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EFFICACY OF DIFFERENT PROTOCOLS FOR ROOT CANAL IRRIGATION ON ULTRASTRUCTURAL ASSESSMENT OF MOLARS' DISTAL ROOT CANAL CLEANING

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ABSTRACT

Effective cleaningofroot canals was evaluated.One hundred distal canals of lower molars were instrumented with R50 and divided into 10 groups with an antimicrobial, a chelator and an agitation device. The groups were: G1 (CHX 2% + EDTA 17% + Canal brush); G2 (CHX 2%+ EDTA 17% + EasyClean); G3 (CHX 2% + EDTA 17% + Irrisonic); G4 (CHX 2%+ EDTA 17% / Canal brush + EasyClean); G5 (CHX 2%+EDTA 17% / Canal brush + Irrisonic); G6 (NaOCl 2,5%+EDTA 17% / Canal brush); G7 (NaOCl2,5%+ EDTA 17%/ EasyClean); G8 (NaOCl 2,5%+ EDTA17%/ Irissonic); G9 (NaOCl 2,5%+EDTA 17% / Canal brush + EasyClean) and G10 (NaOCl 2,5%+EDTA 17% / Canal brush + Irrisonic). Three cycles of 20 second agitation were performed, with 2 ml of each substance. The roots were longitudinally sectioned for scanning electron microscopy analysis.Statistical difference was observed betweenthecervical and apical thirds in G4, G5 and G6 ($p<0.05$); and betweenthe middle and apical thirds in G2 ($p<0.05$). None of the protocols ensured effective cleaning of the root canal, and in all groups the apical portion was more critical to clean.

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INTRODUCTION

The mechanical action of manual or mechanized instruments, along the walls of the root canal during chemical-mechanical preparation, produces scrapings and debris, and these are deposited along the main canal and in its anatomical complexities (Paque *et al*. 2011; Kamel *et al*. 2014; Mendonça *et al*. 2015; Schmidt *et al*. 2015) forming a thick layer, called "smear layer" (Prado *et al*. 2016; Duque *et al*. 2017; Leoni *et al*. 2017). This layer, when not removed, can interfere in the penetration of intra-canal medication, as well as in the sealing action of the root canal sealer, or even in the perpetuation of intra-radicular infections [4], having the potential to cause endodontic failure (Silva *et al*. 2019; Torabinejad *et al*. 2013). As it is not possible to reach all these regions with the mechanical action of the instruments (Pérez *et al*. 2018; Versiani *et al*. 2018), it is necessary to use chemical substances, with disinfectant and lubricant action, and cleaning agents, helping to eliminate and neutralize bacteria and their by-products (Siqueira *et al*. 2013; Justo *et al*. 2014). However, it is already known that the use of conventional irrigation is inefficient fordebris removal (Kamel *et al*. 2014; Schmidt *et al*. 2015), and thatenlargement of the apical thirdaffects directly the penetration of the irrigating agent to reach its full working length (Caron *et al*. 2010; Srikanth *et al*. 2015). Therefore, the antimicrobial action of the irrigating solution (Justo *et al*. 2014; Zargar *et al*. 2015) in association with devices that improve the irrigation is fundamental (Van der Sluis *et al*. 2010; Prado *et al*. 2016; Duque *et al*. 2017; Leoni *et al*. 2017). The anatomy of the distal canals, in the vast majority, presents only a conduit, wide, but quite flattened, often in the form of a tape, with mesial and distal poles of difficult access for instrumentation. Manual or mechanized action just in the canal central area, and the regions that are without instrumentation, around 35%, require solid strategies, aiming at complementing this preparation (Justo *et al*. 2014). Several protocols using agitation devices are being studied, obtaining good results, and the use of ultrasound inserts, plastic devices, adjustable files (Van der Sluis *et al*. 2010; Kato *et al*. 2016; Leoni *et al*. 2017; Silva *et al*. 2019) and canal brushes (Kamel *et al*. 2014) are among them. However, there are no studies in the literature evaluating the use of these devices separately or in association with different irrigation solutions in the cleaning of root canals. Thus, this study

aimed to evaluate final root canal cleaning protocols by scanning electron microscopy, using the chemicals Sodium Hypochlorite 2.5% (NaOCl) (Asfer Chemical Industry, São Caetano do Sul, São Paulo, Brazil), Chlorhexidine gel 2% (CHX) (Pharmacy Biophormula, Fortaleza, Ceará, Brazil) and Ethylenediaminotetraacetic Acid 17% (EDTA) (Biodynamic Pharmacy, Santa Rosa de Viterbo, Paraná, Brazil), associated with the irrisonic agitation devices (Helse Dental Technology, Santa Rosa de Viterbo, São Paulo, Brazil), Easy Clean (EASY Dental Equipment, Belo Horizonte, Minas Gerais, Brazil), and Intracanal Brush (MK Life, Porto Alegre, Rio Grande do Sul, Brazil), used individually or jointly for improvements in the smear layer removal process. The null hypothesis considered was that all protocols present similar cleaning ability.

MATERIALS AND METHODS

This research was approved by the Human Research Ethics Committee.A hundred human permanent lower molars were selected, extracted for reasons unrelated to this research, with complete root formation, without calcifications or resorptions, with only 1 distal root canal (Vertucci's Type I Classification), donated by the CEO-Centro, in the city of Fortaleza-CE. The Vertucci's type I classification were verify two times, with X-ray, and after de acess with a manual hand file. After access with spherical diamond tip in high rotation FG 1014 (KG Sorensen, Cotia, Brazil) and the ENDO ZK drill (JET, France), only canals with foraminal diameter between 200 µm and 300 µm were selected. All root canals were instrumented with the Reciproc® system (VDW®, Munich, Germany) with the R50 file in the forame (0.0), with the VDW Silver engine (VDW®, Munich, Germany), in the RECIPROC ALL program, by the same operator. The root canals were randomly divided into 2 groups (n=50), and protocols for agitation and final cleaning of the root canal were created with an antimicrobial, a chelator and an agitation device. The chemical substance used were Sodium Hypochlorite 2.5% (NaOCl + EDTA 17%) and Chlorhexidine Gel 2% (CHX+ EDTA 17%). Were created them 10 subgroups (n=10) with the final protocols of cleaning of the root canal.

Protocols for agitation and final cleaning of the root canal:

G1 - CHX + EDTA + Canal Brush (CB)
G2 - CHX + EDTA+ Easy Clean (EC)
G3 - CHX + EDTA + Irrisonic (IR)
G4 - CHX + EDTA + Canal Brush/Easy Clean
G5 - CHX + EDTA + Canal Brush/Irrisonic
G6 - NaOCl + EDTA+ Canal Brush
G7 - NaOCl + EDTA + Easy Clean
G8 - NaOCl + EDTA + Irrisonic
G9 - NaOCl + EDTA + Canal Brush/Easy Clean
G10 - NaOCl + EDTA+ Canal Brush/Irrisonic

Passive ultrasonic irrigation (PUI) was performed using the Irrisonic insert (20/0.01), coupled to the Piezon Master 200 ultrasound (EMS, Vallée de Joux, Switzerland) at power 2, and 2 mm from the foramen. Easy Clean (25/0.04) was used in the Reciprocation program, in the VDW Silver electric motor, in the WaveOne function, distancing 2 mm from the foramen. The Canal Brush was used in the Rotary program, in the Dr's function in the same electric motor, with 1.5 N and 350 RPM, in the cervical and middle thirds. Three 20-second cycles (20 s) were performed by each agitation devices, with 2 ml of each chemical substance, totaling 6 ml used (CHX/NaOCl + EDTA) and 2 minutes of agitation. Prior to the use of EDTA, all canals were irrigated with 2 ml of 0.9% saline solution. The use of disposable syringes and the use of the 24 G needle (InjexIndustriasCirurgicas, Ourinhos, Minas Gerais, Brazil) were recommended, distancing 2 mm from the foramen (0.0), and at the end of the protocols, irrigation with 5 ml of 0.9% saline solution was carried out. The root apices were sealed withadhesive wax (Technew, Rio de Janeiro, RJ, Brazil), before the protocols started, to prevent extrusion of irrigant agents during chemical-mechanical preparation.

Evaluation methodology: The analysis methodology used in the present study was based on and analysis reported by Prado *et al*. (2015). Aftercompletingthe final cleaning protocols described, the samples were dried with medium-sized paper points (Odous de Deus, Belo Horizonte, MG, Brazil) and the distal roots were longitudinally cleaved with a hammer and chisel. The most visible root half was selected for further analysis. The halves were metallized in the Denton Desk II sputter coating system (Denton Vacuum, New Jersey, USA) and examined using scanning electron microscopy (SEM) (JOEL JSM model 5410; JEOL Ltd., Tokyo, Japan). The total root length was divided by 3, considering the cervical third 2 mm below the entrance of the canal, middle third to half of the total length and apical third 2 mm above the apical foramen. Three representative images were made, with a 1000x increase, one of the cervical third, another of the middle third, and one apical, and these were arranged in slides in the PowerPoint program® (Windows 10), unidentified and evaluated by 2 independent blinded researchers.Scores were established according to wall cleaning, smear layer removal and dental tubules exposure, being considered score 0 – for cleaning between 100% and 75% of the walls; score 1 - between 75% and 50%; score 2 - 50% and 25% and score 3 - 25% to 0% cleaning and exposure of tubules (Fig. 1).

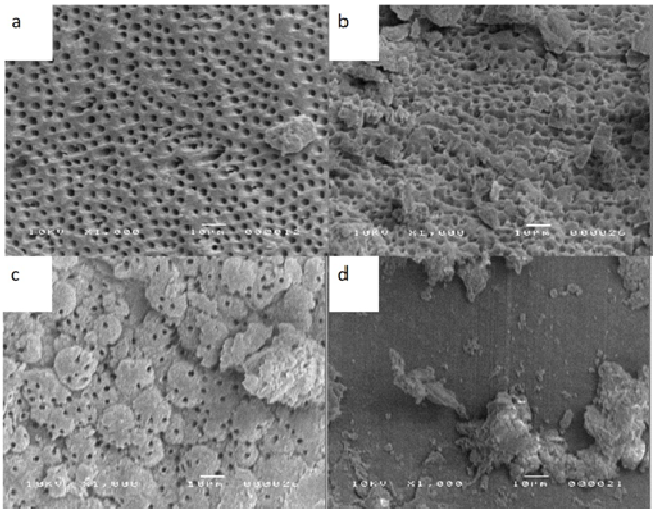

Fig. 1. Representative images of the scores established score 0 (a); 1 (b); 2 (c) and 3 (d) respectively

Statistical analysis: The reliability of the inter-examiner result for the SEM evaluation was assessed using the Cohen kappa coefficient. The data were analyzed using the Kruskal-Wallis and Friedman and Dunn tests with a 5% significance index.

RESULTS

The kappa test showed high agreement between the observers (0.85). The data obtained were analyzed between the groups, taking into account each third, cervical, middle and apical regions. There was no statistical difference between the groups in any of the analyzed thirds, regardless of the chemical substance used or the agitating device used. Maximum and minimum values are expressed in Table 1. In the intra-group analysis, where the thirds were compared within the same group, there was a statistically significant difference ($p<0.05$) in the G2 group, between the middle and apical thirds, where the middle third was cleaner (Fig. 2), considering the score 0, there was no difference between the cervical and middle or cervical and apical thirds. There was also a statistically significant difference between the cervical and apical thirds in groups G4, G5 and G6, with better results for cleaning and exposure of dentinal tubules in the cervical third (Fig. 3). There was no statistical difference between the thirds in the other groups analyzed.

Table 1. Median, Minimum and Maximum root canal cleansing scores, after final cleaning protocol; C (cervical third); M (middle third) and A (apical third)

		G1: CHX +CB	G2: CHX + EC	G3: CHX + IR	G4: CHX +CB/EC	G5: CHX +CB/IR	G6: NaOCl +CB	G7: NaOCl +EC	G8: NaOCl+IR	G9: NaOCl +CB/EC	G10: NaOCl+ CB/IR
C	Median	2,0 $^{A, a}$	1,5 ^{A,ab}	1,5 $^{A, a}$	1,0^{A,a}	2,0^{A,a}	1,0^{A,a}	2,0^{A,a}	1,0^{A,a}	1,5^{A,a}	1,5^{A,a}
	Min/Max	0/3	0/3	0/3	0/3	0/3	0/2	0/3	0/3	0/3	0/3
M	Median	2,0 $^{A, a}$	1,5^{A,a}	2,5 $^{A, a}$	1,5^{A,ab}	3,0^{A,ab}	2,0^{A,ab}	2,0^{A,a}	2,0^{A,a}	2,0^{A,a}	2,0^{A,a}
	Min/Max	0/3	0/3	1/3	0/3	1/3	0/3	1/3	0/3	0/3	0/3
A	Median	1,0 $^{A, a}$	2,5^{A,b}	3,00 $^{A, a}$	2,0^{A,b}	3,0^{A,b}	2,5^{A,b}	3,0^{A,a}	2,0^{A,a}	2,0^{A,a}	2,0^{A,a}
	Min/Max	0/3	1/3	1/3	0/3	2/3	1/2	1/3	0/3	0/3	1/3

Legend: Uppercaseletters represent intergroup differences, and in each third; lowercase letters depict intragroup analysis, between thirds. Different letters represent statistical difference (p>0.05).

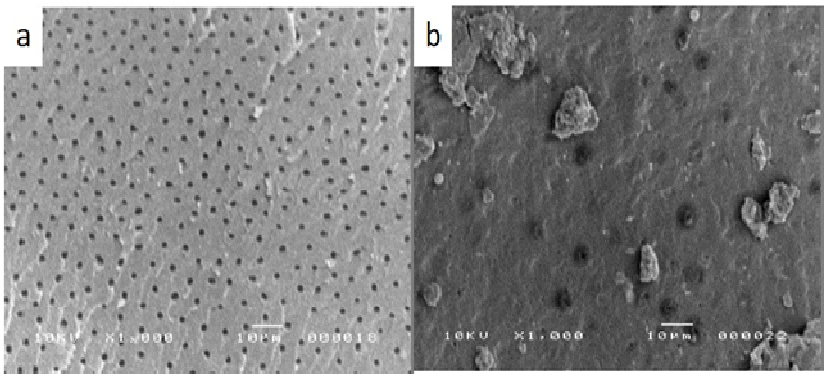

Fig. 2. Representative images of CHX + EC with statistical difference between the Middle (a) and Apical third (b)

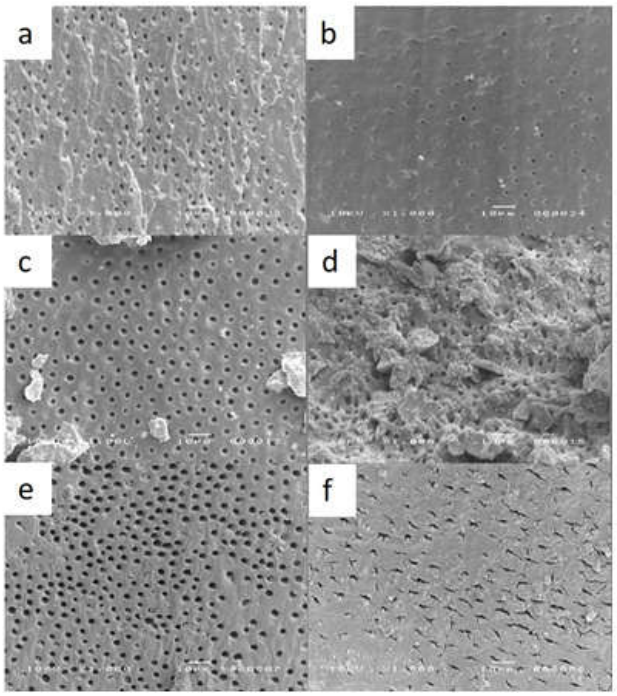

Fig. 3. Representative images of CHX + CB/EC (a,b), CHX + CB/IR(c,d) and NaOCl + CB (e,f), respectively, with statistical difference between cervical (column 1) and apical thirds (column 2).

DISCUSSION

The use of different protocols and cleaning devices is essential to obtain the maximization of root canal cleaning and disinfection. Therefore, the objective of this work was to analyze final cleaning protocols of the root irrigation, using the chemicals NaOCl 2.5% or CHX 2% plus to EDTA 17%associated to the supplementary agitation devices,Irrisonic, Easy Clean and Intracanal Brush, by means of SEM. Similar studies have been previously conducted using a similar number of samples and methodology of analysis by SEM in extracted human teeth (Caron *et al*. 2010; Kamel *et al*. 2014; Mendonça *et al*. 2015; Kato *et al*. 2016; Prado *et al*. 2016). The analysis of the images was performed independently by blind operators, and the scores were established regarding the cleaning of walls, removal of smear layer and opening of dentinal tubules, as well as in other studies already described (Caron *et al*. 2010; Kato *et al*. 2016; Prado *et al*. 2016;Charlie *et al*. 2018). The irrigation protocol used in this study aimed to ensure the standardized volumeand frequency of irrigating solution to ensure a suitable root canal cleaning,and this has been reproduced in numerous studies, proving its efficacy (Justo *et al*.

2014; Cesario *et al*. 2018). This studyused the R50 file during root canal preparationof distal canals of mandibular molars, and 24 G caliber hypodermic needle, ensuring that the irrigant agentreached the middle and apical third of the root canal. Previous studies suggested a minimum enlargement of the apical third, with the file #35 (Van der Sluis *et al*. 2010; Srikanth *et al*. 2015; Silva *et al* 2019). Our study, followed the manufacturer's instruction regarding the sample selection, making use of files smaller or equal to #30 thus the R50 file was selected. The use of an irrigating solution with antimicrobialand bacteriostatic properties is a fundamental condition for endodontic treatment. In the present study, the chemical substances CHX and NaOCl were considered, since both have antimicrobial action proven in the literature (Sassone *et al*. 2008; Zandi *et al*. 2019;), being NaOCl most commonly used, due to its solvent properties (Sassone *et al*. 2008; Charlie *et al*. 2018). CHX, when used, is due to its substantiality and rheological capacity, due to the association of natrosol gel (Caron *et al*. 2010).The most commonly used irrigation protocols for the removal of the smear layer make use of NaOCl in its various concentrations, for removal of the organic component from the layer, and complementation with chelating agents such asEDTA 17% to remove inorganic components. In our study, regardless of the chemical substance used in association with EDTA 17%, there was no statistical difference between the groups, a similar report was also found in the literature (Jimna *et al*. 2017).

Similar results were also observed in other studies in the literature, taking into account the 2 substances, (Justo *et al*. 2014; Charlie *et al*. 2018) or even, using only hypochlorite, in the form of solution and gel (Zandi *et al*.2019) where there was no difference between the groups in the removal of the smear layer in any of the thirds. Different results were found(Charlie *et al*. 2018) when the substances were used independently in the groups, with EDTA 17%, getting the best results, in relation to CHX and NaOCl, demonstrating that EDTA 17% has great value in the final canal cleaning protocols. It should be emphasized that in our study, NaOCl was inactivated with 2 ml of physiological serum, before the use of EDTA 17%. In relation to agitation devices, Irrisonic, Easy Clean and canal brush were used, which were previously described in the literature (Van der Sluis *et al*.2010; Kamel *et al*.2014; Kato *et al*.2016; Silva *et al*.2019), however, these tools were never reported to be used jointly and with variation of the chemical substance. The vast majority of studies concluded that the use of ultrasound (PUI) presents the best results (Paragliola *et al* 2010; Prado *et al*. 2016;) in terms of final cleaning of the canal, with the exception of one study(Kato *et al*.2016), where Easy Clean presented the best results in the apical third, when used in the reciprocal movement, as well as our study. In a recent study (Cesário *et al*. 2018), Easy Clean in rotational motion obtained results similar to those by PUI and when in reciprocal movement its results were similar to those found in conventional irrigation, but with artificial and flattened canals. In our study, in the analysis between the groups, there was no statistical difference between the groups, in any of the analyzed thirds, regardless of the agitating device and the chemical substance employed. Thus, the null hypothesis was accepted, corroborating other similar findings (Silva *et al*. 2019). In the intra-group analysis, that is, comparing the thirds of the canal, within the same group, there was a statistical difference between the middle and apical thirds only in one group (G2), where CHX and EDTA 17% were used in association with Easy Clean, where the middle third presented higher exposure of dentinal tubules and cleaning of the walls. However, there was no difference between the cervical and apical thirds. In groups G4, G5 and G6, there was a relevant statistical difference between cervical and apical thirds, where the last third had the worst scores in the 3 groups, a similar report was also found in the literature (Kamel *et al*. 2014). This result can be explained by the use of the intracanal brush in the 3 groups, since it has its largest area of activity in the cervical third, performing mechanical cleaning, through its friction against the walls of the root canals. The chemical substance did not seem to be relevant, considering that G4 and G5 alsoused CHX and EDTA 17%, while the G6 group usedNaOCl and EDTA 17%. There were no statistical differences in the other groups, between the thirds, however, the apical third presented the worst scores in all groups analyzed. It should be emphasized that the apical third presents many anatomical complexities, as well as a smaller diameter when compared to the other thirds, thus hindering the arrival of the irrigator, cleaning and removal of the smear layer in the region, similar results are already found in the literature (Kamel *et al*. 2014; Prado *et al*. 2016; Silva *et al*. 2019). However, in a study with bovine teeth and enlargement using the File K #80, the use of ultrasound showed the best results, in the apical third regardless of the chemical substance used (Justo *et al*. 2014).

CONCLUSION

Inevitably the instrumentation of the root canal promotes the production of scrapings inside it, and final irrigation protocols are necessary in order to increase the cleaning capacity of the root canal system. Through this study, it was possible to verify that none of the protocols promoted effective cleaning in all thirds, and in all groups the apical portion was more critical, regardless of the agitation technique. The chemical substances used did not interfere in the cleaning of the canals. However, more studies are still necessary to qualify and improve the final cleaning of root canals.

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