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Original Article

Effects of caffeic acid phenethyl ester on root canal dentin: A SEM/EDX study

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Abstract

Aim: To evaluate the effects of Caffeic Acid Phenethyl Ester (CAPE) and its combination with EDTA and NaOCl on the surface of root canal dentine.

Material and Methods: Ninety human extracted central anterior teeth were instrumented. Teeth were divided into six groups according to irrigation regime: (n=15). Group 0 (negative control) saline; group 1: 5.25% NaOCl/ distilled water (positive control); group 2: 0.5% CAPE; group 3: 5.25% NaOCl/17% EDTA; group 4: 5.25% NaOCl/0.5% CAPE and group 5: 0.5% CAPE/17% EDTA. Roots were splitted longitudinally and examined under scanning electron microscopy. Energy Dispersive X-Ray (EDX) method was used to analyse the root dentine mineral content.

Results: A significant difference in smear layer (SR) was found between group 3 and group 4 ($P<0.05$), but no significant difference in SR was found between group 3 and group 5 ($P>0.05$).

Conclusions: In this study, CAPE did not result in better SR removal compared to NaOCl at all levels of the root canals. Overall, Group 5 was as effective as group 3 in SR removal. The mineral content of root dentin has changed.

Keywords: Caffeic acid phenethyl ester; root canal irrigant; smear layer removal; SEM-EDX

INTRODUCTION

During endodontic treatment, root canal instrumentation produces layer of organic and inorganic material called the smear layer (SR). SR can act as a barrier that interferes with the conformation of filling materials to the root canal and can also limit the disinfecting action of intra-canal medicament in dentin tubules (1). Therefore, removal of the SR is an essential step in the success of root canal treatment (2). Various materials and techniques have been proposed for the removal of SR. These include chemicals, lasers and ultrasonic devices. The chemical method is the most commonly used one (3) and typically achieved with solely or combined use of ethylene diamine tetra acetic acid (EDTA) and sodium hypochlorite (NaOCl)(4). Although the combined use of EDTA and NaOCl is the preferred method for removing SR (5), EDTA has disadvantages, including its ability to cause erosion and its limited antibacterial activity (6). Also, when combined with NaOCl, it reduces the tissue dissolving effect of NaOCl(7). NaOCl is a commonly used endodontic irrigant. Its disadvantages are its toxicity on vital tissues and its inability to remove SR (8). Therefore, effective, anti-inflammatory and biocompatible alternative agent are needed. In order to meet the requirements of an ideal irrigant and to overcome the side effects of the above agents, recent studies have been on alternative irrigation agents (e.g. Oregano extract) (9). Natural plant extracts which analgesic, anti-inflammatory, biocompatible and antimicrobial properties have been used as alternatives to NaOCl or EDTA (9). There are many studies in the field of dentistry about Caffeic acid phenethyl ester (CAPE) (10-14). CAPE has become more popular due to its anti-oxidant, antimicrobial, anti-inflammatory activities, biocompatibility (13,14), It encourages bone bone healing (11) and tooth socket healing (12). Although CAPE has advantages such as antioxidant properties, antimicrobial activity, anti-inflammatory and biocompatibility, it is not known whether it removes the SR and if it causes a change in the root dentin mineral content. Therefore, the aim of this study was to investigate the effects of CAPE alone and its combinations (NaOCl/CAPE, CAPE/EDTA) on structural properties (Scanning Electron Microscopy and Energy Dispersive X-Ray (SEM and EDX)) of the root canal dentin surface.

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MATERIAL and METHODS

Preparation of Root Canal

The study was approved by the ethics committee of the Pamukkale University (2018/04-13). Ninety human extracted central anterior teeth were decoronated to a standard root length of 13 mm, then removed 1 mm from the actual root length to determine the working length (WL, 12mm), by inserting a size 10 K-file (Dentsply Maillefer, Switzerland) into each root canal. Then, the root canals were prepared with Reciproc instrument R25 (25/0.08, VDW Reciproc® Munich, Germany) attached to the VDW Gold endodontic motor (WDW Gold, Munich, Germany) according to manufacturer´s instructions. A 27-gauge needle (NaviTip Ultradent Products Inc., South Jordan, Utah, USA) was positioned 1 mm away from the WL by the passive irrigation method(15). According to the manufacturer's instructions, 0.5% CAPE (Aldrich Chemistry, St. Louis, USA), an experimental irrigation agent, was dissolved in 100 ml of 0.01% saline- ethanol solution and prepared 0.5% (w/v) irrigation solution. Commercial forms of other solutions have been used (EDTA: Endo SOlution,Cerkamed, Poland; NaOCl: Chloraxid 5,25%, Cerkamed, Poland). Irrigation protocols shown in Table 1 were applied. Similar to a previous study (16), Grooves were opened to the roots and then the roots were twisted with a chisel and split into two halves, resulting in 15 samples per experimental group and 15 samples for the control.

Table 1. Irrigation procedures for groups (n=15)

Groups	Process	Time	Final Process	Time
G0	Saline	3 min	-	60 sec
G1	3 ml 5.25% NaOCl	3 min	1 ml distilled water	60 sec
G2	3 ml 0.5% CAPE	3 min	1 ml distilled water	60 sec
G3	3 ml 5.25% NaOCl	3 min	1 ml 17 % EDTA	60 sec
G4	3 ml 5.25% NaOCl	3 min	1 ml 0.5 % CAFE	60 sec
G5	3 ml 0.5% CAPE	3 min	1 ml 17 % EDTA	60 sec

SEM Analysis

The scanning electron microscope was used to scan sample areas (Zeiss Supra 40VP, Carl Zeiss, Carl, Germany, magnification: 2000×,10 kv). The amount of remaining SR at all levels of the root canals was scored. The root canals of each specimen were examined, respectively, coronal (10 mm from apex), middle (6 mm from apex) and apical (2 mm from apex). Two independent evaluators, who were unaware of which specimens belonged to which groups, blindly analyzed and scored the degree of SR removal. The irrigation solutions were evaluated using a 5-grade scoring system as described previously(17). Each examiner scored all micro photographs twice at a 2-week interval (Kappa, 0.769).

SEM and EDX Analysis

The middle third of roots from each group (6 mm from apex to coronal) was randomly selected (n=15). The information provided by EDX instrument of Field-Emission Scanning Electron Microscope (FE-SEM) is the surface elemental composition. Also, major elemental composition spectrum of the samples were taken.

Statistical Analysis

All data were processed by SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Statistical analyses of the results were conducted using the Kruskal-Wallis test. Multiple comparisons were adjusted by using Dunn test. Independent evaluators agreement was analyzed by using Kappa statistics.

RESULTS

Table 2 shows scores for evaluation of residual SR (mean ± SD). In all levels of the root canals, there was no significant difference concerning the removal of the SR in between groups the NaOCl and CAPE (P>0.05). In all levels of root canal, statistically significant difference was observed between the NaOCl/EDTA and NaOCl/CAPE groups (P<0.05), No significant difference was observed between the NaOCl/EDTA and CAPE/EDTA groups in the SR scores, except for the apical one-third of the roots (P>0.05). Figure 1 represents the micrographs regarding the SR scores at all groups.

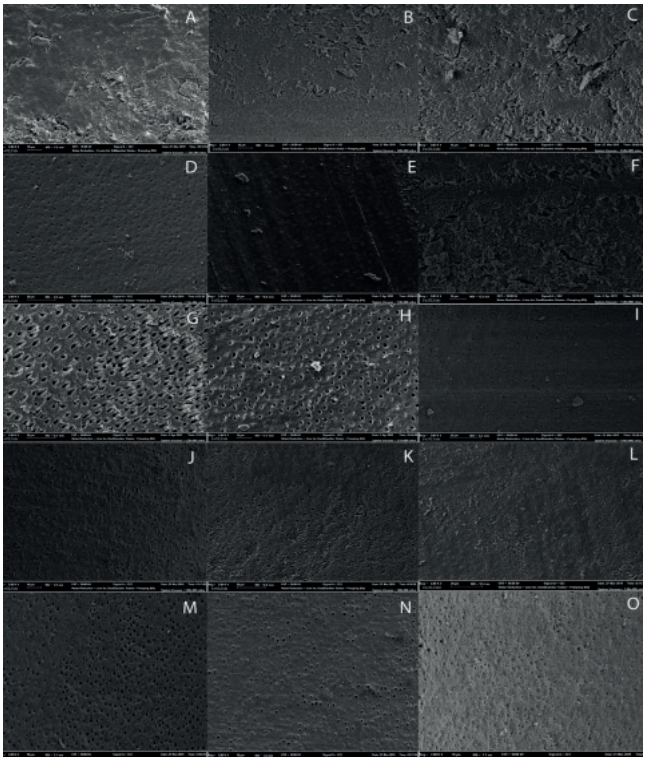

Figure 1. SEM micrographs of residual smear layer from coronal to apical region (from left to right) (2000×, 10 kV). Surfaces: (A-C) NaOCl; (D-F) CAPE; (G-I) NaOCl/EDTA; (J-L) NaOCl/CAPE; (M-O) CAPE/EDTA

Table 2. Scores for evaluation of residual smear layer (mean ± SD)

Groups	Coronal	Middle	Apical	Total
G1	4.1±0.74[a]	4.2±0.57[a]	4.5±0.70[a]	4.26±0.45[a]
G2	2.50±0.70[a]	2.90±0.56[a]	4.43±0.82[a]	3.23±0.56[a]
G3	1.70±0.68[b]	1.90±0.52[b]	2.8±0.63[b]	2.21±0.18[b]
G4	2.80±0.42[a]	3.20±0.42[a]	4.43±0.48[a]	3.43±0.22[a]
G5	1.60±0.51[b]	2.40±0.52[b]	3.90±0.56[a]	2.63±0.29[b]

The same lowercase letter indicates no significant difference (P >0 .05)

Table 3 shows the EDX spectrum and elemental composition table of the middle thirds of the root canal after the irrigation protocol. A statistically significant difference was found between the groups for C, Ca,O, P and Na values (P<0.05). No significant differences were found in the content of K, S and Mg values or the Ca/P ratio (P>0.05). Figure 2 represents SEM micrographs and EDX spectrum of middle root dentin surface.

Table 3. EDX spectrum and elemental composition table of middle region root dentin with different irrigation

Groups	C	O	Ca	P	Na	Mg	K	S	Ca/P
G0	19.45±2.38[c,f]	38.26±2.18	30,45±2.70	10.54±1.11[f]	0.36±0.04[d]	0.57±0.04	0.02±0.01	0.16±0.06	2.59±0.69
G1	17.85±2.79	44.89±2.02[f]	25.65±2.24[f,c]	8.91±0.59[c]	0.29±0.08[d]	0.67±0.08	0.02±0.01	0.07±0.04	2.88±0.28
G2	12.80±2.01[a]	39.44±5.07	32.06±1.50[b]	10.93±0.92[b]	0.36±0.21	0.56±0.23	0.03±0.04	0.15±0.13	2.94±0.25
G3	19.74±1.03	38.86±4.43	30.61±5.17	9.67±0.94[f]	0.64±0.22[a,b]	0.61±0.19	0.02±0.02	0.06±0.06	3.15±0.28
G4	14.86±1.39	42.68±2.60	30.28±2.55	10.39±0.62	0.46±0.10	0.62±0.11	0.04±0.02	0.08±0.05	2.91±0.25
G5	12.85±3.95[a]	36.69±5.72[e]	35.56±3.20[b]	12.49±1.25[a,d]	0.55±0.07	0.78±0.15	0.04±0.05	0.11±0.02	2.86±0.37

[a]Statistically significant difference (P < 0.05) according to group 0 ; [b]Statistically significant difference (P < 0.05) according to group 1
[c]Statistically significant difference (P < 0.05) according to group 2; [d]Statistically significant difference (P < 0.05) according to group 3
[e]Statistically significant difference (P< 0.05) according to group 4; [f]Statistically significant difference (P< 0.05) according to group 5

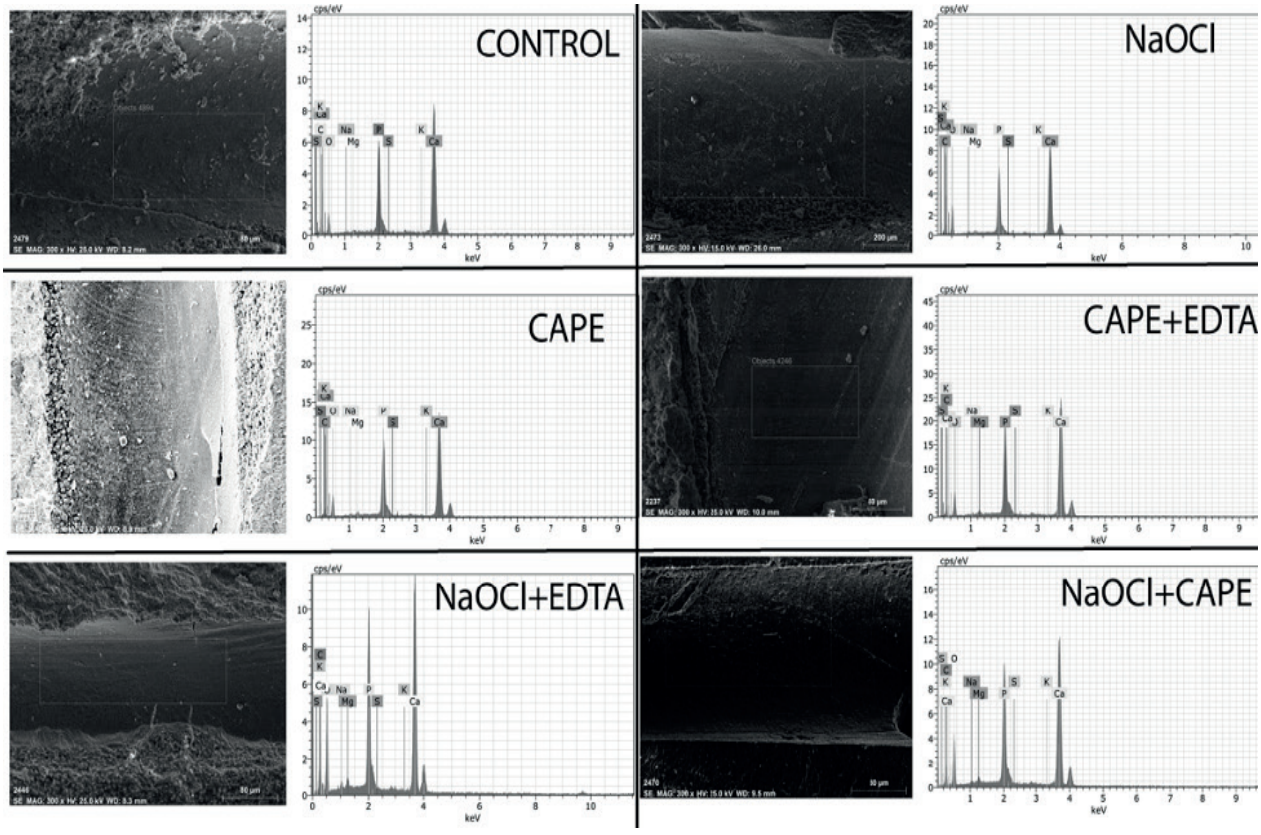

Figure 2. SEM micrographs and EDX spectrum of middle root dentin surface (300 x, 25 kv)

DISCUSSION

Endodontic irrigation agents have three main functions: chemical, biological and aiding the mechanical preparation. During mechanical cleaning with instruments, irrigation agents provide lubrication. Biological goals are to provide antimicrobial activity, to inactivate endotoxins, provide antiseptic properties with minimal toxicity. The Chemical goals are to dissolve organic and inorganic material, remove the SR if formed and to prevent the formation of a SR during instrumentation (18). The clinican should take into account the effect of the choise of root canal irrigant on dentin and its capacity to remove the SR. In this study, all irrigation treatment groups were examined to remove the SR from the root canals and to determine its effect on dentin.

EDTA is used to solve the inorganic part of the SR and is often recommended (6). The success rate of the NaOCl/EDTA solution in removing SR has been presented by previos studies (19,20). In our study, 5.25% NaOCl/17% EDTA (group 3) was found to be effective in removing the SR.

Studies have been conducted to use more efficient and biocompatible chelating agents than EDTA(3, 21). Among these, CAPE has been outstanding due to its antioxidant properties, antimicrobial activity, anti-inflammatory activity and biocompatibility (14,22). Instead of EDTA, it was tested together with NaOCl solution to remove SR using 0.5% CAPE. In all segments, the combination of CAPE and NaOCl did not result in better SR removal compared to NaOCl/EDTA. This may also be due to lack of concentration and time. Therefore, further studies are needed to assess its effectiveness in removing SR at different application durations and in different concentrations.

Although NaOCl is the most widely used irrigation solution in clinical practice, it has some drawbacks such as its toxic effect on surrounding tissues, allergic potential, chemically low stability, corrosion, and ability to remove only the organic part of SR. (4,23). In this study, NaOCl alone, was found insufficient to remove debris and SR.

In our study, Although CAPE was found insufficient to remove the SR, the use of the CAPE at different concentration and time may alter the ability to remove SR. For this reason, further studies are needed to assess the effectiveness of the CAPE in removing the SR at different application durations and in different concentrations. Instead of NaOCl solution, the use of the CAPE was tested to remove the SR along with EDTA solution. When combinations (NaOCl/ EDTA, CAPE/EDTA) were evaluated, the coronal and middle thirds of root canal surfaces treated with G5 removed smear layer, similar to those treated with G3. However, G5 was less able to clean in the apical thirds than in the coronal and middle thirds; this may have been based on differences in anatomy (24).

The effect of irrigation solutions and chelating agents on the mineral content of tooth may vary depending on the different parameters, application time , type of solution, the amount that can invade in the root canal, pH and concentration of the solution (25,26). Irrigation solutions used in endodontic treatment play an important role in the success of treatment and changes mineral content of dentin (27).

Although NaOCl has a significant effect on the organic component of dentin, NaOCl has no effect on the inorganic component of dentin (28). However, studies also report the negative impact of NaOCl. Moreover, NaOCl is a nonspecific oxidant, whose residues and by-products have negative effects (29). On the other hand, other studies have previously shown that antioxidants such as sodium ascorbate and CAPE reverse these negative effects (22, 30). In the present study, the NaOCl group showed a high oxygen (O) value, the O value was significantly reduced when CAPE was used as the final irrigant. The decreased effect of this oxidation can be attributed to the antioxidant effect of the CAPE.

In the NaOCl group, a significant decrease in the Ca value was observed compared with the CAPE and G5 groups (Table 3). Similar to our study, NaOCl was shown to reduce the Ca value in root dentin in another study (26). When single CAPE is used during root canal treatment, Ca values did not statistically different from control group.

In the present study, no significant difference was observed in the Ca/P ratio of all groups (P >0.05). Findings of Altundaşar et al. (31) and Topcuoglu & Koseoglu (32) regarding to Ca / P ratio change are similar to our results; however, there is study that reported a change in the Ca/P ratio (33). Different concentrations of solutions, methods of application and duration of the solutions used in those studies may have led to different findings. It is also important how these irrigation regimes can affect the micro-stiffness of the root canal dentin. More researches are needed to determine the duration and concentration of CAPE with the purpose of SR removal.

CONCLUSION

Within the limitations of this study, it has been proven that CAPE/EDTA effectively remove the SR in the coronal and middle third. Used as single irrigant, the CAPE was found to not effectively remove the SR. The mineral content of root dentin has changed. As a result, further studies are needed by changing the concentrations and pH of the solution to achieve an effective SR removal.

Competing interests: The authors declare that they have no competing interest.

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