UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

Flavia Alfredo Plazza

Avaliação de protocolos de Irrigação Ultrassônica Passiva na remoção de detritos do canal radicular

Araçatuba

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Orientador: Prof. Tit. Dr. João Eduardo Gomes Filho

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(John Ruskin)

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RESUMO

Introdução: O objetivo deste estudo foi avaliar a eficácia de diferentes protocolos de Irrigação Ultrassônica Passiva (PUI) por meio de microscopia eletrônica de varredura (MEV).

Métodos: Seis cavidades foram confeccionadas ao longo da seção vestibular de 20 canais radiculares de pré-molares inferiores humanos e preenchidas com detritos. 10 grupos foram criados de acordo com o protocolo final de irrigação: Grupo 1- controle positivo (sem detritos); Grupo 2- controle negativo (com detritos); Grupo 3 - irrigação convencional com hipoclorito de sódio a 2,5% (NaOCl); Grupo 4 - irrigação convencional com 17% de ácido etilenodiaminotetracético (EDTA) seguido de NaOCl; Grupo 5- PUI com 3 ciclos de 20 segundos (NaOCl-NaOCl-NaOCl); Grupo 6- PUI com 3 ciclos de 20 segundos (NaOCl-EDTA-NaOCl); Grupo 7- PUI com um ciclo de 60 segundos (NaOCl); Grupo 8 - PUI com ciclo de 180 segundos (NaOCl); Grupo 9 - PUI com 2 ciclos de 60 segundos (EDTA-NaOCl); Grupo 10 - PUI com 2 ciclos de 60 segundos (NaOCl-EDTA). Os espécimes foram observados em MEV para a análise da remoção dos detritos (scores 1 a 5) e exposição dos túbulos dentinários (score 1 ou 2) dentro das cavidades. O teste de Kruskal-Wallis foi realizado para a análise estatística, com significância de 5%.

Resultados: Os grupos PUI apresentaram melhores resultados na limpeza de detritos do que os grupos de irrigação convencionais nas cavidades 1, 2 e 3. As cavidades 4, 5 e 6 foram limpas em todos os grupos experimentais. Em relação à exposição dos túbulos dentinários, os grupos PUI foram capazes de alcançar um resultado melhor do que os grupos de irrigação convencional.

Conclusões: A PUI mostrou-se mais eficaz na remoção de detritos do que a irrigação convencional nas cavidades localizadas na região apical do canal radicular, e os grupos PUI promoveram maior exposição dos túbulos dentinários independentemente do uso de EDTA como agente quelante para tecidos inorgânicos.

Palavras-chave: irrigação ultrassônica passiva, detritos, hipoclorito de sódio, MEV.

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ABSTRACT

Introduction: The aim of this study was to evaluate the effectiveness of different Passive Ultrasonic Irrigation (PUI) protocols by means of scanning electron microscopy.

Methods: Six hemispherical cavities were made along the buccal section of 20 root canals of human lower premolars and filled with debris. 10 groups was created according to the final irrigation protocol: Group 1- positive control (no debris); Group 2- negative control (with debris); Group 3- conventional irrigation with 2.5% sodium hypochlorite (NaOCl); Group 4- conventional irrigation with 17% ethylenediamine tetracetic acid (EDTA) followed by NaOCl; Group 5- PUI with 3 cycles of 20 seconds (NaOCl-NaOCl-NaOCl); Group 6- PUI with 3 cycles of 20 seconds (NaOCl-EDTA-NaOCl); Group 7- PUI with a 60 seconds cycle (NaOCl); Group 8- PUI with a 180 seconds cycle (NaOCl); Group 9- PUI with 2 cycles of 60 seconds (EDTA-NaOCl); Group 10- PUI with 2 cycles of 60 seconds (NaOCl-EDTA). Scores were given for the analysis of debris removal in SEM, as well as for the exposure of dentinal tubules within the cavities. Kruskal-Wallis test was performed for statistical analysis.

Results: The PUI groups presented better results in cleaning of debris than the conventional irrigation groups in cavities 1, 2 and 3. Cavities 4, 5 and 6 were well cleansed in all experimental groups. Regarding the exposure of the dentinal tubules, the PUI groups were able to achieve a better result than the conventional irrigation groups.

Conclusions: Passive ultrasonic irrigation proved to be more effective in the removal of debris than conventional irrigation in the cavities located in the apical region of the root canal, and the PUI groups promoted greater exposure of the dentinal tubules independently of the use of EDTA as a chelating agent for inorganic tissues.

Keywords: passive ultrasonic irrigation, debris, sodium hypochlorite, SEM

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Evaluation of Passive Ultrasonic Irrigation protocols in the removal of root canal debris.

1 Introduction

The removal of root canal debris is a challenge in endodontics, with the irrigation stage being fundamental to reach this objective, favoring the cleaning of regions that the instruments can not reach[1]. Conventional irrigation is the most used method, but it is inefficient for cleaning the apical portion of the root canal and regions such as isthmus[2]. Passive Ultrasonic Irrigation has been used as an attempt to increase cleaning of the root canal system [3].

Passive Ultrasonic Irrigation consists in the use of an ultrasonic device to promote an increase of the movement of the solution within the root canal through ultrasonic waves produced by acoustic energy[3]. The effect of this movement facilitates the penetration of the irrigating solution into the irregularities and the apical portion of the root canal, since these debris are more easily displaced, thus providing greater cleaning[4].

Although PUI promotes a potentially beneficial effect on cleaning the apical third of root canals by facilitating the removal of debris, the literature shows work with conflicting results, since removal was more efficient than conventional removal in some studies[5,6], while it showed no difference in others[7,8]. One of the possibilities to explain these findings is the fact that the PUI protocol is not yet standardized, ranging from the type of irrigant used, the concentration of the solution to the time of application of the ultrasonic device.

Some authors used the PUI with an intermittent activation of 3 cycles of 20 seconds for each cycle[9,10,11]. On the other hand, others used a continuous activation of only one cycle of 60 seconds[12,13,14]. Continuous activation of the 3-min ultrasound has also been proposed[6,15]. In addition, there is still no standard regarding the use of EDTA in the ultrasonic activation protocol. There are studies that did not include it in the PUI protocol[7,16]. Differently from others who included it in the protocol[17,18]. The efficacy of the use of EDTA in the PUI protocol has been

controversial in literature. For some authors, the efficiency of the debris removal with the use of EDTA in the PUI protocols[8,19] was increased, while other authors did not obtain the same results, showing no difference in results with and without EDTA.[17,20].

The aim of this study was to evaluate the effectiveness of the various types of protocols with the use of PUI in removal of debris in artificial indentations.

The null hypothesis was proposed that the action of Passive Ultrasonic Irrigation would not be more effective than conventional irrigation, and there would be no difference between the different types of PUI protocols evaluated.

2 Methods

Twenty healthy human lower premolars with roots with curvature between 15º and 20º[21] were extracted from patients requiring orthodontic treatment or periodontal disease. All teeth presented only one root and root canal.

Teeth preparation was done according to the modified Kato methodology[22]. All teeth were worn horizontally with a diamond disk to a standard length of 19 mm. The canals were instrumented with the ProDesign Logic (Easy Dental Equipment, Belo Horizonte, Brazil) rotary system, with a working length of 18 mm. Initially a K#10 file was inserted into the working length, followed by a Glide Path 40/01 file in the same length using the rotating motor (Easy Dental Equipament, Belo Horizonte, Brazil) and finalized with a final modeling file 40/05. 5ml of 2.5% NaOCl was irrigated with each instrument change.

After instrumentation, two longitudinal grooves were made with a 0.08 diamond disk under a magnification of eight times in a dental operating microscope (DF Vasconcelos, São Paulo, Brazil) to a depth close to the root canal. The roots were embedded in a heavy-bodied silicone (Optosil Comfort Putty, Heraeus Kulzer GmbH, Hanau, Germany), which prevented the extrusion of the irrigant and simulated a closed irrigation and aspiration system up to the cement-enamel junction level. After being embedded, a #24 spatula (SSWhite Duflex, Rio de Janeiro, Brazil) was used in the previously made groove and a

vertical force was applied to divide the sample into two halves. The vestibular part was removed and 6 hemispherical cavities of predefined levels (C1, C2, C3, C4, C5 and C6) of approximately 0.05 mm deep, spaced 1 mm from the apex of the tooth with a flame-shaped amalgam polishing drill in low speed (Wilcos Dental Products, Petrópolis – Rio de Janeiro, Brazil). The specimens were washed for 1 minute in running water to remove the debris.

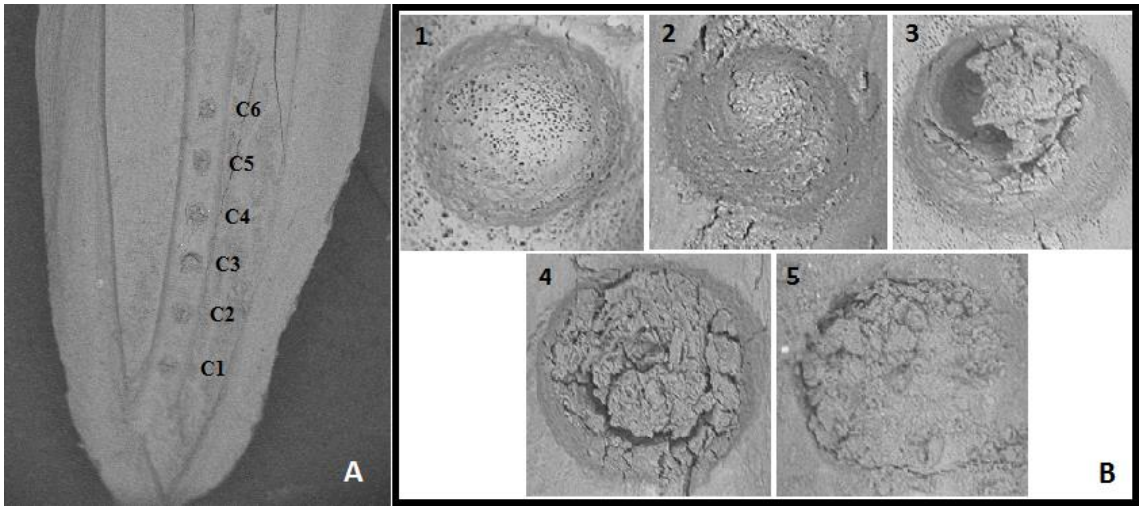

Figure 1: A – SEM image of the cavities. All hemispherical cavities shown are 1mm apart. B – Representative images of scores (1, 2, 3, 4 and 5).

The specimens were mounted on the silicone system after the cavities were filled with the debris. The debris was prepared according to the modified protocol of Martins Justo[19] with a mixture of 0.025g of dentin debris to 0.1ml of 2.5% NaOCl for 5 minutes.

The study was composed of 10 groups elaborated from the final irrigation protocols most evidenced in the literature, described as follows.

Group 1: Positive control. The specimens were immersed in an ultrasonic bath containing 2.5% NaOCl solution for 3 minutes and then in 17% EDTA for 3 minutes. The samples were then washed with distilled water for 1 minute and dried in the oven at 80°C for 3 minutes. In the SEM, the cavities were confirmed to be completely free of debris.

Group 2: Negative control. The specimens were prepared in the same manner as the Positive Control Group and the artificial cavities were filled with debris and, without receiving any type of irrigation, were analyzed in the SEM.

Group 3: Conventional irrigation (CI) with 2.5% NaOCl. Irrigation of 15 ml of 2.5% NaOCl was performed.

Group 4: CI with 17% EDTA and 2.5% NaOCl. A 5ml irrigation of 2.5% NaOCl was performed, followed by 5ml of 17% EDTA and 5ml of 2.5% NaOCl.

Group 5: PUI with 3 cycles of 20 seconds using 2.5% NaOCl. In this group, all cycles were performed with 5ml of 2.5% NaOCl.

Group 6: PUI with 3 cycles of 20 seconds using 2.5% NaOCl and 17% EDTA. The first cycle was performed with 5ml of 2.5% NaOCl, the second cycle with 5ml of 17% EDTA followed by a new cycle with 5ml of 2.5% NaOCl.

Group 7: PUI with 1 cycle of 60 seconds, using 15ml of 2.5% NaOCl.

Group 8: PUI with 1 cycle of 180 seconds, using 15ml of 2.5% NaOCl.

Group 9: PUI with 2 cycles of 60 seconds, the first cycle being performed with 5ml of 17% EDTA and the second cycle with 10ml of 2.5% NaOCl.

Group 10: PUI with 2 cycles of 60 seconds, the first cycle being carried out with 10ml of 2.5% NaOCl followed by a new cycle with 5ml of 17% EDTA.

PUI was performed with modification of the previously described technique[23]. An Irrisonic Power tip (Helse Industria e Comercio, Santa Rosa de Viterbo, Brazil) was mounted on a Gnatus ultrasonic handpiece (Medical-Dental Equipment, Brazil), adjusted at power 1, and placed 1mm short of the working length. The technique was modified by the oscillation of the ultrasound that was performed in the vestibular-lingual direction, based on previous work demonstrating the greater cleaning efficiency[23].

The experimental groups received a final rinse of 5 ml of saline. Groups 1, 4, 5, 8 and 9 were made in ten teeth, randomly selected. Ten other teeth were used to perform the protocols of groups 2, 3, 6, 7 and 10 (figure 2). Therefore, each tooth was reused five times, based on previous methodologies that showed

that the same specimen can be used more than 5 times without causing any damage to dental structure[23].

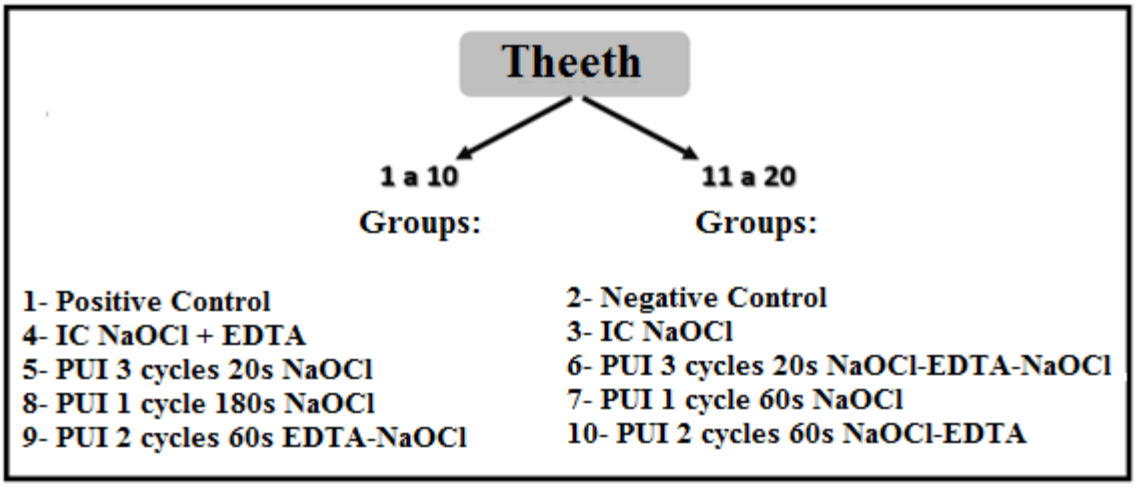

Figure 2: Distribution of the teeth in relation to the control and experimental groups.

The analyzes were performed on the images obtained in a SEM of low vacuum (PSEM, Express™, Aspex Corporation, Delmont, PA) of indentations with a 500-fold increase and 20kV. The images were classified by score according to the amount of debris present in each cavity, based on methodology already established[22]: score 1- without debris with exposure of the dentinal tubules; score 2- without debris without exposure of the dentinal tubules; score 3 - present debris covering an area smaller than 50% of the dentinal tubules; score 4- present debris covering an area greater than 50% of the dentinal tubules; score 5- dentinal tubules entirely covered by debris.

Each image obtained was coded according to the group (negative control, positive control and experimental groups), the tooth (from 1 to 10) and the level at which the reading was performed (C1, C2, C3, C4, C5 or C6). All images from the control and experimental groups were shown to two independent examiners, previously calibrated and blinded for the study, in which they marked the images according to the evaluation criteria described previously.

For the statistical analysis, the kappa test was used in the inter-examiner concordance analysis. The Kruskal-Wallis test was used to compare data on cleaning effectiveness. Statistical calculations were performed using GraphPad Prism 5.0 software. The level of significance was 5%.

3 Results

3.1 Systems x Samples

A statistical analysis was performed of the cleaning ability of the protocols in relation to the sample, including all cavities in the analysis (table 1). The conventional irrigation groups (groups 3 and 4) were superior to the negative control (group 2). All groups using passive ultrasonic irrigation (groups 5, 6, 7, 8, 9 and 10) were superior to the negative control groups (group 2) and conventional irrigation (groups 3 and 4), and similar to the positive control (group 1).

Concerning the exposure of the dentinal tubules (Table 1), conventional irrigation groups (groups 3 and 4) did not promote their cleaning. In contrast, the PUI groups (groups 5, 6, 7, 8, 9 and 10) were efficient in this respect, regardless of the use of EDTA.

Table 1: Analysis of the cleaning achieved with each protocol in relation to the samples and in relation to the dentinal tubules ($p < 0.05$).

	Cleaning (debris removal)	Cleaning (opening of tubules)
1 Positive Control	1^{b}	1^{bc}
2 Negative Control	4^{c}	2^{a}
3 (IC NaOCl)	2^{a}	2^{a}
4 (IC EDTA)	3^{a}	2^{a}
5 (3x20s NaOCl)	1^{b}	1^{b}
6 (3x20s EDTA)	1^{b}	1^{c}
7 (1x60s NaOCl)	1^{b}	1^{c}
8 (1x180s NaOCl)	1^{b}	1^{bc}
9 (2x60s EDTA+NaOCl)	1^{b}	1^{bc}
10 (2x 60s NaOCl+EDTA)	1^{b}	1^{c}

* The numbers are related to the median of each group. Different overlapping letters indicate statistical difference between groups (analysis by column).

3.2 Systems x Cavities

Each of the 6 cavities for each sample was evaluated according to the degree of cleaning of the debris obtained with the different protocols (table 2).

Table 2: Analysis of each cavity in relation to the protocols used (p <0.05).

	CAV1	CAV2	CAV3	CAV4	CAV5	CAV6
1 (controle positivo)	1^{Ab}	1^{Aa}	1^{Ab}	1^{Aa}	1^{Aa}	1^{Aa}
2 (controle negativo)	4^{Ac}	4^{Ab}	4^{Aa}	4^{Ab}	4^{Ac}	4^{Ab}
3 (IC NaOCl)	3^{Aac}	2.5^{ABabc}	2^{ABa}	2^{ABab}	$1,5^{Bab}$	1^{Ba}
4 (IC EDTA)	3^{Aac}	3^{ABbc}	3^{ABa}	1^{Ba}	2^{Bbc}	$1,5^{Ba}$
5 (3x20s NaOCl)	1^{Aab}	1^{Aac}	1^{Ab}	1^{Aa}	1^{Aa}	1^{Aa}
6 (3x20s EDTA)	1^{Ab}	1^{Aa}	1^{Ab}	1^{Aa}	1^{Aa}	1^{Aa}
7 (1x60s NaOCl)	1^{Ab}	1^{Aa}	1^{Ab}	1^{Aa}	1^{Aab}	1^{Aa}
8 (1x180s NaOCl)	1^{Ab}	1^{Aa}	1^{Ab}	1^{Aa}	1^{Aa}	1^{Aa}
9 (2x60s EDTA+NaOCl)	1^{Ab}	1^{Aa}	1^{Ab}	1^{Aa}	1^{Aa}	1^{Aa}
10 (2x60s NaOCl+EDTA)	1^{Ab}	1^{Aa}	1^{Ab}	1^{Aa}	1^{Aa}	1^{Aa}

* Analysis was performed vertically (lower case letters) and horizontally (upper case letters). Different overlapping letters indicate statistical difference between the groups.

By comparing the cavities, it was observed that all the PUI protocols provided better cleaning than the negative control group and the CI groups (p <0.05). Cavities 1 and 2 have a higher cleaning with PUI protocols. The group of 3 cycles of 20 seconds using only NaOCl (group 5) showed no significant difference in relation to the CI groups (groups 3 and 4). In addition, CI groups were similar to the negative control group (group 2). Thus, for cavity 1 and 2, the sequence of the best for the worst group was 10=9=8=7=6=5=1<3=4<2. In cavity 3 all PUI groups were superior to the conventional irrigation groups (groups 3 and 4), which in turn were similar to the negative control group (group 2). Cavities 4, 5 and 6 showed effective cleaning with all groups, including conventional irrigation groups. The conventional irrigation group with NaOCl (group 3) did not show statistical difference of the negative control group (group 2) in cavity 4, but did not show difference between the other groups. The PUI groups (groups 5, 6, 7, 8, 9 and 10) obtained a similar result to the positive control group (group 1) in all cavities.

Regarding the exposure of the dentinal tubules of the cavities, the groups using the PUI were effective independent of EDTA being statistically similar to the positive control group (p> 0.05). CI groups were not effective in clearing the dentinal tubules of the wells and were statistically similar to the negative control group (p> 0.05) (Table 3).

Table 3: Analysis of the dentin tubule cleavage of each protocol in relation to the samples ($p < 0.05$).

	CAV1	CAV2	CAV3	CAV4	CAV5	CAV6
1 (controle positivo)	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}
2 (controle negativo)	2^{a}	2^{a}	2^{a}	2^{a}	2^{a}	2^{a}
3 (IC NaOCl)	2^{a}	2^{a}	2^{a}	2^{a}	2^{a}	2^{a}
4 (IC EDTA)	2^{a}	2^{a}	2^{a}	2^{a}	2^{a}	2^{a}
5 (3x20s NaOCl)	$1,5^{ab}$	1^{ab}	1^{ab}	1^{ab}	1^{ab}	1^{ab}
6 (3x20s EDTA)	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}
7 (1x60s NaOCl)	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}
8 (1x180s NaOCl)	1^{ab}	1^{ab}	1^{b}	1^{ab}	1^{ab}	1^{b}
9 (2x60s EDTA+NaOCl)	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}
10 (2x60s NaOCl+EDTA)	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}

*At the opening of the tubules, the number 1 indicates exposure of the tubules, the number 2 indicates that the tubules were not exposed. Different overlapping letters indicate statistical difference between groups (analysis by column).

The following image shows the cleaning characteristics of each group.

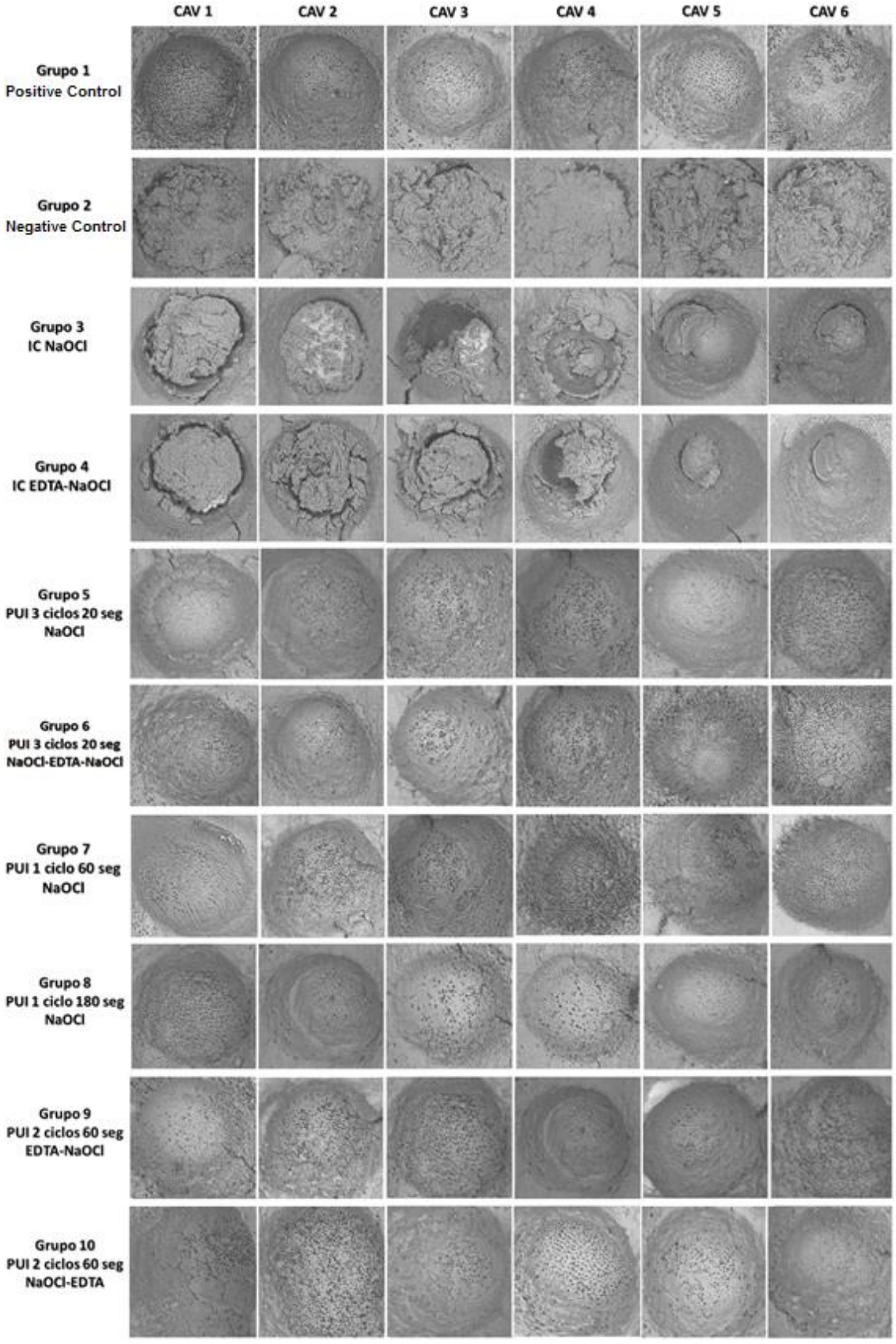

Figure 3: Images representative of each group in 500x magnification in the Scanning Electron Microscope of low vacuum.

4 Discussion

In the present study, newly extracted human teeth were used. The same tooth was used in five different groups in order to reduce the interference of anatomical variation in the results. In addition, it has been shown that the same dental element can be used more than five times without damaging its structure[23,24,25]. Tooth instrumentation was performed untill a # 40 gauge file to facilitate better penetration of the irrigant into the apical third of the root canal[26,27]. The cavities were performed according to the methodology proposed by Kato[22], with the objective of standardizing the amount of debris for cleaning, as well as the location of the analyzes in SEM.

The results of the present study showed that, regardless of the protocol used, PUI was more effective in cleaning the debris than in conventional irrigation. These results are consistent with other authors[5,6], stressing the importance of finding alternatives to conventional methods. Many studies have been performed using passive ultrasonic irrigation with different types of protocols and the results have presented in a divergent way. Some authors did not observe differences between the methods[7,8]. This can be explained by non-standardization of the method, such as specimen preparation, ultrasound power, presence or absence of cavities and area of choice for analysis.

According to the individual cavity analysis it was possible to infer that conventional irrigation was inferior in the removal of debris in relation to the PUI in the cavities located in the most apical region (1, 2 and 3). In the cavities closest to the middle third (cavities 4, 5 and 6), there was adequate cleaning among all experimental groups (conventional irrigation and PUI). Passive ultrasonic irrigation was performed with an apparatus that converts electrical energy into ultrasonic waves by means of a transducer that emits acoustic waves above 20,000Hz[3]. These waves, in addition to their function of cleaning by the action of fluid movement, suffer the effect called cavitation[3]. Cavitation is the ability that

ultrasound has to aggregate gases and vapor into bubbles at a very low density. Once these bubbles rupture in the walls of the root canal, there is a great release of energy, and may also act as a phenomenon that helps to clean the debris adhered to the wall of the root canal[3]. This way, the superiority of the PUI protocols used in comparison with the usual method can be justified by the physical action caused by the movement of the fluids inside the root canal, associated with the cavitation property that the system produces[3], promoting greater penetration of the irrigant in the apical region of the root canal.

Tubal exposure was observed in all PUI groups regardless of EDTA use. EDTA has the ability to dissolve inorganic tissues by reacting the chelating ion with calcium present in hydroxyapatite crystals, removing them from the root canal[28,29].As a result, increased permeability of the dentinal tubules occurs and contributes to the removal of debris produced by root canal instrumentation[30,31]. The ultrasound protocols showed to be more effective in the removal of the smear layer than the conventional irrigation associated with EDTA, showing the importance of the physical action of irrigation of the root canals. Some authors proved the effectiveness of EDTA in root canal cleaning, emphasizing its need for better treatment[8,19]. However, other studies have not identified the effectiveness of EDTA, evidencing the importance of new studies focusing on the protocols and systems improvement[17,20]. Moreover, it was used Irrissonic Power ultrasound tip with 0.02 mm in diameter and 0.01 in taper, according to the manufacturer. Such tip is the second generation of Irrisonic ultrasound tip that was changed in the overall length of the workpiece and bending angle in order to improve the frequency and mechanical quality of vibration. The tip improvements can have positively influenced the results regarding the quality of cleaning regardless the use of EDTA.

5 Conclusion

Based on this study, it can be concluded that the use of PUI, regardless of the protocol used, was more effective in the removal of debris than conventional irrigation in the apical third. It is also concluded that the PUI groups promoted greater exposure of the dentinal tubules independently of the EDTA use.

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