001$). However, thegroup3, group4, and group 5 did not differ significantly (r = -9.051, $P = 0.261$). In the apical region, the use of group 2showed significantly greater smear layer removal rate compared to other groups, followed by group 3 and group 1, which led to significantly greater removal of the smear layer. Additionally, the group 3, group 4, and group 5 showed no significant differences (Table 3). Intergroup comparisons were also illustrated in figure 2, 3 and 4 in coronal, middle and apical segments respectively.

Table 2. Comparison of the average smear layer removal in the 5 studied groups.

	Coronal			Middle		Apical	
	N	Median	IQR	Median	IQR	Median	IQR
5.25% NaOCl	26	2	1	3	1.25	3	1
5.25% NaOCl + PUI	26	1	1	2	1	2	1.25
Ozone	26	3	1	4	1	4	1
Ozone + PUI	26	3.5	1	4	1.25	5	2
Control	26	3	1	4	1	4	1
Control	<0.001			<0.001		<0.002	

P-value: Kruskal-Wallis test, NaOCl: Sodium hypochlorite, PUI: Passive ultrasonic irrigation, IQR: Intraquartile range.

Table 2. Comparison of the average smear layer removal in the 5 studied groups.

(I)	(J)	Coronal		Middle		Apical	
		Statistics	P-value	Statistics	P-value	Statistics	P-value
NaOCl	NaOCL + PUI	0.001	1.000	39.808	0.001	25.365	0.012
NaOCl	Ozone	=59.13	<0.001	-63.635	<0.001	-33.327	0.014
NaOCl	Ozone + PUI	-60.231	<0.001	-80.058	<0.001	-56.895	<0.001
NaOCl	Control	-68.712	<0.001	-23.927	0.261	-65.269	<0.001
NaOCL + PUI	Ozone	-59.231	<0.001	-24.577	0.213	-7.492	1.000
NaOCL + PUI	Ozone + PUI	-60.231	<0.001	-40.250	0.001	-31.5	0.027
NaOCL+ PUI	Control	-68.712	<0.001	0.75	1.000	-39.885	0.001
Ozone	Ozone + PUI	-1.096	1.000	16.423	1.000	-23.559	0.264
Ozone	Control	-9.577	1.000	14.442	1.000	-31.942	
Ozone + PUI	Control	8.451	1.000	=15.673	1.000	8.365	1.00

P-value: Kruskal Wallis, Bonferroni test, NaOCl: Sodium hypochlorite, PUI: Passive ultrasonic irrigation.

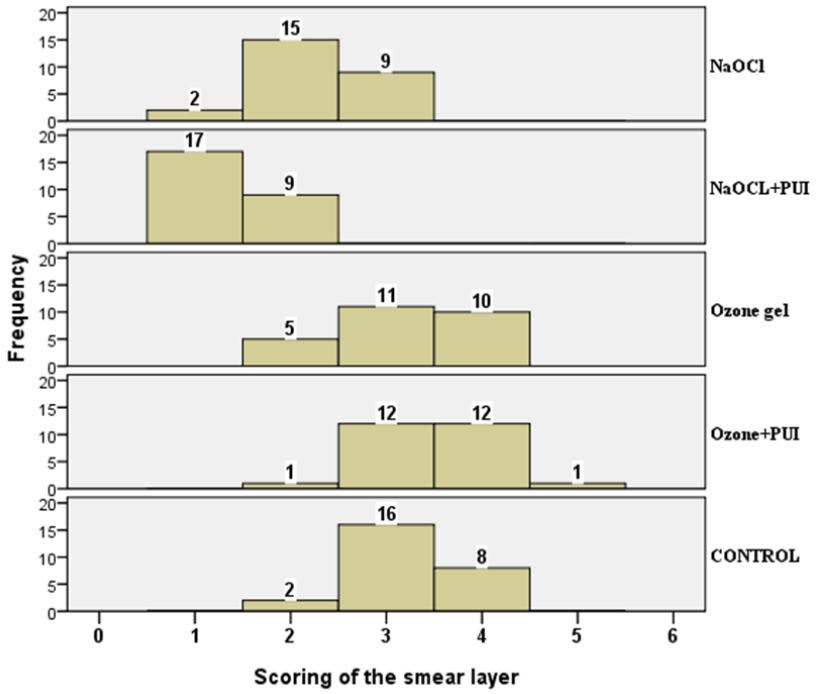

Figure 2. Intergroup comparison of smear layer removal in the coronal segment.

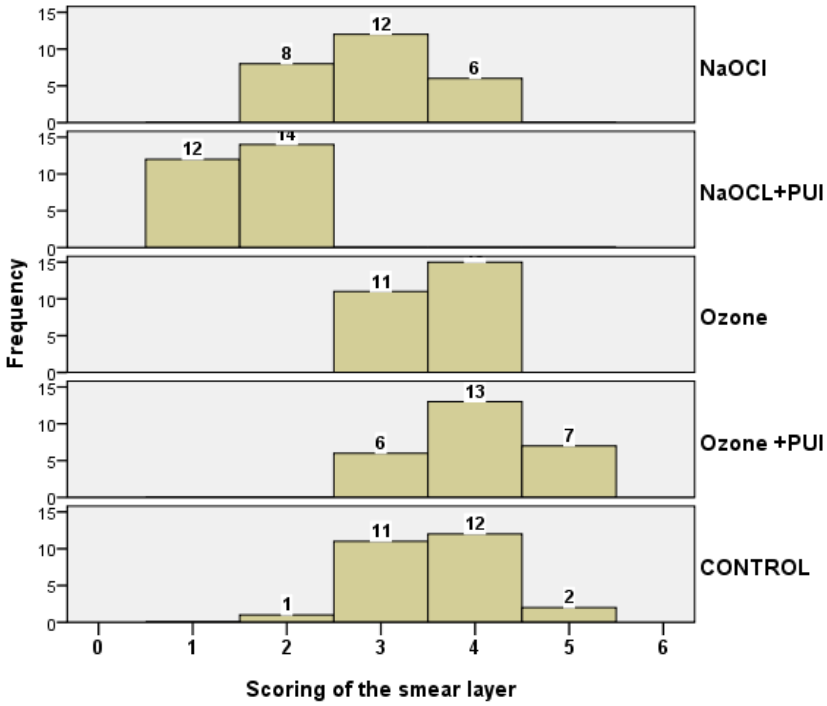

Figure 3. Intergroup comparison of smear layer removal in the middle segment.

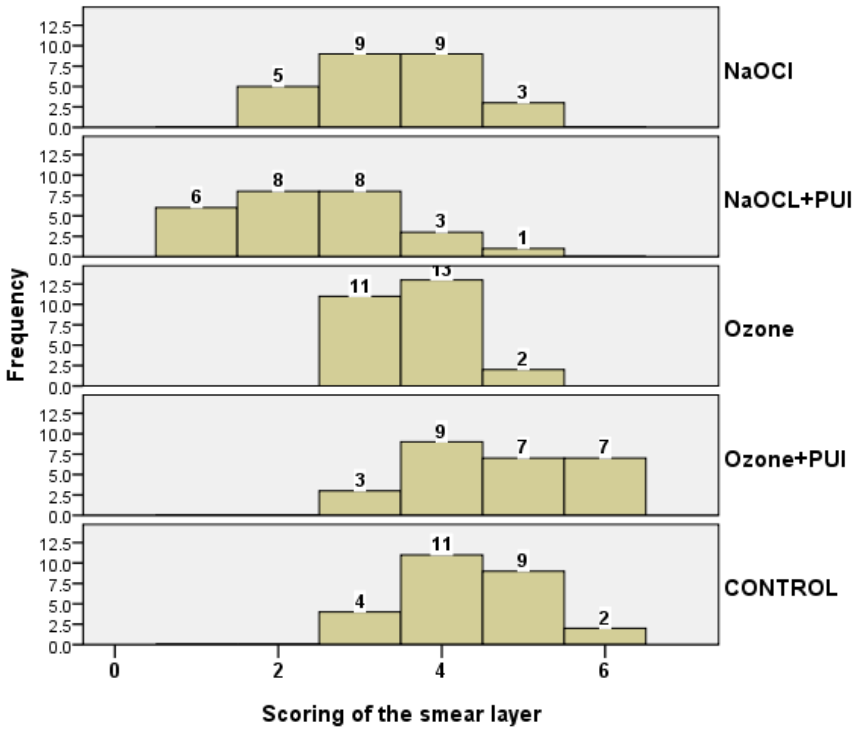

Figure 4. Intergroup comparison of smear layer removal in the apical segment.

4. Discussion

Removing the smear layer from infected root canals is essential for disinfecting the entire root canal. Despite the availability of a wide range of intracanal irrigants, efforts to achieve an ideal irrigant continue. The current study aimed to compare the efficacy of sodium hypochlorite and ultrasonic-activated ozone gel in smear layer removal as a root canal irrigant.

In the present study, of the four study groups, the sodium hypochlorite and ultrasonically activated sodium hypochlorite groups exhibited increased smear layer removal compared to the control group. A possible explanation for this phenomenon could be that the antimicrobial effect of 5.25% NaOCl relies on chemical reactions that lead to the non-specific destruction of microbial cells. Furthermore, ultrasonic activation creates mechanical properties and cavitation bubbles that help disrupt the smear structure and enhance the penetration of sodium hypochlorite. Supporting the findings of the present study, Estrela et al. showed that sodium hypochlorite increases smear layer removal by disrupting the extracellular matrix of the smear and bacterial proteins (27). Additionally, Whitbeck et al. showed that the ultrasonic technique improved the penetration of NaOCl into deeper layers through cavitation (28). Van der Slius et al. and Zehnder et al. reported that ultrasonically activating irrigation solutions could significantly enhance smear layer removal, especially in apical areas with limited mechanical access (29,30). In a study by Macini et al., an ultrasonic-activated endoactivator effectively removed the smear layer in the apical part of the root canal (31). Moreover, Karunakar et al. found that using ultrasonics in conjunction with sodium hypochlorite improved smear layer removal (32). In contrast to the results of the present study, Schmidt et al. showed that using ultrasonics did not increase the efficacy of sodium hypochlorite (33). Additionally, Suman et al. demonstrated that none of the activation systems completely removed the smear layer from the dentinal walls (34).

Based on the findings of the present study, the ozone and ultrasonic-activated ozone gel groups did not show a significant change in smear layer removal compared to the control group. To justify this finding, it can be pointed out that while ozone has high antibacterial properties and is used in dental treatments to control microorganisms, it appears to lack the necessary characteristics to dissolve the organic and inorganic structure of the smear layer. This has also been confirmed by other studies (35); for example, a study by Huth et al. demonstrated that although ozone can inhibit resistant bacteria at high concentrations, it does not have a significant mechanical effect on the smear layer (19). Additionally, a study indicate the potential of ozone to improve antibacterial efficacy without completely removing the smear layer, suggesting that ozone may play an auxiliary role alongside other irrigants, but alone or even when activated, it lacks the efficacy required to remove the smear layer (27). Some studies indicate that ozone significantly reduces bacterial levels; however, it cannot achieve similar results to NaOCl 5.25% when used alone (36-38). Therefore, from this perspective, ozone treatment should not replace

conventional chemomechanical methods using the most commonly used root canal irrigants, such as 5.25% NaOCl. Murugesan et al. showed that it is advisable to use ozone in combination with other root canal irrigants for the synergistic effect of its antibacterial properties and smear layer removal (39). In contrast, Case et al. stated that although combining ozone gas with irrigation solutions may be beneficial for disinfecting root canals in endodontic treatments, the high cost of ozone solutions and gases makes it difficult to recommend for general use (40).

The findings of this study showed that the use of ultrasound did not affect the potential for smear layer removal of the ozone gel, indicating that although ultrasound, as a physical activation method, can enhance the effectiveness of many irrigants, this effect was not observed with the ozone gel. The main reason might be the ozone gel's chemical nature. Ozone primarily has antibacterial and oxidizing properties and has much less efficacy in removing the smear layer compared to solutions like EDTA. Although ultrasound can increase the penetration of the solution into the dentinal tubules by creating cavitation bubbles and improving flow, this mechanical stimulation is effective only when the irrigant used has an intrinsic ability to dissolve or detach the smear layer. Since the ozone gel lacks this ability, the mechanical stimulation caused by ultrasound cannot independently increase its efficacy. This finding is consistent with a study by Murugesan et al. (39).

Despite the limitations of this study, the ozone gel exhibited less potential for smear layer removal compared to sodium hypochlorite, and the ultrasonic technique did not affect its potential for smear layer removal in all root canals. In contrast, sodium hypochlorite showed increased smear layer removal potential with ultrasound. One of the limitations of this study is that it did not consider the role of blood and residues of the irrigant solutions in the potential for smear layer removal. Additionally, smear layer removal might be more challenging in curved root canals. Further clinical studies should be conducted in this area.

5. Limitations

Despite its methodological strengths and standardized experimental design, this *in-vitro* study has several inherent limitations. The use of extracted single-rooted teeth with relatively straight canals may limit the generalizability of the findings to more anatomically complex clinical scenarios, including curved or multi-rooted canals. In addition, *in-vitro* conditions cannot fully replicate the clinical environment, particularly the presence of vital tissues, blood, and mature polymicrobial biofilms, which may influence irrigant behavior and smear layer removal. Although efforts were made to standardize instrumentation and irrigation protocols, natural variability in dentin structure and canal morphology may still have affected the outcomes. Furthermore, smear layer assessment using SEM is semi-quantitative and may be subject to observer-related variability despite calibration procedures. Finally, the study focused on short-term smear layer removal without evaluating long-term antimicrobial efficacy or the interaction of irrigants with organic tissue remnants, which may have clinical relevance.

6. Conclusion

Despite our limitations, our findings suggested that the combination use of 5.25% NaOCl and EDTA was more effective than ozone gel and EDTA in removing the smear layer. Also, ultrasonic activation enhances NaOCl/EDTA removal efficacy. Our findings highlighted that ozone gel cannot be used as an alternative to 5.25 % NaOCl and should be used as an adjuvant therapy.

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Ethics

The study protocol was approved by the ethics committee at Tabriz University of Medical Sciences (IR.TBZMED.VCR.REC.1402.152).

Using artificial intelligence (AI)

No artificial intelligence (AI) tools were used at any stage of this study, including study design, data collection, data analysis, manuscript preparation, or editing.

Author contributions

Hadi Mokhtari: Conceptualization, Methodology, Project administration, Supervision
Amin Salemmilani: Methodology
Shaghayegh Ghadimi: Writing – Review & editing
Mohsen Nabavi: Investigation, Data curation
Touraj Nejatian: Writing – Review & editing
Elham Behrouzpour: Writing – Original draft

Conflict of interest:

The authors declare no conflict of interest.

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