Effect of different protocols of final irrigation on smear layer removal

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ABSTRACT

Introduction: This study evaluated the effect of different final irrigation protocols using EDTA and QMix on smear layer removal. **Methods:** The root canals of 40 single-rooted human teeth were prepared with ProTaper Universal SX - F3. The substances used were 5.25% sodium hypochlorite (NaOCl), 2% chlorhexidine gel (chlorhexidine), 2% chlorhexidine solution and distilled water. The teeth were divided into 8 groups, according to the irrigant used: G1: 5.25% NaOCl + 17% EDTA + 5.25% NaOCl; G2: 5.25% NaOCl + 17% EDTA + distilled water; G3: 5.25% NaOCl + QMix + 5.25% NaOCl; G4: 5.25% NaOCl + QMix + DW; G5: 2% chlorhexidine gel + 17% EDTA + 2% chlorhexidine solution; G6: 2% chlorhexidine gel + 17% EDTA + distilled water; G7: 2% chlorhexidine gel + QMix + 2% chlorhexidine solution; and G8: 2% chlorhexidine gel + QMix + distilled water. Samples were evaluated under environmental scanning electron microscopy (ESEM) before and after irrigation. ESEM images were classified according to smear layer scores, and the Kruskal-Wallis and Mann-Whitney tests were used for statistical analyses. **Results:** Group 1 had the best results for smear layer removal ($p<0.05$). Smear layer formation was lower when 2% chlorhexidine gel was used after cleaning and shaping than when the irrigant was 5.25% NaOCl, and 5.25% NaOCl had the best cleaning ability as a final irrigant. **Conclusion:** 5.25% NaOCl combined with EDTA was the best final irrigant for smear layer removal.

Keywords: Endodontics. Microscopy. Electron. Scanning. Smear Layer.

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Introduction

The success of an endodontic treatment depends primarily on cleaning and shaping of the root canal system. However, although shaping techniques have advanced substantially along the years, no cleaning technique achieves complete disinfection of root canals.[1] Several chemical substances have to be used for that purpose during and after root canal shaping.[2]

Sodium hypochlorite (NaOCl) is the most common irrigant during root canal instrumentation because of its broad antimicrobial spectrum, its ability to dissolve tissue remnants and its low cost.[3] However, this solution is unstable and may cause allergic reactions and tissue damage when in contact with the mucosa and periapical tissues.[4] For that reason, other solutions have been suggested to complement or substitute for NaOCl.

Chlorhexidine is used as an irrigant because of its antimicrobial action, substantivity and biocompatibility. However, differently from NaOCl, it does not dissolve tissue remnants.[5] Chlorhexidine is recommended as a final irrigant because it promotes wettability of endodontic cements on the dentin surface and delays coronal microinfiltration.[6-8]

The smear layer, a compact layer of inorganic debris, dentin particles and organic matter, such as bacteria and their byproducts, forms during cleaning and shaping of a root canal system. Removal of this layer is recommended because it may harbor bacteria and their byproducts and compromise the root canal disinfection. Moreover, the smear layer reduces dentinal permeability, which affects the diffusion of intracanal medication and the penetration of endodontic sealers into the dentinal tubules.[9-11]

As NaOCl and chlorhexidine are unable to remove the smear layer, a chelating agent should be used as an adjuvant substance. The most common irrigant for that purpose is ethylenediaminetetraacetic acid (EDTA)[3] at different concentrations.

QMix (DENTSPLY Tulsa Dental, Tulsa, OK) is a compound formed by EDTA, chlorhexidine, a surfactant agent and deionized water.[12] QMix may be used as a solution for smear layer removal and a substitute for EDTA.[13,14] In addition to its ability to remove smear layer, it has antimicrobial activity, a low level of toxicity and does not precipitate when interacting with the remaining NaOCl, if used according to instructions for the final rinse.[15]

Thus, the aim of this study was to evaluated, under scanning electron microscopy, the effect of different final irrigation protocols combined with 17% EDTA and QMix on smear layer removal.

Methods

Sample selection and preparation

This study was approved by the Ethics Committee of the Pedro Ernesto University Hospital, State University of Rio de Janeiro, Brazil (Opinion no. 561,682). Forty single-rooted human teeth with fully formed apices were used. The presence of a single canal was determined on buccolingual and mesiodistal radiographs of each tooth. After that, teeth were immersed in 5.25% sodium hypochlorite for one minute for disinfection and then stored in 1% thymol. Before the beginning of the trial, specimens were pre-rinsed under running water for one hour to remove thymol.

The access was prepared using a #1014 round bur 1014 (KG Sorensen, Zenith Dental ApS, Agerskov, Denmark) and Endo Z (Maillefer Instrument, Baillaigues, Switzerland). Canal patency was checked with a #15 Kerr Flexofile (Dentsply-Maillefer Instruments, Baillaigues, Switzerland), and working length was established at 1 mm short of the apical foramen.

Root canals were prepared using a SX, F1, F2 and F3 sequence (ProTaper Universal, Dentsply-Maillefer Instruments, Baillaigues, Switzerland). After the use of each instrument, 1 mL of 5.25% NaOCl (Mil Fórmulas, Rio de Janeiro, Brazil) or 2% chlorhexidine gel (Mil Fórmulas, Rio de Janeiro, Brazil) was used as an irrigant, at a total of 5 mL (Phase 1).

For smear layer removal, 17% EDTA (Mil Fórmulas, Rio de Janeiro, Brazil) or QMix 2 in 1 (Dentsply Tulsa Dental, Tulsa, OK) was applied for three minutes (1 mL/min). Finally, one mL/min of 5.25% NaOCl, 2% chlorhexidine solution or distilled water (control) was applied for three minutes (Phase 2).

Specimens were divided into groups (Table 1) according to initial and final irrigation protocols.

Environmental Scanning Electron Microscopy (ESEM)

After cleaning and shaping (Phase 1), the specimens were sectioned longitudinally using a double-

Table 1. Irrigation protocols

Groups	Irrigant	Smear layer removal	Final irrigant
1	NaOCl 5,25%	EDTA 17%	NaOCl 5,25%
2	NaOCl 5,25%	EDTA 17%	Distilled water
3	NaOCl 5,25%	QMix	NaOCl 5,25%
4	NaOCl 5,25%	QMix	Distilled water
5	chlorhexidine gel 2%	EDTA 17%	chlorhexidine solution 2%
6	chlorhexidine gel 2%	EDTA 17%	Distilled water
7	chlorhexidine gel 2%	QMix	chlorhexidine solution 2%
8	chlorhexidine gel 2%	QMix	Distilled water

faced, diamond-coated disk (DP1522, DHPro Tecnologia Profissional, Paraná, Brazil) and a low speed electric motor. Two superficial buccolingual cuts that did not reach the canal were made on each root. Marks were made on the dentin to divide the canal into three thirds: coronal, middle and apical. After that, each root was split into two equal halves using a spatula.

The roots were place on the stubs with the internal surface up and taken to the environmental scanning electron microscope (ESEM) (FEI, Quanta 400, Thermo Fisher Scientific, Hillsboro, OR). The canals were first examined along the entire root at a 50x magnification. After that, the coronal, middle and apical thirds were scanned at a 100x magnification, as described by Prado et al,[16] and three regions were scanned in each third at a 2000x magnification.

The roots were removed from the scanner and etched according to the protocols and volumes described above using 5-mL disposable syringes (Ultradent Products Inc., South Jordan, UT) and 30-gauge NaviTips needles (Ultradent Products Inc., South Jordan, UT). One half of each root received a final irrigation with 5.25% NaOCl or 2% chlorhexidine solution and the other half, with distilled water (control) (Phase 2).

The root canal was fully dried with absorbing paper tips (Dentsply, Tulsa Dental Specialties, OK) and the roots were taken to the ESEM scanner again to obtain images after etching. The regions to be scanned were selected again, as described above.

Three previously calibrated endodontists, blinded to irrigation protocols, evaluated all images. The cleanliness of root canals was evaluated according to a scoring system described by Prado et al.[16] (Fig 1):

» Score 1 – no smear layer, all tubules are clean and open;

» Score 2 – few areas covered by smear layer, most tubules clean and open;

» Score 3 – smear layer covers most of the surface, few tubules are open;

» Score 4 – smear layer covers all surface.

Statistical analysis

Data were tabulated and analyzed statistically. The kappa test was used to evaluate agreement between observers in the classification of ESEM images ($p<0.001$). The Kruskal-Wallis and Mann-Whitney U tests ($p<0.05$) were used for the comparisons between groups and between each 2 groups. Scores were compared before and after etching with the chelating solutions using the Wilcoxon test to evaluate differences between two paired variables, such as the two halves of the same root in the ESEM trial.

Kappa results indicated excellent agreement in the classification of smear layer removal ($p<0.001$).

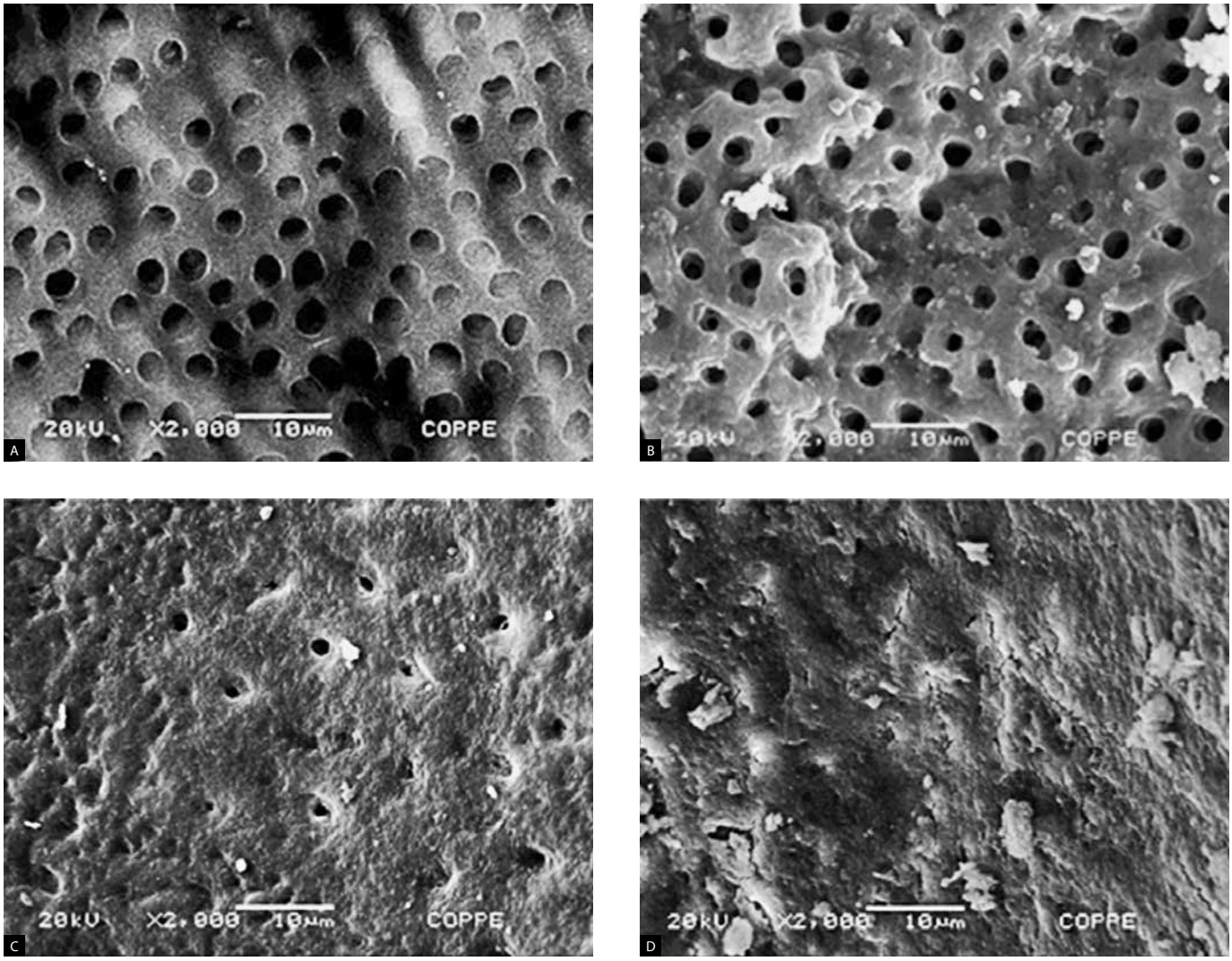

Figure 1. Images illustrating the scoring system used for evaluation under ESEM: (**A**) score 1, (**B**) score 2, (**C**) score 3 and (**D**) score 4.

Results

Initial (after cleaning and shaping) and final (after the use of chelating agents or final irrigation) results were statistically different for all irrigation protocols under evaluation ($p < 0.05$) (Table 2).

The irrigation protocols for the experimental groups were statistically different ($p<0.001$). Group 1 (5.25% NaOCl + 17% EDTA+ 5.25% NaOCl) had the best results, whereas Group 5 (2% chlorhexidine gel + 17% EDTA+ 2% chlorhexidine solution), the worst ($p<0.05$) (Table 3).

There was a significant difference in smear layer formation between 5.25% NaOCl and 2% chlorhexidine gel, as the amount of smear layer at the end of preparation was smaller for 2% chlorhexidine gel than for 5.25% NaOCl. ($p<0.05$).

The analysis of final irrigation protocols revealed a significant difference between the solutions under evaluation, and smear layer removal with 5.25% NaOCl was better than with 2% chlorhexidine solution ($p<0.05$).

Table 2. Mean scores before and after etching.

Irrigation protocols	Before	After
5.25% NaOCl+17% EDTA+5.25%NaOCl	3.79 ± 0.41^{B}	2.08 ± 0.87^{A}
5.25%NaOCl + 17% EDTA + Distilled water	3.48 ± 0.52^{B}	2.33 ± 0.87^{A}
5.25% NaOCl+ QMix + 5.25% NaOCl	3.8 ± 0.4^{B}	2.56 ± 0.81^{A}
5.25% NaOCl+ QMix + Distilled water	3.74 ± 0.46^{B}	2.46 ± 0.71^{A}
2% chlorhexidine gel + 17% EDTA + 2% chlorhexidine solution	3.57 ± 0.53^{B}	3.02 ± 0.59^{A}
2% chlorhexidine gel + 17% EDTA + Distilled water	3.57 ± 0.49^{B}	2.63 ± 0.77^{A}
2% chlorhexidine gel + QMix + 2% chlorhexidine solution	3.16 ± 0.59^{B}	2.56 ± 0.61^{A}
2% chlorhexidine gel + QMix + Distilled water	3.14 ± 0.52^{B}	2.58 ± 0.64^{A}

Note: Different superscript letters within rows indicate statistically significant differences (Wilcoxon, $p<0.05$)

Table 3. Scores for different irrigation protocols.

Group	Irrigation protocols	Mean ± SD
1	5.25% NaOCl+17% EDTA+5.25% NaOCl	2.08 ± 0.87^{A}
2	5.25% NaOCl+ 17% EDTA + Distilled water	2.33 ± 0.87^{B}
3	5.25% NaOCl+ QMix + 5.25% NaOCl	2.56 ± 0.81^{BC}
4	5.25% NaOCl+ QMix + Distilled water	2.46 ± 0.71^{BC}
5	2% chlorhexidine gel + 17% EDTA + 2% chlorhexidine solution	3.02 ± 0.59^{D}
6	2% chlorhexidine gel + 17% EDTA + Distilled water	2.63 ± 0.77^{C}
7	2% chlorhexidine gel + QMix + 2% chlorhexidine solution	2.56 ± 0.61^{BC}
8	2% chlorhexidine gel + QMix + Distilled water	2.58 ± 0.64^{C}

Note: Different superscript letters within columns indicate statistically significant differences (Mann-Whitney, $p<0.05$)

Discussion

This study evaluated, under ESEM, the effect of different final irrigation protocols combined with 17% EDTA or QMix on smear layer removal.

The most common method to evaluate smear layer and the dentinal surface is SEM. However, SEM requires sputter coating, sanding and dehydration, which result in biological damage to the samples. To avoid it, De Deus et al.[17] recommended the use of techniques in which observations may be conducted under low vacuum conditions, without the need for sample coating. This study used ESEM and, therefore, preserved the characteristics of the dentinal structure and of the smear layer, something not possible when using conventional SEM evaluations.

The irrigation protocol using NaOCl+EDTA+NaOCl (Group 1) had the best results. Several studies have demonstrated that this protocol, the most common in clinical practice, has a good smear layer removal ability, and that its results are superior to those obtained when using other solution or combinations as potential substitutions for NaOCl and EDTA.[18-25]

QMix had a poorer smear layer removal ability than EDTA when combined with NaOCl. However, Aranda-Garcia et al.[25] and Dai et al.[15] did not find any differences between QMix and EDTA when combined with NaOCl. This may be explained by the chemical interaction between NaOCl and the chlorhexidine found in QMix composition,[26-30] which results in the formation of a precipitate that covers the dentinal tubules.[29] This chemical layer may compromise the reliability of score classifications and mislead observers into misclassifying the cleanliness of the dentinal wall.[28]

Table 4. Smear layer formation after cleaning and shaping.

Final irrigant	Mean ± SD
NaOCl 5,25%	3.7 ± 0.47[B]
CHX 2% gel	3.36 ± 0.57[A]

Note: Different superscript letters within columns indicate statistically significant differences (Mann-Whitney, p<0.05)

Table 5. Smear layer removal after final irrigation with NaOCl or chlorhexidine.

Final irrigant	Mean ± SD
NaOCl 5,25%	2.31 ± 0.88[A]
CHX 2% solution	2.79 ± 0.64[B]

Note: Different superscript letters within columns indicate statistically significant differences (Mann-Whitney, p<0.05)

The negative result of the chlorhexidine gel + EDTA + chlorhexidine solution (Group 5), which had higher scores than all other groups, may be explained by the chemical interaction resulting from the combination of chlorhexidine and EDTA and the consequent formation of a white-milky precipitate, a product of the acid-base reaction between the two chemicals.[19] The accumulation of this chemical layer on the dentinal wall prevents the visualization of the dentinal tubules and is confused with the mechanical smear layer that results from endodontic instrumentation.

Group 6 (2% chlorhexidine gel + 17% EDTA + distilled water) had better results than Group 5 (chlorhexidine gel + EDTA + chlorhexidine solution). This may be explained by the fact that the chemical interaction between EDTA and chlorhexidine solution, which would lead to the formation of a chemical smear layer, was blocked because distilled water was used in place of the chlorohexidine solution. Therefore, distilled water provided a better cleaning of the dentinal walls than the QMix solution.[19,27]

In this study, QMix used with chlorhexidine (Group 7) produced better smear layer removal than EDTA (Group 5). The information provided by QMix manufacturers does not include the exact concentration and proportion of the chemicals in the solution. EDTA concentration, according to the manufacturer, is lower than 15% (Material Safety Data Sheet). A lower EDTA concentration and amount may be sufficient to reduce the precipitate produced by its interaction with chlorhexidine.

Magro et al.[18] suggest that canal aspiration and drying might be enough to inhibit the interaction between irrigants and, consequently, precipitate formation. However, in this study precipitate might have formed on the dentinal surface in all the groups in which chlorhexidine was used in combination with EDTA, which may explain the poorer results of these groups than those of the groups in which NaCl was used. Therefore, an unreactive solution should be used as irrigation between the applications of different substances during endodontic treatment, as previously described in the literature.[19]

The evaluation of smear layer at the end of cleaning and shaping revealed that the dentinal surfaces treated with 2% chlorhexidine gel had less smear layer resulting from the mechanical action of instruments than those treated with 5.25% NaCl (Table 4). This result is in agreement with those reported in other studies, which reported that gel viscosity and rheological characteristics are the main reasons why 2% chlorhexidine gel keeps instrumentation debris in suspension and results in cleaner dentinal walls.[27,30]

The cleaning ability scores for 5.25% NaOCl and 2% chlorhexidine solution as final irrigants were significantly different and indicated that 5.25 % NaOCl resulted in better cleaning of the dentinal walls. Some authors have reported similar results.[27,29] Important to note that distilled water, as a final irrigant, had lower or similar scores to those of NaOCl, but equal or higher than those of chlorhexidine, and varied according to type of irrigant used for smear layer removal. The use of distilled water as a final irrigant may inhibit the continuation of the chemical reactions between substances that may interact in the root canal.

Conclusion

The use of 5.25% NaOCl combined with 17% EDTA as a final irrigant resulted in better smear layer removal.

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