Effectiveness of Final Irrigant Protocols for Debris Removal from Simulated Canal Irregularities

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Abstract

Introduction: The aim of this *in vitro* study was to compare the effectiveness of saline, 2.5% sodium hypochlorite, and 2% chlorhexidine, with or without passive ultrasonic irrigation (PUI), in debris removal from simulated canal irregularities within prepared root canals. **Methods:** Ninety bovine lateral incisors were randomly divided into 3 main groups (n = 30) based on the irrigant and prepared with hand files attached to an oscillating handpiece (NSK, Tokyo, Japan) up to a size #80 K-file. Next, the teeth were split longitudinally, and a standardized groove was prepared into the apical third and filled with dentin debris. After the halves were reassembled, they were placed in a muffle. Each main group was randomly subdivided into 2 groups (n = 14) and was treated with different final irrigation protocols. In the sodium hypochlorite/PUI, chlorhexidine/PUI, and saline/PUI groups, the solution was ultrasonically activated 3 times for 20 seconds. In the remaining groups, PUI was not performed. Specimens were scored for debris removal and analyzed under a scanning electron microscope. **Results:** An association was observed between the score of debris removal and protocols using PUI ($P < .05$). No association was observed between the scores of debris removal and the irrigants (P = .87). **Conclusions:** Final irrigation protocols that used PUI were more effective in removing debris from simulated canal irregularities into the apical third than those that did not use it. *(J Endod 2014;40:2009–2014)*

Key Words

Dentin debris, endodontics, irrigation systems, passive ultrasonic irrigation, root canal irrigation

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The success of endodontic therapy is dependent on the proper preparation, disinfection, and root canal filling, providing optimal conditions for periapical tissue repair (1). The root canal system is highly complex, impairing cleaning and disinfection procedures as well as the removal of root canal dressing. The root canal shaping, performed by different manual and rotary instruments, acts just in the central area of the root canal, especially those with oval (2) and C-shaped cross-sections (3). Therefore, isthmuses, apical deltas, accessory canals, and anatomic irregularities may be filled with debris, necrotic pulp remnants, and microorganisms and their by-products, which will contribute to the persistence of apical periodontitis (2, 4).

According to Peters et al (5), at least 35% of the root canal surface remains uninstrumented regardless of the canal preparation technique. Therefore, the use of irrigants is vital for chemomechanical preparation because they remove pulp tissue, microorganisms, the smear layer, and debris; neutralize endotoxins; and lubricate the canal walls in addition to disinfecting the areas that are inaccessible to instruments (6). Sodium hypochlorite (NaOCl) and chlorhexidine (CHX) have an antibacterial effect against microorganisms present in the root canal system (7). Additionally, NaOCl is able to dissolve organic tissue (8), and CHX shows a residual effect or substantivity (9).

The following 2 factors are directly related to efficient irrigation: the chemical properties of the irrigant and the delivery system used for irrigation, especially if it is able to reach areas that cannot be reached by instruments (10). Numerous techniques and irrigation devices have been tested to improve cleansing and disinfection of the root canal system (11, 12). Among these techniques, passive ultrasonic irrigation (PUI) has been described as an excellent aid in the final cleaning process of the root canal system (13). Several authors have reported the cleaning potential of PUI and its ability to increase the effectiveness of irrigants to remove the smear layer and debris from inaccessible areas of the root canal (14–18). The ultrasonic tips used in PUI must oscillate freely and toward the irregularities to promote better cleaning (19). Therefore, acoustic microstreaming and hydrodynamic cavitation (the formation and implosion of vapor bubbles) have been even more intense and contributed to the maximal effectiveness of the irrigants (19, 20).

The aim of this *in vitro* study was to assess the effectiveness of saline, 2.5% NaOCl, and 2% CHX with or without PUI in debris removal from simulated canal irregularities into the apical third of prepared root canals. The null hypothesis was that there would be no differences among the debris scores of simulated canal irregularities regardless of the irrigant and whether PUI was performed.

Materials and Methods

Tooth Selection

The study was approved by the Ethics Committee of the Federal University of Rio Grande do Sul. Ninety bovine lateral incisors were extracted and stored in 0.2% thymol solution (Spengler Pharmacy Manipulation, Porto Alegre, RS, Brazil). All roots were examined under a magnifying glass (×8), and roots with fractures, lacerations, incomplete root formation, or resorption were excluded from the study. Only roots with an initial apical diameter equal to the size of a 50 K-file were included in this study.

All crowns were removed with a double-sided diamond disc (Komet, Santo André, SP, Brazil) using a low-speed straight handpiece (Kavo, Joinville, SC, Brazil). The root size of each specimen was standardized to a length of 16 mm. Then, 2 longitudinal grooves were created along the mesial and distal external roots without reaching the root canal (Fig. 1A). The teeth were randomly divided into 3 main experimental groups (n = 28) and a control group (n = 6) using random table numbers generated by the www.randomizer.org website.

Specimen Embedment in a Muffle System

Utility wax was placed at the apex of each sample (Wilson; Polidental Indústria e Comércio Ltda, Cotia, SP, Brazil). Then, each sample was molded with condensation silicone (Clonage; DFL, Rio de Janeiro, RJ, Brazil) and embedded in the center of a metallic muffle (Fig. 1A) to prevent leakage from the irrigant during the chemomechanical preparation simulating a closed irrigation system.

Chemomechanical Preparation

Chemomechanical preparation was performed up to a size #80 K-file (Dentsply Maillefer, Ballaigues, Switzerland) attached to an oscillatory handpiece (16:1 reduction, 750 rpm) (NSK, Tokyo, Japan). The working length was set to 15 mm, which was 1 mm shorter than the root length. A set of endodontic files was used for each group of 5 specimens. The specimens of each group were flushed with 2 mL of the respective irrigant at each file change and at the end of the preparation process.

Group 1 was irrigated with saline (Texon, Viamão, RS, Brazil), group 2 with a 2.5% solution of NaOCl (Marcela Manipulation Pharmacy, Porto Alegre, RS, Brazil), and group 3 with 2% CHX (Marcela Manipulation Pharmacy). A 30-G irrigation needle was used to deliver the solutions into the root canals (Navitip; Ultradent, South Jordan, UT). The needle was positioned 2 mm shorter from the working length and attached to a 10-mL disposable syringe (Injex, Ourinhos, SP, Brazil), which was properly identified with the irrigant's name.

Simulated Canal Irregularities

After chemomechanical preparation, the samples were removed from the muffles and cleaved longitudinally using a chisel (SS White Duflex, Rio de Janeiro, RJ, Brazil). A longitudinal groove (4-mm long, 0.2-mm wide, and 0.54-mm deep) was created on the internal surface of the canal. A graphite mark was made 2 mm away from the apex indicating the apical end of the groove (Fig. 1B). A second graphite mark was

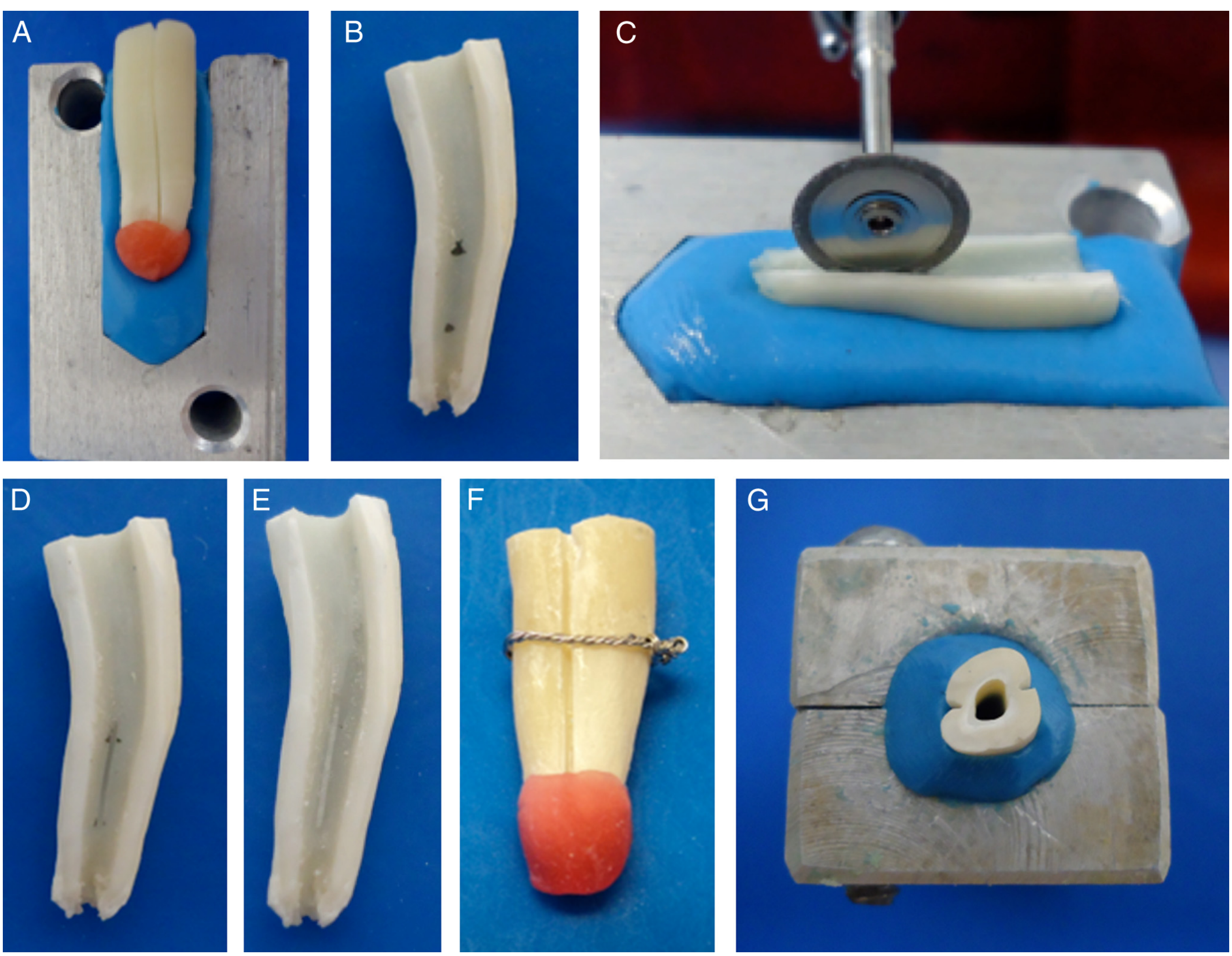

Figure 1. (*A*) Longitudinal grooves along the root without reaching the canal and embedding in a metallic muffle with condensation silicone. (*B*) After chemomechanical preparation, the roots were cleaved, and graphite marks were performed to delimitate the limits of the groove. (*C*) A double-sided diamond disc (0.8-cm diameter and 0.2-mm thickness) was used to create the groove. (*D*) A longitudinal groove in the apical portion of the root. (*E*) Grooves completely filled with debris. (*F*) The root halves were joined together with a ligature wire (0.20-mm diameter). (*G*) The roots were placed in the mold, and the muffle was positioned.

made 4 mm apart from the first mark indicating the other end of the groove. This maneuver was performed using an electronic digital caliper (Starrett, Athol, MA) with graded ends that were fixed 4 mm from one another to standardize the groove size and location.

The groove was created with a double-sided diamond disc (0.8-cm diameter and 0.2-mm thickness) (Komet, Santo André, SP, Brazil) that was attached to a straight handpiece (Kavo, Joinville, SC, Brazil) mounted on a table clamp (Forjasul, Canoas, RS, Brazil) (Fig. 1*C*). The root canal halves were secured on a metal surface with dense condensation silicone (Clonage). The low-speed bur penetrated the canal wall until the graphite marks disappeared, cutting a longitudinal groove that was 4 mm in length (Fig. 1*D*).

The Pythagorean theorem was used to calculate the exact groove depth (Fig. 2). According to this theorem, in a right triangle, the sum of the squares of the 2 legs equals the square of the hypotenuse. Consider "R" the circle radius equal to 4 mm and "a" as half of the total length of the groove equal to 2 mm. After applying the formula, the "b" value was found to be 3.46 mm, and the exact value of "x" was determined by subtracting 3.46 mm from 4 mm (R), which resulted in 0.54 mm.

Debris Production and Insertion into the Groove

Debris was produced from the wear of the remaining root dentin after standardization of root length with spherical steel burs no. 8 (KG Sorensen, Cotia, SP, Brazil) at low speed. Debris was weighed on an analytical balance (Sertorius AG, Goettingen, Germany), separated into 0.025-g portions, and packed in an aluminum foil bag. The debris was mixed with 0.1 mL of the respective irrigant during the irrigation protocol using a 0.5-mL syringe (BD, Franklin Lakes, NJ). This mixture

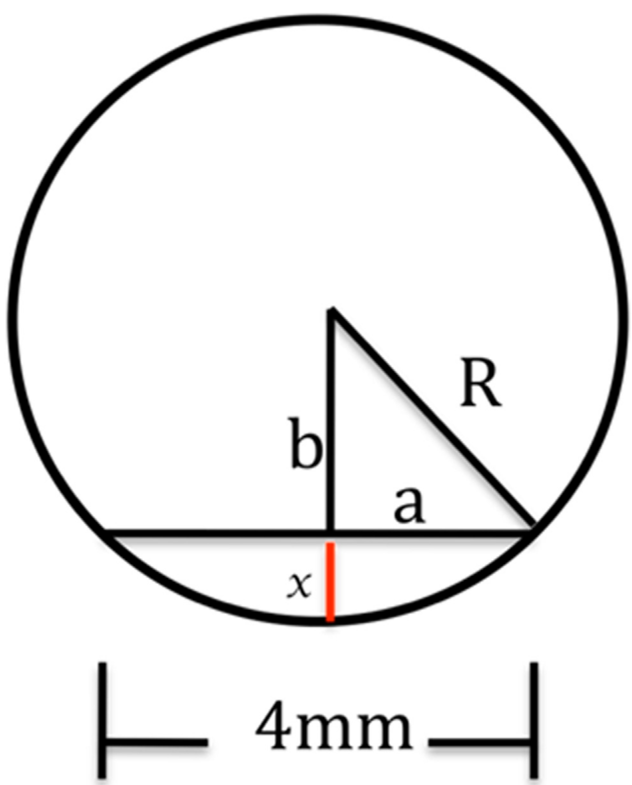

Figure 2. chematic diagram of the dimensions of the disc used to create the groove and calculations to measure the exact groove depth. Ø = 8 mm; R = 4 mm; a = 2 mm; R2 = a2 + b2 ⇒ 42 = 22 + b2 ⇒ b2 = 16 − 4 = 12 ⇒ b = $\sqrt{12}$ = 3.46; R = b + x ⇒ x = 4 − 3.46 ⇒ x = 0.54.

was placed into the grooves using a mineral trioxide aggregate carrier (Angelus, Londrina, PR, Brazil) (Fig. 1*E*). Excess debris was removed from the groove with a superfine microbrush (FGM, Joinville, SC, Brazil).

Irrigation Protocols

The root halves were joined together, secured with ligature wire that was 0.20 mm in diameter (Morelli, Sorocaba, SP, Brazil), and interlocked using straight orthodontic pliers no. 121 (Golgran, São Paulo SP, Brazil) (Fig. 1*F*). The apexes were sealed using utility wax (Wilson), the roots were placed into the mold, and the muffle was repositioned (Fig. 1*G*). The 3 main groups of 28 samples were divided into 2 subgroups based on the presence or absence of PUI, resulting in 6 groups with 14 specimens each as follows:

1. Saline: 6 mL saline + 5 mL 17% EDTA + 2 mL saline
2. NaOCl: 6 mL 2.5% NaOCl + 5 mL 17% EDTA + 2 mL 2.5% NaOCl
3. CHX: 6 mL 2% CHX + 5 mL 17% EDTA + 2 mL 2% CHX
4. Saline/PUI: 6 mL saline + PUI for 1 minute (3 activations of 20 seconds) + 5 mL 17% EDTA + 2 mL saline
5. NaOCl/PUI: 6 mL 2.5% NaOCl + PUI for 1 minute (3 activations of 20 seconds) + 5 mL 17% EDTA + 2 mL 2.5% NaOCl
6. CHX/PUI: 6 mL 2% CHX + PUI for 1 minute (3 activations of 20 seconds) + 5 mL 17% EDTA + 2 mL 2% CHX

Each group was irrigated with 26 mL of the respective solution (18 mL during chemomechanical preparation and 8 mL during the final irrigation protocol) and 5 mL 17% EDTA, resulting in 31 mL of total solution. PUI was performed with an NAC Plus device (Adiel Ltda, Ribeirão Preto, SP, Brazil) by inserting a smooth ultrasonic tip (E1 Irrisonic; Helse, Capelli e Fabris, São Paulo, Brazil) with a tip diameter equivalent to a #20 K-file at a frequency of 30 kHz 1 mm shorter from the working length.

Six specimens were used as controls: 2 for each irrigant, 1 positive control, and 1 negative control. The positive controls had the grooves filled with debris and received no final irrigation protocol. The negative controls had empty grooves, and the final irrigation protocol was not performed.

The root half of each sample that contained the groove was secured onto a metal stub and stored in an oven for 48 hours at 36°C. Next, the samples were gold plated (SCD 050; Bal-tec, Tokyo, Japan) to be examined by scanning electron microscopy (JEOL 6060; JEOL, Tokyo, Japan) operated at 10 kV and ×20 magnification.

Assessment Criteria

The criteria for debris removal assessment used the following scores: 1, the groove is empty; 2, <50% of the groove filled with debris; 3, >50% of the groove filled with debris; and 4, the groove is completely filled with debris (21). The evaluations were performed by 2 calibrated examiners blinded to the experimental groups. Ten representative score images not used for this study analysis were presented to the examiners for calibration. After 24 hours, the investigators received images numbered 1–84 for evaluation together with a separate sheet, which contained the numbering of the images, and the scores were registered.

Data Analysis

The collected data were organized into a database and statistically analyzed using the SPSS 19.0 software (SPSS Inc, Chicago, IL). The Cohen kappa test was used to assess interrater reliability. The Kolmogorov-Smirnov normality tests revealed that the distribution was not in accordance with normal standards, with all variables showing significant results ($P < .05$).

The chi-square distribution and the adjusted Pearson residual analysis were used to assess the frequency distribution of events among the groups and the association among them. The frequency scores of debris removal from the groove were analyzed by the nonparametric Kruskal-Wallis test followed by Dunn multiple comparison tests. A significance level of 5% was set for all statistical analysis.

Results

Debris Removal

The Cohen kappa coefficient of interrater reliability was 0.77. Divergent scores occurred in 15 cases, which were reassessed to reach a consensus. After all of the samples were scored, inferential statistical analysis was performed.

An association was observed among the debris removal scores and protocols that used PUI ($P < .05$). The adjusted residual analysis revealed that in the groups that underwent PUI, the frequency of scores 1 and 2 was significantly higher than expected, whereas the frequency of score 4 was significantly lower than expected. The data are shown in Table 1.

No association was found among the scores of debris removal and the irrigants ($P = .870$). Debris removal scores were not dependent on the type of irrigant used. The data are shown in Table 1.

Scanning electron microscopic images represent the median scores for debris removal in each group. Figure 3*A–L* shows scanning electron microscopic images from groups with and without PUI, respectively.

Discussion

Because of the difficulty of obtaining sound single-rooted human teeth, bovine teeth have been used as an alternative for *in vitro* studies because they have physical properties that are similar to human dentin (22), are easily available, and have a low prevalence of caries. Furthermore, it is possible to obtain specimens of the same age and dentinal features, enabling samples of the same animal to be distributed within different experimental groups and, thus, minimizing sample variability. According to Camargo et al (23), bovine root dentin has a higher amount of dentinal tubules than human dentin, but no difference was found between them regarding the tubule diameter from all root thirds.

The experimental design of this study is comparable with the designs of previous studies (21, 24, 25). The present study was performed to assess dentine debris removal from simulated canal irregularities in the apical third. In the previous studies (18), a rectangular-shaped groove was created, whereas the groove in this study was similar in size but was semicircle in shape. Thus, the anatomic features found in isthmuses, irregularities, extensions of oval canals, and untouched canal areas during chemomechanical preparation where dentinal debris accumulates were represented with greater fidelity and precision than in rectangular-shaped grooves (4).

The groove standardization enabled the insertion of equal amounts of debris before the final irrigation protocols because all of them had the same dimensions in terms of length, width, and depth. The groove length was defined by the action of the dental disc along the 4-mm extension determined by a digital caliper. The groove width was determined by the thickness of the dental disc working without oscillations, and the depth was determined using the Pythagorean theorem as described earlier. This method allowed technical reproducibility and standardization in creating grooves that simulate isthmuses. One study examined the amount of debris only after preparation and irrigation, without reporting any standardization of debris before irrigation and, therefore, making it impossible to effectively compare the cleaning capability between different irrigation protocols (26).

In the present study, PUI was performed for 1 minute in 3 periods of 20 seconds each, with renewal of the irrigant between activations. According to van der Sluis et al (21), ultrasonic activation of the irrigant combined with 3 refreshment/activation cycles produces a cumulative effect on debris removal. In addition, short periods of activation facilitate the maintenance of the ultrasonic tip in the center of the root canal, thereby preventing its contact with the dentinal wall (27). During PUI, the use of a smooth tip did not cause any damage to the canal wall and has been shown to be as effective as a K-file for debris removal (24). Jensen et al (28) also recommended the use of ultrasonic tips or even K-files with small diameters to avoid the contact of these instruments with the canal walls. Therefore, these instruments can freely oscillate and promote increased cavitation effects in the irrigant (19, 29). The oscillating direction of the ultrasonic tip was parallel to the groove, corroborating the study by Jiang et al (19); they found a smaller amount of debris compared with oscillations perpendicular to the groove.

Previous studies have shown that the volume of the irrigant affects root canal cleaning (25, 30). In this study, the overall volume of irrigants in each group was standardized (31 mL). Van der Sluis et al (25) observed that syringe delivery of 6 mL and 12 mL 2% NaOCl were as effective as continuous delivery of 50 mL 2% NaOCl during PUI. However, conventional irrigation and increasing the irrigating volume have not been enough to remove debris completely, especially into the apical third and in anfractuosities of the root canals (25). When PUI is used, the energy generated during the ultrasonic activation is transmitted to the ultrasonic tip and then to the irrigant (19). These factors induce 2 physical phenomena, acoustic microstreaming and hydrodynamic cavitation, which are responsible for the cleaning findings of this study.

Four scores were established to assess debris removal (21) based on quantitative criteria (<50% or >50%), whereas other authors (31, 32) have used subjective criteria (low, moderate, or severe).

TABLE 1. Frequency Distribution of Debris Removal Scores with and without Passive Ultrasonic Irrigation (PUI) and after Using the Irrigants

	Final irrigating system (%)			Irrigants (%)			
Scores	PUI	Without PUI	Overall	Saline	NaOCl	CHX	Overall
1	15* (17.8)	1† (1.2)	16 (19)	4 (4.8)	5 (5.9)	7 (8.3)	16 (19)
2	11 (13)	3† (3.7)	14 (16.7)	4 (4.8)	4 (4.8)	6 (7.1)	15 (16.7)
3	12 (14.4)	13 (15.4)	25 (29.8)	10 (11.9)	8 (9.5)	7 (8.3)	25 (29.8)
4	4 (4.8)	25* (29.7)	29 (34.5)	10 (11.9)	11 (13.1)	8 (9.5)	29 (34.5)
N	42 (50)	42 (50)	84 (100)	28 (33.4)	28 (33.3)	28 (33.3)	84 (100)

Pearson chi-square test ($P < .05$) for the final irrigating systems; Pearson chi-square test ($P = .870$) for the irrigants.

*Frequency of scores significantly higher than expected measured by adjusted residual analysis.

†Frequency of scores significantly lower than expected measured by adjusted residual analysis.

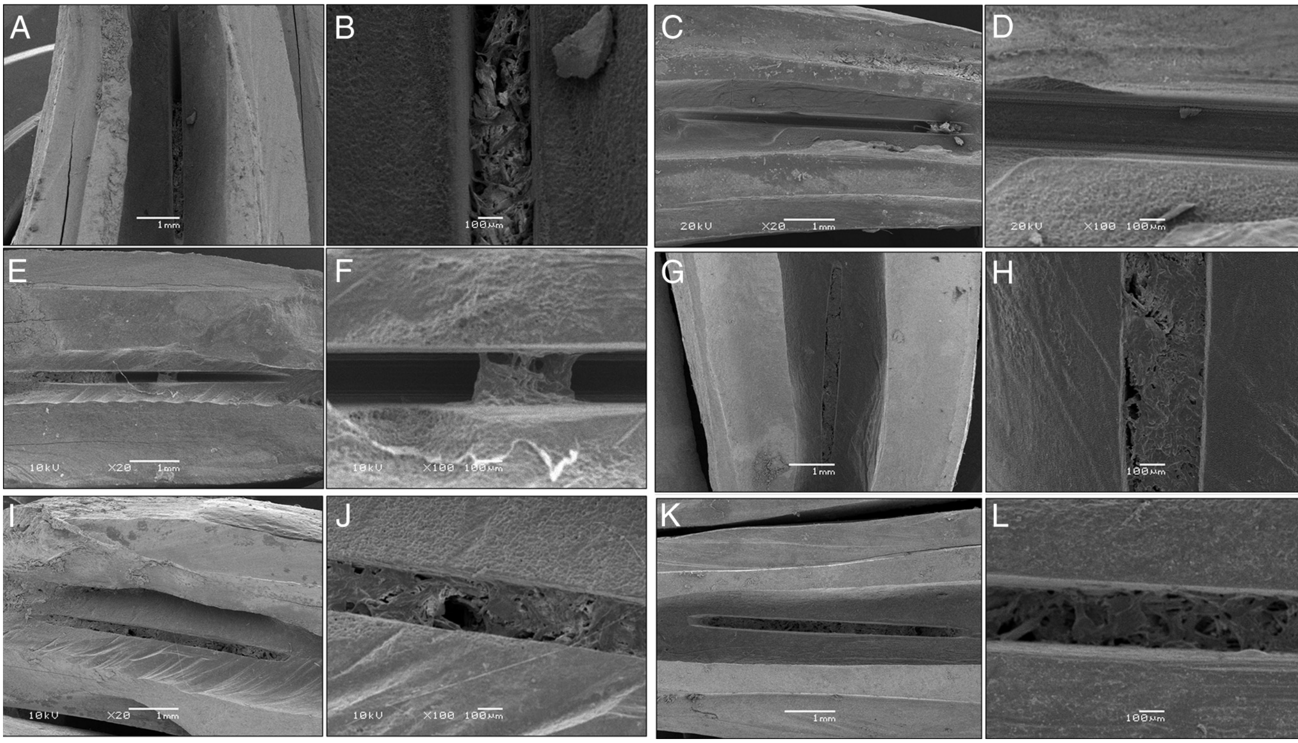

Figure 3. Scanning electron microscopic images (×20 and ×100) representing the median scores for debris removal from groups with and without PUI. Saline/PUI: more than 50% of the groove filled with debris (*A* and *B*), NaOCl/PUI: less than 50% of the groove filled with debris (*C* and *D*), CHX/PUI: less than 50% of the groove filled with debris (*E* and *F*), saline: the groove is completely filled with debris (*G* and *H*), NaOCl: the groove is completely filled with debris (*I* and *J*), and CHX: the groove is completely filled with debris (*K* and *L*).

This factor may contribute to the decrease in intraobserver and interobserver agreement. Qualitative data assessment does not have a distribution within the normal range and requires a nonparametric analysis of the data. Therefore, the Kruskal-Wallis test was used for comparison among the groups, and the Pearson chi-square test was used to assess the frequency distribution of events among the groups and whether there was an association among them.

Results showed that all groups had significantly lower scores when PUI was used. These findings are similar to those from previous studies (14, 31, 32) that showed that debris removal was related more closely to the mechanical activation than the type of irrigant used. However, van der Sluis et al (21) suggested that there was a chemical effect of the irrigant on debris removal. NaOCl activated ultrasonically had better results than distilled water. Previous studies have not shown a difference between the protocols with or without PUI, most likely because those studies used low-power ultrasound (33) or PUI was performed in curved canals (34). The present findings reject the hypothesis that there is no difference between the debris scores of simulated canal irregularities regardless of the irrigant and whether PUI was performed.

Conclusion

Given the experimental conditions of this study and the results obtained, it can be concluded that the final irrigation protocols using PUI were more effective in removing debris from the apical third than those that did not use PUI. The longitudinal groove using a mathematic equation appears to be an interesting alternative for the standardization of simulated canal irregularities and the standardization of the amount of debris inserted into the groove.

Acknowledgments

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

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