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ORIGINAL ARTICLE

The effects of various irrigation protocols on root canal wall adaptation and apical microleakage in primary teeth

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ABSTRACT

Objective: The objective of this study is to evaluate the wall adaptation and apical microleakage values following the application of various irrigation protocols in primary teeth.
Material and methods: For the two parts of the study, extracted upper incisor primary teeth were randomly included to the 1% sodium hypochlorite (NaOCl), 10% ethylenediaminetetraacetic acid (EDTA)+1% NaOCl, 6% citric acid (CA)+1% NaOCl and 0.9% physiological saline (PS) groups. Canal wall adaptation and apical microleakage were assessed by scanning electron microscopy (SEM) and stereomicroscope, respectively.
Results: 6% CA + 1% NaOCl group was found to be the most successful irrigation protocol in providing strong canal wall adaptation and less apical microleakage, followed by 10% EDTA +1% NaOCl. 6% CA +1% NaOCl was significantly superior regarding apical microleakage ($p < .05$).
Conclusions: Due to the ability to provide appropriate changes in the root canal walls to make a well-adapted and leak-proof canal filling, 6% CA + 1% NaOCl can be recommended as an irrigation protocol in primary teeth.

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Dental leakage; dental adaptation; primary teeth; root canal irrigants; scanning electron microscopy

Introduction

Successful root canal treatment requires complete obturation of the root canal system. Three-dimensional obturation should be provided by the penetration of root canal filling material throughout all the dentinal surfaces of the root canal system [1]. The smear layer which could act as a barrier between the filling material and the canal walls is a layer in the amorphous structure formed due to the mechanical preparation of root canals, composed of inorganic and organic contents [1–4]. Besides the ideal wall adaptation, a tight apical seal is also necessary to obtain successful root canal treatment. In the absence of apical sealing, the bacteria and their by-products that remain persistent in the root canal system may cause reinfection of the root canal system. Many researchers reported the relationship between the presence of the smear layer and the apical microleakage. In the presence of this layer, the penetration of the root canal filling materials to the dentine tubules is limited and an adaptation deficiency between the canal wall and the filling material [1–6]. Therefore, the removal of the smear layer is recommended to reach better clinical results in root canal treatment in primary teeth [1,4,7,8].

Various irrigation solutions have been used for smear layer removal in root canal treatment of primary teeth, and NaOCl has been used as the gold standard for many years due to its high antibacterial effect [9]. It was reported that NaOCl has a potent antibacterial effect that can rapidly induce bacterial inactivation even at a low concentration of 0.5% [9–12], so it was stated that using 1% NaOCl is satisfactory in primary teeth [7,13,14]. However, NaOCl is not effective enough to remove the inorganic content of the smear layer [3,9,15,16], despite its advantage of the high antibacterial effect. It is known that the smear layer in primary teeth can be removed more easily due to increased organic and water content, the reduced hardness of the tissue, increased density of dentine tubules when compared to permanent teeth [17]. EDTA, which acts as an inorganic tissue solvent through dentine chelation, effectively removes the inorganic part of the smear layer [5,9,18] and the concentration of EDTA in primary teeth varies between 10% and 17% [3,13,17,19–22]. In primary teeth, it is preferred to use 10% of EDTA, a lower concentration than its conventional use in permanent teeth. Also, although EDTA is successful in removing the smear layer, its low antibacterial activity and insufficient strength as a tissue solvent make this agent necessary to be combined with NaOCl, which has both antibacterial effect and high ability to dissolve organic tissue [3,5,9]. In this context, 10% EDTA was used in combination with 1% NaOCl in this study. Even though it is known that EDTA removes the smear layer efficiently, researchers mentioned the findings as dentinal tissue damage, erosion in the peritubular dentine, destruction in the intertubular dentine and junctions in the tubules with the combined use of EDTA and NaOCl [3,17].

CA, a member of organic acids, enables removal of the inorganic part of the smear layer through dentine

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decalcification [3,23,24]. When CA studies were reviewed, it was observed that a 6% solution of CA was used more frequently in primary teeth, that the smear layer was very effectively removed at such concentration [3,15,23,25] and that the normal structure of the dentine tissue was not damaged [3]. CA has been used in primary teeth with a lower concentration compared to permanent tooth studies, combined with 1% NaOCl for additional antibacterial and organic tissue dissolving effect.

As described above, various root canal irrigants have different levels of effectiveness in the removal of the smear layer [26,27]. Because varying efficiency of smear layer removal of each of these irrigation agents, the wall adaptation and apical microleakage are also expected to be at different levels. The objective of this study is to evaluate the canal wall adaptation of filling material and apical microleakage in primary teeth by scanning electron microscopy (SEM) and stereomicroscope following the application of various irrigation protocols. The null hypothesis tested in this study was that there would be no difference among various irrigation protocols regarding wall adaptation and apical microleakage.

Material and methods

Tooth selection criteria

For the root canal wall adaptation and the apical microleakage parts of the study, 40 and 50 extracted single-rooted upper primary central incisor teeth were used, respectively. Teeth in which the root resorption level did not exceed 1/3 root length macroscopically and that the apical opening was not wider than a #50 K-file (G-star Medical Co., Ltd., Guangdong, China) were included in the study. Teeth with abnormalities such as internal or external resorption, abnormal root fracture, calcification or obliteration in the canal detected by obtained preprocedural periapical radiographs were excluded from the study. Selected teeth, stored in 0.9% PS until the beginning of the process, were immersed in 2.5% NaOCl solution for 48 h to remove organic debris, and tissue remnants and debris were brushed and cleaned under running water.

Preparation of the samples

All the teeth are embedded 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 an excavator and the pulp tissue residues in the canal were removed by a barbed broach. After determining the estimated working length of the specimens by using a file on the periapical radiographs of the samples, and taking this measurement as a reference, the definite working length was determined as 2 mm coronal to the radiologic apex by placing a #15 K-file (G-star Medical Co., Ltd., Guangdong, China) into the root canal. In all groups, each hand-file set was used for only one sample group, and the root canal preparations were made by a single operator, using K-files sized from #15 to #45. During the preparation process, a file with a larger diameter was used after the small file was provided to move freely in the canal, while the irrigation procedures associated with the groups were performed. All the root canals were irrigated with a 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 experimental groups

The teeth were randomly divided into four groups, with 10 samples per group for both the root canal wall adaptation and the apical microleakage evaluation parts of the study. Besides, positive ($n = 5$) and negative ($n = 5$) control groups were constituted for the evaluation of apical microleakage.

Common experimental procedures for both canal wall adaptation and apical microleakage assessments

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.

Irrigation groups specified for apical microleakage assessment

Negative Control Group (Group 5): 10 ml of 0.9% PS was used between each instrumentation and as the final irrigation.

Positive Control Group (Group 6): 10 ml of 0.9% PS was used between each instrumentation and as the final irrigation.

The root canals of the teeth (except for Group 6) were air-sprayed after drying with paper points, ensuring that no air bubbles were left. Then, all canals were filled with Endoflas (Sanlor and Cia, S. en C.S., Cali, Colombia) filling material by using a low-speed handpiece and with the lentulo spiral method (ISO size 25) to be one mm shorter than the working canal length. At the end of the procedure, radiographs were obtained to confirm that the canal filling material reached apical third, and then access cavities were sealed with strengthened zinc oxide eugenol (Intermediate Restorative Material; IRM; Dentsply, Milford, DE). In the evaluation of apical microleakage, only the samples of the positive control group were not filled, and all the samples were kept in distilled water at 37 °C in the incubator for 24 h to allow the filling material to set before the samples were removed from the wax blocks.

Assessment of root canal wall adaptation (SEM)

The divided root surfaces were visually assessed for the presence of any gap between the filling material and the dentine walls and the penetration of the material to dentine tubules in the coronal, middle and apical third of each root separately by 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 aluminium stubs and sputter-coated with gold-palladium particles at 135 A thickness. Coronal (8–10 mm from the apex), middle (5–7 mm from the apex) and apical (1–3 mm from the apex) thirds of each specimen were examined, photographs were taken at 3.50 K magnification and labelled by an independent SEM technician. Representative images were evaluated visually by two calibrated examiners who were blinded to the experimental groups.

Assessment of apical microleakage (stereomicroscope)

In the apical microleakage assessment groups, for stereomicroscopic examination, root surfaces of experimental groups (Groups 1–4 and 6) were painted with 2 coats of nail polish, except 1 mm from the apical foramen and entire root length was covered with nail polish in Group 5. The samples were then incubated for 7 d in a 2% methylene blue solution in glass bottles, as would be individual for each group. At the end of this period, the teeth removed from the solution were rinsed with water and dried. After splitting into two halves with the guidance of superficial grooves previously prepared at the root surface, the more leaking half was selected, and the depth of leakage was assessed according to the deepest point. After all these procedures, the apical regions of all samples were measured in millimetres under the stereomicroscope by two calibrated examiners who were blinded to the experimental groups.

Statistical analysis

Intra- and inter-examiner validity for SEM analysis was verified by a kappa test. The kappa test values ranged from 0.8 or above, demonstrating good reliability. The ANOVA test ($p < .05$) was used to analyse whether there was any significant difference between the irrigation agent groups regarding the evaluation of apical microleakage.

Results

In canal wall adaptation part of this study, the strongest adapted root canal filling was determined in 6% CA + 1% NaOCl group, followed by 10% EDTA + 1% NaOCl, 1% NaOCl and 0.9% PS groups, respectively, in the coronal and the middle thirds of the root canal (Figure 1). Regardless of the irrigation procedures, the wall adaptation of the apical portions of the root canals was found to be less successful when compared to the middle and coronal regions.

Similarly, in the assessment of apical microleakage, statistically, significantly less microleakage values were found in 6% CA + 1% NaOCl group ($p < .05$) when compared to the 0.9% PS and 1% NaOCl groups. As in the assessment of the wall adaptation, 6% CA + 1% NaOCl group was followed by 10% EDTA + 1% NaOCl, 0.9% PS and 1% NaOCl groups (Figure 2, Tables 1 and 2).

Discussion

A successful root canal treatment of primary teeth depends on the elimination of infectious microorganisms and the sealing capability of the filling material [1,28–30]. The hermetically sealed obturation of the root canal is possible by the strong adaptation of the canal filling material to the root dentine [3]. However, it is difficult to perform a hermetic canal filling due to the necessity of using resorbable and non-condensable filling material in primary teeth. Considering the possibility of the lack of adaptation between the canal wall and the filling material, the removal of the smear layer in primary teeth is thought to be an important step in the prognosis and eventual success [1–4]. In this respect, the purpose of this study was to assess canal wall adaptation and apical microleakage following the application of various irrigation protocols in primary teeth.

Considering the root canal anatomy of primary teeth, it is known that morphological deviations are seen more frequently particularly in primary molar teeth whereas primary incisors have relatively simple and uniform morphology with a single root and canal. Since atypical variations in primary molars may adversely affect standardization and assessment conditions during the investigation of the effects of irrigation agents on canal wall adaptation and apical microleakage, we preferred to use single-root and single-canal upper incisors as in other studies [31,32].

In this study, the coronal, middle and apical thirds of the canal were examined separately during the determination of the effectiveness of irrigation agents in canal wall adaptation and microleakage evaluation was performed only in the apical region. Because it is known that, in primary teeth, the apical region of the root canals has strategic importance due to possible morphological variations, having difficulties in reaching and instrumentation, increased apical opening with root resorption and the necessity to protect the underlying permanent tooth germ [23].

In this study, SEM -the gold standard of examination methods- was used to assess the adaptation of the filling material with the canal wall, whereas the stereomicroscope was used to evaluate apical microleakage. In the SEM investigation, the visual observation was performed for identifying the gaps between the canal wall and the filling material together with the penetration quality of the filling material whereas the millimetric measurement of dye penetration into the canal was preferred for the evaluation of apical

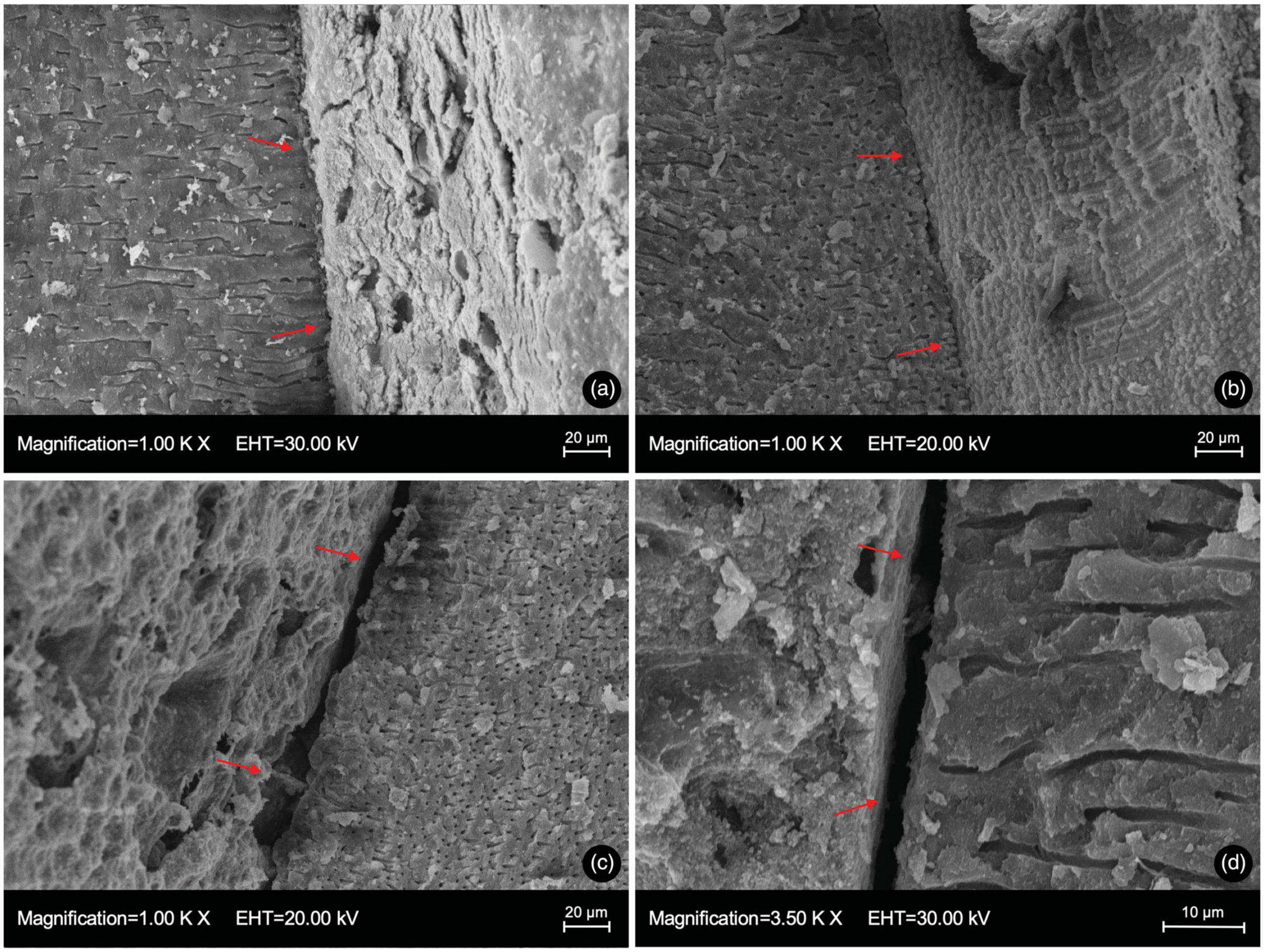

Figure 1. SEM images of the adaptation between the filling material and the root canal dentine. a: CA Group b: EDTA Group c: NaOCl Group d: PS Group (The red arrows indicate the joining line between the canal wall and the filling material together with the adaptation in this field).

microleakage. In the evaluation of apical microleakage, despite the disadvantages mentioned in the literature [33], methylene blue could be used for the evaluation of apical microleakage as a dye due to its properties such as easy handling, high degree of staining, molecular weight lower than that of bacterial toxins and lower cost [34]. In both evaluation parts, the assessment was made on the images obtained by taking the longitudinal section of the root.

In the assessment of canal wall adaptation, the most successful results were in the CA group, followed by the EDTA, NaOCl and PS groups, respectively, in the coronal and middle thirds of the root canals. On the other hand, regardless of the irrigation procedures, canal wall adaptation was found to be less successful in the apical portion of the root canal when compared to the coronal and middle thirds. This finding could be attributed to the inability of irrigation solutions to reach the apical region and, therefore, dentinal penetration of the filling material in this region could not be at the desired level due to the presence of the smear layer.

In the evaluation of apical microleakage, like the results above, minimal leakage values were determined in the CA group. Likewise, this irrigation agent was followed by EDTA, NaOCl and PS. The superiority of the same irrigation protocol in both evaluation parts of the study was a finding that supports the notion that effective sealing could be achieved by an effective wall adaptation.

CA was found to be the most successful irrigation agent in apical microleakage and wall adaptation concerning the ability of efficient removal of the smear layer by the agent. Among the experimental groups, in the protocol where NaOCl was applied alone, the worst findings of the adaptation and the microleakage values led to the need for adding another solution as a final irrigant to solve the inorganic component of the smear layer. In the preparation stage of this study, no similar studies have been found in the literature on root canal wall adaptation and apical microleakage following the smear layer removal in primary teeth. However, as the result of this study, in routine clinical conditions, it is considered that the well-adapted and leak-proof canal filling following the removal of the entire contents of the smear layer will increase the eventual success rate of root canal treatment in primary teeth. The null hypothesis that there are no differences among the efficacies of all irrigation solutions regarding wall adaptation and apical microleakage was rejected. As a conclusion of this study, it is possible to say that 6% CA + 1% NaOCl could be preferred as an alternative irrigation protocol because of its potential to provide strong wall adaptation and hermetic sealing.

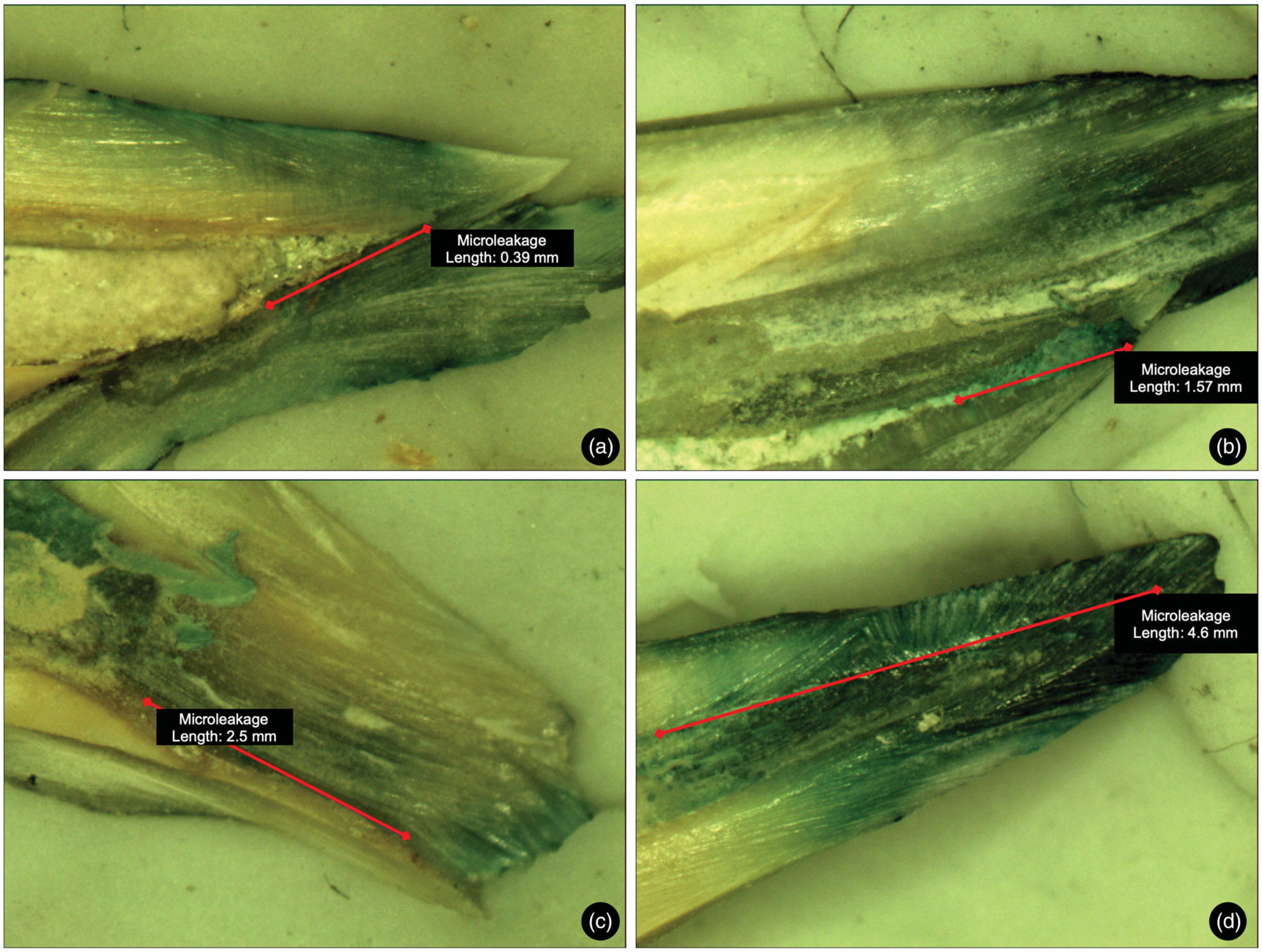

Figure 2. Stereomicroscope images of apical microleakage. a: CA Group b: EDTA Group c: NaOCl Group d: PS Group (Microleakage values in the apical area were shown in millimetres).

Table 1. Mean apical microleakage values of the irrigation groups.

Irrigation groups	*n*	Mean apical microleakage values	Standard deviation	Standard error
Group 1	10	2639.2	707.89	223.85
Group 2	10	1205	829.32	262.25
Group 3	10	735.5	727.34	230
Group 4	10	2115.7	1538.36	486.47

Table 2. Multiple comparisons of the apical microleakage values of the irrigation groups.

Irrigation groups	Mean difference	Std. error	*p* Value
Groups 1–2	**1434.2***	451.9	**.003***
Groups 1–3	**1903.7***	451.9	**.000***
Groups 1–4	523.5	451.9	.254
Groups 2–3	469.5	451.9	.306
Groups 2–4	910.7	451.9	.051
Groups 3–4	**1380.2***	451.9	**.004***

*The mean difference is significant at the .05 level.
The bold values indicate the statistical significance between the related irrigation groups. ($p < .05$)

Disclosure statement

No potential conflict of interest was reported by the authors.

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