Effect of Ultrasonic Activation of Irrigants on Smear Layer Removal

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Abstract

Introduction: The objective of this study was to evaluate the efficacy of passive ultrasonic irrigation (PUI) with 17% EDTA and 1% NaOCl solutions on smear layer removal. Methods: Root canal preparations of 32 human teeth were performed with the ProTaper system. Next, they were longitudinally fractured to permit quantitation of smear layer creation from the cervical, middle, and apical thirds of the roots by using scanning electron microscopy. After reassembling the fractured tooth halves, they were divided into 4 groups according to different final irrigation protocols: group 1, EDTA + NaOCl; group 2, EDTA with PUI + NaOCl; group 3, EDTA + NaOCl with PUI; and group 4, EDTA + NaOCl, both with PUI. After irrigation, the tooth halves were separated to permit imaging the same areas by scanning electron microscopy, and a percentage of opened dentinal tubules in irrigated areas as a percent of the total area was obtained. The results were submitted to Kruskal-Wallis, analysis of variance, and Bonferroni tests ($p = 0.05$). Results: The cervical third of the samples from all groups showed higher percentage of smear layer removal and open dentinal tubule areas, followed by the middle and apical thirds. Among the irrigation groups, there were statistically significant differences in cervical third between group 2 and group 4 samples, with the highest and lowest percentage of smear layer removal, respectively. Conclusions: PUI by using 1% NaOCl and ultrasonic tip placed within 1 mm of the apical foramen did not show higher efficacy in smear layer removal compared with conventional irrigation. (J Endod 2015;41:1359–1363)

Key Words

Irrigation, passive ultrasonic irrigation, SEM, smear layer

The removal of smear layer produced after root canal instrumentation has been recommended because its presence can have deleterious effects on the endodontic treatment. The presence of bacteria and their by-products and necrotic residue in endodontic smear layers compromise the disinfection process (1). In addition, smear layer decreases dentin permeability, interfering with diffusion of antimicrobial agents from irrigants and intracanal medications into root dentin (2, 3). Smear layers also block tubular entry of endodontic sealers and act as a barrier between obturation materials and canal walls, compromising root canal sealing and increasing chances of reinfection (4).

The alternating use of EDTA and sodium hypochlorite (NaOCl) solutions is used to remove the inorganic and organic portions of the smear layer, respectively (5). To be effective, the solutions must come into contact with root canal walls (6). Irrigation methods that use syringe and needle have been shown to be incapable of reaching difficult access areas such as apical and isthmus regions. Thus, the activation of irrigating solutions applied by various methods has been proposed to enhance their action and penetration (7, 8). Studies have shown that ultrasonic activation of irrigating solutions as passive ultrasonic irrigation (PUI) promotes better removal of the smear layer in the apical region and isthmus regions (9–11).

However, despite the publication of many articles on smear layer removal, there is no well-established protocol for PUI (11). In addition, the scoring of dentin only after final irrigation and qualitative analysis of smear layer removal by scores have been reported to be technical failures (12–14).

Because of these technical problems, it is necessary to establish final irrigation protocols for PUI. For this reason, this study evaluated longitudinally and quantitatively the efficacy of PUI on 17% EDTA and 1% NaOCl solutions to remove the smear layer.

Materials and Methods

The protocol used in the present study was approved by the Human Research Ethics Committee, Federal University of Santa Catarina. Thirty-two human premolars with single straight or slightly curved canals and fully formed roots were collected. Radiographs were taken to confirm straight single canal and the canal space size. Those teeth were extracted from young adult patients between 13 and 17 years old for orthodontic reasons. After accessing the root canal, the tooth length was obtained by introducing #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) into the canal to the point of displaying its tip at the apical foramen. The working length (WL) was obtained by subtracting 1 mm from the tooth length.

The apical region of each root was covered with a layer of heavy condensation silicone impression material (Zetaphus; Zhermack, Polesine Badia, Italy) to avoid...
extravasation of irrigating solutions and simulate the clinical condition of the presence of periapical tissues (15).

Root canal preparations were performed by the same operator with rotary instruments by using nickel-titanium ProTaper Universal files (Dentsply Maillefer) up to an F4 file. At each file change, the canals were irrigated with 2 mL 1% NaOCl by using a 5-mL syringe and NaviTip 30-gauge tip (Ultradent Products Inc, South Jordan, UT) calibrated to stop –2 mm from the WL, with back-and-forth movements of 2–3 mm. Simultaneously, suction was accomplished by using a metal cannula. Apical patency was maintained at each change of instrument by inserting #10 K-file up to the apical foramen. At the end of the procedure, canals were irrigated with 3 mL distilled water and dried with absorbent paper points.

A gutta-percha cone was introduced into the canal, and longitudinal grooves were made on the buccal and lingual external surfaces of each tooth by using diamond double-sided disks with a diameter of 22 mm and a thickness of 0.1 mm (ref. 7020; KG Sorensen, Cotia, Brazil) operated at low speed until the presence of the pink gutta-percha cone was seen, thereby avoiding accidental contamination and invasion of the canal by cutting debris. A small cotton pellet was placed in the access opening to prevent entry of cutting debris.

After the teeth were cleaved with the aid of a chisel, 1 of the 2 halves was selected for preirrigation evaluation by scanning electron microscopy (SEM). Three external markings were made on this half with a fine-tip pen on the external root surface, perpendicular to the long axis, to divide it into cervical, middle, and apical thirds of the same length. The markings served as references to make 3 grooves in the canal wall, delimiting the root into thirds (cervical, middle, and apical). The grooves were created by using double-sided diamond micro-disks, 7 mm in diameter and 0.1 mm in thickness, deep enough to be satisfactorily viewed by SEM. A no. 11 scalpel blade was used to create a new mark approximately 5 mm in length on the axial grooves. Thus, an image similar to a cross could be visualized on the root canal wall of each of the thirds (Fig. 1 A).

The fractured tooth samples were kept in an incubator at 37°C for 48 hours. Then the samples were placed in a vacuum desiccator containing anhydrous silica for the same period to remove any moisture. Without any coating or additional preparation, the samples were evaluated by SEM operated at low vacuum (JCM-6390LV; JEOL, Peabody, MA).

After locating the cross-shape markings on the canals, the most well-defined area completely covered by smear layer in each of the thirds was chosen at ×100 (Fig. 1 B). Another image was obtained at ×500, with its edges coinciding to the limits of the marks (Fig. 1 C). Then a third image was obtained at ×1000 without changing the position of the sample in SEM (Fig. 1 D). In total, 9 images were obtained per sample before irrigation, 3 images for each third. These initial images were used to evaluate the condition of the root canal walls before the final irrigation.

Next, the halves of each tooth were placed back together, and the grooves previously created for cleavage were filled with resin (Topdam; FGM, Joinville, Brazil) to stabilize the parts. The reassembled tooth root was inserted into heavy condensation silicone impression material to increase stability and prevent leakage of the solutions used in the final irrigation protocols.

Thirty-two teeth were randomly divided into 4 groups (n = 8) according to the final irrigation protocol used (Table 1).

All canals were irrigated with the same techniques used during the chemical-mechanical preparation. PUI was performed with a specific tip without cutting power, with apical diameter #20, taper 0.1 (Irrisonic E1; Helse, Santa Rosa de Viterbo, Brazil) calibrated to 1 mm short of the WL, activated by ultrasound (JetSonic; Gnatus, Ribeirão Preto, Brazil) at a power of 20% indicated by the manufacturer, avoiding contact with the walls of the root canal. The canals of all groups received 3 mL EDTA for 3 minutes and 3 mL NaOCl for 3 minutes.

At the end of the procedure, the canals were irrigated with 3 mL distilled water to remove possible salt residues from the irrigation solutions. Then they were dried with paper points.

After the experiment, the teeth were separated into their 2 halves and were dried, coated with gold, and analyzed by conventional SEM (high vacuum). New images were obtained from the same preselected and pre-photographed areas, following the methodology described.

**Figure 1.** (A) Marks to determine the evaluation area. Black arrows: grooves perpendicular to long axis of root canal made with diamond disk. White arrows: marks made with scalpel blade in direction of long axis of the canal. (B) “Cross” at the center of the canal wall. LL, lower left area; LR, lower right area; UL, upper left area; UR, upper right area. (C) Image obtained from apical third of the root, after root canal preparation showing the smear layer formed (original magnification, ×500). Arrows indicate marks made on root canal wall. (D) Image obtained after final irrigation. Note marks (arrows) that enabled to re-evaluate the same area of the image (original magnification, ×500). (E) Image of smear layer taken at ×1000. (F) The ×1000 image showing effect of final irrigation. (G) Image processing by ImageJ software (cervical third). Dark areas correspond to open dentinal tubules. (H) Identification of open dentin tubules (in red) by ImageJ software.
above (Fig. 1D and F). The images taken at ×1000 were evaluated by using Image J version 1.47 to enable identification and expression of the percentage of area of open dentinal tubules in relation to the total area of the analyzed image (Fig. 1G and H).

### Statistical Analysis

The Kolmogorov-Smirnov test was used to analyze the normality of the continuous variables, and the Levene test was used to analyze the homogeneity of variances among the groups. The combinations of variables “group” and “canal thirds” were statistically shown to have a normal distribution ($P > .05$). Because of the significant differences among the variances of the groups, a nonparametric analysis of variance test (Kruskal-Wallis) was used ($P = .05$). The analysis of variance was then used for multiple comparisons, with Bonferroni correction, to isolate the differences, which reduced the $P$ value to .005.

### Results

The average ± standard deviations of the percentages of open dentinal tubule areas in relation to the total area of the image are summarized in Table 2. In all groups, the highest percentage of opened dentinal tubule areas was found in the cervical third, followed by the middle and apical thirds.

When the thirds were compared, the results of different groups were similar ($P > .05$) except for the cervical third ($P < .001$), where a greater percentage of open tubule areas was observed in samples of group 2 compared with group 4 ($P = .02$).

When the average percentages of open tubule areas in all thirds of all groups were compared, a significant difference was observed only among groups 2 and 4 ($P = .018$).

### Discussion

Although PUI use has been reported to optimize smear layer removal (9, 10), there is no set protocol for quantity of irrigating solution, working time, or choice of solution to be activated. Furthermore, the methodology applied in most studies of smear layer removal has been questioned (14). This SEM study was designed to quantitatively and longitudinally evaluate the efficacy of PUI on removing the smear layer from cervical, middle, and apical thirds of instrumented root canals.

To replicate clinical conditions, a closed apical system was created to simulate clinical conditions because the presence of periapical tissues and possible entrapment of air in the apical region can hinder the penetration of solutions in this region (15).

To ensure that enough space was allowed for adequate flow of irrigating solutions to the WL, the canal was prepared up to ProTaper F4, with an apical diameter #40 (16).

One percent NaOCl was chosen in this study because when it is associated with the use of EDTA, it has been shown to be effective in the removal of smear layers (5, 9, 17). Moreover, the 1% NaOCl solution showed the same antibacterial effect of higher concentrations (18, 19). Its use can minimize the adverse effects of accidental extrusion (20) and deleterious changes in the structural properties of the dentin (21).

Techniques that promote activation of irrigating solutions have been used to allow better dispersion and penetration, enhancing its effects (7, 8). Among those techniques, PUI has been the subject of several studies (7–11). The term PUI (passive ultrasonic irrigation) is considered inappropriate by some researchers because it is impossible to prevent the ultrasonically activated instrument from touching the canal walls. The term is used to differentiate ultrasonic irrigation procedures from ultrasonic root canal preparation that is aimed at cutting or shaping dentin walls (11). In this study, a smooth-edge tip with no cutting power was used, with an apical diameter corresponding to #20 instruments, to avoid undesirable morphologic changes in already prepared canal walls (22).

In studies that used PUI with EDTA followed by NaOCl to remove the smear layer, there is no consensus on which solution should be ultrasonically activated. Some investigators used EDTA (17, 23), only NaOCl (24), or both (9, 25). Furthermore, there is no comparison

### Table 2. Average (A) and Standard Deviations (SD) of Open Dentinal Tubule Areas in Relation to Total Area of the Image, According to the Groups and Canal Thirds

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cervical</th>
<th>Medium</th>
<th>Apical</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>SD</td>
<td>A</td>
<td>SD</td>
</tr>
<tr>
<td>1: Conventional</td>
<td>21.14</td>
<td>3.40</td>
<td>12.51</td>
<td>6.16</td>
</tr>
<tr>
<td>2: PUI EDTA</td>
<td>25.22**</td>
<td>3.23**</td>
<td>15.70</td>
<td>8.48*</td>
</tr>
<tr>
<td>3: PUI NaOCl</td>
<td>20.12*</td>
<td>6.21**</td>
<td>13.65</td>
<td>6.75*</td>
</tr>
<tr>
<td>4: PUI EDTA + PUI NaOCl</td>
<td>18.60*</td>
<td>2.81</td>
<td>9.66</td>
<td>7.33a</td>
</tr>
<tr>
<td>Total</td>
<td>21.27*</td>
<td>4.66**</td>
<td>12.73</td>
<td>7.29a</td>
</tr>
</tbody>
</table>

A, average; SD, standard deviation.

Groups identified by different letters in each vertical column are significantly different ($P < .05$).

*Values with statistically significant difference ($P < .05$).
in the same study between the effects of PUI in each solution separately or together, as was done on this study.

Likewise, activation times of PUI, which vary from 20 seconds to 5 minutes, can be found in the literature (26–28). Activation time in this study was 1 minute because it is sufficient to remove the smear layer from the apical region, and it has been used in other studies (9, 17, 25).

The majority of studies on the removal of smear layer are performed by using conventional SEM, which means that it requires high vacuum and metalized specimen surfaces to allow visualization of the area to be evaluated. This type of analysis allows only acquisition of post-treatment images in a single moment in the study, which are acquired after the final irrigation because the sample, once desiccated and metalized, does not allow new experimental interventions. This model has been criticized as not allowing for longitudinal evaluations. Root canal areas not touched by instrumentation could be erroneously scored as areas of smear layer removal and lead researchers to mistaken conclusions by assigning maximum cleaning values to areas previously free of smear layer (14).

The SEM used in the current experiment can operate in low vacuum without metal coating. This allows pretreatment observations of samples that cannot be evaluated at high vacuum because of the presence of excessive water, either because there was no conductive surface or because they could not have a metal coating. A pilot study was conducted to verify the possibility of identifying the presence or absence of smear layer in samples without metallic coating by using backscattered electrons, with satisfactory results.

The qualitative analysis of open dentinal tubule scores is the most used method in research (14, 23, 25). To avoid observer bias and allow for quantitative analysis, automated evaluation by using software has been recommended (14, 29). It allows identification of the number and corresponding areas of opened dentinal tubules (30–32).

The results showed relatively high values for open dentin tubule areas on cervical thirds in all irrigation groups, followed by middle and apical thirds. This result was expected because the number and surface area of dentinal tubules decrease in the apical direction (35).

When multiple comparisons were made on regional smear layer removal, the only significant differences were found in the cervical third that was irrigated with PUI EDTA or with PUI NaOCl + PUI EDTA.

One reason that may explain this result is that a larger number of samples showed dentin erosion in the cervical and middle thirds in group PUI EDTA + PUI NaOCl. Niu et al (34) demonstrated dentin erosion occurs when NaOCl is applied after EDTA. Thus, some authors contraindicate NaOCl application after chelator use (7). Moreover, Calt and Serper (35) assert that to prevent erosion, which could lead to dentin physical and chemical changes, EDTA (17%) should not remain in contact with root canal walls for more than 1 minute. In this work, the irrigation time for both EDTA and NaOCl was 3 minutes. Moreover, specimens in the PUI EDTA + PUI NaOCl group had a longer ultrasonic activation time than the other groups (1 minute EDTA + 1 minute NaOCl), and ultrasonic activation of NaOCl may have contributed to dentin erosion. The eroded dentin surface showed significant irregularities, making it harder to identify dentinal tubules by software because such identification uses differences in the gray scale of images.

Despite the fact that no significant regional differences were identified in the apical region of the different groups, on samples with PUI usage, the percentages of open dentinal tubule areas were higher than in the conventional group. This finding corroborates results of other studies where PUI use has promoted better removal of the smear layer (9, 25).

According to the results of the current study, it can be concluded that by using 1% NaOCl and ultrasonic tip placed within 1 mm of the apical foramen, PUI did not show higher efficacy in smear layer removal compared with conventional irrigation.

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The authors deny any conflicts of interest related to this study.

References


