Original Article

Evaluation of pulp tissue dissolution capacity through different sodium hypochlorite agitation protocols

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Abstract

Aims: This *ex vivo* study aimed to assess the dissolving capacity of 2.5% sodium hypochlorite using eight agitation protocols within swine pulp tissue.

Subjects and Methods: Twelve lower first premolars were prepared and split into the fragments with a groove housing porcine dental pulp. Groups were assigned based on agitation systems: manual, passive ultrasonic, Easy Clean and XP-Endo Finisher. Two agitation time protocols were applied: One min (3 s × 20 s cycles) and 2 min (6 s × 20 s cycles). Wilcoxon Mann–Whitney *U* test was used to compare the groups.

Results: Both time frames demonstrated superior results compared to manual group ($P > 0.5$). However, in the two min groups, no significant differences were observed among the other protocols ($P < 0.5$). Intriguingly, increasing cycle numbers significantly improved results within each group ($P > 0.5$).

Conclusion: Extending the chemical agitation time during final irrigation enhances tissue removal, regardless of the irrigation protocol employed.

Keywords: Agitation; dental pulp; endodontics; pulpitis; root canal preparation; sodium hypochlorite

INTRODUCTION

Disinfection of the root canal system is one of the main steps toward successful treatment.[1] To achieve good disinfection, it is necessary to remove necrotic and pulpal remains from the interior of the root canal system.[2] Sodium hypochlorite is the most commonly used endodontic irrigant,[3,4] being commercialized with concentrations ranging from 0.5% to 5.25%, affecting its properties, such as antimicrobial capacity and tissue dissolution.[3] However, irrigation using needles does not allow it to reach the entire length of the root canal.[4-6]

The activation of this chemical substance has been the object of the study, as it optimizes the properties of the irrigant and promotes better performance inside the root canals.[7] This activation can be performed in several ways, such as manually with gutta-percha cones, ultrasonic inserts, rotating or ultrasonic plastic tips, lasers, and mechanized instruments.[8-12] The ideal time that sodium hypochlorite at a given concentration needs to remain in the channel system is a problem to be solved.[13,14]

Passive ultrasonic irrigation uses smooth inserts that oscillate at high frequencies, enhancing the action of irrigant through the formation of micro-acoustic currents

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and the phenomenon of cavitation.[15] The Easy Clean plastic file (Easy Equipamentos Odontológicos, Belo Horizonte, Brazil) produces a hydrodynamic phenomenon through the movement of the tip, which is highly flexible and does not suffer interference from contact inside the channel.[11] XP-Endo Finisher files (FKG Dentare SA, La-Chaux de Fonts, Switzerland) using MaxWires technology, employing the shape memory principle, which, when activated inside the canal, assume a semicircular shape causes that touches the region of the isthmus and mechanical preparation irregularities.[12]

This study evaluated and compared the tissue removal efficiency of 2.5% NaOCl when using different agitation protocols.

SUBJECTS AND METHODS

This study was approved (register no. 3,190,481) by the Ethics and Research Committee of the Dental Research Center São Leopoldo Mandic SS, Campinas, Brazil. For the sample size calculation, a Type I error (α) of 5% and a Type II error (β) of 20% were adopted, with a power of 80%, and based on the effect found in the pilot study, an estimated number necessary sample size of 10 samples per group for the present study, which, adding 20% to the calculated value, to consider possible losses, the final sample value necessary for the study was 12 samples.

Twelve ($n = 12$) mandibular first premolars were selected, single-rooted, with a single canal and fully formed apices, maximum root curvature of 10°,[16] without perforations or previous endodontic treatment, and with foramina that had an initial diameter of a #15 file.[17]

Endodontic access was performed by grinding with 1014 HL and 3083 drills (Sorensen KG, São Paulo, Brazil), and exploration was performed with a #15 K-file (Dentsply Maillefer Instruments, Ballaigues Switzerland) until its tip was visible through the apical foramen obtaining the actual length of the tooth. Root curvature was verified by radiography using the proposed Schneider method.[16]

The size of the samples with 15.0 mm was standardized through occlusal wear of the elements with a double-sided diamond disk with a thickness of 0.15 mm (Sorensen KG, São Paulo, Brazil). Confirmation of the sample measurement was performed with a caliper (Astro Mix, São Paulo, Brazil), and the working length (WL) was set at 14.0 mm.

The modeling of the samples was standardized with a Reciproc R40 instrument (VDW GmbH, Munich, Germany), powered by X-Smart Plus (Dentsply Maillefer Instruments, Ballaigues, Switzerland) engine configured for use in Reciproc mode. Instrumentation was performed with small "down and back" movements with a maximum amplitude of 3 mm. Irrigation of 3 ml of 2.5% NaOCl was performed at each advanced third, with 3 ml disposable syringes attached to a 27 G (Endo-Eze Navitip, Ultradent, Brazil) side outlet needle, totalizing an average of 15 ml of irrigant.

After preparation, two longitudinal grooves were created on the buccal and lingual walls of the specimens with a diamond disk with a thickness of 0.15 mm (Sorensen KG) mounted on a straight piece of low rotation, without communication with the main canal.

The specimens were then placed in a muffle made of Zetaplus System (ZhermackSpA, Badia Polesine, Italy) condensation silicone and Eppendorf type plastic flask, creating a system for fixing the parts and also to simulate a closed suction and irrigation system.

After the material had hardened, the sample was removed from the muffle and cleaved into two fragments, using a number 11 scalpel blade, as previously described by Estevez *et al*.,[17] with a 0.15 mm thick diamond disc (Sorensen KG), a channel with a length of 1 mm and a thickness of 0.15 mm was made perpendicular to the root canal at 3 mm from the WL.

Porcine pulp tissue was obtained from lower incisors within a maximum period of 12 h after the animal was slaughtered for the food industry. The tissue samples were stored in saline until use, within a maximum period of 1 h. The samples were dissected into smaller fragments with a scalpel blade number 15C and prepared to adapt to the grooves created. Later, they were stained with 1% sodium fluorescein for 30 s to improve the contrast with the dentinal tissue. All preparation is shown in Figure 1.

After inserting the pulp tissue into the groove, the root fragments were photographed with a T4i camera and a 17 mm × 55 mm lens (Canon, Tokyo, Japan), mounted on a fixed stand to standardize the photos, serving as initial parameters for the later stages. The roots were regrouped, and a layer of dental wax was adapted to the apical 5 mm of the sample to promote apical sealing. The samples were repositioned on the silicone base as described above. The confirmation of pulp tissue adaptation quality was performed in two ways.

Initially, optical verification was conducted through the analysis of the image captured by the camera. Subsequently, the root fragments were regrouped, and a size #15 K-type instrument (Dentsply Maillefer Instruments, Ballaigues, Switzerland) was inserted to the WL to check for any interferences. In the event of inadequate pulp tissue adaptation being detected in the region, the pulp tissue insertion steps would be repeated to ensure satisfactory adaptation.

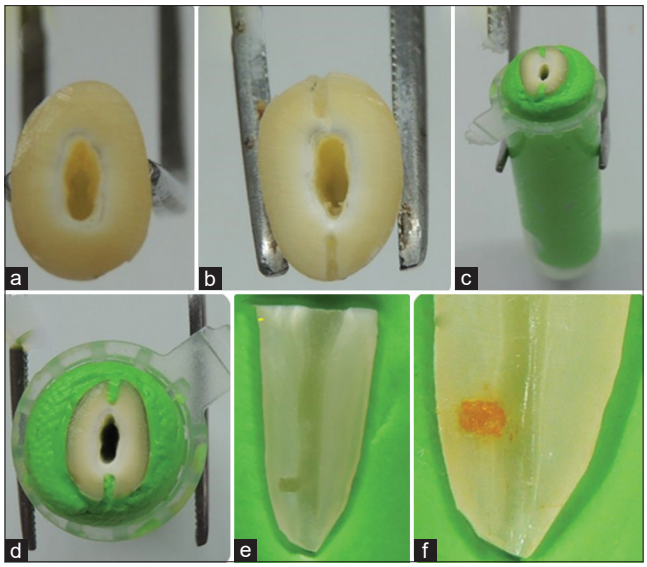

Figure 1: Sample preparation. (a) Wear of the occlusal surface, (b) Preparation of wear on the vestibular and lingual surfaces without reaching the main canal lumen, (c and d) Root adaptation in a custom-made tray using impression material, (e) Creation of a stopper in the apical third, (f) Adaptation of porcine pulp tissue

The same 12 root elements were used for all groups. In each sample, irrigation was carried out with 3 ml of NaOCl 2.5% with the needle tip coupled to 1 mm of the WL with a default time of 10 s. After that, each group had its protocol.

Positive pressure group 1 min

The NaOCl was left inside the channel for another 20 s. This cycle was repeated three times, totalizing 1 min of irrigation.

Positive pressure group 2 min

The cycle was repeated six times, totalizing 2 min of irrigation.

Passive ultrasonic irrigation group 1 min

The NaOCl was activated for 20 s with a smooth E1 ultrasonic tip (Helse, Brazil), which has an ISO 20 gauge and 0.1 taper, positioned 2 mm from the WL, coupled to a P200 piezo ultrasound unit (EMS, Injecta, Brazil) configured at 20% power current. This cycle was repeated three times, totalizing 1 min of activation.

Passive ultrasonic irrigation group 2 min

It was performed similarly to passive ultrasonic irrigation group 1 min (PUIG1), only repeating the cycle six times, totalizing 2 min of activation.

Easy clean group 1 min

Activation of NaOCl occurred through Easy Clean plastic instruments (Easy, Brazil), which have an ISO 25 gauge tip and 0.4 taper, coupled to the X-Smart Plus motor (Maillefer Instruments) in continuous rotation movements with a speed of 1200 rpm and torque 1. Was used one instrument for the sample, inserting it into WL, and the sequence and stirring time were similar to PUIG1.

Easy clean group 2 min

It was performed in a similar way to the easy clean group 1 min, only repeating the cycle six times, totalizing a time of 2 min of activation.

XP-Endo group 1 min

Activation of NaOCl was performed with an XP-Endo Finisher file (FKG Dentare SA, La-Chaux de Fonts, Switzerland), which has a diameter of ISO 25 and taper 0, coupled to the X-Smart Plus motor in continuous rotation mode with a speed of 800 rpm and torque 1. Was used one instrument for the sample, inserting it into WL, and the sequence and stirring time were similar to PUIG1.

XP-Endo group 2 min

It was performed similarly to the XP-Endo group 1 min, only repeating the cycle six times, totalizing 2 min of activation.

At the end of each experimental stage, final irrigation with 10 ml of distilled water was performed, and the conduits were dried with absorbent paper points. After that, the teeth were removed from the muffle, opened, and the groove with tissue photographed similarly to the initial image. Before commencing the subsequent experiment, the remaining pulp tissue was meticulously removed using a #15 K-file (Dentsply Maillefer Instruments, Ballaigues, Switzerland), supplemented by irrigation with distilled water until visually confirming the absence of any remaining pulp tissue. This step ensures through the removal of pulp remnants, maintaining the integrity of the experimental procedure.

The pre- and postexperiment images were inserted into the Image J program (Bethesda, Maryland, USA), and the volumetric amount of initial and remaining tissue was obtained. With the total area of the initial tissue and the remainder, obtaining the percentage of tissue removal was possible after the activation process. The data were statistically evaluated.

Wilcoxon Mann–Whitney *U* test was used to compare the groups to detect the differences between irrigation protocols. All statistical calculations were performed using the R Software (Bell Labs, Berkeley Heights, USA), with an assumed significance level of 5%.

RESULTS

All the groups tested showed tissue removal; however, no group could completely dissolve the tissue of the samples.

Table 1 shows the reduction values of each group compared to the initial area value evaluated. All activation groups showed better results when compared to the PPG groups ($P < 0.05$). Among the 1-min groups, the best tissue removal protocols were due to the PUIG and XPG ($P < 0.05$). However, when the time was increased to 2 min, the ECG group reached the highest values of the research ($P < 0.05$), together with PUIG and XPG, with no difference between them ($P > 0.05$). The intragroup comparison shows a significant improvement in tissue removal when the number of activation cycles increased ($P < 0.05$). Figure 2 shows the de box spot of all the values.

DISCUSSION

Effective removal of pulpal tissue is essential to eliminate the potential sources of infection and guaranteeing thorough disinfection of the root canal system. This underscores the importance of precise and meticulous protocols to ensure the success of the endodontic procedure.[4,7]

In this study, no significant difference in tissue removal was found between the experimental groups with 2 min of activation; in this sense, the null hypothesis was partially accepted.

The chosen methodology allowed using the same roots in all experimental groups, ensuring homogeneity and discarding the anatomical factor as a variable.[18] Standardization of samples, such as standardization of WL, the establishment of a standard depth for irrigation with needles, similar volumes of the porcine pulp tissue, same diameter and depth of grooves, and obliteration of foramina through the use of was, avoided possible methodological biases and simulated the conditions of clinical use as closely as possible.[10,17]

The ultrasonic activation of NaOCl, through smooth inserts, is the most adopted protocol in the literature and presents satisfactory results when used in straight canals,[7,8,14] as confirmed by this study. The XP-Endo Finisher instrument proved effective concerning tissue removal, showing no statistical difference with the group with ultrasonic irrigation, as observed in previous studies.[9,12]

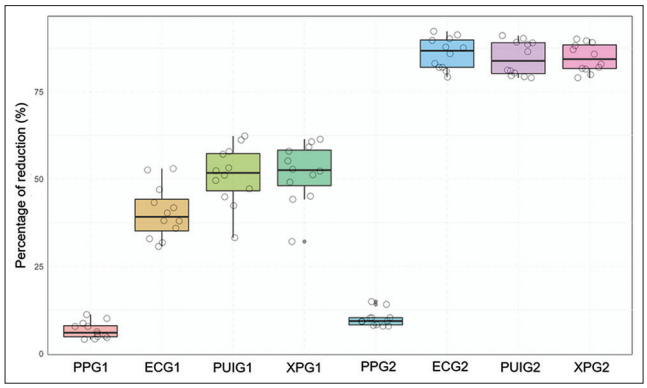

Figure 2: Box plot of the tested groups in the research, observing the percentage of reduction for each group. PPG: Positive pressure group, PUIG: Passive ultrasonic irrigation group, ECG: Easy clean group, XPG: XP-Endo group

The Easy Clean, despite showing acceptable results in the removal of debris in studies,[11] showed lower results concerning tissue removal when compared to the other two experimental groups in the time of 1 min. The justification for this result may be related to the fact that it's made of acrylonitrile butadiene styrene plastic and does not promote temperature increase similarly to the ultrasonic insert and the XP-Endo Finisher metallic instrument. Moreover, it is important to mention that protocols employing instrument agitation tend to exert greater mechanical forces than dissolution alone.[11]

An important point is the "time-volume" relationship regarding tissue removal within the root canal.[14,19] With the increase of time and in this way, using a larger volume of NaOCl 2.5% efficiently improved the cleaning of the root canal system,[20] as evidenced in this study. It can be observed that all agitation groups obtained similar performances, with no statistical difference between them, when they underwent 2 min of agitation.

The tissue dissolution capacity by the irrigating solution is related to the increase in the contact time of the solution with the tissues and their renewal.[4,7] This highlights the importance of NaOCl in tissue dissolution, as even at a concentration of 2.5%, demonstrates satisfactory tissue dissolution capacity.[21,22] In the intra-group analysis with 1 and 2 min, the improvement in the degradation capacity of the porcine pulp tissue was evident, regardless of the device used, where all experimental groups presented similar results.

So far, no irrigation protocol has demonstrated efficiency in the total removal of pulp tissue remains from the root canal system, especially in areas of recesses, isthmuses, and curved canals,[23,24] it is encouraging to continue research

Table 1: Mean, standard deviation, minimum value, maximum value, and intergroup *P* value of the research

Groups	Percentage of reduction			Intergroup (*P*)
	Mean±SD	Minimum	Maximum	
PPG 1	6.71±2.39^{A}	4.10	11.20	0.0032
PPG 2	9.99±2.29^{B}	7.90	14.90	
PUIG 1	51.04±8.35^{C}	33.20	62.30	0.0000
PUIG 2	84.60±4.88^{D}	79.00	91.10	
ECG 1	40.45±7.49^{E}	30.70	53.00	0.0000
ECG 2	86.00±4.45^{D}	79.20	92.30	
XPG 1	51.77±8.40^{C}	32.10	61.40	0.0000
XPG 2	84.74±4.01^{D}	79.00	90.10	

Different letters mean statistical differences ($P<0.05$). PPG: Positive pressure group, PUIG: Passive ultrasonic irrigation group, ECG: Easy clean group, XPG: XP-Endo group, SD: Standard deviation

for the elaboration of an ideal protocol for activating irrigation solutions and systems currently available.

The findings of the study emphasize the superiority of irrigation protocols over passive methods in clinical practice, especially those utilizing higher cycle numbers. However, it is important to acknowledge that pulp tissue within root canals is typically confined to smaller volumes and firmly attached to the tooth. In our study, efforts were made to ensure comparability by standardizing all groups consistently.

CONCLUSION

It can be concluded that the longer agitation time of the chemical solution during the final irrigation stage promoted more significant tissue removal regardless of the agitation system used.

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Conflicts of interest
There are no conflicts of interest.

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