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Apically Extruded Debris Using Passive Ultrasonic Irrigation Associated with Different Root Canal Irrigants

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Introduction
Microbial reduction from root canals is of major relevance to achieve success in endodontic treatment. During the chemo-mechanical preparation (CMP), endodontic files and root canal irrigants are used to eliminate organic/inorganic tissues, which may harbor bacteria within the root canal system (1). As a consequence, organic and inorganic debris, bacteria and irrigants may extrude into the periapical tissues (2), resulting in severe pain (3).

Sodium hypochlorite (NaOCl) is the most used chemical substance during the root canal treatment (4). Chlorhexidine (CHX) has been recently proposed as an alternative to NaOCl due to its properties, such as lubricating action, broad spectrum, substantivity and lower toxicity compared to NaOCl (5,6). In addition, the rheological action of CHX-based gel maintains the debris in suspension (5), and as a result it prevents apical extrusion of debris (6).

In order to enhance the effectiveness of root canal irrigants, several studies have suggested its agitation. Passive ultrasonic irrigation (PUI) is a technique that relies on the ultrasonic activation of irrigants for efficient removal of debris and microorganisms (7). An ultrasonic tip is activated inside the root canal, along the working length (WL), and moved passively in up-and-down motions to prevent it from binding to the root canal walls (8).

PUI associated with different substances have been used to improve the effectiveness of the root canal treatment. However, literature is scanty regarding whether PUI can prevent debris extrusion. Therefore, the aim of the present study was to evaluate the amount of apically extruded debris after chemo-mechanical preparation associated with passive ultrasonic irrigation using four different root canal irrigants, namely: 6% sodium hypochlorite (NaOCl), 2% chlorhexidine gel + saline solution (2% CHXg + SS), 2% chlorhexidine solution (2% CHXs) and SS alone. The null hypothesis was that there were no differences in the amount of extruded debris between the irrigants tested.

Material and Methods
Sample Selection

The present study was approved by the Human Research Ethics Committee of the Piracicaba Dental School, State University of Campinas – UNICAMP, São Paulo, SP, Brazil.

Previous studies (9,10) were used to identify an effect size of 0.50 required to calculate the total sample size for this study. \( \alpha \) -type error = 0.05 and power \( \beta \) = 0.80 were also input. A total of 48 samples were indicated as the
minimum to observe differences between the systems (F test family, ANOVA, G*Power for Windows). A minimum of 12 teeth per group should be used. Therefore, 60 human mandibular premolars extracted for reasons not related to this study were selected. Prior to the experiments, the teeth were disinfected with 0.5% chloramine T, kept in distilled water at 4°C and used within 6 months after extraction. Soft tissue remnants and/or calculi on the external root surface were ultrasonically removed under constant and copious irrigation.

All the specimens selected for this study presented similar root length (19±1 mm) confirmed by using a digital caliper (American Dental Systems, Vaterstetten, Germany). The inclusion criteria were as follows: single-rooted teeth with one straight root canal, one apical foramen with mature apex (radiographically confirmed) and absence of fractures, caries and resorptions. Digital radiographs from the buccolingual and mesiodistal angles were taken of each tooth to standardize the root canal anatomy and curvature. Image analysis software (AxioVision 4.5; Carl Zeiss Vision, Hallbergmoos, Germany) was used to evaluate the root canal anatomy and to measure the angle of curvature of each root canal. Teeth with single oval-shaped root canals, with a cross-section diameter ratio of ≥2.5:1 at 5 mm from the apex were included in the study. Curvature angle was measured at the coronal aspect of the apical third of the canal (11), and only those teeth with root canal curvature < 10° and initial apical size equivalent to a #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) were selected (12).

Traditional endodontic access cavities were prepared on the occlusal surfaces by using high-speed diamond burs under copious water-cooling. To achieve apical patency, a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was introduced until its tip reached the apical foramen. This procedure was performed under an operating microscope (DFV Comercial e Ind. Ltda, Valença, RJ, Brazil) at 20X magnification. The working length (WL) was established at 1 mm short from the apical foramen and the foramen diameter of all teeth was standardized by using a #20 K-file (Dentsply Maillefer, Ballaigues, Switzerland) along the WL. After the initial apical diameter (0.20 mm) standardization, Reciproc R25 single-files were used for the mechanical instrumentation (not a tested variable in this study). Therefore, a compatible file with the initial diameter was used for minimum interference as possible in the passive ultrasonic irrigation results.

Root Canal Preparation

The specimens were randomly assigned into 4 experimental groups (n=15) according to the root canal irrigating solution used during CMP. A computer algorithm (www.random.org) was used for specimen randomization as follows: G1 (PUI + 6% NaOCl) ; G2 (PUI + 2% CHXg + SS) ; G3 (PUI + CHXs) ; G4 (PUI + SS).

A single experienced and previously trained operator performed CMP and passive ultrasonic irrigation of all specimens. Reciproc® R25 files (25/0.08, VDW, Munich, Germany) were used with the RECIPROC ALL program (VDW) in a slow in-and-out pecking motion and 3-mm amplitude limit combined with brushing motion. After 3 pecking motions, the endodontic file was withdrawn from the root canal, cleaned and inspected before being reused (6).

Irrigation Protocol

All chemical substances were prepared by the same manufacturer (Drogal, Piracicaba, SP, Brazil) and used just after their manipulation.

Passive ultrasonic irrigation (PUI) was used as irrigation protocol in all groups. The root canal irrigant was delivered with a syringe and a 30-gauge needle (NaviTip, Ultradent Products Inc, South Jordan, UT, USA).

Initially, the root canal was rinsed with 5 mL saline solution for 1 minute in all groups. Immediately before CMP with R25 Reciproc files, the teeth were rinsed with 1 mL of each solution and after 3 pecking motions the root canals were again irrigated with 5 mL of each solution. A final irrigation was performed with 5 mL of each irrigating solution, and in the 2% CHXg + SS group, irrigation was completed with 5 mL of SS. As for group 2, before each preparation, the root canals were rinsed with 1 mL of CHXg.

The total volume used in groups 1, 3 and 4 was 35 mL. For group 2, 5 mL of 2% CHXg and 30 mL of SS were used for final volume standardization, as previously described.

Root canals were rinsed with each irrigation solution before 0.20-mm ultrasonic tip (0.01 taper) (E1, Helse, Santa Rosa de Viterbo, SP, Brazil) was placed 1 mm short of the WL, which was activated at a frequency cycle of 40-kHz per second for 30 s. After last irrigation, it was used for 1 minute (Obtura Spartan Endodontics, Algonquin, IL, USA).

Initially, the root canals were rinsed with 5 mL saline solution for 1 minute in all groups. Next, each tooth was filled with 1 mL of the correspondent irrigant solution (6% NaOCl, 2% CHXg, 2% CHXs or SS). After 3 pecking-motions, the root canals were rinsed with 5 mL of the correspondent irrigant solution, and in the 2% CHXg it was rinsed with SS. This procedure repeated until the R25 Reciproc file reached the WL.

Following, passive ultrasonic irrigation was carried out with ultrasonic tip E1 (Irisonic, Helse Dental Technology, Santa Rosa de Viterbo, São Paulo, Brazil) mounted on a piezoelectric ultrasonic unit (Piezon 150, Electron Medical Systems, Nyon, Switzerland). The ultrasonic tip was inserted 1 mm short of the WL and the irrigant present within the
root canal space was passively activated using a power setting of 30% of the ultrasonic device. This procedure was performed in three cycles of 20 s each (total activation of 60 s). Short in-and-out motions (2–3 mm) were performed without touching lateral walls. Replenishment of the irrigant was performed using conventional syringe/needle irrigation. The protocol was completed within 5 min using a total of 5 mL per tooth.

Debris Collection
The method used for the collection of apically extruded debris was adapted from previous studies (6,9,13,14).

Empty Eppendorf tubes were pre-weighted by using a 10-5-g precision analytic microbalance (SP Labor, São Paulo, SP, Brazil). Three consecutive weights were obtained for each tube, and the mean value was considered to be its initial weight.

A 27-G needle was inserted alongside the stopper to be used as a drainage cannula and to equalize the air pressure inside and outside the tubes. Next, each stopper with tooth and needle was attached to its Eppendorf tube, and the tubes were fitted into vials.

The operator was blinded from seeing the root apex during the instrumentation procedures by a rubber dam overshadowing the vial.

Immediately after the instrumentation, the Eppendorf tube was removed from the vial. Each tooth was gently removed from the Eppendorf tube and the debris adhered to the root surface were collected by washing off the apex with 1 mL of distilled water into the Eppendorf tube. The tubes were stored in an incubator at 68°C for 5 days to evaporate the moisture before weighing the dried debris (14). Weighing was carried out again and three consecutive weights were obtained for each tube, and the mean was calculated. The dried weight of the extruded debris was calculated by subtracting the weight of the empty tube from that of the tube containing debris.

Statistical Analysis
Statistical analysis was performed by using one-way analysis of variance (ANOVA). Tukey’s post hoc test was used for multiple comparisons. The alpha-type error was set at 0.05.

Results
Apical Extrusion of Debris
Table 1 provides an overview of the mean values and standard deviation in each group. Debris extrusion was observed in all groups, irrespective of the root canal irrigant used during CMP. The use of 2% CHXg + SS was associated with lower debris extrusion compared to their irrigants (p<0.05). No significant differences were observed between 6% NaOCl, 2% CHXs and SS irrigation protocols.

Discussion
The present study was undertaken to evaluate the amount of apically extruded debris after chemomechanical preparation associated with passive ultrasonic irrigation using four different root canal irrigants (6% NaOCl, 2% CHXg + SS, 2% CHXs and SS alone). PUI has been associated with different substances (i.e. sodium hypochlorite or Chlorhexidine) to enhance microbial reduction in the root canal system, consequently increasing the effectiveness of the endodontic therapy. In the present study, the use of 2% CHXg + SS minimized the apical extrusion of debris compared to other root canal irrigants tested (p<0.05). Therefore, the null hypothesis was rejected.

For assessment of apically extruded debris, an already-established protocol described by Myers and Montgomery (13) was used in the presented study as well as elsewhere (6,9,14). Although this method was also recommended by Tanalp and Güngor (3), the limitation of the present study is related to the lack of an apparatus simulating the periodontal tissue. Thus, the foramina of the specimens were suspended in air (zero back pressure). The results obtained in our study may differ from a clinical study where the periodontium acts as a natural barrier, possibly limiting the extrusion of debris (6,9,15).

Teeth were carefully selected taking into consideration their type and length, standardization of the initial foramen diameter and working length, number of canals and canal curvature. This procedure guaranteed that debris extrusion was a result of the study variables (i.e. root canal irrigants). Mechanical instrumentation of the specimens was carried out by using reciprocating single files, as they allow less apical extrusion of debris than conventional multiple-file rotary systems (6,16). Moreover, reciprocating single file systems are simple, faster and efficient compared to conventional rotary systems (2,6).

Most of the studies have evaluated different endodontic files and/or systems regarding the amount of apically extruded debris (9,17). With regard to assessment of

<table>
<thead>
<tr>
<th>Root canal irrigants</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>6% Sodium hypochlorite</td>
<td>0.00422 ± 0.00154 B</td>
</tr>
<tr>
<td>2% Chlorhexidine gel + saline solution</td>
<td>0.00147 ± 0.00061 A</td>
</tr>
<tr>
<td>2% Chlorhexidine solution</td>
<td>0.00405 ± 0.00174 B</td>
</tr>
<tr>
<td>Saline solution</td>
<td>0.00539 ± 0.001921 B</td>
</tr>
</tbody>
</table>

Different superscript letters represent significant differences (p<0.05).
the amount of extruded debris using different irrigating systems, only a few studies have been published (6,18). There are a few studies evaluating different root canal irrigation solutions for preventing debris extrusion. Barbosa-Ribeiro et al. (6) evaluated extruded debris by using positive (conventional irrigation) and negative pressure (EndoVac) irrigation systems in association with different irrigants. In our study, sodium hypochlorite and chlorhexidine (gel and solution) were used due to their great acceptability for use in the endodontic practice, whereas saline solution was used as control.

The use of 2% CHXg + SS prevents, though not completely, apical extrusion of debris compared to 6% NaOCl, 2% CHXs and SS alone (p<0.05). The current results may be the consequence of the viscosity and rheological action of chlorhexidine in gel formulation, which keeps debris in suspension during CMP (5,6). Our findings agree with a previous study (6), which showed that 2% CHXg + SS was associated with less debris extrusion compared to 6% NaOCl, 2% CHXs and SS alone (p<0.05) using conventional irrigation with syringe and needle. However, no difference was observed between the irrigating solutions using negative pressure irrigation system (EndoVac, SybronEndo, Orange, CA, USA), suggesting that mechanical action was the main responsible for preventing extrusion of debris.

Debridement of the root canal system with minimum extrusion of debris is one of the main goals of the endodontic treatment. Apical extrusion of organic and inorganic debris, bacteria and irrigants may cause damage to the periradicular tissues and result in severe pain, particularly in root canals with complex anatomies (2,6,19,20). The results of this work confirmed that PUI and the tested auxiliary chemical substances could not completely prevent extrusion of debris. Therefore, this study indicates a need for complementary irrigation approaches to enhance the cleaning of canals instrumented with single-files and minimizing the risks of pain due to the extrusion of toxic substances into the periapical region. Further studies should be conducted to evaluate whether different cycles and frequency of the ultrasonic irrigation, device setting power and type of activation (passive or active) can prevent the amount of extruded debris. This is of clinical importance, since modern techniques for root canal treatment require less clinical time. Therefore, additional disinfection protocols are needed for better prognosis.

Although it was not possible to completely avoid the extrusion of debris through the apical foramen, it is mandatory the use of passive ultrasonic irrigation or other irrigation protocols to complement the disinfection of the root canal system and consequently increase the success rates of the endodontic therapy, clinically.

In conclusion, passive ultrasonic irrigation did not completely prevent apical extrusion of debris. However, PUI with 2% Chlorhexidine gel + saline solution was found to significantly reduce debris extrusion compared to 6% sodium hypochlorite, chlorhexidine solution and saline solution.

Resumo
O presente estudo avaliou a quantidade de debris extrudidos apicalmente após o preparo químico-mecânico (PQM) associado à irrigação ultrassônica passiva (IUP) em associação com quatro diferentes irrigantes – hipoclorito de sódio 6% (NaOCl), clorexidina gel 2% + solução salina (CLXg 2% + SS), solução de clorexidina 2% (CLXs 2%) e SS. Sessenta pré-molares inferiores com canais radiculares únicos e retos foram selecionados e aleatoriamente distribuídos em 4 grupos (n=15) de acordo com o irrigante utilizado: G1 (IUP + NaOCl), G2 (IUP + CLXg + SS), G3 (IUP + CLXs) e G4 (IUP + SS). Limas Reciproc® R25 (25/0.08) foram utilizadas durante o PQM e os debris extrudidos de cada dente foram coletados em tubos Eppendorf pré-pesados e secos. O peso médio de debris foi avaliado através de microbalança analítica, e os dados foram analisados estatisticamente utilizando ANOVA e teste de Tukey post hoc (α=0.05). Extrusão de debris foi observada em todos os grupos, independente do irrigante. CHXg 2% + SS foram associados a menor extrusão de debris comparado aos demais irrigantes (p<0.05). Não foram observadas diferenças estatisticamente significativas entre NaOCl 6%, CLXs 2% e SS. Concluindo, irrigação ultrassônica passiva não preveniu completamente a extrusão apical de debris, entretanto, IUP realizada com CLXg 2% + SS minimiza significativamente a extrusão de debris comparado ao NaOCl 6%, CLXs 2% e SS.

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