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ORIGINAL ARTICLE

The effect of different irrigation protocols on smear layer removal in root canals of primary teeth: a SEM study

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ABSTRACT

Objective: The purpose of this study was to compare the efficacy of different irrigation protocols on smear layer removal in root canals of primary teeth by scanning electron microscopy (SEM).

Material and methods: The study was conducted with 40 extracted maxillary primary incisor teeth divided into four groups ($n = 10$) as 1% sodium hypochlorite (NaOCl), 10% ethylenediaminetetraacetic acid (EDTA)+1% NaOCl, 6% citric acid (CA)+1% NaOCl, and 0.9% physiological saline (PS). After the irrigation procedures, root canal walls were examined by SEM and the efficacies of irrigation solutions in smear layer removal were scored and compared. Data were analyzed using Kruskal–Wallis, Friedman and Siegel Castellan tests.

Results: The smear layer removal was found to be statistically more effective in groups of 10% EDTA + 1% NaOCl and 6% CA + 1% NaOCl when compared with the other groups ($p < .05$). Smear removal efficacy was statistically significantly higher in coronal and medium thirds when compared with the apical regions in the experimental groups.

Conclusions: It was concluded that 10% EDTA + 1% NaOCl and 6% CA + 1% NaOCl could be alternative irrigation protocols regarding smear layer removal. However, due to the absence of erosive dentinal changes, it might be suggested that using 6% CA + 1% NaOCl can be recommended compared to 10% EDTA + 1% NaOCl in primary root canals.

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Introduction

The success of the root canal treatment depends on the chemo-mechanical debridement of infective microorganisms in the canal [1–3]. A smear layer composed of organic and inorganic contents is formed following the instrumentation procedure of the root canals. It contains hard tissue remains, coagulation proteins, necrotic pulp tissue residues, various blood cells, and microorganisms that infect dentin tubules [1,4]. This layer prevents antibacterial irrigants and medicaments from reaching the dentin tubules, as well as complete sealing of the root dentin with the root canal filling material, causing microleakage. Another significant disadvantage of this structure is that it provides an environment in which microorganisms can maintain their vitality in the root canals [1,4–8].

The remaining surface area without instrumentation is significant due to the complex anatomical structure, morphological variations, accessory canals, and ramifications of the root canals of primary molar teeth [2,3]. In addition, the difficulty of hermetic sealing as a result of enlarged apical opening due to physiological root resorption, lack of lateral condensation due to the necessity of using soluble bases because of physiological resorption, and the inability to use pressure-based methods to avoid extension of sealer from the apical foramen lead to additional disadvantages in long-term outcome of root canal treatments [9–11]. Because of these adverse features, although there is no consensus in the literature, the opinion that removal of the smear layer is necessary for the success of endodontic treatment is more common [5].

Physiological saline (PS), sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA) and its combinations, and citric acid (CA) are frequently used as irrigation solutions for root canal treatment in primary teeth. However, these agents have varying levels of effects on smear layer removal [10,12]. Although NaOCl has been used for many years as a gold standard for irrigation due to its high antibacterial effect, many studies have shown that it is not sufficient to solve the inorganic component of smear layer of primary root dentin. Therefore, combined use of irrigation solutions is recommended to remove all the components of the smear layer [10,13]. EDTA which is a chelating agent was used to solve especially the inorganic components of the smear layer in primary teeth; however, researchers were reported erosive defects in dentin [6]. CA, a member of organic acids, is known as an agent that also helps removal of the inorganic part of the smear layer claiming to have less destructive effect by causing decalcification of dentinal walls and tubules [6,14–16].

Since the risks of periapical overflow due to enlargement of the apical opening and positional change because of

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physiological root resorption are present [10], there is a need to obtain to effective irrigation protocol that could safely remove all the components of the smear layer in primary teeth.

Based on this view point, the purpose of this study was to evaluate the efficacies of different irrigation protocols used in root canal treatment of primary teeth in removing the smear layer by scanning electron microscopy (SEM). The null hypothesis tested in this study was that there would be no difference among various irrigation solutions regarding effectiveness in removing the smear layer in primary teeth root canals following hand instrumentations.

Material and methods

Teeth selection criteria

Primary incisor teeth extracted for various reasons were selected from patients who presented to the pediatric dentistry clinic. The written consent forms were signed by the parents. The study followed the principles of the Declaration of Helsinki. Forty extracted, single-rooted, maxillary primary incisor teeth of which the root resorption level did not exceed one-third of root length macroscopically and that the apical opening was not wider than the #50 K file diameter were used in this study. Teeth which had internal or external resorption, root fracture, calcification, or obliteration were excluded from the study. The teeth, which were stored in 0.9% PS solution until the beginning of the study, were left in 2.5% NaOCl solution for 48 h to remove organic debris, and the residues were cleaned by brushing under water.

Procedure

All teeth were buried in wax blocks leaving the clinical crowns out. Following the preparation of the access cavity, the tissue residues in the pulp chamber were removed by excavators, and the residues in the root canal were removed by a barbed broach (Medin Barbed Broach, Vlachovice, Czech Republic). The estimated working length was determined by using a hand file on the periapical radiographs of the specimens. With reference to this measurement, the correct working length was determined by a #15 K file (G-star Medical Co., Ltd., Guangdong, China) placed 2 mm coronally from the apical foramen. In all groups, each hand file set was used for only one group of samples, and instrumentations were made by the same operator (BNY), starting from the K file #15 to #45. The large diameter hand files were used after the small file could move easily in the root canal during the preparation steps. Instrumentation was done according to the conventional preparation using a pull-back movement. All root canals were irrigated with 27-gauge tip diameter needle attached to 2 ml syringe (Ayset Medical Products, Adana, Turkey) and the needle tip was inserted 2 mm shorter than the working length.

Design of the study groups

To evaluate the efficacies of various irrigation protocols in smear layer removal, the teeth were randomly divided into four groups, each group consisting of 10 samples:

1% NaOCl (Group 1): Between each instrumentation, the root canals were irrigated with 10 ml of 1% NaOCl. Final irrigation was performed using 10 ml of 1% NaOCl.
10% EDTA + 1% NaOCl (Group 2): Between each instrumentation, the root canals were irrigated with 10 ml of 1% NaOCl. Final irrigation was performed using 10 ml of 10% EDTA and 10 ml of 1% NaOCl. The root canals were dried with paper points between the use of two different solutions.
6% CA + 1% NaOCl (Group 3): Between each instrumentation, the root canals were irrigated with 10 ml of 1% NaOCl. Final irrigation was performed using 10 ml of 10% CA and 10 ml of %1 NaOCl. The root canals were dried with paper points between the use of two different solutions.
0.9% PS (Control) (Group 4): Between each instrumentation, the root canals were irrigated with 10 ml of 0.9% PS. Final irrigation was performed using 10 ml of 0.9% PS.

In all groups, the root canals were dried with paper points, and the access cavities were closed with reinforced zinc oxide eugenol (Intermediate Restorative Material, IRM; Dentsply, Milford, DE). The roots removed from the wax blocks were divided into two halves with the guidance of superficial grooves mesio-distally along their long axis in their outer walls.

SEM analysis

The efficacy of smear layer removal on the walls of the root canal halves was evaluated by scoring according to the presence of smear layer in the coronal, middle, and apical thirds in examination with SEM (Leo 440 Computer Controlled Digital, UK). Each specimen was dehydrated in graded ethanol series 25%, 50%, 75%, and 90% for 25 min, and finally in 100% ethanol for 60 min. The specimens were critically point-dried, mounted on aluminum stubs, and sputter-coated with gold–palladium particles at 135 A° thickness. Coronal (8–10 mm from apex), middle (5–7 mm from apex), and apical (1–3 mm from apex) thirds of each specimen were examined, photographs were taken at ×3.50 K magnification, and labeled by an independent SEM technician. Two independent examiners who were unaware of which specimen belonged to which group blindly analyzed and scored for the degree of smear layer removal. Evaluation of the presence of smear layer was performed in accordance with the following criteria [12,17,18]:

Score 0: absence of smear layer (majority of dentinal tubules open)
Score 1: moderate presence of smear layer and outline of tubules partially visible (majority of dentinal tubules partially obliterated)

Score 2: presence of abundant smear layer (majority of dentinal tubules completely obliterated)

Intra- and inter-examiner validity was assessed by Kappa statistics. Kappa values ranged from 0.8 to 1, demonstrating good reliability.

Statistical analysis

The differences among the efficacies of irrigation agents were assessed by the Kruskal–Wallis test, and the differences among the thirds (coronal, middle and apical) were assessed by the Friedman test. The Siegel Castellan ($p < .01$) test was used for bilateral comparisons both within and between the groups when significant differences were detected ($p < .001$).

Results

It was seen that, in the experimental groups, smear removal efficacies were statistically significantly higher in the coronal and medium thirds compared to the apical (Table 1). In the coronal and middle thirds of the root canals, both Group 3 and 2 irrigations were found to significantly reduce the amount of smear layer compared with the other groups ($p < .05$). There was no statistically significant difference between Groups 1 and 4. On the other hand, in the coronal and middle thirds, there was no statistically significant difference between Groups 2 and 3 (Figures 1–4).

When the differences between the scores of the apical thirds were evaluated, it was determined that only Group 2 irrigation resulted in more effective cleaning, statistically significantly different when compared to Group 1. No difference was found in the other groups (Table 1) ($p < .05$). Moreover, erosive defects were observed around peritubular and intertubular dentin in the coronal and middle thirds of the roots of some samples in Groups 1 and 2.

Discussion

NaOCl has a potent antibacterial effect that can provide rapid bacterial inactivation even at low concentrations [13,19–21]. It is usually used at ranges of 0.005–5.25%; however, as the concentration increases, the destructive effect on the vital tissues increases [22,23] and, therefore, it is stated that the use of 1% NaOCl in primary teeth is sufficient [9,10,12,24]. In this study, the use of 1% concentration of NaOCl was preferred to use because NaOCl in high concentration does not increase antibacterial activity and also creates a risk of overflow from periapical tissues in primary teeth with enlarged apical opening due to physiological root resorption. However, it is also known that this agent is not active enough to remove the inorganic content within the smear layer, despite its advantage of high antibacterial effect [6,13,18,25].

EDTA and its combinations, which are used as one of the most important members of the chelating agents, solubilize especially the inorganic part of the smear layer by forming a chelate with the calcium within the dentin tissue, and the concentration of 17% is usually used in root canal treatments of permanent teeth [7,13,26]. EDTA concentrations used in primary teeth range from 10 to 17% [6,12,16,27–30]. In the primary teeth, organic contents and water content are higher than those of permanent teeth. Therefore, the tissue stiffness is reduced, the dentin tubule density is higher, and the primary tooth dentin is more reactive to chemical irrigants when compared to the permanent tooth. Consequently, the smear layer can be removed more easily than permanent teeth and so, it is preferred to use 10% EDTA. In addition,

Table 1. The scores and the median values of the smear layer scores at the coronal, middle, and apical levels of various irrigation protocols groups.

	1% NaOCl (Group 1)	10% EDTA + 1% NaOCl (Group 2)	6% CA+ 1% NaOCl (Group 3)	0.9% PS (Group 4)	(Between groups) *p*	
Coronal third	1 [1–2]	0 [0–1]	0 [0–1]	1 [1–2]	<.001*	Group 1–Group 2: <.05* Group 1–Group 3: <.05* Group 2–Group 3: >.05 Group 2–Group 4: <.05* Group 3–Group 4: <.05* Group 4–Group 1: >.05
Middle third	2 [1–2]	1 [0–1]	0 [0–1]	2 [1–2]	<.001*	Group 1–Group 2: <.05* Group 1–Group 3: <.05* Group 2–Group 3: >.05 Group 2–Group 4: <.05* Group 3–Group 4: <.05* Group 4–Group 1: >.05
Apical third	2 [2–2]	1 [1–2]	1.5 [1–2]	2 [2–2]	.002*	Group 1–Group 2: <.05* Group 1–Group 3: >.05 Group 2–Group 3: >.05 Group 2–Group 4: >.05 Group 3–Group 4: >.05 Group 4–Group 1: >.05
Inter-group (*p*)	.006 Coronal-middle: >.05 Coronal-apical: <.05* Middle-apical: <.05*	<.001* Coronal-middle: >.05 Coronal-apical: <.05* Middle-apical: <.05*	<.001* Coronal-middle: >.05 Coronal-apical: <.05* Middle-apical: <.05*	.011* Coronal-middle: <.05* Coronal-apical: <.05* Middle-apical: >.05		

The differences among the efficacies of various irrigation agents were assessed by the Kruskal–Wallis test. The differences among the thirds (coronal, middle, and apical) were assessed by the Friedman test. The Siegel Castellan ($p < .01$) test was used for bilateral comparisons both within and among groups when significant differences were detected ($p < .001$).
*indicates the statistically significance.

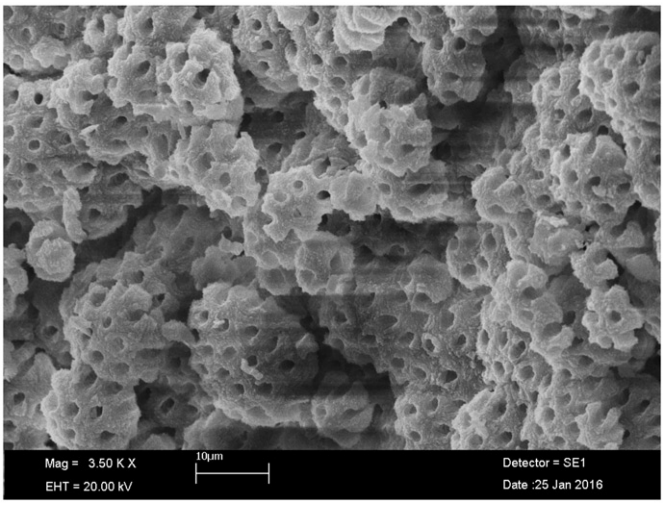

Figure 1. SEM images of the coronal third (score 0) for the NaOCl group. Representative images of erosive defects seen in coronal third (x3.50 K).

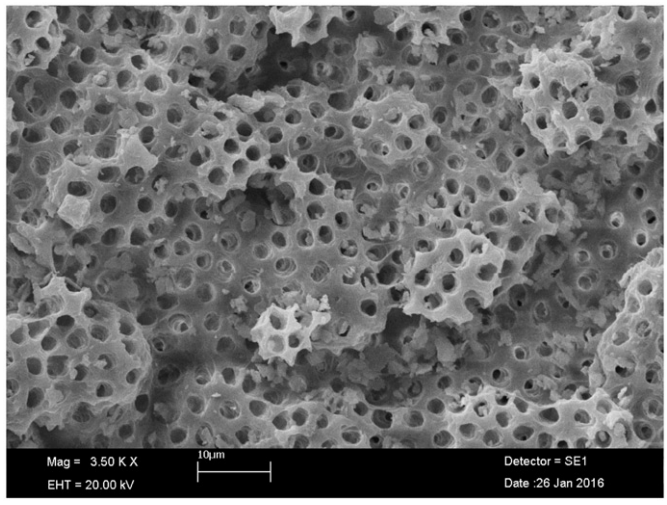

Figure 2. SEM images of the coronal third (score 0) for the EDTA group. Representative images of erosive defects seen in coronal third (x3.50 K).

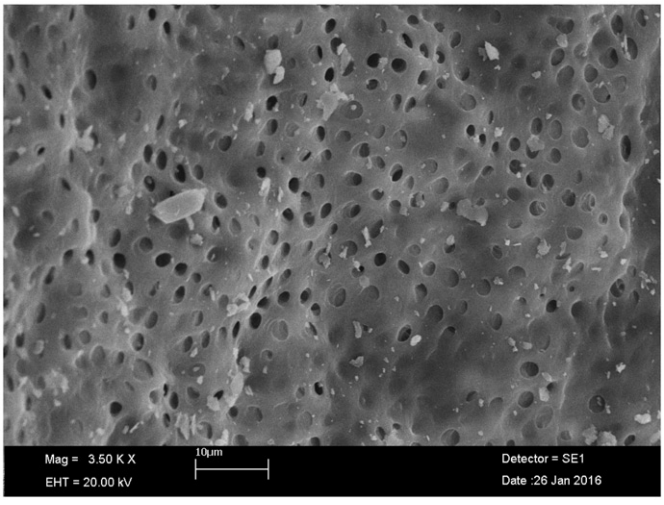

Figure 3. SEM images of the coronal third (score 0) for the CA group (x3.50 K).

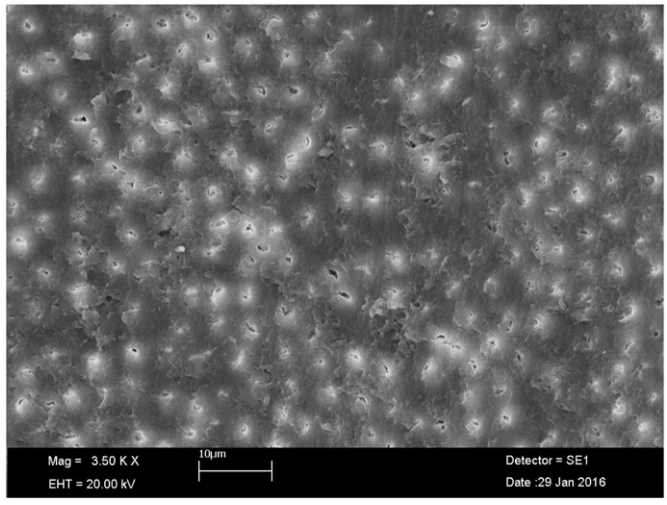

Figure 4. SEM images of the coronal third (score 1) for the PS group (x3.50 K).

although EDTA is successful and biocompatible in removing the smear layer, its low antibacterial activity and insufficient organic tissue solubility make this agent necessary to be combined with NaOCl, which has both an antibacterial effect and a high ability to degrade organic tissue [6,7,13]. For this reason, in the present study 10% EDTA was combined with 1% NaOCl.

CA, which is a member of organic acids and acts as a chelator like EDTA, allows removal of the inorganic part of the smear layer through dentin decalcification [6,14,15]. CA concentrations have been reported to vary between 1% and 50%, but it was reported that the use of 6% was sufficient to remove the smear layer [6,10,18,31] and does not cause destructive effects on dentinal structures [6]. The use of CA in combination with 1% NaOCl was also recommended for the mentioned additional antibacterial and organic tissue-dissolving effects of NaOCl [12]. About 91.2% success rate was found in an *in vivo* study in which the effect of smear layer removal by CA was evaluated for the success of root canal treatment in primary teeth [32].

When the root canal anatomy of primary teeth is considered, it is known that atypical morphological variations are observed mainly in posterior teeth, while incisors have a uniform and straightforward morphology with relatively single root and single root canal [33,34]. In this study, we preferred to use single-rooted and single-canal maxillary primary incisor teeth as in other studies to provide standardization in examining the effect of irrigation agents [6,30].

Because of morphological variations, different dentinal characteristics, and divergency of canals in the primary teeth mostly observed in the apical region compared to the other parts of the canals, this region has the difficulty of reaching and instrumentation. In addition, due to increased apical opening with root resorption, to protect the underlying permanent tooth germ, pressure-irrigation cannot be performed in this region. Therefore, it is necessary to evaluate the apical region separately in root canal treatment [10]. Due to these specific conditions, efficacies of irrigation agents were evaluated by comparing the three different regions of the canal as coronal, middle and apical, like in other studies [16,31].

In this study, CA and EDTA results were similarly superior in the middle and coronal thirds of the root canals compared with other groups. These findings are similar to those of previous studies [12]. Even though it is known that EDTA removes the smear layer at a satisfactory level, it was reported that it causes dentinal tissue damage, such as erosion in the peritubular dentin, destruction in the intertubular dentin and junctions in the tubules [6,16]. Researchers also reported that EDTA should be used with caution because of its potentially toxic effect on the underlying permanent tooth germ [35]. In the present study, erosive dentinal damage was observed in some samples of the EDTA and NaOCl groups. Our results confirmed the similar findings in the previous studies which suggest CA may be a relatively safer agent compared to EDTA [6,16]. The limitation of this study is that the number of samples is insufficient for the comparative evaluation of erosive dentin defects by SEM analysis.

In the present study, smear layer removal in the coronal and middle thirds were found to be statistically significantly higher than that of the apical region in all experimental groups. The results of our study are like those of the studies that attribute high smear scores to the inaccessibility of the apical region [16,31].

Although it is reported that the presence of the smear layer has negative effects on the root canal of primary teeth in *in vitro* studies, the detection of high success rates in various clinical studies where the smear layer has not been removed makes the necessity of removing the smear layer contradictory [36,37]. Similarly, in a 3-year follow-up *in vivo* study, the effect of removing smear layer on the success of pulpectomy was 82% in the group that they removed the smear layer and 88% in the group without removal [9]. In contrast to those findings, high success rates were also reported with the smear layer removal [32]. Therefore, further clinical studies are needed to clarify the survival effect of the smear layer removal in the root canal treatment of primary teeth.

The null hypothesis that there are no differences among the efficacies of all irrigation solutions regarding smear layer removal was rejected.

Conclusions

Although 6% CA + 1% NaOCl irrigation did not yield a significant superiority compared to 10% EDTA + 1% NaOCl irrigation in the coronal and middle thirds of the root canals, within the limitations of this study. However, due to erosive changes seen in EDTA samples, CA may be an appropriate alternative for the removal of smear layer in root canal treatment.

Due to the insufficient smear layer removal of apical thirds in all groups, it should be considered to use of more effective irrigation techniques for this region.

Disclosure statement

No potential conflict of interest was reported by the authors.

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