

# Cleaning of long oval canals with WaveOne Gold system associated with different irrigant agitation protocols

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**Aim:** The aim of the study was to evaluate the cleaning of mandibular incisors with WaveOne Gold® (WO) under different preparation techniques. **Methods:** A total of 210 human mandibular incisors were selected and divided into seven groups (n = 30), prepared by WO single-files (Small 20/.07 – WOS; Primary 25/.07 – WOP; Medium 35/.06 – WOM; or Large 45/.05 - WOL) and sequential-file techniques (WOS to WOP; WOS to WOM; and WOS to WOL). Further subdivision was made according to irrigation protocol: control group (manual irrigation - CON), E1 Irrisonic® - EIR, and EDDY® - EDD. Debris removal and the smear layer were evaluated by scanning electron microscopy. Data were analyzed by using Spearman's correlation test. The significance level was set at 5%. **Results:** For debris and smear layer removal, WOS and WOP, EIR differed from CON and EDD (p <0.05). **Conclusion:** Regardless of the instrumentation used, the agitation of the irrigant solution provided better cleanability. These findings reinforce the need for agitation techniques as adjuvants in cleaning root canal systems in mandibular incisors.

**Keywords:** Endodontics. Root canal irrigants. Root canal preparation. Microscopy, electron, scanning.



## Introduction

Endodontic therapy aims to promote the shaping of the root canal system, eliminating microorganisms, tissue remains and debris, promoting a sanitized place for obturation<sup>1</sup>.

In all dental groups cleaning of the apical region is a challenge, as reported by many authors<sup>2,3</sup>, but some teeth have anatomical characteristics that make this process more difficult, such as the mandibular incisors. These teeth are especially flatted and narrow, and it is known that the excessive dentin wall wear due to the use of large instruments can lead to root weakening, while the use of less tapered instruments can lead to non-instrumented areas<sup>4</sup>.

In the literature, findings indicate significant results regarding the reduction of root canal microorganisms using the combination of chemical and mechanical preparation<sup>5</sup>. Better results in terms of disinfection are found proportionally with the root canal enlargement, especially in the apical region<sup>6</sup>, when the benefits of the action of sodium hypochlorite solution (NaOCl) appear to be significantly increased<sup>7</sup>.

New systems and techniques aimed at improving the root canal preparation have been commercially introduced regularly. Currently, most are presented as single-use instruments with the proposal of cost and operating time reduction<sup>8</sup>. The WaveOne Gold® (WO) system was presented in this context and featured in Small (20/.07), Primary (25/.07), Medium (35/.06), and Large (45/.05) (Dentsply Sirona Endodontics, Ballaigues, Switzerland).

Despite technological advances related to endodontic instruments, tips to agitate the irrigant solution have been proposed to enhance the disinfection process<sup>9</sup>. These techniques are detrimental to the conventional methods performed with a syringe and fine-caliber needles<sup>10</sup>. Thus, the EDDY® sonic tip (VDW GmbH, Munich, Germany) as well as the E1 Irrisonic® (20/.01) (Helse Ultrasonic, Santa Rosa de Viterbo, Brasil) are aimed at agitating the irrigant solutions<sup>11</sup>.

Regarding the cleaning capacity, scanning electron microscopy (SEM) has been used to analyze the presence of debris and smear layer adhered to the inner walls of the root canal after preparation<sup>12,13</sup>.

Therefore, the present study aimed to analyze the cleaning of mandibular incisors with the WO system under different preparation protocols. The null hypothesis tested was there is no difference between the preparation techniques.

## Material and Methods

This study was approved by the Institutional Ethics Committee of Universidade Positivo (Approval number 2,420,082). Only one trained operator performed the experimental procedures. For the analysis of the images, two researchers were trained and calibrated ( $\kappa = 0.83$ ).

### Sample size estimation and characterization

Initially, sample data from previous studies were used as reference<sup>14,15</sup>. Subsequently, the power observed in the sample was calculated, considering the value of  $\alpha = 5\%$  and

the rejection of the null hypothesis (existence of difference between treatments) by the nonparametric ANOVA test, which resulted in a power value > 99%.

A total of 210 freshly extracted human mandibular incisors were selected, which at the digital radiographic examination towards bucco-lingual and mesio-distal directions, presented a single root canal, complete rhizogenesis, and had no endodontic treatment, calcifications, internal and external resorption, lacerations, or fractures. These teeth remained in 0.9% sodium chloride (NaCl) solution (Eurofarma, São Paulo, Brazil) until the beginning of the experiment, with the solution being replaced every seven days.

The crowns were sectioned with a double-sided diamond disc (KG Sorensen, Cotia, Brasil) with the remaining standardized at a length of 15 mm. The patency of all root canals was established with a 10 K-file (Dentsply Sirona Endodontics, Ballaigues, Switzerland), at a working length (WL) of 14 mm. Initially the specimens were divided into four groups according to the instrument used for the preparation (n = 30): WO Small (20/.07) – WOS; WO Primary (25/.07) - WOP; WO Medium (35/.06) - WOM; WO Large (45/.05) - WOL. This allocation was based on the manual files used to negotiate the root canals, according to Van der Vyver et al.<sup>16</sup>.

The surpluses of this division were distributed in the other groups, until each reached n = 30.

### Specimen preparation

Then the root apices were isolated with composite resin (Filtek® Z350; 3M ESPE Saint Paul, EUA). The specimens were divided as follows (n = 30): WOS, WOP, WOM, WOL, WOS to WOP, WOS to WOM, WOS to WOL.

In this study, the WO system, which has different conicities, was tested in a single-file technique (each specimen was prepared with only one instrument according to the initial apical diameter) and sequential-file techniques (specimen preparation was performed sequentially using the entire system).

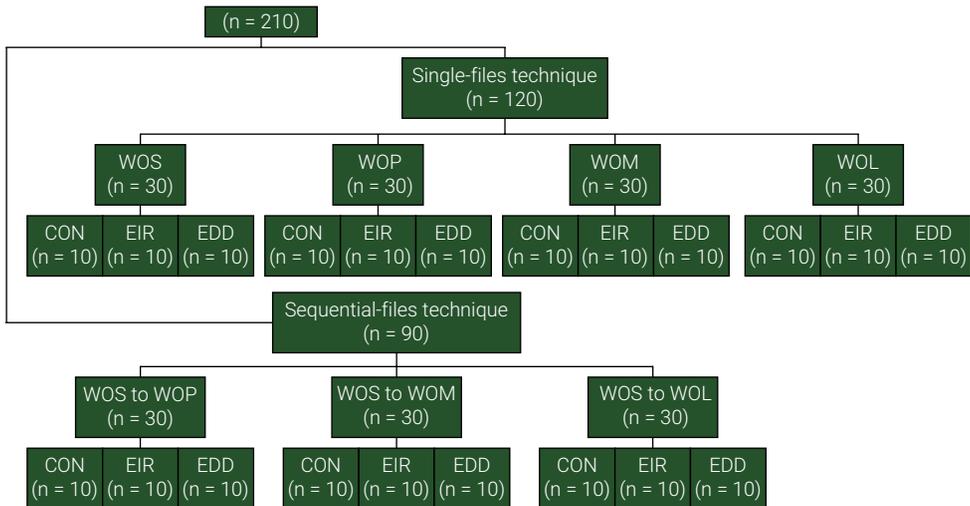
The instruments were coupled to the X-Smart Plus® electric motor (Dentsply Sirona Endodontics, Ballaigues, Switzerland) and were used according to the manufacturer's instructions.

Regarding the use of instruments reproducing clinical reality, in this study each instrument was used three times (three specimens) with the consideration that the instrumentation of molar teeth (with three to four root canals) with a single instrument is safe.

### Irrigation protocols and solution agitation

Irrigation during preparation for all groups was performed with a 5 mL Luer Lock syringe (BD®, Curitiba, Brasil) coupled to a 30 G needle irrigation tip (Navitip; Ultradent, South Jordan, EUA), calibrated at 11 mm. At each 3 mm advance of the instrument inside the root canal, 2 mL 2.5% NaOCl was used. Between advances, the WL was verified with a 10 K-file.

After preparation, the specimens were divided into three subgroups (n = 10) according to the irrigation protocol (Figure 1). The control group (CON) received conventional irrigation, as described above. The E1 Irrisonic® (20/.01) tip (EIR) (Helse Ultrasonic, Santa Rosa de Viterbo, Brasil), and the EDDY® tip (EDD) (VDW GmbH, Munich, Germany) were used for agitation. The EIR tip was coupled to the Piezon® Master 200 ultrasound



**Figure 1.** Flowchart of the division of experimental groups. WOS, WaveOne Gold® Small; WOP, WaveOne Gold® Primary; WOM, WaveOne Gold® Medium; WOL, WaveOne Gold® Large; CON, control group; EIR, E1 Irrisonic®; EDD, EDDY®.

device (EMS, Nyon, Switzerland) power 1; EDD was activated in a handpiece (Sonic Borden 2000N, Kavo Dental Ltda., Joinville, Brazil) at a power of 5000 Hz to 6000 Hz. Both instruments were calibrated at 1 mm from the WL (13 mm).

In all specimens the methodology presented by Plotino et al.<sup>13</sup> was followed to both, EIR and EDD. The irrigation was performed at 1 mm from the WL with 2.5 mL of 2.5% NaOCl, activated for 20 s three times, renewed with 1 mL of fresh 2.5% NaOCl between them. Again, the root canals were irrigated with 2.5 mL of 2.5% NaOCl. Final irrigation was performed with 17% EDTA for a total of 2 min as follows: the activation occurred during 20 s three times, with solution renovation (1 mL of fresh 17% EDTA), and then another irrigation with 2 mL of 17% EDTA continuously for 1 min. For the CON group, the irrigation was performed, without agitation, with 8 mL of NaOCl and 10 mL of EDTA. A final flush was performed with 2.5 mL of sterile saline solution in all canals. The final aspiration was done with 0.36 mm Capillary Tip tips (Ultradent, South Jordan, EUA).

### Preparation of specimens for SEM exam

For the cleavage of all of the specimens into two halves, two longitudinal grooves were made along the entire root length in the buccal and lingual walls with double-sided diamond discs (KG Sorensen). An Ochsenbein No. 1 micro chisel (Quinelato®; Schobell Industrial Ltda., Rio Claro, Brazil) was introduced into the grooves, and lever movements were made throughout the root extension to avoid an uneven fracture of the apical third, which was the focus of the of analysis.

For dehydration, the sample was placed in Petri dishes, stored in a sterilization and drying oven, for 48 h at 36 °C. Prior to image acquisition, the specimens were subjected to gold sputtering.

## Analysis of dental wall cleaning by SEM

The root walls of the apical third were analyzed at 3 mm from the apex using a scanning electron microscope (JSM 6010; JEOL, Peabody, USA) at a power of 20 Kv. Magnifications of 100× and 1000× allowed evaluation of debris and smear layer, respectively<sup>13</sup>. For the acquisition of the images, the microscope's own software measurement application was used. The wall submitted to analysis was the one with the highest thickness.

The criteria used to quantify these variables followed the classification of Gutmann et al.<sup>17</sup>: score 1, absence or little debris covering up to the 25% of the surface; score 2, little to moderate debris covering from 25% to 50% of the surface; score 3, moderate to high presence of debris covering from 50% to 75% of the surface; score 4, high amount of aggregated or scattered debris covering over 75% of the surface. For smear layer the criteria was: score 1, absence or little smear layer, covering less than 25% of the specimen with tubules visible and patent; score 2, little to moderate or patchy amounts of smear layer, covering from 25% to 50% of the specimen with many tubules visible and patent; score 3, moderate amounts of scattered or aggregated smear layer, covering from 50% to 75% of the specimen with minimal tubules visible or patent; score 4, high amount of smear layer covering over 75% of the specimen with no tubule orifices visible or patent.

Thus, debris and smear layer were considered criteria to evaluate the cleaning capacity in the different forms of irrigant solution agitation, compared to the manual irrigation technique in the apical region only.

All analyses were performed using SPSS (IBM® Statistics v. 25.0, SPSS Inc, Chicago, USA) with a significance level of 5%. The Spearman correlation test was used for debris to test the correlation of the different scores between groups.

## Results

The sequential use of WOS to WOP instruments resulted in greater removal of debris and smear layer, with a statistically significant difference when compared to WOP ( $p < 0.05$ ). Differences were also observed in smear removal in single use between WOM and WOS ( $p = 0.025$ ), and sequentially between WOS to WOM and WOS to WOP ( $p = 0.035$ ) (Table 1).

**Table 1.** Results of debris and smear layer assessment, according to group and irrigation/agitation (median: minimum-maximum).

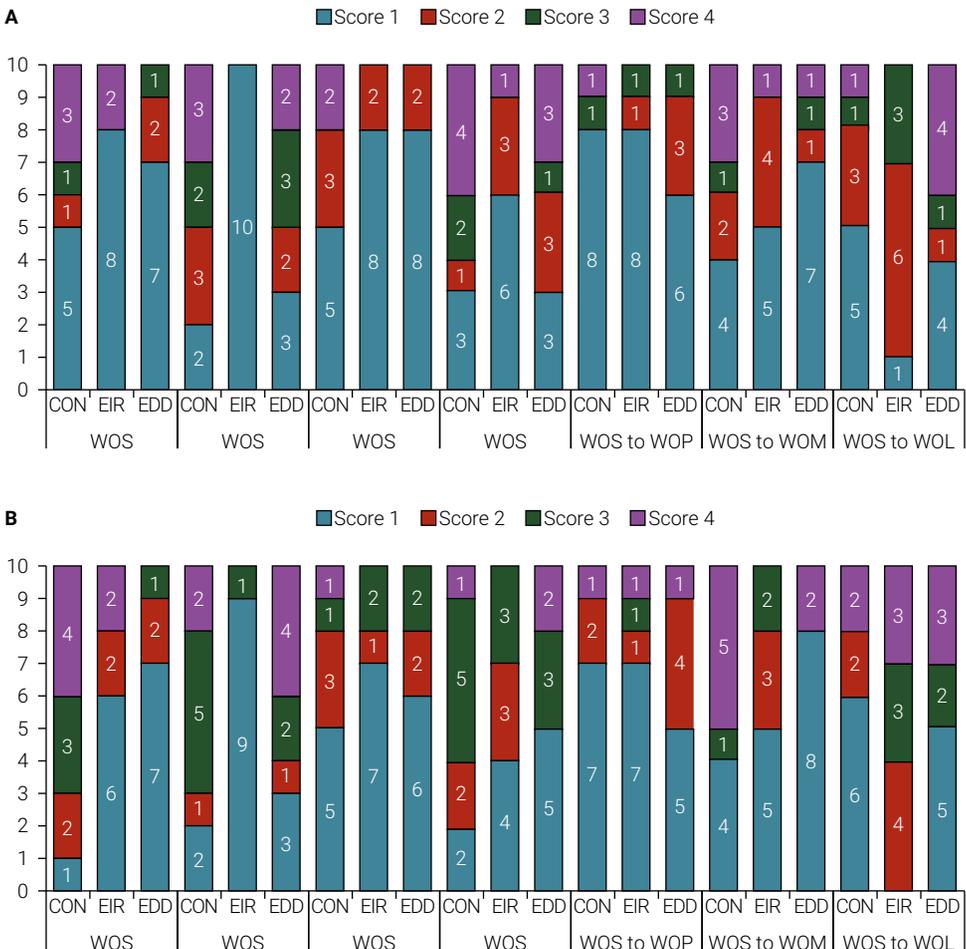
Group	Debris			Smear layer		
	CON	EIR	EDD	CON	EIR	EDD
WOS	1.50 (1-4) <sup>A,B,C</sup>	1.00 (1-4) <sup>A,B</sup>	1.00 (1-3) <sup>A,B</sup>	3.00 (1-4) <sup>B,b</sup>	1.00 (1-4) <sup>A,a</sup>	1.00 (1-3) <sup>A,a</sup>
WOP	2.50 (1-4) <sup>B,C,b</sup>	1.00 (1-1) <sup>A,a</sup>	2.50 (1-4) <sup>C,D,b</sup>	3.00 (1-4) <sup>B,C,b</sup>	1.00 (1-3) <sup>A,a</sup>	3.00 (1-4) <sup>B,b</sup>
WOM	1.50 (1-4) <sup>A,B,C</sup>	1.00 (1-2) <sup>B</sup>	1.00 (1-2) <sup>A</sup>	1.50 (1-4) <sup>A,C</sup>	1.00 (1-3) <sup>A</sup>	1.00 (1-3) <sup>A</sup>
WOL	3.00 (1-4) <sup>B</sup>	1.00 (1-4) <sup>A,B,C</sup>	2.00 (1-4) <sup>C</sup>	3.00 (1-4) <sup>B,C</sup>	2.00 (1-3) <sup>A,B</sup>	3.00 (1-4) <sup>A,B</sup>
WOS to WOP	1.00 (1-4) <sup>A</sup>	1.00 (1-3) <sup>A,B</sup>	1.00 (1-4) <sup>A,C</sup>	1.00 (1-4) <sup>A</sup>	1.00 (1-4) <sup>A</sup>	1.50 (1-4) <sup>A,B</sup>
WOS to WOM	2.00 (1-4) <sup>A,B</sup>	1.50 (1-4) <sup>A,B,C</sup>	1.00 (1-4) <sup>A,B,D</sup>	3.50 (1-4) <sup>B,C</sup>	1.50 (1-3) <sup>A</sup>	1.00 (1-4) <sup>A</sup>
WOS to WOL	1.50 (1-4) <sup>A,B</sup>	2.00 (1-3) <sup>C</sup>	2.50 (1-4) <sup>B,C</sup>	1.00 (1-4) <sup>A,C</sup>	3.00 (2-4) <sup>B</sup>	2.00 (1-4) <sup>A,B</sup>

Note: Capital letters indicate statistically significant differences in the column. Lower case letters indicate statistically significant differences in the line.

Kruskal-Wallis followed by Dunn's post hoc test ( $p < 0.05$ ).

Concerning agitation, for EIR differences were observed in the comparisons between groups for WOS a WOP and WOS a WOL ( $p = 0.012$ ); in the intra-group comparison, for WOP, EDD and CON were the same ( $p = 0.682$ ), but different from EIR ( $p < 0.05$ ). These differences occurred for both debris and smear layer. EDD only showed significant differences when dealing with a single-use instrument. For debris, WOS and WOM were the same ( $p = 0.654$ ), but differed from WOP and WOL ( $p < 0.05$ ). For the removal of smear layer, there was a difference between WOP and WOS ( $p = 0.017$ ), and WOP and WOM ( $p = 0.049$ ) (Table 1).

The assessment of debris and smear layer scores is shown in Figure 2. Of a total of 210 specimens analyzed, 114 had a score of 1, i.e., more than 50% of the sample obtained satisfactory cleaning. This image also reveals that, in absolute numbers, EDD performed at or above EIR, based on the number of specimens recorded as score 1.



**Figure 2.** (A) Number of specimens recorded for each score in relation to the apical third of root canals in the evaluation of residual debris of the different treatments (100×), and (B) smear layer (1000×). WOS, WaveOne Gold® Small; WOP, WaveOne Gold® Primary; WOM, WaveOne Gold® Medium; WOL, WaveOne Gold® Large; CON, control group; EIR, E1 Irrisonic®; EDD, EDDY®.

## Discussion

This study aimed to evaluate the cleaning of mandibular incisors with WO under different preparation techniques. The results found in the present study revealed that even with the technological advances related to endodontic instruments, their use alone does not have efficient root canal cleaning capacity, and the combination of the use of irrigation solutions concomitantly with their agitation is necessary<sup>3,9</sup>.

The failure of endodontic therapy is related to the maintenance of pathogens into the root canal system<sup>18</sup>, thus the chemical and mechanical combination in preparation is so important<sup>5</sup>. In this context, the apical region is a challenge, being the place with the largest amount of debris<sup>2,3</sup>. The mechanical debridement of the last millimeters of the root canals is not as effective as cervical region, and some authors attribute this fact due to caliber of the dentinal tubules or the narrower diameter of the canal in this region<sup>19</sup>, therefore less penetration and contact occurs between the canal walls and the irrigators<sup>2</sup>.

Once the literature reveals that limitations in endodontic treatment are also related to the variation of internal root anatomy<sup>20</sup>, the study of the apical region of mandibular incisors is opportune, because this dental group has root canals especially flatted and narrowed. Care during preparation of these teeth is necessary, because the greater enlargement of the apical third promotes more cleaning<sup>6</sup>, but it know that excessive instrumentation can lead the root weak<sup>4</sup>. Other justification to the method adopted here is due to the fact that protocols with an association of different instruments and irrigants are necessary to compare the performance in removing debris and smear layer<sup>21</sup>.

The techniques (single or sequential instruments) used in this study to assess the cleaning capacity of the root canals with WO instruments showed differences between WOP versus WOS to WOP. Thus, the null hypothesis was rejected, with a significant difference regarding the removal of debris and smear layer on the root canal walls. With the same purpose in mandibular incisors, De-Deus et al.<sup>22</sup>, revealed that better cleaning was observed when preparation was performed with sequential instruments. Similar results were also highlighted in another study, although performed on molars, the differences occurred when the canals were also prepared in sequential mode<sup>23</sup>.

Considering the presence of regions in mandibular incisors that can provide the accumulation of debris and microorganisms<sup>24</sup>, the role of irrigant solutions comes to the fore<sup>25</sup>. Given the ability to dissolve, neutralize and remove organic and inorganic matter<sup>26</sup>. Among the most used are NaOCl, due to its bactericidal power, low surface tension, and antibacterial action, spreading throughout the deproteinization canals<sup>27</sup>. As for the concentration of NaOCl, a 2.5% liquid was adopted, based on the study of Duque et al.<sup>14</sup>. Another irrigant used in this study, 17% EDTA, aimed to dissolve inorganic material<sup>28</sup>. Based on the chemical action of the aforementioned, an association of these irrigants was adopted, justified by the presence of organic and inorganic tissues within the root canal<sup>28</sup>. In addition to the chemical properties of these solutions, it is known that the way they are applied in difficult regions influences their effectiveness, due to the trapping of air such occurs in the apical region<sup>29</sup>, which impairs or prevents the flow of irrigants.

Despite the advances in the field of instruments, and the persistence of non-instrumented areas after preparation<sup>30</sup>, it was proposed to use ultrasonic inserts to enhance the cleaning of root canals, under agitation of the irrigant solution<sup>14</sup>. In the present study differences occurred. Corroborating these findings, a recent systematic review also indicated an association, with ultrasonic activation being more effective than syringe irrigation in removing tissue debris and fragments of hard tissue<sup>31</sup>.

Regarding the removal of remnants, EIR showed a lower score when compared to CON and EDD. The same tip was also evaluated by Duque et al.<sup>14</sup>. The authors demonstrated that the agitation of the irrigant solution promoted a better cleaning of the canal and isthmus areas when compared to the syringe irrigation technique. Evaluating the reduction of microorganisms within the root canals, the effectiveness of EIR was tested against irrigation with syringe. Although the NaOCl used was 6%, the clinical trial revealed a greater reduction in the amount of microorganisms the group where ultrasonic activation was instituted<sup>32</sup>. Both mentioned studies corroborate the results of the present study, where EIR was superior to CON in removing debris and smear layer.

The EDD tip was commercially introduced as a sonic system for agitating irrigant solutions. Here, this tip not showed differences in comparison to CON and EIR. According to the manufacturer it is made of polyamide and has a flexible tip, giving it the same effectiveness as ultrasonic. Its vibration, with great amplitude, are due to the high flexibility, which promotes a three-dimensional movement that generates the same physical effects of cavitation and acoustic streaming achieved by ultrasonic. In comparison, syringe irrigation and EDD, decontaminating root canals infected with *Enterococcus faecalis*, the first one was significantly less efficient in eliminating microorganisms in single canals<sup>33</sup>. Zeng et al.<sup>34</sup> also evaluated the reduction in bacterial load, and identified that EDD was superior to manual irrigation in relation to bacterial death in the intratubular region in the cervical and middle thirds. With regard to the use of EDD, the present results differed from those mentioned above, since statistical differences were not identified in comparison to CON.

Based on the results of this research, a satisfactory cleaning regarding the removal of debris and smear layer can be observed. Even though there were no significant differences, between and within groups for some associations. Such observation is due to the greater number of specimens registered for score 1, in both quantifications. A possible justification for this is associated with the homogeneity of the initial anatomy achieved in the specimens during the allocation between groups. For that, manual files were used to negotiate the canals and consequently indicated the preparation instrument, according to Van der Vyver et al.<sup>16</sup>. As a result, canals with smaller diameter were prepared with smaller taper instruments, as were the larger ones. Critically, it was still possible to observe that in the EDD groups, the number of specimens registered with score 1 was higher than the CON group, and equally or higher than EIR. Therefore, it is noteworthy that in the quantification of debris and smear layer, score 1 is classified, with no or slight presence of surface debris, covering up to 25% of the dentin surface, and for smear layer, little or no smear layer, covering less than 25% of the specimen with visible tubules, respectively<sup>17</sup>.

Currently, this quantification has been proposed by the SEM, through the presence of debris and smear layer, in different increases<sup>12</sup>. In this study, magnifications of 100x and 1000x were used, respectively, based on the study by Plotino et al.<sup>13</sup>. It is known that this methodology presents restrictions, as it evaluates a limited area of the canal; therefore, the standardization of the studied region was adopted in an attempt to always analyze the same region, in relation to the root apex. Thus, image acquisition was performed at a distance of 3 mm from the root apex, and defined at the central point between the dentin walls in the mesio-distal direction. The chosen face was always the one with the least irregularities resulting from the neckline, as the teeth were thin. A total of 420 images (210 samples versus two enlargements, versus one third) were analyzed by two blind, trained and calibrated observers.

In reference to the enlargement of the preparation of the canals for the institution of the agitation protocols, considering that the taper of EDD (25/.04) and EIR (20/.01), there would be a need for preparation with a 60 taper instrument to allow the movement the tip of the instrument. According to Ahmad et al.<sup>35</sup> for ultrasonic irrigation to be effective and for acoustic streaming to occur, it must operate within a space three times larger than the diameter of the tip used. However, the anatomical characteristics of the dental group used, the apical preparation was performed with a maximum 45 taper instrument, which may have had an impact on the conduction of ultrasonic energy. Within the limitations of this study with respect to cleaning, none of the preparation techniques associated with tips produced a debris-free dentin surface and smear layer. However, it is indicated that for the preparation of mandibular incisors with manual irrigation, a protocol with sequential file, with the association of the WOS and WOP instruments. In the presence of tips of the irrigant solutions, EIR can be used after WOP and WOM instruments as well as WOS to WOP association. While the use of EDD is indicated, it can be used after WOM and WOS to WOM instruments.

In conclusion, the agitation of the irrigant solution provided better cleaning capacity, with better results for the EIR. These findings reinforce the need for agitation techniques as aids in cleaning the root canal system, especially in cases of canals with a tendency to flatten.

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