

# Effect of irrigation protocols on root canal wall after post preparation: a micro-CT and microhardness study

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**Abstract:** The aim of this study was to investigate the effects of different post space irrigation protocols for removing residual filling material from dentin walls, by using microcomputed tomography (micro-CT), and the influence of these protocols on dentin microhardness. Bovine incisors (n = 35) were filled with the single-cone technique and MTA Fillapex (Angelus, Londrina, PR, Brazil). Post space preparation (PSP) was performed 7 days after filling, using the Odous Touch electrical system (Odous De Deus Ind. e Com., Belo Horizonte, MG, Brazil), followed by post space irrigation using manual irrigation, passive ultrasonic irrigation, or Easy Clean, together with 2.5% sodium hypochlorite (NaOCl), or with 2.5% NaOCl and 17% EDTA (NaOCl/EDTA). Micro-CT scans were performed at three time points. The residual filling material was evaluated at three levels: cervical, middle and apical. The Knoop test was measured with four indentations around the canal lumen at three dentin depths: X (100  $\mu$ m), Y (200  $\mu$ m) and Z (400  $\mu$ m). Statistical analysis was performed using ANOVA ( $p < 0.05$ ). The effects of the activation method ( $p < 0.001$ ), and the root level ( $p = 0.013$ ), as well as the interaction between the irrigant and the activation method ( $p = 0.041$ ), led to different percentages of residual filling material. Lower amounts of residual filling material were observed at the cervical versus the middle and apical levels ( $p < 0.05$ ). No significant differences were observed in dentin microhardness ( $p > 0.05$ ). The best removal of the residual filling material was performed using the Easy Clean tip and NaOCl/EDTA, regardless of the activation methods.

**Keywords:** Hardness Tests; X-Ray Microtomography; Root Canal Irrigants.

## Introduction

Post space preparation (PSP) leads to the formation of a smear layer that contains the residual filling material used to fill the root canal,<sup>1,2</sup> and this layer on the root canal wall may influence the retention of fiberglass posts.<sup>3</sup> Considering that the adhesive failure of fiberglass posts is mainly related to debonding at the resin cement/dentin interface,<sup>2-5</sup> the residual filling material bonded to the canal walls should be removed to improve fiberglass post retention.<sup>3,6</sup>

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Several solutions and protocols have been proposed for post space irrigation,<sup>4,7,8</sup> including sodium hypochlorite (NaOCl)<sup>9</sup> and acid ethylenediaminetetraacetic acid (EDTA).<sup>4,6,8,10</sup> NaOCl acts on the organic part of dentin and modifies collagen fibers, whereas EDTA acts on the inorganic part and chelates calcium ions.<sup>11</sup> Both chemical irrigants can change the structural properties of dentin, such as its microhardness.<sup>12,13</sup>

The effects of irrigating solutions can be enhanced by activation protocols such as passive ultrasonic irrigation (PUI).<sup>1,10</sup> Easy Clean (EC) (Easy Dental Equipment, Belo Horizonte, MG, Brazil) was developed as a simple alternative to PUI.<sup>14,15,16</sup> Neither of these strategies has been studied at length in regard to irrigant activation in the root canal after PSP, but both have been shown to improve debris removal and bond strength of fiber posts.<sup>3,10,16</sup> The effect of EC used in continuous rotation was observed for debris removal from the main canal and the isthmus of the mesial root of mandibular molars, and was found to be adequate.<sup>17,18</sup> However, no studies have investigated the effect of EC under low-speed continuous rotation as a post space irrigation (PSI) strategy.

The aim of this study was to investigate the effects of different post space irrigation protocols for removing the residual filling material on dentin walls, using microcomputed tomography (micro-CT), and the influence of these protocols on dentin microhardness. The null hypotheses were as follows: a) the solutions and the activation methods used for post space irrigation would not improve the outcome of residual filling material cleaning; b) the solutions and activation methods would not affect dentin microhardness.

## Methodology

### Tooth selection and root embedment

The sample size was calculated using the BioEstat 5.3 (Mamirauá Institute, Tefé, Brazil) statistical software program.<sup>15,19</sup> Five teeth per group were required to detect a 90% chance of finding significant differences at the 5% level (2-sided test), with a minimum detectable mean difference of 13.54, and an expected standard deviation of 3.58 with regard to microhardness, the secondary outcome.

Thirty-five bovine incisors with a single root canal, completely formed apices, and similar external morphology were selected. The root dimensions were measured with a digital caliper (Mitutoyo Sul Americana, Suzano, Brazil) in the buccolingual and mesiodistal directions for the 3 thirds of the root. The teeth were also scanned using digital radiography (Sensor Fit T1, Micro Imagem, Indaiatuba, Brazil), so that the mean area of the root canals could be measured by the ImageJ software program (National Institutes of Health, Bethesda, USA). Maximum deviations of 10% from the mean value were included. The teeth were cleaned and then stored in distilled water to prevent the effects of confounding factors on the microhardness tests. The crowns were removed with a diamond disc (KG Sorensen, Barueri, Brazil), and the mean root length was standardized at 15.0 mm. The working length was determined with a #10 K-file (Dentsply Sirona, York, PA, USA), which was introduced until its tip was visible at the apical foramen, and 1 mm was subtracted from this measurement. The roots were embedded in polystyrene resin (Cristal, Piracicaba, Brazil) to simulate the alveolar bone,<sup>20</sup> and allow the samples to be inserted in the same position during the micro-CT scan (Figure 1A).

### Endodontic treatment and post space preparation

All the procedures were performed by different experienced endodontic specialists. The root canals were prepared using a reciprocating nickel-titanium #50.05 file (Reciproc system - VDW, Munich, Germany) with an endodontic motor (Dentsply Sirona, York, USA). Irrigation was performed using 12 mL of 2.5% NaOCl (Asfer Indústria Química, São Caetano do Sul, Brazil) for all the specimens. Next, the root canals were rinsed with 3 cycles of 1 mL of 17% EDTA (Biodinâmica, Ibiporã, Brazil) for 1 minute, followed by 5 mL of 2.5% NaOCl, and a final flush with 5 mL of distilled water. The canals were dried with paper points (VDW), and obturated with the single-cone technique using gutta-percha points R50 (VDW) and MTA Fillapex (batch no. #40006 and #40063; Angelus, Londrina, Brazil).

The specimens were stored at 37°C in a moist environment for 7 days to allow the sealer to set completely. PSP was performed with thermoplasticized gutta-percha for each sample, using M and FM tips from Odous Touch (Odous De Deus Ind. e Com., Belo Horizonte, Brazil); 10 mL of distilled water was used to irrigate a space of 10 mm in length, leaving 4 mm of the apically sealed region untouched.

### Post space irrigation protocols

The roots were randomly divided (www.random.org) into two experimental groups ( $n = 15$ ), according to the irrigant: 2.5% NaOCl and 2.5% NaOCl followed by 17% EDTA (NaOCl/EDTA). Each group was further divided into 3 subgroups, according to the following irrigating activation methods ( $n = 5$ ): manual irrigation (MI), PUI, or EC tip, yielding the final groups of NaOCl/MI, NaOCl/PUI, NaOCl/EC, NaOCl/EDTA/MI, NaOCl/EDTA/PUI, and NaOCl/EDTA/EC. As for the controls, the micro-CT evaluations considered manual irrigation as the control for each subgroup (NaOCl/MI and NaOCl/EDTA/MI), and the dentin microhardness assessment considered the root that was obturated and subjected to PSP ( $n = 5$ ) as the control group.

Manual irrigation ( $n = 10$ ) was performed with a 30-g open-ended needle (NaviTip, Ultradent, South Jordan, UT, USA) introduced with an “in-and-out” motion. PUI ( $n = 10$ ) was performed with an Irrisonic E1 (20/01) tip (Helse Dental Technology, Santa Rosa de Viterbo, Brazil) attached to the ultrasonic unit of a Piezon Master 200 (EMS, São Bernardo do Campo, Brazil), on a power level of 2. The EC ( $n = 10$ ) had a 25.04 tip attached to a low-speed contra-angle handpiece (KaVo Kerr Group, Charlotte, USA), running at approximately 20,000 continuous rotations per minute. In all subgroups, the devices were placed 1 mm short of the remaining gutta-percha.

The irrigation sequence for using NaOCl was 1 mL of solution for 20 seconds in 6 cycles.<sup>15,16</sup> In the NaOCl/EDTA group, 3 mL was used for each solution,<sup>10</sup> with 3 cycles of 1 mL of NaOCl followed by 3 cycles of 1 mL of EDTA, resulting in the same volume and time of irrigation. All irrigants were applied with a 30-g open-ended needle (NaviTip, Ultradent, South Jordan, USA). A final flush with

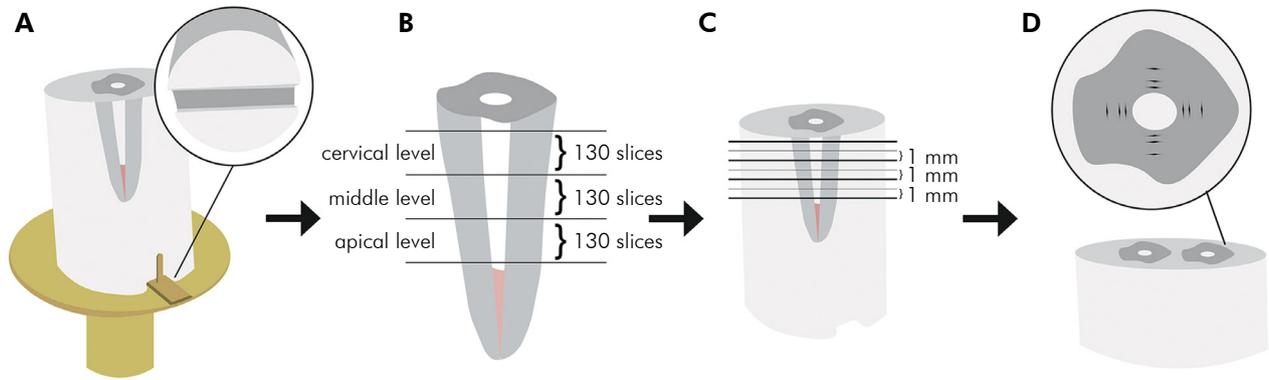
10 mL of distilled water was performed to remove any chemical solution residue.<sup>13</sup>

### Micro-CT analysis

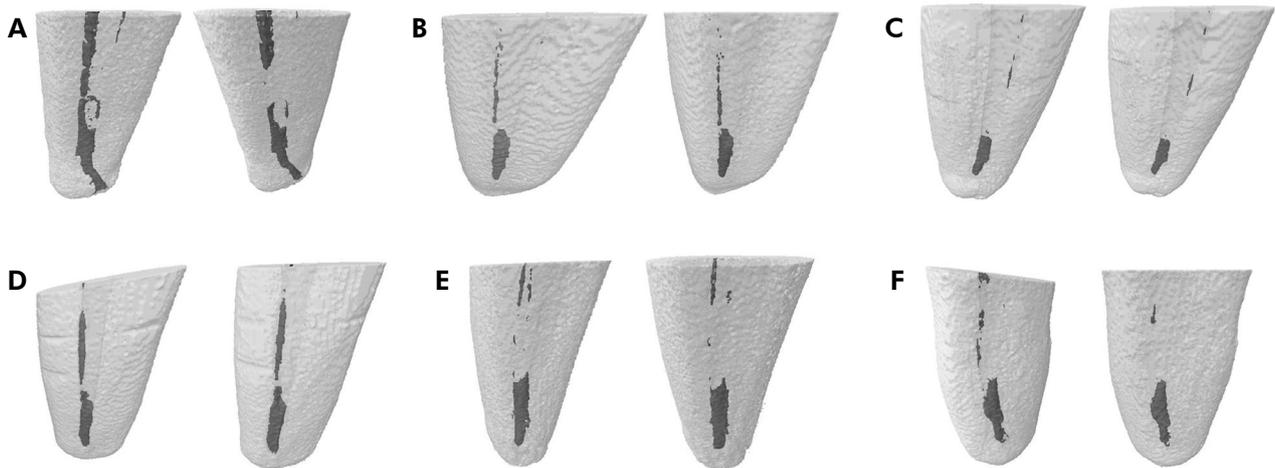
The specimens were scanned in a micro-CT device (SkyScan 1272; Bruker micro-CT, Kontich, Belgium) at three time points to evaluate the residual filling material: after obturation, at PSP and at post space irrigation. Scanning was conducted under the following conditions: 100 KV and 100  $\mu$ A, 21.7  $\mu$ m pixel size, 2000 ms exposure time, 180° rotation angle at a rotation step of 0.8, frame averaging of 2, random movement of 20, and Cu filter of 0.11 mm. The images acquired by micro-CT were reconstructed by a NRecon software program (version 1.6.3.3, Bruker micro-CT) with a beam hardening correction of 2%, a smoothing level of 1, and a ring artifact correction level of 7.

A CTAn (version 1.14.4.1, SkyScan, Bruker, Belgium) was used to identify the region of interest (ROI), corresponding to the amount of residual filling material. The ROI selected for each slice was the area around the root canal, used to calculate the volume of residual filling material ( $\text{mm}^3$ ). The presence of residual filling material was processed with the original grayscale in the cervical, middle and apical levels, with 130 slices for each level (Figure 1B). The residual filling material values obtained before applying the irrigating activation method were recorded in  $\text{mm}^3$  and converted into a percentage using the following formula:  $V_{A-PSP} * FP / V_F$  where  $V_{A-PSP}$  indicates the object volume after PSP ( $\text{mm}^3$ ), FP indicates the filling percentage (%), and  $V_F$  indicates the filling object volume ( $\text{mm}^3$ ). After the post space irrigation protocols were performed, the  $V_{A-CP} * V_{A-PSP} / V_{PSP}$  formula was used, where  $V_{A-CP}$  indicates the object volume after performing the irrigating activation method ( $\text{mm}^3$ ),  $V_{A-PSP}$  indicates the residue after PSP (%), and  $V_{PSP}$  indicates the PSP object volume ( $\text{mm}^3$ ).

A CTAn software program was used to generate 3D models for the residual filling material before and after applying the irrigating activation method (Figure 2). One examiner previously calibrated and blinded to the study groups performed all the measurements.



**Figure 1.** Schematic figure of the methodologies for micro-CT and Knoop test: (A) Sample placed in a standardized position during the micro-CT scans, using a custom device; (B) the region to be analyzed was divided to represent the cervical, middle and apical levels in the PSP, with 130 slices at each level; (C) two 1-mm-thick root slices were obtained from each PSP level; (D) four indentations were made around the canal lumen at three dentin depths.



**Figure 2.** 3D models for residual filling material before and after the cleaning protocols: (A) NaOCl/MI, (B) NaOCl/PUI, (C) NaOCl/EC, (D) NaOCl/EDTA/MI, (E) NaOCl/EDTA/PUI, (F) NaOCl/EDTA/EC.

### Dentin microhardness assessment - Knoop test

The roots previously used for micro-CT analysis were prepared according to previously described methods (Figure 1C).<sup>20</sup> The Knoop indentation values were determined with a microhardness tester (FM700; FutureTech, Kawasaki, Japan) at the cervical, middle and apical levels, after a load of 50 g was applied for 15 seconds.<sup>21</sup> Four indentations were made around the canal lumen at three dentin depths (Figure 1D). The average values of dentin microhardness were recorded.

### Statistical analysis

The normality of the data was tested using the Shapiro-Wilk test, and homoscedasticity was tested using the Levene test, after which parametric statistical tests were performed. The micro-CT analysis used three-way ANOVA with Tukey's and Dunnett's tests to evaluate the overall effects of the irrigant activation method and the root level (cervical, medium and apical). The subgroup analysis used post hoc Tukey's test for two-by-two comparisons. Two-way repeated measures ANOVA was used to compare the irrigants and activation methods at the cervical, medium and apical levels.

The dentin microhardness analysis used two-way ANOVA to compare the irrigants and activation methods for each dentin depth of the canal lumen at the same root level, after which Tukey's and Dunnett's tests were performed. The significance level was set at  $\alpha = 0.05$  for all the tests. Statistical analysis of the data was conducted by using SigmaPlot, version 13.1 (Systat Software, San Jose, USA).

## Results

### Residual filling material

Three-way ANOVA showed that the activation methods ( $p < 0.001$ ), the root levels ( $p = 0.013$ ), and the interaction between the irrigants and the activation methods ( $p = 0.041$ ) led to significant differences in the percentage of residual filling material. Overall, the samples cleaned with EC showed less residual filling material than those cleaned with PUI or MI ( $p < 0.05$ ). Significantly more residual filling material was removed at the cervical than middle and apical levels ( $p < 0.05$ ). Table 1 presents the mean and standard deviation of each post space irrigation subgroup, regardless of the third level.

**Table 1.** The mean  $\pm$  standard deviation values of residual filling material (%) for each PSI subgroup, regardless of the third level.

Variable	NaOCl	NaOCl/EDTA
MI (control)	8.28 $\pm$ 2.08 Cb	3.88 $\pm$ 1.64 Aa
PUI	3.23 $\pm$ 1.54 Ba	3.66 $\pm$ 1.12 Aa
EC	0.41 $\pm$ 0.21 Aa	1.02 $\pm$ 0.48 Aa

Uppercase letters were used to compare each irrigant among the activation methods (columns); lowercase letters were used to compare the irrigants using the same activation method (rows). Different letters indicate significant differences based on the Tukey test ( $p < 0.05$ ).

**Table 2.** The means and standard deviations of dentin microhardness at the three root levels (cervical, middle and apical).

PSI level	Cervical			Middle			Appical		
	X	Y	Z	X	Y	Z	X	Y	Z
Control group	37.07 $\pm$ 3.44	39.05 $\pm$ 3.83	41.80 $\pm$ 2.71	35.33 $\pm$ 2.31	37.94 $\pm$ 2.16	41.29 $\pm$ 1.62	35.56 $\pm$ 4.23	37.85 $\pm$ 5.11	40.22 $\pm$ 5.56
NaOCl/MI	33.50 $\pm$ 0.79	35.77 $\pm$ 0.52	39.13 $\pm$ 0.53	34.42 $\pm$ 4.03	37.83 $\pm$ 4.95	40.61 $\pm$ 4.82	32.45 $\pm$ 3.98	33.79 $\pm$ 4.94	37.38 $\pm$ 5.85
NaOCl/PUI	33.86 $\pm$ 4.92	37.87 $\pm$ 6.54	41.91 $\pm$ 7.89	31.93 $\pm$ 3.81	36.64 $\pm$ 2.73	41.62 $\pm$ 3.35	31.75 $\pm$ 2.63	35.59 $\pm$ 3.09	40.15 $\pm$ 3.70
NaOCl/EC	33.98 $\pm$ 2.08	36.82 $\pm$ 2.29	42.43 $\pm$ 2.91	32.48 $\pm$ 4.35	35.15 $\pm$ 4.10	39.21 $\pm$ 4.85	33.90 $\pm$ 2.99	37.02 $\pm$ 4.36	44.03 $\pm$ 2.72
NaOCl/EDTA/MI	38.46 $\pm$ 4.35	40.83 $\pm$ 4.61	43.55 $\pm$ 4.96	34.89 $\pm$ 3.13	37.48 $\pm$ 2.20	40.32 $\pm$ 3.62	34.92 $\pm$ 4.74	37.53 $\pm$ 5.26	42.05 $\pm$ 6.19
NaOCl/EDTA/PUI	34.85 $\pm$ 4.20	38.43 $\pm$ 3.49	43.48 $\pm$ 2.34	33.28 $\pm$ 2.41	36.13 $\pm$ 2.59	39.64 $\pm$ 3.30	33.11 $\pm$ 1.10	35.61 $\pm$ 1.03	41.31 $\pm$ 2.02
NaOCl/EDTA/EC	32.04 $\pm$ 2.51	35.74 $\pm$ 3.74	40.56 $\pm$ 4.74	33.60 $\pm$ 3.91	37.65 $\pm$ 5.84	41.70 $\pm$ 8.57	30.65 $\pm$ 2.22	34.04 $\pm$ 3.12	39.26 $\pm$ 3.84

The repeated measures did not present statistically significant differences among the irrigant solutions at the cervical, middle and apical levels ( $p = 0.991$ ;  $p = 0.699$ ;  $p = 0.750$ , respectively). The EC group showed lower residue values at the middle and apical levels than the MI group (control;  $p < 0.027$  and  $p = 0.044$ , respectively).

### Dentin microhardness

The means and standard deviations of dentin microhardness at the three root levels (cervical, middle and apical) are summarized in Table 2. Two-way ANOVA and Dunnett's test at the cervical, middle and apical root levels, and at X (100  $\mu$ m), Y (200  $\mu$ m) and Z (400  $\mu$ m) distances did not detect any differences in dentin microhardness among the irrigants, the activation methods or the interactions ( $p > 0.05$ ).

## Discussion

Based on the results observed in this study, the null hypotheses were partially rejected, because a statistically significant difference was observed in the cleaning outcome, resulting from the particular activation method used. In contrast, the dentin microhardness values were not affected by the irrigating solutions, or the activation protocols. The effects of PSP on the roots filled using the single-cone technique have been previously evaluated regarding apical displacement and residual filling material.<sup>22,23</sup> The assessment of residual filling material using micro-CT<sup>9,22</sup> provides a three-dimensional view of the root canal without damaging the samples.<sup>9</sup> This assessment made it possible to use the same roots in the microhardness test. However, two different control groups were used for the micro-CT<sup>24</sup> and the dentin microhardness analyses.

Regarding that of microhardness, a positive control group was established with no additional irrigation protocols, since the use of chemical solutions can affect dentin microhardness.<sup>12</sup>

The residual filling material was removed with NaOCl or NaOCl/EDTA, both of which have often been recommended as post space cleaning solutions.<sup>6,8,9,25</sup> The volume of irrigants and the number of activation cycles were selected based on preliminary studies that used EC.<sup>14,15,16</sup> In the present study, EC was inserted using a low-speed pneumatic contra-angle, enabled by its wide-ranging availability, at no additional cost to dentists. Moreover, this method can be considered more advantageous than that indicated by the manufacturer, which requires reciprocating movement.<sup>14,16</sup> The lack of a significant difference in the cleaning achieved by EC and PUI, among the subgroup samples irrigated with NaOCl/EDTA, confirms that EC can be used in continuous rotation without affecting the performance of the tips, as previously demonstrated.<sup>17</sup>

The E1 ultrasonic insert has a tip size of 20.01, and promotes cavitation, acoustic microstreaming and shock waves<sup>1</sup> when used properly; it may not have had extensive contact with the canal walls.<sup>16</sup> The contact area could not be covered in the present study, because the Reciproc 50.05 device was used for instrumentation, and its file body has a larger diameter than its ultrasonic tip, thus allowing it to work free of interference. The reduction in the residual filling material adhered to root dentin after application of PUI for post space irrigation corroborates the results of a previous study.<sup>1</sup> However, there are no previous data on EC, regarding its ability to perform adequate cleaning for post installation. Its aircraft wing design and flexibility<sup>14,15</sup> probably helped clean the gutta-percha and endodontic sealer from the root canals, as suggested by the results of previous studies evaluating its debris removal<sup>14,16,17,26</sup> and irrigant penetration capabilities in lateral canals.<sup>24</sup>

A concern regarding EC is that erosion may occur when it is used with EDTA.<sup>15</sup> Although this issue was not evaluated in the current study, the sequential use of NaOCl and EDTA enhanced by the activation methods may result in the depletion of collagen and apatite in the dentin walls,<sup>11</sup> and a reduction in the calcium/phosphorus ratio,<sup>27</sup> which affects properties such as microhardness.<sup>12,28</sup> However, the lack of differences observed between Reciproc and EC, regardless of the root level and depth, suggests that the microhardness value eventually became equal to that of the control, when the NaOCl was neutralized by the hydroxyapatite, or when the hydroxyapatite crystals were reprecipitated onto the dentin surface.<sup>11</sup> Nevertheless, microhardness is a complementary parameter, and should not be assessed independently. Although this *in vitro* study had some limitations, such as the use of bovine teeth, non-inclusion of instrument aging, and inability to use the same sample before and after the post space irrigation protocols for dentin microhardness, the results are promising for EC under continuous rotation.

## Conclusion

In conclusion, irrigation with Easy Clean under continuous rotation showed a lesser amount of residual filling material than PUI or manual irrigation. The cleaning outcomes of the two systems were equivalent when NaOCl/EDTA was used with Easy Clean, PUI and manual irrigation. Dentin microhardness was not affected by the post space irrigation protocols.

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