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THE EFFECT OF DIFFERENT IRRIGATION PROTOCOLS ON APICAL MICROLEAKAGE IN ROOT CANAL TREATMENT: AN IN VITRO STUDY

ВЛИЈАНИЕТО НА РАЗЛИЧНИ ПРОТОКОЛИ ЗА ИРИГАЦИЈА ВРЗ АПИКАЛНАТА МИКРОПРОПУСТЛИВОСТ КАЈ ТРЕТМАН НА КОРЕНСКИОТ КАНАЛ: ИН ВИТРО СТУДИЈА

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Abstract

Aim: The aim of this in vitro study was to evaluate the effect of different irrigation protocols on apical microleakage in root canals obturated with the single-cone technique. **Materials and Methods:** Eighty extracted single-rooted human teeth were randomly divided into four groups according to the irrigation protocol. The protocols included sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) with ultrasonic activation, NaOCl with ultrasonic activation, NaOCl with EDTA without activation, and a control group irrigated with saline solution. After irrigation and obturation using the single-cone technique, apical microleakage was evaluated by the methylene blue dye penetration method under light microscopy. **Results:** Ultrasonic activation significantly enhanced the performance of irrigants, producing lower microleakage values compared with non-activated protocols. The NaOCl + EDTA protocol with ultrasonic activation achieved the lowest apical microleakage, whereas the saline control group exhibited the highest values. Statistical analysis confirmed statistically significant differences among the groups, demonstrating the strong influence of irrigation protocols on apical microleakage. **Conclusion:** Activated irrigation, particularly with NaOCl and EDTA, creates cleaner canal walls and provides more favorable conditions for sealer adaptation. Incorporating such protocols into clinical practice can help reduce apical microleakage and support the long-term success of endodontic treatment. **Keywords:** root canal irrigation, ultrasonic activation, apical microleakage, single-cone obturation, methylene blue dye penetration.

Апстракт

Цел: Целта на оваа in vitro студија беше да се оцени влијанието на различни протоколи за иригација врз апикалната микропропустливост кај коренските канали оптурирани со техниката single-cone. **Материјал и методи:** Осумдесет екстрахирани еднокорени хумани заби беа случајно поделени во четири групи според применетиот протокол за иригација: натриум хипохлорит (NaOCl) и етилендиаминтетраоцетна киселина (EDTA) со ултразвучна активација; NaOCl со ултразвучна активација; NaOCl со EDTA без активација; и контролна група иригирана само со физиолошки раствор. По завршената иригација и оптурација со техниката single-cone, апикалната микропропустливост беше евалуирана со методот на пенетрација на метиленско сино и беше анализирана со светлосна микроскопија. **Резултати:** Ултразвучната активација значително ја зголеми ефикасноста на иригансите, овозможувајќи пониски вредности на микропропустливост во споредба со неактивираните протоколи. Протоколот со NaOCl + EDTA со ултразвучна активација постигна најниска апикална микропропустливост, додека контролната група третирана со физиолошки раствор имаше највисоки вредности. Статистичката анализа потврди значителни разлики помеѓу групите, укажувајќи на значајното влијание на протоколите за иригација врз апикалната микропропустливост. **Заклучок:** Активираната иригација, особено со NaOCl и EDTA, обезбедува почисти ѕидови на каналите и создава поповолни услови за адаптација на материјалот за оптурација. Вклучувањето на вакви протоколи во клиничката практика може да помогне во намалување на апикалната микропропустливост и да придонесе за долгорочниот успех на ендодонтскиот третман. **Клучни зборови:** иригација на коренски канал, ултразвучна активација, апикална микропропустливост, single-cone оптурација, пенетрација на метиленско сино.

Introduction

Predictable and long-term success in endodontic treatment depends primarily on two key objectives: the complete elimination of microorganisms from the root canal system and the effective sealing of the canal space to prevent reinfection. Despite thorough mechanical instrumentation, complex anatomical areas such as lateral canals, isthmuses, and apical deltas often remain untouched, allowing residual pulp tissue, debris, and microbial biofilm

to persist[1–5]. Although mechanical shaping is essential for preparing the main canal, it alone cannot ensure complete debridement of these intricate regions[6]. Therefore, a well-designed irrigation protocol that complements mechanical instrumentation is crucial.

One of the main obstacles to achieving an adequate apical seal is the smear layer that forms on the canal walls during preparation. This layer, consisting of both organic and inorganic materials as well as microorganisms, can hinder sealer adhesion to dentinal surfaces and provide a substrate for bacterial proliferation[7]. Effective removal of this layer is thus a critical step in the cleaning and shaping process, as it directly affects the quality of the apical seal and, consequently, the long-term success of the treatment[8].

Modern endodontic irrigation has evolved from simply choosing suitable chemical agents to optimizing their method of delivery. Sodium hypochlorite (NaOCl) remains the irrigant of choice due to its strong tissue-dissolving and antimicrobial properties[9]. Ethylenediaminetetraacetic acid (EDTA), on the other hand, is typically used to chelate and eliminate the inorganic portion of the smear layer, enhancing the interface between dentin and obturation materials[7]. However, both agents have limitations when used with conventional passive irrigation techniques, especially in the apical third of the canal.

To overcome these limitations, clinicians increasingly employ activation systems such as passive ultrasonic irrigation (PUI), which enhances the dynamics of irrigant flow within the root canal. Ultrasonic activation produces acoustic streaming and cavitation effects that allow irrigants to penetrate more deeply into complex spaces, dislodging debris and disrupting biofilms more efficiently[10,11].

The present study evaluates how different irrigation protocols influence apical microleakage. The results confirm that activated irrigation-particularly when NaOCl and EDTA are used in combination with ultrasonic activation-achieves a more effective reduction of apical leakage than non-activated methods. These findings reinforce the importance of selecting an appropriate irrigation protocol to achieve a reliable apical seal and emphasize its critical role in the long-term success of endodontic treatment.

Materials and methods

This in vitro study was conducted on 80 extracted single-rooted human teeth. Immediately after extraction, all specimens were cleaned of soft tissue residues and disinfected in 3 % sodium hypochlorite (NaOCl) for 24 hours[9]. The teeth were then stored in distilled water at 4 °C until use. To standardize root length, the crowns were sectioned at the cemento-enamel junction using a low-speed diamond disc under continuous water cooling. Working length was determined by inserting a size #10 K-file into the canal until the tip was visible at the apical foramen, and then subtracting 1 mm from that measurement.

Canal preparation was performed with ProTaper Universal rotary instruments (Dentsply Maillefer), completing instrumentation up to size F2. After each file, 5 mL of the respective irrigant was delivered using a 27-gauge side-vented needle inserted passively into the canal without binding to the canal walls.

The specimens were randomly assigned to four experimental groups according to the irrigation protocol used (Table I):

- **Group 1:** 2.5 %NaOCl + 17 % EDTA with ultrasonic activation[7,10],
- **Group 2:** 2.5 %NaOCl with ultrasonic activation[10],
- **Group 3:** 2.5 %NaOCl + 17 % EDTA without activation[7],
- **Group 4:** (Control): physiological saline solution

Following instrumentation, all canals were obturated using the single-cone technique with F2 gutta-percha cones and AH Plus resin-based sealer[12].

The samples were incubated at 37 °C in 100 % humidity for 7 days to ensure complete setting of the sealer.

Once set, the external root surfaces were coated with two layers of nail varnish, leaving a 2 mm window around the apical foramen uncoated to allow dye penetration. The teeth were immersed in 2 % methylene blue for 24 hours, then rinsed under running water and sectioned longitudinally in a bucco-lingual direction using a low-speed diamond disc.

Table 1. Group Division and Protocols

Group	Irrigation Protocol	Obturation Method	Sealer
1	NaOCl+EDTA+ UA	Single-cone	AH Plus
2	NaOCl + UA	Single-cone	AH Plus
3	NaOCl+EDTA	Single-cone	AH Plus
4	Saline	Single-cone	AH Plus

The extent of apical dye penetration was observed under a light microscope at 10× magnification, and measurements were recorded in millimeters. Statistical analysis was performed using SPSS software (version 25.0; IBM Corp., Armonk, NY, USA). Descriptive statistics (minimum, maximum, mean, and standard deviation) were calculated for each group. Differences among the groups were analyzed using one-way ANOVA, followed by Tukey's post hoc test. Statistical significance was set at $p< 0.05$.

Results

Descriptive analysis demonstrated clear differences in the extent of apical dye penetration among the experimental groups. The lowest level of microleakage was recorded in Group 1 (mean value: 1.05 mm), where canals were irrigated with NaOCl and EDTA with ultrasonic activation. This protocol consistently provided the most effective apical seal, reflected by minimal dye penetration values.

By contrast, the highest microleakage was observed in Group 4(mean value: 2.45 mm), the control group irrigated only with physiological saline solution, which highlighted the limited capacity of simple saline to adequately clean and prepare the canal walls for sealing.

Intermediate microleakage values were noted in Groups 2 and 3. Specifically, ultrasonic activation of NaOCl Group 2(mean value: 1.12 mm) produced lower microleakage values compared to NaOCl + EDTA without activation Group 3 (mean value: 1.78 mm), underscoring the beneficial role of activation in enhancing irrigant effectiveness.The inclusion of standard deviation (SD) values in the results provides a clearer understanding of how consistent the data were within each experimental group. When the standard deviation is small, it shows that most of the measured values are close to the average, meaning the samples behaved in a fairly consistent way. On the other hand, a larger standard deviation means that the values were spread out more widely, indicating greater differences in how the samples responded. In the present study, the groups with ultrasonically activated irrigation showed the lowest SD values, suggesting that activation contributed not only to lower mean microleakage but also to more stable and reproducible outcomes across specimens. (Table II).

Table 2. Micro leakage results-minimum, maximum, mean values and sfor each group

Group	N	Min (mm)	Max (mm)	Mean (mm)	SD (mm)
1	20	0.2	2.0	1.05	0.51
2	20	0.5	2.1	1.12	0.45
3	20	1.1	2.5	1.78	0.39
4	20	2.2	2.7	2.45	0.25

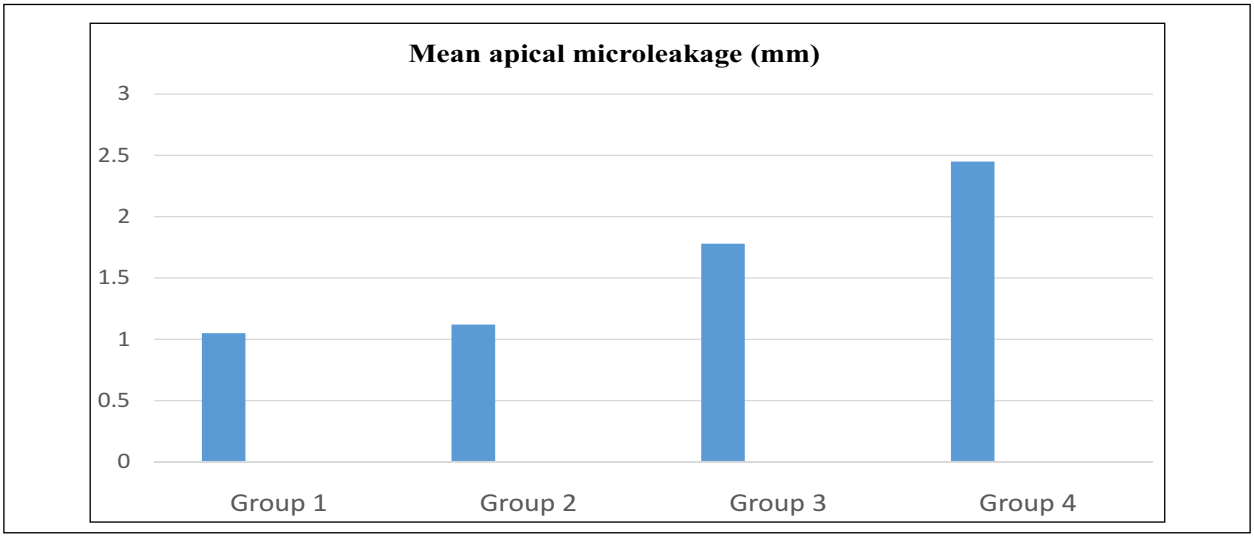

Figure 1. *Mean apical microleakage (mm) across all experimental groups*

Table 3. Micro leakage results-minimum, maximum, mean values and sfor each group

Comparison	Mean Difference (mm)	p-value	Significance	Interpretation
Group 1 vs Group 2	0.205	0.287	NS	No significant difference
Group 1 vs Group 3	0.760	<0.001	S	Group 1 lower leakage
Group 1 vs Group 4	1.390	<0.001	S	Group 1 much lower leakage
Group 2 vs Group 3	0.555	<0.001	S	Group 2 lower leakage
Group 2 vs Group 4	1.185	<0.001	S	Group 2 much lower leakage
Group 3 vs Group 4	0.630	<0.001	S	Group 3 lower leakage

Statistical comparisons confirmed that the differences among groups were significant, indicating that the type of irrigant used and its activation protocol exert a decisive influence on the degree of apical microleakage. Taken together, the results indicate that activated irrigation, especially with NaOCl and EDTA, not only reduces apical microleakage but also creates cleaner canal walls, thereby supporting improved adaptation and sealing effectiveness of the root canal sealer (Figure 1).

The post hoc Tukey test revealed clear and statistically significant differences among the experimental groups (Table III).

Group 1 (NaOCl + EDTA with ultrasonic activation) demonstrated the lowest level of apical microleakage, showing a statistically significant difference compared to all other groups. Group 2 (NaOCl with ultrasonic activation) also showed favorable results, though slightly less effective than the combined NaOCl + EDTA protocol.

Group 4 (control with physiological saline) recorded the highest microleakage values, confirming that irrigation with saline alone cannot provide an adequate apical seal.

Overall, the intergroup comparisons underline the pivotal role of ultrasonic activation and the combined use of NaOCl and EDTA in minimizing apical microleakage. These findings emphasize that the irrigation protocol plays an important role in reducing apical microleakage by improving canal cleanliness and dentin surface conditions, thereby facilitating a more effective apical seal and contributing to the long-term success of endodontic treatment.

Discussion

The results of this study clearly demonstrate that the irrigation protocol plays an important role in the degree of apical microleakage in teeth obturated with the single-cone technique. Among the tested regimens, the combination of sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) with ultrasonic activation provided the lowest levels of microleakage, underscoring the importance of using an enhanced irrigation strategy to optimize canal cleanliness and sealer adaptation. This finding is consistent with previous research that highlights how ultrasonic activation increases the penetration and efficacy of irrigants through cavitation and acoustic streaming, facilitating the removal of smear layer and debris even in anatomically complex areas of the root canal system[10,11,13].

In contrast, canals irrigated with physiological saline solution showed the highest levels of microleakage, confirming that the absence of active chemical agents significantly compromises the sealing ability of the obturation. Furthermore, when NaOCl and EDTA were applied without ultrasonic activation, apical microleakage values remained comparatively higher than those observed in the activated groups. This finding suggests that the chemical action of irrigants on its own cannot ensure ideal sealing conditions; activation is necessary to improve canal cleanliness and surface preparation, thereby facilitating a more effective apical sea[7,8,14].

Interestingly, NaOCl with ultrasonic activation alone also achieved favorable results, showing significantly less

microleakage compared to non-activated regimens. This suggests that, in certain clinical situations, a simplified irrigation strategy based solely on activated NaOCl could provide a balance between effectiveness and practicality, particularly when access to multiple irrigants is limited[9,15].

Taken together, these results highlight that the irrigation protocol plays an important role in reducing apical microleakage. Because persistent microleakage at the apex is one of the leading causes of endodontic failure, our findings underscore the clinical importance of incorporating irrigant activation into routine practice. In particular, ultrasonically activated NaOCl combined with EDTA proved to be the most effective irrigation strategy, providing superior canal cleanliness and minimizing apical microleakage, thereby contributing to more predictable, long-term outcomes in root canal therapy.

Clinically, persistent apical microleakage remains one of the leading causes of endodontic failure. By showing that ultrasonic activation of irrigants, especially NaOCl combined with EDTA, markedly reduces microleakage, this study offers practical implications for everyday dentistry: adopting activated irrigation protocols can support cleaner canals, more effective sealing, and ultimately more predictable, long-term success for patients.

Conclusion

This study demonstrated that the irrigation protocol has a significant impact on apical microleakage in root canal therapy. The combination of sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) with ultrasonic activation produced the lowest levels of apical microleakage, consistently outperforming other regimens. This outcome reflects the effectiveness of activated irrigation in enhancing canal cleanliness and creating favorable conditions for reliable sealing.

These findings underline that effective irrigation, particularly when supported by ultrasonic activation, improves cleaning and debridement of the canal system and reduces the risk of persistent microleakage. By doing so, it supports the long-term predictability and success of endodontic treatment. Incorporating such strategies into routine clinical practice is therefore strongly recommended to optimize treatment outcomes.

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