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Evaluation of Smear Layer Removal using Different Irrigation Protocols: An *In Vitro* Sem Study

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Abstract

Objectives: *Sodium hypochlorite (NaOCl) and decalcifying agents are common irrigation solutions used in endodontic therapy. The aim of this study was to compare solutions of ethylenediaminetetraacetic acid (EDTA) and 1-hydroxyethane-1,1-diphosphonic acid (HEDP) in combination with sodium hypochlorite to observe their abilities to remove smear layer using scanning electron microscope (SEM).* ***Results:*** *SEM evaluation showed the smallest significant amount of smear layer in the coronal part of the canals irrigated with 2.5% NaOCl + 18% HEDP compared with the apical part (P = 0.014). Root canals irrigated with NaOCl + EDTA and NaOCl + HEDP showed a greater ability to remove the smear layer when compared with the canals irrigated with 2.5% NaOCl alone or saline water in all canal thirds (P < 0.05). No significant difference was found between groups 1 and 4, or between groups 2 and 3, in all canal thirds (P > 0.05).* ***Conclusion:*** *A chelator used together with NaOCl can reduce but not completely remove a smear layer from root canal dentin during rotary root canal instrumentation.*

Keywords*: Ethylenediaminetetraacetic acid, irrigation, smear layer, etidronic acid*

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Introduction

One of the main purposes in endodontic therapy is to disinfect the root canal, which is accomplished by removing the pulp tissue or necrotic pulp remnants, as well as microorganisms present in the root canal system and infected dentin. Pulp remnants are removed by using a variety of instruments and techniques during the preparation of root canal systems, which includes enlarging and shaping the canal together with its disinfection.[1] However, the use of hand or rotary instruments during the preparation of the root canal results in the production of a considerable amount of smear layer that forms on instrumented dentin surfaces and debris.[2] Debris is a superficial layer that covers dentinal walls, and the smear layer occludes dentinal tubules. Removing the smear layer and debris allows for more thorough cleaning and disinfection of root canal walls and better adaptation of root canal filling materials.[3] Various methods have been used to remove the smear layer and debris.

Sodium hypochlorite (NaOCl) is a common irrigation solution used in endodontic therapy because it has bactericidal properties and the ability to dissolve organic tissues.[4] Its effectiveness has been shown to depend on its concentration, temperature, pH, and storage conditions.[5] The main disadvantage of NaOCl is its inability to remove the inorganic portion of smear layer and debris. For this reason, using a combination of NaOCl with decalcifying solutions is advised. The widely used decalcifying solution is ethylenediaminetetraacetate (EDTA), which has the ability to dissolve inorganic residues and is

generally used at 17% concentration. EDTA seems to reduce the antibacterial and solvent activity of hypochlorite, but these liquids should not be used together in the canal at the same time. Furthermore, EDTA may weaken the dentinal structure and erode the tubular dentine if it remains in the root canal for a prolonged period.[6,7] This reduction can adversely affect the physical and mechanical properties of the dentin, bonding, and sealing and ultimately increase the risk of root fracture.[8] For this reason, during mechanical preparation abundant and frequent washing with sodium hypochlorite is used, whereas EDTA is used at the end of the preparation phase to remove the inorganic debris and smear layer completely from the canal walls.[9]

To facilitate the irrigation protocol and avoid the problems mentioned above, a need arises for a decalcifying agent that can be combined with NaOCl and does not aggressively decalcify the dentin but removes inorganic residues.

According to Paqué et al,[10] etidronic acid is a mild chelator that is compatible with NaOCl in the short term and should be used as an additive in NaOCl-irrigating solutions during the entire endodontic treatment. This combination is favorable because this solution balances the hypochlorite – hypochlorous acid equilibrium toward hypochlorite, which has better tissue dissolution capacity than hypochlorous acid has and less cytotoxicity. A combined NaOCl and hydroxyethane 1,1-diphosphonic acid (HEDP; the salt is called etidronate) solution not only prevents smear layer and hard tissue debris accumulation during root canal instrumentation, but also reduces torsional load on rotary instruments.[10-13] Research has also shown HEDP in fresh mixtures with NaOCl does not reduce the antibacterial effect of the NaOCl and can even improve the NaOCl's disinfection effect in the presence of a smear layer or hard tissue debris.[14] HEDP is a less destructive agent in terms of its effects on root dentine and does not erode dentin and expose collagen.[12] The tetrasodium salt of HEDP has a high NaOCl compatibility and can be directly dissolved in a NaOCl solution, which then remains useful for at least 1 hour.[15,16]

Moreover, research has been proven that conventional needle-delivery irrigation solutions are only effective 1 to 2 mm beyond the needle tip,[17] which is insufficient for cleaning the apical area completely, as well as for inaccessible areas of the root canal system. Therefore, many techniques have been involved in sufficient cleaning of the apical area, isthmus, and anastomosis, including the use of sonic/ultrasonic instruments and laser to increase the efficacy of irrigant solutions.[18] XP-endo Finisher as a finishing file for irrigant activation has been developed to improve the penetration and effectiveness of solutions inside the root canal system.[19]

The aim of this study is to compare solutions of EDTA and HEDP acids used together with sodium hypochlorite regarding their ability to remove smear layer using scanning electron microscopy (SEM).

Materials and Methods

The study was performed in the Department of Dental and Oral Pathology, Lithuanian University of Health Sciences, and was approved by the Lithuanian University of Health Sciences ethics committee.

Forty single-rooted mandibular incisors extracted for reasons not related to this study were used. The teeth were cleaned and stored in 1× phosphate-buffered saline solution at room temperature after extraction. Inclusion criteria were as follows: permanent teeth, intact fully formed apices, no extensive restorations or previous root canal treatment, and no root caries, cracks, or fracture lines. The presence of a single canal with an intact and closed apex was verified by taking both the buccolingual and mesiodistal views of radiographs. The same operator (HHW) manipulated all experimental procedures. Working lengths were established 1 mm short of the anatomical apex by visually identifying a #10 K-file (KaVo Kerr, Glendora CA, USA) at the apical foramina. To achieve a closed system, the apices were closed with softened wax (Morsa Dental, Krumbach, Germany) to prevent any flow of irrigants and a #10 K-file was inserted before the apex was sealed to prevent the materials from entering the canal.

K-file sizes 10 and 15 (KaVoKerr, Glendora CA USA) were used to create a glide path. Root canals were prepared with XP-endo Shaper (FKG Dentaire, Switzerland) to size 30.4% taper using a torque-controlled motor (X-smart,

Dentsply Maillefer, Ballaigues, Switzerland) according to the manufacturer's recommendations. After each file, the root canals were rinsed with an endodontic syringe and 30-gauge Navi Tip side-vented needle (Ultradent Products Inc., South Jordan, UT, USA). The needle was introduced 2 mm short of the working length by adjusting the rubber stopper on the needle to the desired length. Four irrigation solutions were prepared: (1) 2.5% NaOCl (Chloraxid; CERKAMED Medical Company, Poland), (2) 17% EDTA (i-EDTA Solution; i-dental, Lithuania), (3) 18% HEDP (Dual Rinse HEDP; Medcem GmbH, Switzerland) dissolved in 2.5% NaOCl, and (4) saline water (sterile NaCl 0.9 mg/ml solution; Polifarma, Lithuania).

According to the respective irrigation protocol, the teeth were randomly divided into 3 experimental groups ($n = 10$) and 1 control group ($n = 10$).

- Group 1: Irrigation of the canals with 2.5% NaOCl. The final irrigation used 2.5% NaOCl activation with XP-endo Finisher for 1 minute.
- Group 2: Irrigation of the canals with 2.5% NaOCl. The final irrigation used 2.5% NaOCl activation with XP-endo Finisher for 1 minute, 17% EDTA irrigation, and activation with XP-endo Finisher for 20 seconds, leave for more than 40 seconds.
- Group 3: Irrigation of the canals with 2.5% NaOCl + 18% HEDP. The final irrigation used 2.5% NaOCl + 18% HEDP, activation with XP-endo Finisher for 1 minute.
- Group 4: Irrigation of the canals using saline solution.

The canals were rinsed with saline solution and dried with paper points (FKG Dentaire, Switzerland).

SEM Preparation

The teeth were decoronated using diamond discs (Yeti Dental, Germany) at the enamel-cemental junction to obtain a standardized root canal length of 9 mm. Then all the roots were split longitudinally into two halves. Initially, to facilitate fracture into two halves, all roots were grooved longitudinally on the buccal and lingual surfaces with a diamond disc, avoiding penetration into the cavity. Then the roots were split with a small chisel into two halves. One-half of each root was chosen randomly. In total, 40 sections were obtained. The sections were allowed to dry at room temperature for 24 hours and sputter-coated with gold before being observed with SEM (SEM Zeiss Ultra Plus, Zeiss, Oberkochen, Germany). Photomicrographs at ×2000 for the smear layer evaluation were taken, and scoring was performed in the apical, middle, and coronal thirds of one longitudinal half of each root.

Specimen Grading

An operator who was experienced in the analysis of teeth specimens and blinded to the aim of the study obtained scanning electron photomicrographs of the teeth at ×2000 to evaluate smear layer. All specimens were divided into apical, middle, and coronal thirds using a marker to draw horizontal lines. A certain field of each third of each specimen was chosen randomly and assessed using a 4-point scoring system, according to the classification of Gutmann et al,[20] and the mean value was calculated. A score of 1 indicated little or no smear layer, covering less than 25% of the specimen with tubules visible and patent. A score of signaled little to moderate or patchy amounts of smear layer, covering between 25 and 50% of the specimen, with many tubules visible and patent. A score of 3 indicated moderate amounts of scattered or aggregated smear layer covering between 50% and 75% of the specimen, and minimal to no tubule visibility or patency. A score of 4 meant heavy smear layering covering more than 75% of the specimen, with no tubule orifices visible or patent.

A preliminary series of 4 teeth, not included in this study, served for training and calibration of the procedure, both for the operator and observers. Four photomicrographs, taken as a representative of the 4-point scoring system for smear layer and debris, served as visual reference standards throughout the evaluation [Figure 1].

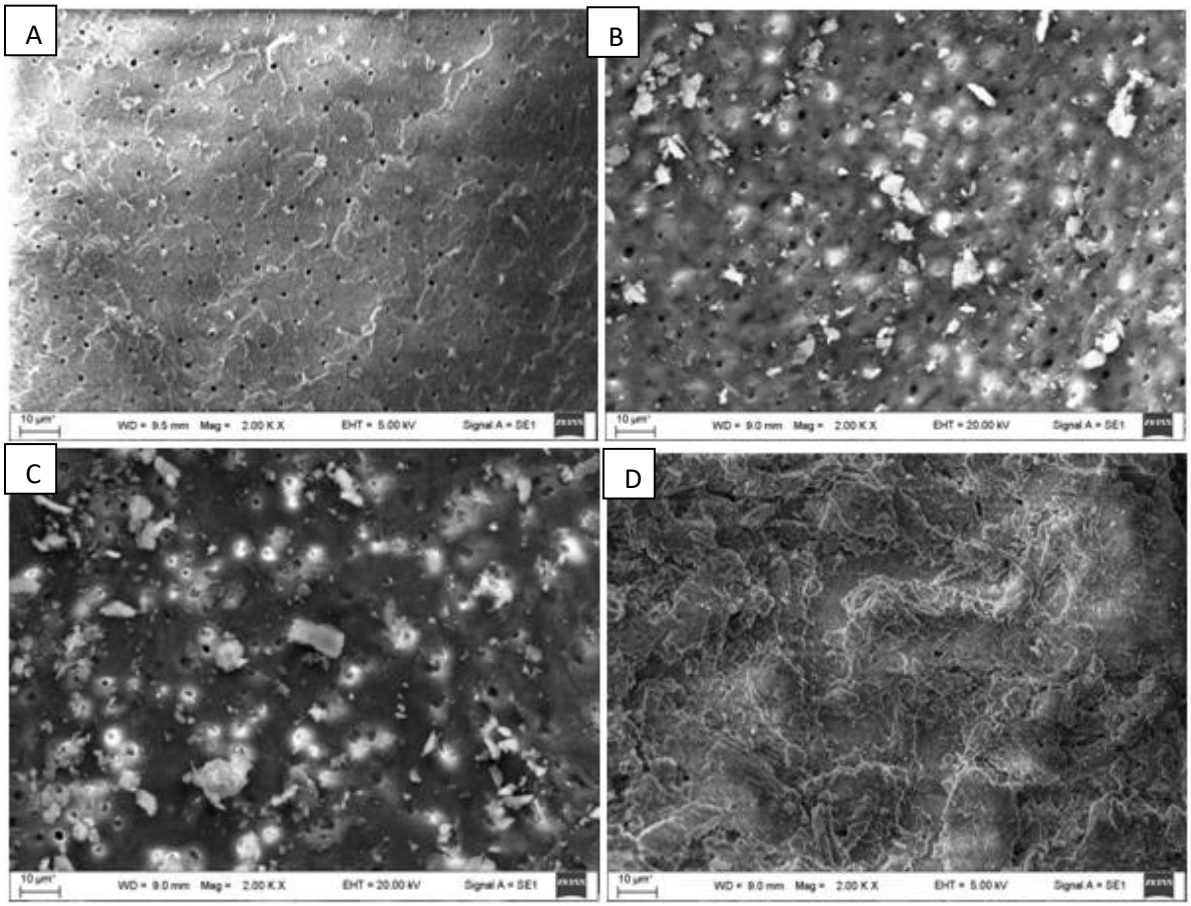

Figure 1: Figure 1: Standardized gradations of smear layer used for specimen evaluation A – score 1, B –score 2, C – score 3, D – score 4. Original magnification x2000.

Evaluation

Two independent observers evaluated 120 scanning electron photomicrographs for smear-layer removal in the coronal, middle, and apical thirds of 1 longitudinal half of each root, using the previously described 4-point scoring system. Both observers scored 10% of the photomicrographs (12) together and then scored the remaining 90% of the photomicrographs (108) independently. After 4 weeks, each observer performed a second reading on a randomly selected 50% of the photomicrographs (60). Photomicrographs given different scores by the 2 observers were discussed until an agreement was achieved.

Statistical Analysis

Data were collected and statistically analyzed using the SPSS version 22. $P < 0.05$ was considered statistically significant. The quantitative variables were described as the arithmetic M and SD. One-sample Kolmogorov–Smirnov test calculated from data showed that test distribution was normal. Kruskal–Wallis analysis of variance followed by the nonparametric Mann–Whitney U test were used for intergroup comparison, and nonparametric Wilcoxon Signed Ranks test was used for intragroup comparison. Sample size was calculated after the results were obtained and were considered statistically significant.

Results

The smallest significant amount of smear layer was found in the coronal third of the canals irrigated with 2.5% NaOCl + 18% HEDP compared with the apical third ($P = 0.014$); however, there was no significant difference compared with the middle third ($P > 0.05$). Evaluating intragoup comparison in smear-layer removal between groups 1, 2, and 4, no statistically significant difference was found in all canal thirds ($P > 0.05$) [Table1].

Table 1: Mean score and SD for smear layer of coronal, middle and apical thirds.

	Group 1 2.5% NaOCl	Group 2 2.5% NaOCl + 17% EDTA	Group 3 2.5% NaOCl + 18% HEDP	Group 4 Saline water
Coronal third	3.9 ± 0.316	1.90 ± 1.287	1.30 ± 0.483	4 ± 0.000
Middle third	4.0 ± 0.000	2.20 ± 1.317	1.70 ± 0.940	4 ± 0.000
Apical third	4.0 ± 0.000	2.40 ± 1.265	2.30 ± 1.059	4 ± 0.000

Statistical analysis revealed a significantly higher ability to remove smear layer in root canals irrigated with 2.5% NaOCl + 17% EDTA and 2.5% NaOCl + 18% HEDP compared with the canals irrigated with 2.5% NaOCl alone or saline water in all canal thirds ($P < 0.05$). No significant difference was found in the ability to remove a smear layer between groups 1 and 4, or between groups 2 and 3, in all canal thirds ($P > 0.05$) [Table 2,3,4].

Table 2: Statistical analysis between groups in coronal third.

Groups	Mean Rank	Z	P	Mann Whitney U test
Group 1- Group 2	14.35 – 6.65	-3.239	0.001	11.500
Group 1- Group 3	15.50 – 5.50	-4.065	0.000	0.000
Group 1- Group 4	10.00 – 11.00	-1.000	0.317	45.000
Group 2- Group 3	11.45 – 9.55	-.847	0.397	40.500
Group 2- Group 4	6.50 – 14.50	-3.472	0.001	10.000
Group 3- Group 4	5.50 – 15.50	-4.147	0.000	0.000

Table 3: Statistical analysis between groups in middle third.

Groups	Mean Rank	Z	P	Mann Whitney U test
Group 1- Group 2	14,50 - 6,50	-3,453	0,001	10,000
Group 1- Group 3	15,00 - 6,00	-3,775	0,000	5,000
Group 1- Group 4	10,50 - 10,50	,000	1,000	50,000
Group 2- Group 3	11,35 – 9,65	-,692	0,489	41,500
Group 2- Group 4	6,50 - 14,50	-3,453	0,001	10,000
Group 3- Group 4	6,00 - 15,00	-3,775	0,000	5,000

Table 4: Statistical analysis between groups in apical third.

Groups	Mean Rank	Z	P	Mann Whitney U test
Group 1- Group 2	14,50 - 6,50	-3,446	0,001	10,000
Group 1- Group 3	15,00 - 6,00	-3,749	0,000	5,000
Group 1- Group 4	10,50 - 10,50	,000	1,000	50,000
Group 2- Group 3	10,80 - 10,20	-,240	0,810	47,000
Group 2- Group 4	6,50 - 14,50	-3,446	0,001	10,000
Group 3- Group 4	5,50 - 15,50	-3,749	0,000	5,000

The obtained results are detailed as follows: the mean smear layer removal score for apical thirds was 4 for the group without chelators and 2.35 for the group with chelators. Type 1 error was <0.05 and statistical power >0.9, which is considered significant, and type 2 error was <0.01, which is considered that 40 specimens is significant.

Discussion

Elimination of the smear layer is a very important step in optimizing endodontic treatment. Therefore, penetration of intracanal medicaments and root canal sealers into dentinal tubules can improve, so they could exert their antimicrobial activity and obtain a tighter seal.[21] The present study showed that the smear layer could be effectively reduced in instrumented root canals by a combination of 2.5% NaOCl with a decalcifying agent. Namely, these agents are either 17% EDTA or 18% HEDP. In our study, the difference in smear-layer removal

between EDTA and HEDP was not statistically significant, but both protocols performed significantly better than the control and NaOCl groups. Irrigation protocols with both chelating agents could be clinically advantageous over the use of 2.5% NaOCl alone, which is in an agreement with Lottani et al[22] and Kuruvilla et al.[23] They reported that irrigation protocols, where NaOCl was used with either HEDP or EDTA, left similar amounts of smear layer without a significant difference between them. The current study showed no significant difference in removing the smear layer between coronal, middle, and apical thirds in all groups, except in the 18% HEDP + 2.5% NaOCl group, in which significantly less smear layer was found in coronal thirds compared with apical thirds. This is in an agreement with Lottani et al,[22] in which the HEDP group in the apical area showed slightly more smear layer compared to the coronal area. However, Kuruvilla et al[23] found a significantly better ability to remove smear layer in coronal versus apical thirds in both groups with chelating agents. These results may be explained by the fact that they managed to prepare coronal canal parts wide enough with Gates-Glidden drills and K files and, due to insufficient traditional manual activation with side-vented needle, irrigants were unable to reach the apical area effectively. Analyses of the smear layer left in apical thirds of the canals showed no significant difference between groups with chelating agents. Our results contrast with those shown in Hegde Vibha and Thakkar Pranav,[24] who displayed that at apical third, only the continuous soft chelating irrigation protocol with HEDP showed an improved removal of the smear layer. The results might differ because different mechanical preparations were used in both studies, so it might be that a newly designed instrument (XP-endo Shaper) and promoted activation with XP-endo Finisher used in the current study showed that the conventional irrigation protocol with EDTA is as effective as the continuous chelating irrigation protocol with HEDP in apical third.

The results of the present study offer a new approach regarding the 2.5% NaOCl + 18% HEDP solution as a sole irrigant in smear-layer removal during the entire chemomechanical preparation, which would effectively replace the use of EDTA. Furthermore, in contrast with EDTA, which interferes with the organic tissue dissolution properties and antimicrobial efficacy of NaOCl, HEDP shows no short-term interference with NaOCl, even though it increases its antimicrobial effect in dentinal tubules and surface tension.[25, 26] Although the current experiment was performed in a simulated clinical environment, the application of these results to the clinical situation is not straightforward. Further studies testing the smear-layer removal of the combined NaOCl/HEDP solution under conditions closer to the clinical situation may serve to confirm these preliminary findings.

Conclusion

Chelators used together with NaOCl can reduce but not completely remove a smear-layer from root canal dentin during rotary root canal instrumentation.

***Conflict of Interest*: None**
***Source of support*: Nil**
***Ethical Clearance*: Obtained**

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