Spectrophotometric Analysis of Apical Extrusion of Sodium Hypochlorite using Different Irrigation Protocols in an *Ex Vivo* Model of Immature Teeth

Keywords

Sodium Hypochlorite
Activation
Extrusion
Immature Teeth

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Received: 02.10.2023
Accepted: 31.01.2024

doi: 10.1922/EJPRD_2635Vieira07

ABSTRACT

Objective: To evaluate the effect of different agitation methods on apical extrusion of 1.5% sodium hypochlorite (NaOCl) in an ex vivo model of immature teeth. Methods: Sixty extracted human inferior incisors were prepared to simulate immature teeth and embedded in an artificial root socket made of silicone impression material. The teeth were then divided into four groups: Conventional needle irrigation (CNI) alone, CNI supplemented with Ultrasonic Irrigant Activation (UIA), EasyClean (EC), or XP-endo Finisher (XPF). Extruded NaOCl was collected, reacted with m-cresol purple, and its absorbance values were measured. The data were statistically analyzed using One-way analysis of variance with a significance level of 5%. Results: All groups showed apically extruded irrigating solution, and the mean volumes of extruded NaOCl did not differ significantly between any of the test groups (p>0.05). Conclusion: The activation of 1.5% NaOCL by UIA, EC, or XPF as supplementary to CNI does not promote greater apical extrusion when compared to CNI alone in simulated immature teeth.

INTRODUCTION

Regenerative Endodontic Therapy (RET) represent a biological approach for treating teeth with incomplete root formation and pulpal necrosis. The goal of RET is to eliminate signs and symptoms while promoting the development of dentin-pulpal complex structures, such as dentin, root structures, and cells.[1] However, effective bacterial decontamination protocols are critical to achieve these goals.[2,3] Microbiological studies have shown that the microbiome of endodontic infections in immature teeth is composed of a high diversity of bacterial genera, species, and phyla that organize into complex biofilms adhered to the root canal walls.[4,5] A limitation for the adequate elimination of the infectious content in these teeth is the absence of conventional instrumentation of the root canal, depending only on the decontamination promoted by irrigating solutions and intracanal medicaments.[5,6]

To increase the efficacy of the irrigant solutions used in endodontic therapy, supplementary activation techniques have been proposed.[7] Ultrasonic activation (UA) involves transmitting acoustic energy from an oscillating insert to activate the irrigating solution.[8] When compared to conventional irrigation techniques, studies have demonstrated that UA activation improves the elimination of bacterial biofilms[9,10] and virulence factors.[11] In addition to UA, electric motor-driven instruments with rotatory and reciprocating movements have been proposed as alternatives for activating irrigation solutions and to remove bacterial biofilms. The XP-endo Finisher (XPF; FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) and the EasyClean® (Easy Equipamentos Odontológicos, Belo Horizonte, Brazil) are two examples of such instruments. These instruments are advantageous solutions for improving the activity of irrigation solutions in RET since they do not mechanically damage the root canal walls.[9,11-13]

However, during the irrigation process of immature teeth, a concern is the extrusion of NaOCl into the periapical tissues[14] due to its caustic effects.[15] Immature teeth have wide canals and foramen with diameters greater than 1mm, making them more susceptible to NaOCl extrusion and possible repercussions to periapical tissues,[16–18] such as cytotoxicity effects on mesenchymal stem cells of the apical papilla,[19] as well as pain or intra- and postoperative complications.[20]

The impact of activation on apical extrusion of NaOCl in mature teeth is widely discussed in the literature, with some studies reporting lower extrusion in teeth undergoing agitation,[21–23] while other studies observed opposite results depending on the activation system used[24] and apical diameter.[16] Recent studies[14,25] compared NaOCl extrusion in simulated immature teeth after activation by different methods, including UIA, XPF, and EC, and found no differences between them or when compared to CNI. However, none of these studies used a sensitive method to quantify the real volume of extruded solution.

Therefore, this study aimed to evaluate the effects of different activation methods on NaOCl extrusion in an *ex vivo* model of an immature tooth using a spectrophotometric analysis to quantify the volume of extruded solution. We tested the null hypothesis that there would be no difference between the experimental groups.

MATERIAL AND METHODS

This study was conducted in accordance with the Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines[26] and was approved by the local institutional research ethics committee. The PRILE 2021 flowchart is illustrated in Figure 1.

SAMPLE SIZE CALCULATION

To determine the appropriate sample size for evaluating NaOCl apical extrusion, we performed a calculation based on a previous study with similar methodology.[24] We utilized G*Power v. 31 for Windows and selected the ANOVA one-way test (a priori, F test family option), with an effect size of 0.53, a type I (alpha) error of 0.05, and a type II (beta) error of 0.90. The minimum sample size required was estimated to be 14 sampling units per experimental group, and we used 15 teeth per allocation group, considering possible losses (resulting in a total sample of 60 teeth).

TEETH SELECTION AND PREPARATION OF ROOT CANAL ORGANOTYPE MODEL AND ARTIFICIAL ROOT SOCKET

Sixty human inferior incisors were selected for this study and stored in distilled water at 4°C until use. Only single-rooted teeth with a minimum length of 12 mm, no history of root canal therapy or root caries, and without pronounced curvature, cracks, fractures, or root canal calcification were included.

The teeth were decoronated 2 mm below the cemento-enamel junction using a slow-speed diamond saw (ISOMET, Buhler, Ltd. Lake Buff, NY, USA) under continuous irrigation. Subsequently, a second cut was made 7 mm from the first cut to standardize the root length to approximately 7 mm.

To prepare the root canal organotypic model, the root canal was enlarged using retrograde instrumentation with the ProDesign file with a #30/0.10 taper (Bassi, Belo Horizonte, Brazil)[14] and irrigated with 5 mL of 1.5% NaOCl until the apical opening was standardized to 1.3 mm, simulating an immature apex, which is commonly seen in regenerative cases.[27] Finally, the root segments were rinsed with distilled water, and the external surface of the roots was covered with nail polish to prevent irrigant extrusion through lateral canals[23] *(Figure 2A)*.

The artificial root sockets were prepared according to a previous study.[24] Briefly, after instrumentation, the roots were wrapped in hydrophobic impression material (Speedex, Vigodent-Coltene, Rio de Janeiro, Brazil) so that simulates root socket and the periapical tissue resistance during irrigation. The tooth was then removed, and the patency was checked to ensure there was no blockage of the apical foramen by impression material. Next, the roots were inserted in the artificial socket and fixed with a light-cure gingival barrier to seal the spaces between the roots and the impression material. Finally, the set formed by the impression material and specimen was fixed in a glass plate for stabilization and submitted to the irrigation procedures *(Figure 2B)*. The irrigant extruded during the following procedures was captured in the silicone socket.

IRRIGATION AND ACTIVATION PROTOCOLS

For the evaluation of the irrigant extrusion, the teeth were divided into four groups (n=15) according to the type of the irrigation protocol (CNI, UIA, EC, and XPF). The sample were

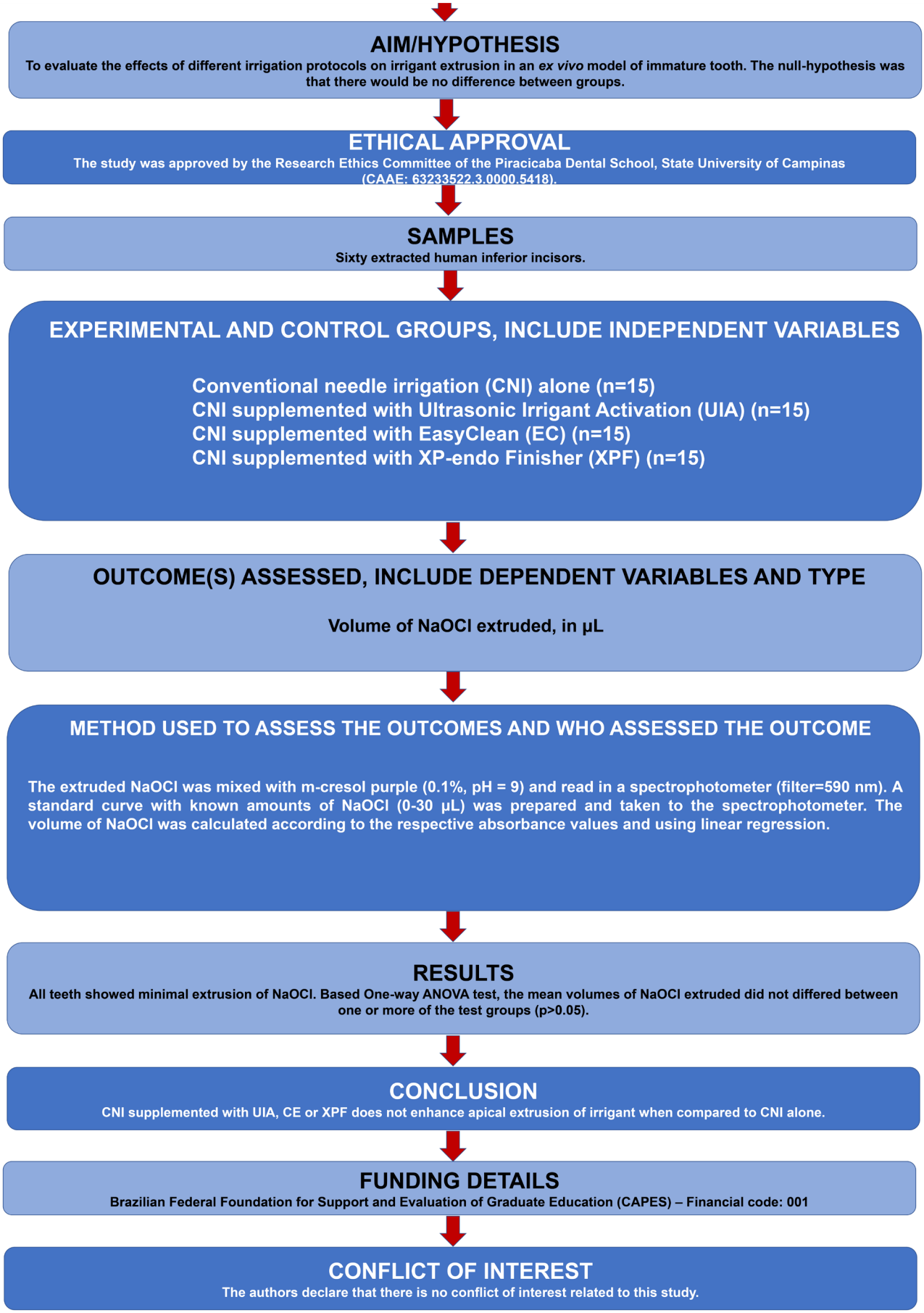

Figure 1: PRILE 2021 flowchart. From: Nagendrababu et al. (2021).

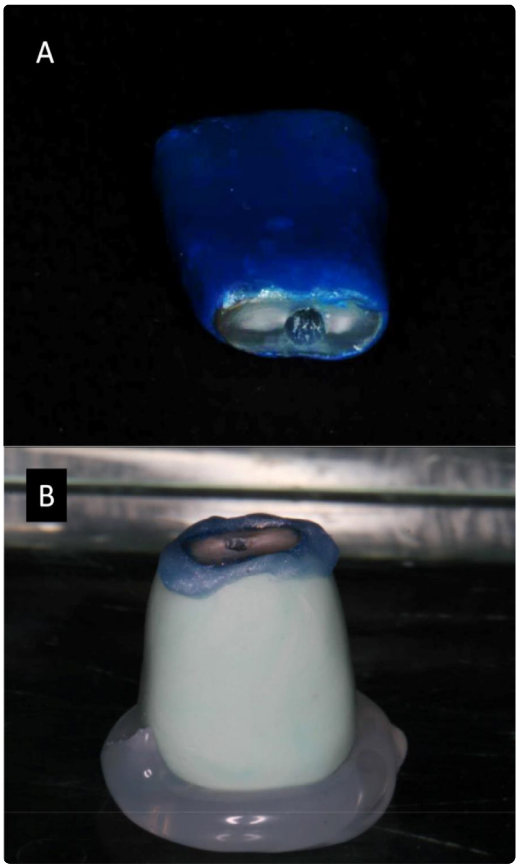

Figure 2: Preparation of root canal organotype model and artificial root socket. A) Root fragment after instrumentation. B) Artificial root socket made of hydrophobic impression material fixed in a glass plate.

randomly assigned to the experimental groups using a computer-generated random number in the Excel software (Microsoft, Redmond, United States of America).

Experimental Groups

- CNI group: 20 mL (four 5-mL needles) of 1.5% NaOCl (pH = 11) at room temperature was used with a 24G-hypodermic needle (0.55x20mm) (Becton Dickinson, Franklin Lakes, NJ, USA) positioned 2 mm from the apex. Each 5 mL was dispensed for 75 seconds (total flow rate of 20 mL / 5 minutes). Simultaneously, the fluid was aspirated with a sterile metal cannula associated with a Capillary Tip (0.014 mm diameter) (Ultradent Products, Indaiatuba, Brazil) connected to a vacuum suction pump and positioned at the entrance of the root canal during the entire irrigation process. The last 1.5 mL of that amount was dispensed in three cycles (0.5 ml for 10 sec) and left in the root canal for 20 sec without any activation.
- UA group: 20 mL of NaOCl was dispensed following the same protocol and flow rate of the CNI group; however, the last 1.5 mL of that amount was dispensed in three cycles (0.5 ml for 10 sec) and agitated for 20 s after each irrigation cycle with an Irrisonic tip (Helse, Riberão Preto, Brazil) coupled to an ultrasound unit (Satelec Booster, Acteon, Indaiatuba, Brazil) at a power of 2 (10%), with 1-min agitation of the solution inside the root canal.
- EC group: The instrument was coupled in an endodontic motor (X-Smart Plus, Dentsply, Munich, Germany) and activated using reciprocating motion (150º counterclockwise followed by a 30º clockwise turn), and the irrigant was activated following the same protocol as UIA group.
- XPF group: The instrument was coupled in an endodontic motor in rotary motion (800 rpm and 1.0-Ncm torque), and the irrigant was activated following the same protocol as UIA and EC groups.

A stopper was placed in all instruments at the required length for length control (2 mm from the apex). A unique operator calibrated for the flow rate was responsible for all irrigation process.

SPECTROPHOTOMETRIC ANALYSIS OF NaOCL EXTRUSION

After completing the irrigation procedures, the roots were removed from the silicone and the NaOCl that had extruded into the root socket was collected for analysis. To collect the fluid, 100 µL of deionized water was introduced into the artificial root socket and mixed with the extruded NaOCl. The entire contents were then transferred into an Eppendorf tube containing 900 µL of deionized water and vortexed thoroughly. Next, 10 µL of each sample was pipetted into a 96-well plate (Costar, New York, NY, USA) that contained 160 µL of m-cresol purple (0.1%, pH = 9) (Quimlab Produtos de Química Fina Ltda, SP, Brazil). The mixtures were mixed on a plate shaker and read using a spectrophotometer (ELx808; BioTek, Austria) (filter=590 nm)[23] after three hours.

To quantify the amount of extruded NaOCl, a standard curve was prepared with known amounts of 1.5% NaOCl (0-30 µL) in duplicate, using the same protocol as described above *(Figure 3A)*. The volume of NaOCl was determined by calculating the respective absorbance values and using linear regression *(Figure 3B)*. The standard curve was prepared on the same day as the reading of the experimental groups, and the amount of NaOCl extracted was reported in µL. Each sample was analyzed in duplicate by a blinded investigator who performed the spectrophotometer analysis and calculated the volume of extruded NaOCl.

STATISTICAL ANALYSIS

Data analysis was carried out using the Prism 8.01 software program (GraphPad Software Inc, San Diego, CA). The normality and homoscedasticity of the data were checked using the Shapiro-Wilk and Bartlett tests, respectively. The extruded NaOCl data were compared using one-way ANOVA with a significance level of 5%.

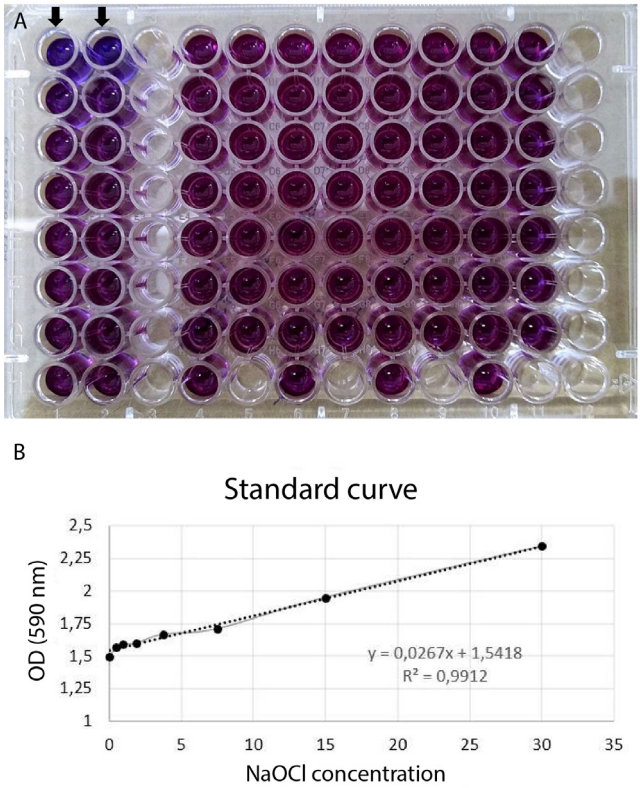

Figure 3: NaOCl standard curve preparation. A) 96-well plate containing the standard curve with known amounts of NaOCl (black arrows) and the extruded NaOCl mixed with m-cresol. B) The standard curve for NaOCl extrusion from 0–30 µL. The R^2 was 0.9912.

RESULTS

All 60 teeth showed minimal extrusion of NaOCl. The mean volumes of NaOCl extruded did not differ among the experimental groups, according to the One-way ANOVA test ($F(3, 56) = 0.6272$, $p = 0.6004$) (*Figure 4*): UIA (6.34 ± 1.8 µL) (mean ± SD), XP-F (5.54 ± 2.87 µL), CNI (5.36 ± 1.8 µL), and EC (5.34 ± 2.24 µL).

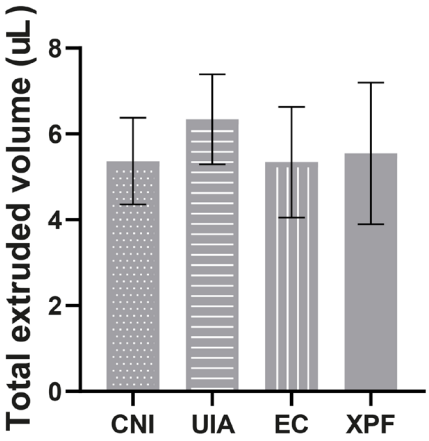

Figure 4: The mean extruded volume ± the 95% confidence interval (CI) for each of the four irrigation protocols. One-way ANOVA test ($F(3, 56) = 0.6272$, $p = 0.6004$).

DISCUSSION

This study aimed to evaluate how activation methods affected the NaOCl extrusion in an *ex vivo* simulated immature teeth. The results demonstrated that the activation methods did not enhance the volume of apical extrusion of NaOCl when compared to CNI alone.

Apical extrusion of NaOCl is a relevant problem during endodontic procedures, and its adverse effects have been widely documented in the literature.[20,28] Some authors suggest that the size of the apical foramen increases the risk of irrigant extravasation, making the irrigation of immature teeth a delicate step in passive decontamination.[16-18] In this study, all samples showed apical extrusion of NaOCl, regardless of the irrigation protocol. This finding agrees with previous studies that used *ex vivo* models of simulated immature teeth.[14,25] Moreover, the amount of irrigant extruded in this study was comparable to that reported in others *ex vivo* studies that used mature teeth.[23,24] Azim *et al.*[24] demonstrated that both XP-Endo finisher and CNI extruded a comparable volume of NaOCl (7.8 ± 4.1 µL and 7.4 ± 3.4 µL, respectively), closely aligning with the findings of the present study. In addition to activation techniques, irrigant extrusion may be influenced by various variables, including flow rate, needle type, and insertion depth,[21] which could explain the occurrence of some degree of extrusion in all samples.

Although the extruded volume among the experimental groups was small (ranging from 1 µL to 12 µL), it is challenging to determine whether this amount is sufficient to negatively impact the clinical and histological outcomes of teeth undergoing regenerative endodontic procedures due to the deleterious effect of NaOCl on undifferentiated mesenchymal cells even at low volumes and concentrations.[19,29,30]

The current study results show no significant difference in the amount of NaOCl extruded from the apex of teeth between different activation methods and the control group. This aligns with findings from a previous study by dos Reis *et al.*,[14] which employed volumetric analysis through micro-computed tomographic imaging. The study observed no statistical difference between groups that utilized Easy Clean (median 3.15 mm³), Ultrasound (median 3.14 mm³), XP-endo Finisher (median 0.67 mm³), or positive pressure with no agitation (median 0.76 mm³). The lack of difference in extrusion volume could be explained by two factors. Firstly, the instruments were positioned 2 mm away from the apical foramen, which has been shown to minimize the risk of irrigant extrusion during irrigation/activation protocols.[21] Secondly, agitation methods may generate a similar apical pressure to that of CNI, which may result in comparable extrusion volumes.[31]

To assess the volume of extruded NaOCl, we employed a spectrophotometric analysis method that utilizes a pH-sensitive indicator. This method was first described by Rodriguez-Figueiroa *et al.*[23] and offers the advantage of highly sensitive

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result analysis.[32] However, we made two modifications to the original methodology in our study. During pilot testing, we found that using m-cresol purple at pH 7 led to an irregular reading of absorbances in the spectrophotometer due to the color change from yellow to purple after contact with NaOCl (pH 11). This was also noted in a previous study.[24] To address this, we opted to use m-cresol purple at pH 9 as it produced a linear pattern of color changes in response to known volumes of NaOCl (0-30 µL) that was appropriate for creating a standardized curve after 3 hours of reaction between NaOCl and the pH indicator.

Our second modification involved dissolving NaOCl in deionized water before coming into contact with m-cresol. In the study by Rodriguez-Figueiroa *et al*.,[23] extruded NaOCl was directly transferred to wells containing 170 µL of m-cresol. However, we observed a progressive discoloration and reduction in the absorbance levels of m-cresol over time, indicating an unstable reaction. Furthermore, the calibration curve without water produced an opposite pattern to the expected one, where higher NaOCl concentrations resulted in lower absorbance. We speculate that this result may be due to NaOCl degrading the pH indicator. To mitigate this issue, we found that initially dissolving NaOCl in deionized water provided stability in the reaction with m-cresol, producing precise and stable readings without interfering with the sensitivity to pH alteration. Our standard curve showed that by adding water, we were able to alter the absorbance standard, resulting in a proportional relationship between concentration and absorbance.

Another methodological aspect in evaluating the extruded irrigant is the use of an artificial root socket made of hydrophobic silicone. Rodriguez-Figueroa *et al*.[23] and Azim *et al*.[24] used this methodology, which has proven effective in collecting extruded NaOCl for subsequent reading in the spectrophotometer. In addition to serving as a collection medium, the artificial root socket aims to simulate the resistance of periapical tissues to extravasation.

The primary limitation of this study is the inability to confirm whether the threshold pressure required to induce extravasation in this model aligns with that in periapical tissues. Another consideration is that immature teeth may have varying apical diameters, suggesting the need for future studies to explore the impact of activation protocols on irrigant extrusion in teeth with broader apical diameters. Additionally, future investigations could examine the influence of different needle designs on the apical extrusion of NaOCl.

Conversely, this study stands out as the first to assess the extrusion of apical irrigants in teeth with an apical foramen compatible with immature teeth, using a sensitive and reproducible methodology. Furthermore, the utilization of an artificial root socket partially simulated apical resistance, addressing a primary limitation observed in previous studies.

CONCLUSION

Despite the limitations of this study, we can conclude that the activation of 1.5% NaOCl by UIA, EC, or XPF as supplementary to CNI does not result in greater apical extrusion when compared to CNI alone in simulated immature teeth. From a clinical perspective, this study provides evidence that the use of NaOCl activation during the passive decontamination of immature teeth could be a safe complementary procedure with the potential to optimize bacterial elimination without causing damage to periapical tissues.

FUNDING SOURCE

This study was funded by CAPES - Finance Code 001.

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