

Effects of experimental ethylenediaminetetraacetic acid pastes with 0.2% cetrimide on smear layer formation at apical root canal walls prepared by rotary nickel titanium files

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Objectives: To investigate the effects of experimental ethylenediaminetetraacetic acid (EDTA) pastes at 10%, 15%, and 20%, and with 0.2% cetrimide on smear layer formation on apical root canal walls prepared by rotary nickel-titanium (Ni-Ti) files, in comparison to a commercial product (RC-Prep).

Materials and Methods: Experimental EDTA pastes at 10%, 15%, and 20% were prepared with or without addition of 0.2% cetrimide; RC-Prep was used as a control. Eighty-four root canals in maxillary premolars were prepared using rotary Ni-Ti files (WaveOne[®] Gold), with one of the EDTA pastes used as a lubricant, n=12 of each group. NaOCl at 2.5% was used as an irrigation. The roots were sectioned to investigate smear layer formation at apical half using scanning electron microscopy. Smear layer was scored from 1 to 5 following the degree of formation (lowest to highest).

Results: Heavy smear layer formation at the apical root level (scores 4-5) was observed at the root canal walls in all experimental EDTA pastes and RC-Prep, which were not significantly different ($p > 0.05$).

Conclusion: Increase in EDTA paste concentration or addition of 0.2% cetrimide did not have an effect on smear layer removal at apical root canal walls prepared by rotary Ni-Ti files.

Keywords: cetrimide, EDTA paste, root canal wall, rotary Ni-Ti, smear layer.

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Introduction

In endodontic treatment, the infected root canal is cleaned and shaped by mechanical instrumentation. Rotary nickel-titanium (Ni-Ti) files are gaining popularity based on their advantages over the hand files. From the nature of rotating action, high frictional force occurs and may cause fracture of the file. Rotary Ni-Ti files need a chelating lubricant to reduce the friction during root canal preparation. [1]

Root canal preparation using the rotary files produces smear layer left on root canal wall, which is composed of dentin chip, pulpal tissue remnants and microorganisms. [2] Even smear layer can be removed later by using ethylenediaminetetraacetic

acid (EDTA) irrigant, its removal is still difficult at the apical level. [3, 4] Smear layer prevents the penetration of intra-canal medication and interferes the adaptation of root canal sealer. [2, 5] Thus, smear layer should be formed as low as possible, especially at the apical level, during root canal preparation.

A chelating agent, EDTA paste, is widely used as a root canal lubricant when the rotary instrument is utilized. For instance, RC-Prep (*Premier Dental, Plymouth Meeting, PA, USA*) is a commercial EDTA paste composed of 15% EDTA, 10% urea peroxide, and vehicles. The paste lubricates and reduces smear layer formation during root canal preparation. [6-10] Concentrations of EDTA paste range from 10-20%, but the most effective concentration remains unclear.

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Cetrimide is a cationic surfactant that reduces surface tension and contains antimicrobial action. [11-13] Mixing 0.2% cetrimide with EDTA irrigant improved the ability to remove smear layer when used in combination with sodium hypochlorite. [14, 15] The addition of cetrimide into EDTA paste might reduce smear layer formation during root canal preparation. However, there are no current study on the effect of adding cetrimide to EDTA paste on smear layer formation.

Therefore, the purpose of this study is to compare the effects of experimental EDTA pastes at three concentrations, with or without 0.2% cetrimide, on smear layer formation on root canal walls at the apical level after preparation using rotary Ni-Ti files, compared to a commercial product, RC-Prep.

Materials and Methods

This study protocol was reviewed and approved by the ethic committee of Faculty of Dentistry and Faculty of Pharmacy, Mahidol University, MU-DT/PY-IRB 2018/007.1602.

Preparation of experimental EDTA pastes

Vehicle ingredients- propylene glycol, cetyl alcohol, mineral oil, and hydrogen peroxide were weighed and mixed at 60°C using a hot plate until homogenous. EDTA powder were geometrically added into the prepared solution to achieve 10%, 15%, and 20% EDTA pastes. In the groups with cetrimide, 0.2% cetrimide powder was added into the mixed pastes. The mixed product was loaded into a 1-ml syringe and kept at 4 °C until it was used. In addition, pH of the experimental EDTA pastes were measured using a pH meter (*Orion 3-Star pH, ExpotechUSA, Houston, Texas, USA*)

Tooth preparation

Eighty-four first and second intact premolars, which had two separated, straight or slightly-curved roots, were collected in a total of 42 teeth

(84 root canals). The teeth were extracted because of orthodontic reason without any root crack/fracture, and kept in 0.9% normal saline solution. Root surfaces were cleaned to remove calculus and any soft tissue remnants using a hand scaler.

Coronal access was prepared using a high-speed, round diamond bur and taper diamond bur (*Intensiv SA, City of Lugano, Canton, Switzerland*). Coronal access was flushed with normal saline at a total amount of 20 ml during the procedure. Buccal and palatal canal orifices were located using an endodontic explorer (DG16) (*American Eagle, Missoula, Montana, USA*). Canal patency was verified by negotiation with #10 K-file (*Dentsply Maillefer, Ballaigues, Switzerland*). The file was used to determine the apical foramen, and the length of tooth was obtained. Working length for root canal preparation was set by subtracting 0.5 mm from the length. Initial apical file (IAF) size was verified by using a K-file, and the canal with IAF larger than size 25 was excluded. The tooth was embedded into a plastic block using pink wax and acrylic resin.

The samples were divided into 7 groups (12 root canals per group) according to the EDTA pastes used during root canal preparation as follows:

- Group 1: RC-Prep (commercial 15% EDTA, control group)
- Group 2: EDTA-10 (10% EDTA)
- Group 3: EDTA-10C (10% EDTA with 0.2% cetrimide)
- Group 4: EDTA-15 (15% EDTA)
- Group 5: EDTA-15C (15% EDTA with 0.2% cetrimide)
- Group 6: EDTA-20 (20% EDTA)
- Group 7: EDTA-20C (20% EDTA with 0.2% cetrimide).

For group 1, RC-Prep was used in the buccal and palatal canals of 6 teeth (n = 12 canals). For groups 2-7, EDTA paste at the same concentration with or without cetrimide was randomly used in the buccal or palatal canal of the same tooth (12 canals in each group) to control biological variation of root dentin.

In addition, the root canals (n=2) were prepared using the rotary files without use of any EDTA paste, as a *positive* control (full smear layer presented), while the prepared root canals (n=2) with a final irrigation of 17% EDTA at 3 mL for 1 min and 2.5% NaOCl at 5 mL was used as *negative* control (no or few smear layers presented).

Root canal preparation using Ni-Ti files and EDTA pastes

Before each root canal preparation, the assigned EDTA paste at 0.1 mL was injected from a 1-mL syringe using an irrigation needle (size G25) into the canal at the coronal-middle level. The root canal was mechanically instrumented using the rotary Ni-Ti file size 25/.07 (*WaveOne[®] Gold, Primary, Dentsply Sirona, Ballaigues, Switzerland*). NaOCl 2.5% at 2 mL was used as an irrigant after each preparation, and recapitulation by #10 K-file was made. The procedure was repeated 3 times until the rotary file reached the working length.

The canal was further enlarged using the rotary Ni-Ti file size 35/.06 (*WaveOne[®] Gold, Medium*). The root canal preparation was repeated 2 times until the rotary file reached the working length. NaOCl 2.5% at 2 mL was used as a final irrigant. An EDTA irrigant was not used in this study in order to investigate the effect on EDTA paste on smear layer formation.

Evaluation of smear layer formation at the apical level of root canals

The specimens were stored in 100% relative humidity at 37 °C for 1 week. Coronal tooth structure was removed by horizontal sectioning at the CEJ level using a micro-blade (*Isomet 1000, Buhler, Lake Bluff, NY, USA*). The root was then sectioned vertically in the mesio-distal direction with a micro-blade to separate the buccal and palatal roots. The sectioned root was further grounded with silicon-carbide paper grit 600 to remove root dentine, until the root canal was revealed. Buccal wall of the buccal root canal and lingual wall of the lingual

root canal were achieved for scanning electron microscopy (SEM).

The specimens were fixed and dehydrated in 2.5% glutaraldehyde for 24 h. Each specimen was dried, mounted on metallic stubs, and coated with gold-sputter coater. Formation of smear layer at the apical third level of the root canal was initially scanned using a scanning electron microscope (*JSM-6610 Series SEM, JOEL Ltd., Akishima, Tokyo, Japan*) at a low magnification of 50x. Since homogeneity of smear layer formation at the apical area was observed in SEM image, a representative area of each specimen was then selected and photographed at a high magnification of 1,000x.

The amounts of smear layer formation on the prepared apical canal walls were calibrated with the reference images of smear layer formation and rated into the scoring system (modified from Plotino G et al, 2014) [16], according to the area covered by smear layer, as follows (Fig.1):

Score 1- no smear layer with all patent dentinal tubules were present.

Score 2- less than $\frac{1}{4}$ of total area

Score 3- more than $\frac{1}{4}$ of total area, but less than or equal to $\frac{1}{2}$

Score 4- more than $\frac{1}{2}$ of total area, but less than or equal to $\frac{3}{4}$.

Score 5- more than $\frac{3}{4}$ of total area.

Normality and homogeneity of data were checked using Shapiro-Wilk test and Levene's test. Due to the scores did not show normal distribution and homogeneity of variance. Kruskal-Wallis and Mann-Whitney U test were used to determine the difference among group. A significant level was set at p-value <0.05.

Results

The pH of experimental EDTA pastes- 10% without or with cetrimide; 15% without or with cetrimide; and 20% without or with cetrimide, were 5.00, 5.12, 4.74, 4.89, 4.69, and 4.88 respectively.

Smear layer formation at the apical root level of the different EDTA pastes at 10%, 15% and 20% with or without cetrimide are presented in Fig. 2 and Table 1. *Median* scores of smear layer formation in all experimental EDTA pastes were 5 (highest) while the score of RC-Prep was 4. All EDTA groups including RC-Prep showed *mode* of smear layer formation at score 5 (Fig. 2). No statistically significant difference in score of the smear layer formation was observed among the groups ($p=0.093$).

From SEM images of root canal walls at the apical-level, the root canal wall in the negative control was minimally covered by smear layer, with

patent dentinal tubules were partially observed while smear plugs partly remained (Fig. 1A). The root canal wall in the positive control was heavily covered with smear layer (Fig. 1E). After root canal preparation using the rotary Ni-Ti files and irrigation with NaOCl, the majority of RC-Prep showed thick smear layer formation on root dentine, similar to the positive control. However, patent dentinal tubules without smear layer covering were partially observed in RC-Prep (Fig. 1B-1D). Most of all experimental EDTA groups demonstrated smear layer covering almost the entire canal walls (Fig. 1E), similar to the positive control.

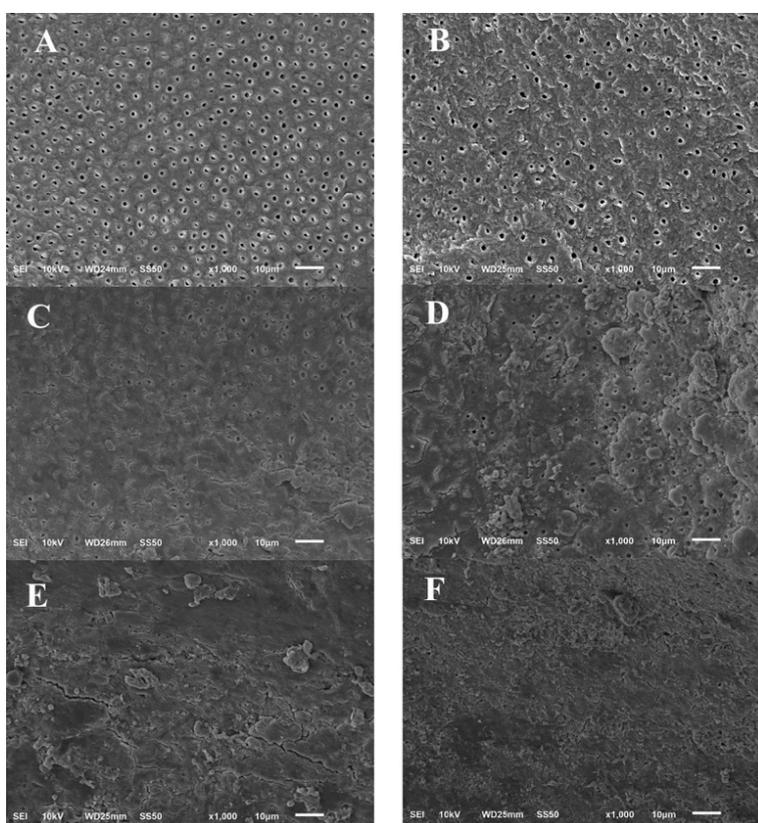
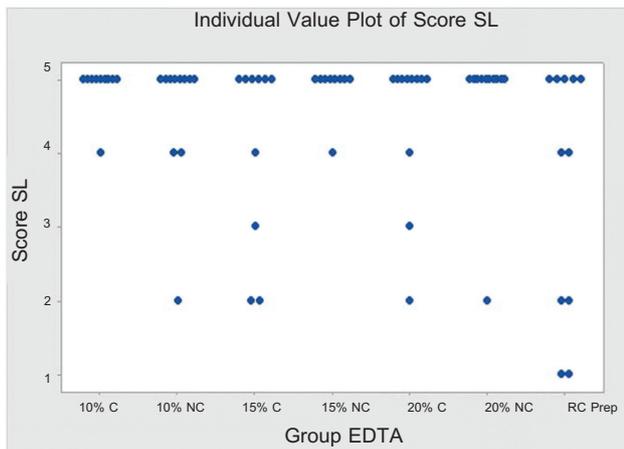


Figure 1 Representative SEM micrographs of smear layer formation at the apical areas: (A) score 1 (negative control); (B) score 2 (RC-Prep); (C) score 3 (RC Prep); (D) score 4 (RC Prep); and (E-F) score 5 (experimental EDTA paste and positive control).

Table 1 Median, mode and range (minimum and maximum) of smear layer scores from the experimental groups (C- with 0.2% cetrimide, NC- without cetrimide).

Smear layer formation score	RC Prep	With 0.2% Cetrimide			Without 0.2% Cetrimide		
		10% EDTA	15% EDTA	20% EDTA	10% EDTA	15% EDTA	20% EDTA
Minimum	1	4	2	2	2	4	2
Maximum	5	5	5	5	5	5	5
Median	4	5	5	5	5	5	5
Mode	5	5	5	5	5	5	5
N for mode	5	9	6	10	9	8	10

**Figure 2** Individual plot of smear layer (SL) score in each experimental group (C- with 0.2% cetrimide, NC- without cetrimide).

Discussion

Smear layer formation on root canal walls at the apical root level after preparation with the Ni-Ti files were not significantly different among the experimental EDTA pastes. Theoretically, higher concentration of EDTA might improve the chelation effect on the inorganic contents of smear layer, and then the ability of NaOCl penetration to remove organic contents. [17] However, increase in concentration of EDTA from 10% to 20% did not help in the reduction of smear layer formation in this study. Using 17% EDTA irrigant in a combination with 2.5% NaOCl did not totally remove smear layer and plugs in the negative control, which corresponded to the other studies. [18] Without

the EDTA irrigant, the experimental EDTA paste used with the NaOCl irrigant did not reduce smear layer formation, and thick smear layer was still formed. [19]

Viscosity of the EDTA paste that is markedly different to the EDTA irrigant may be a crucial factor. The lower viscosity of the paste, the better cleanliness of the root canal is expected due to the flowing capacity into root canal, especially in the apical region. The experimental EDTA pastes were more viscous than the irrigant or even RC-Prep, and might not flow well in the apical level of root canals. The experimental pastes contain propylene glycol and mineral oil as the vehicles. The mixing ratio of these vehicles should be modified to improve the flow of paste.

In this study, the addition of 0.2% cetrimide into the EDTA paste did not improve the results. Comparison between the pastes with or without the cetrimide at the same concentration, smear layer formation was the same. Theoretically, a surfactant should help in cleaning and lubricating root canals during preparation. [1] Mixing 0.2% cetrimide into the EDTA irrigant improved the ability to remove smear layer on root canal wall. [14, 15] However, a cetrimide concentration at 0.2%, similar to what was added into the irrigant form, was only used in this study. Increase in concentration of cetrimide may improve the results and should be further tested.

With a trend of lower smear layer formation of RC-Prep, urea peroxide in RC-Prep, which disintegrated into hydrogen peroxide and urea, might have a determinant effect to reduce smear

layer formation. Urea peroxide promotes in emulsifying the organic components of smear layer and increasing penetration of NaOCl irrigation. [20] The experimental EDTA pastes in this study contains hydrogen peroxide at 10%, but its effect on smear layer reduction was inferior to urea peroxide.

Conclusion

In this study, various concentrations at 10-20% of the experimental EDTA pastes or addition of 0.2% cetrimide did not reduce smear layer formation at the apical level on the root canal walls prepared by the rotary Ni-Ti files. Further development of the experimental EDTA paste, in terms of smear layer reduction, is considered.

Conflict of Interest

The authors declare no conflict of interest.

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