Influence of Irrigation Protocol on Peroxide Penetration into Dentinal Tubules Following Internal Bleaching: A Confocal Laser Scanning Microscopy Study

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Introduction: *Discoloration of anterior teeth can result in cosmetic impairment in young children. The walking bleach technique stands out because of its esthetic results with minor side effects. Little information is available regarding the influence of various irrigation solutions on peroxide penetration. The aim of this study is to evaluate the influence of different irrigation protocols on peroxide penetration into dentinal tubules using confocal laser scanning microscopy (CLSM).*

Study design: *Cavity preparations were made in 50 extracted permanent premolars. The teeth went through different irrigation sequences: A. control B. saline C. EDTA, NaOCl D. phosphoric acid E. EDTA, NaOCl, phosphoric acid. Then, mixture of fluorescent dyed sodium perborate paste was placed along the pulp chamber and the coronal access cavity, and was refilled at days 7, 14 and 21.*

Results: *The minimal and maximal penetration depths were 324 and 3045 μm, respectively, with a mean of 1607μm. The stained areas were significantly larger in the buccal and lingual directions ($P<0.05$). Groups B and C showed significantly larger penetration in weeks 2 and 3 compared to week 1 ($P<0.05$). Group D and E showed significantly larger penetration compared to groups B and C at all times ($P<0.05$).*

Conclusion: *Bleaching agents penetrate to the extra-radicular region of teeth; however, the level of peroxide penetration is significantly higher when the irrigation sequence consists of phosphoric acid prior the bleaching agent placement.*

Keywords: *Discoloration, Bleaching, Sodium perborate, Peroxide, CLSM*

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INTRODUCTION

Dental trauma and pulpal infections are part of the routine pediatric dentistry. Common consequences in these cases are alterations in dental color[1–3] compromising patients' esthetics and their interactions in social environment [4–6]. Bleaching intends to preserve dental structure already weakened and to show immediate esthetic results.

Materials used for bleaching pulpless teeth are evaluated for the speed and efficacy in re-establishing the natural color of the teeth and for their potential (or lack thereof) of causing damage to surrounding structures [3,7,8] An adverse effect that has been reported following internal tooth bleaching is cervical root resorption (an inflammatory mediated external resorption of the root)[9].

One of the most important properties of a bleaching material is its ability to allow penetration of the bleaching agent through dentinal tubules permeability [3,7]. The deeper the penetration, the more pigment that causes chromatic alteration of the dental tissues can be reversed by the oxidation reaction[3,7].

Several procedures have been reported to increase dentinal tubules permeability for the bleaching agents, including phosphoric acid etching of tooth structure before placing bleaching agents [10], smear layer removal [11] and heat application [12,13]. However, thermocatalytic bleaching techniques have recently been questioned due to the deleterious effects that may be produced on dentinal structures [14,15]. As to the agents used for bleaching of non-vital teeth, sodium perborate paste has shown improved esthetic results when prepared with either hydrogen peroxide or distilled water as the liquid vehicle. The 10% carbamide peroxide has provided similar performance to sodium perborate for internal bleaching [16,17] The water-based sodium perborate paste has been reported to have less potential to harm dental tissues [18-20]. Different types of sodium perborate (mono-, tri- or tetra-hydrated) may be used with similar results [18,21]. Sodium perborate is an oxidizing agent available as a powder. It is stable when dry; however, in the presence of acid, warm air or water, it breaks down to form sodium metaborate, hydrogen peroxide, and nascent oxygen. H2O2 is released during the decomposition of perborate[22].

Sodium perborate is easier to control and safer than concentrated hydrogen peroxide solutions[1] The first description of the walking bleach technique with a mixture of sodium perborate and distilled water was mentioned in a congress report by Marsh and published by Salvas[23]. In this procedure, the mixture was left in the pulp cavity for a few days, and the access cavity was sealed with provisional cement.

The penetration of peroxide into dentin has been previously presented [7,24–26] However, little information is available regarding the influence of various irrigation solutions on sodium perborate penetration. Therefore, the aim of this study is to evaluate the influence of different irrigation protocols on sodium perborate penetration into dentinal tubules using confocal laser scanning microscopy (CLSM).

MATERIALS AND METHOD

50 fully developed human permanent premolar teeth were used in this study, following the approval of the Tel Aviv University ethics committee. All teeth were freshly extracted for periodontal reasons from male and female patients (age range 30–45 years). Teeth with previous root canal treatment, root resorption, or caries and teeth with root fractures were excluded from the study. Once the tooth was extracted, the debris and soft tissue remnants were removed from the root with a sharp scalpel. Then, the teeth were stored in phosphate-buffered saline until used for the study.

Occlusal access openings were prepared using Endo-Access bur (Dentsply-Maillefer, Ballaigues, Switzerland). The root canals were prepared to a standard shape using Gates Glidden burs (Kerr Dental, Orange, CA, USA) to the apical foramen. The teeth were then randomly divided into five experimental groups of 10 teeth, each group went through a different irrigation sequence:

Group A (10 teeth): negative control, saline irrigation without a following bleaching agent.

Group B (10 teeth): saline irrigation

Group C (10 teeth): 17% EDTA followed by 3% NaOCl and final saline irrigation

Group D (10 teeth): 37% phosphoric acid followed by saline irrigation

Group E (10 teeth): 17% EDTA followed by 3% NaOCl, 37% phosphoric acid and final saline irrigation.

After the irrigation sequence, the bleaching agent–sodium perborate (Merck KGaA, Darmstadt, Germany) mixed with distilled water in a ratio of 2 g of powder to 1 mL of liquid–was used. In order to analyze fluorescence under confocal laser microscopy, Rhodamine B dye (Sigma-Aldrich, St. Louis, MO, USA) isothiyocyanate fluorescent was added (maximum absorption = 570 nm, maximum emission = 720 nm) to an approximate concentration of 0.1%. The mixture of fluorescent dye and sodium perborate paste (Merck KGaA, Darmstadt, Germany) was placed along the pulp chamber and the coronal access cavity.

A dry cotton pellet was placed inside the pulp chamber in all groups, and the access cavities were sealed with glass ionomer cement (GC Fuji IX GP; GC America Inc, Alsip, IL). The teeth were then stored at 100% humidity and 37°C. During the experiment, the teeth were re-rinsed and the pulp chamber was refilled with fresh bleaching paste at days 7, 14 and 21.

Preparation of Specimen for Microscopy

The specimens were embedded in a self-cure acrylic repair material (UNIFAST Trad, GC America) and cut perpendicularly to the long axis of the root under water cooling with a diamond saw rotating at 500 rpm (Isomet, Buehler Ltd., Lake Bluff, IL, USA) in order to obtain five slabs for each specimen of 1 mm thickness.

Confocal Microscopy Evaluation

Fluorescence from the stained bleaching agents was observed under a confocal laser scanning microscope (CLSM) (Leica TCS SP5, Leica Microsystems CMS GmbH, Germany). Single-channel imaging was used to display red fluorescence. The CLSM images were acquired at a resolution of 1024×1024 pixels and analyzed by the LAS AF software (version 2.6.0.7266; Leica Microsystems CMS GmbH). The specimens observed using a ×4 lens. The mesial, distal, buccal, and lingual areas of the specimens were evaluated by the software as follows:

1. The size of fluorescent staining within the evaluated areas, as calculated by the software.
2. The depth of penetration into the dentinal tubules was measured and recorded considering the canal wall as the starting point (figure 1f).

Statistical analysis

The results were evaluated statistically using Mann Whitney U test to compare the proportion between the buccal/lingual/mesial/distal areas. Kruskal-Wallis test was used to evaluate the fluorescence staining within the evaluated areas. $P<0.05$ was considered as statistically significant.

RESULTS

No fluorescence was observed in the control group, and fluorescence was found in all specimens of the other groups (figure 1 a-f). The minimal and maximal penetration depths into the dentinal tubules were 324 and 3045 µm, respectively, with a mean of 1607µm. Table 1 presents the fluorescence stained sodium perborate penetration depths into the dentinal tubules in the different groups. The extent and amount of the stained areas were significantly larger in the buccal and lingual directions compared to the mesial and distal directions in all groups (figure 1d, Table 1, $P<0.05$).

Groups B and C showed significantly larger penetration in weeks 2 and 3 compared to week 1 ($P<0.05$). Group D and E showed significantly larger penetration compared to groups B and C at all times ($P<0.05$) (Figure 2, 1a,d,e).

DISCUSSION

This study compared dentinal peroxide penetration levels after different irrigation sequences. Confocal laser scanning microscopy was used in the present study to analyze the flow of peroxide penetration because it is a technology which combines optical microscopy, physical-chemical principles and computing resources for acquisition and processing of images[28–30]. The system uses a laser source to promote excitation of fluorophores. The laser beams may diffuse through the dentin, enamel and biofilms, thus detecting their inner structures and forming several two-dimensional images[31].

Confocal laser scanning microscopy has some advantages compared to scanning electron microscopy, including histological evaluation and other methodologies for assessing penetration of endodontic materials[29,31,32]. This technology may also be used in microbiological studies for quantifying bacteria within dentinal tubules[33,34]. Previous ex-vivo studies attempted to evaluate peroxide penetration [7,24–26] However, these studies were limited since they were using indirect models, which are incapable of evaluating the actual routes of peroxide penetration. Unlike the previous traditional

Figure 1: Confocal laser scanning microscopy (CLSM) images of the different groups. Penetration of peroxide (red) inside the dentinal tubules is clearly visible (a-f), in lower (a) and Higher (b,c) magnifcations. A butterfly-like appearance is seen on the root cross section (d) that occurs as a result of increased sclerosis along the tubules located on the mesial and distal sides of the canal lumen. Penetration depth was measured and recorded considering the canal wall as the starting point (f). The peroxide penetration was significantly higher when the irrigation sequence consists of phosphoric acid prior the bleaching agent placement (d) when comparing the other groups (a,e).

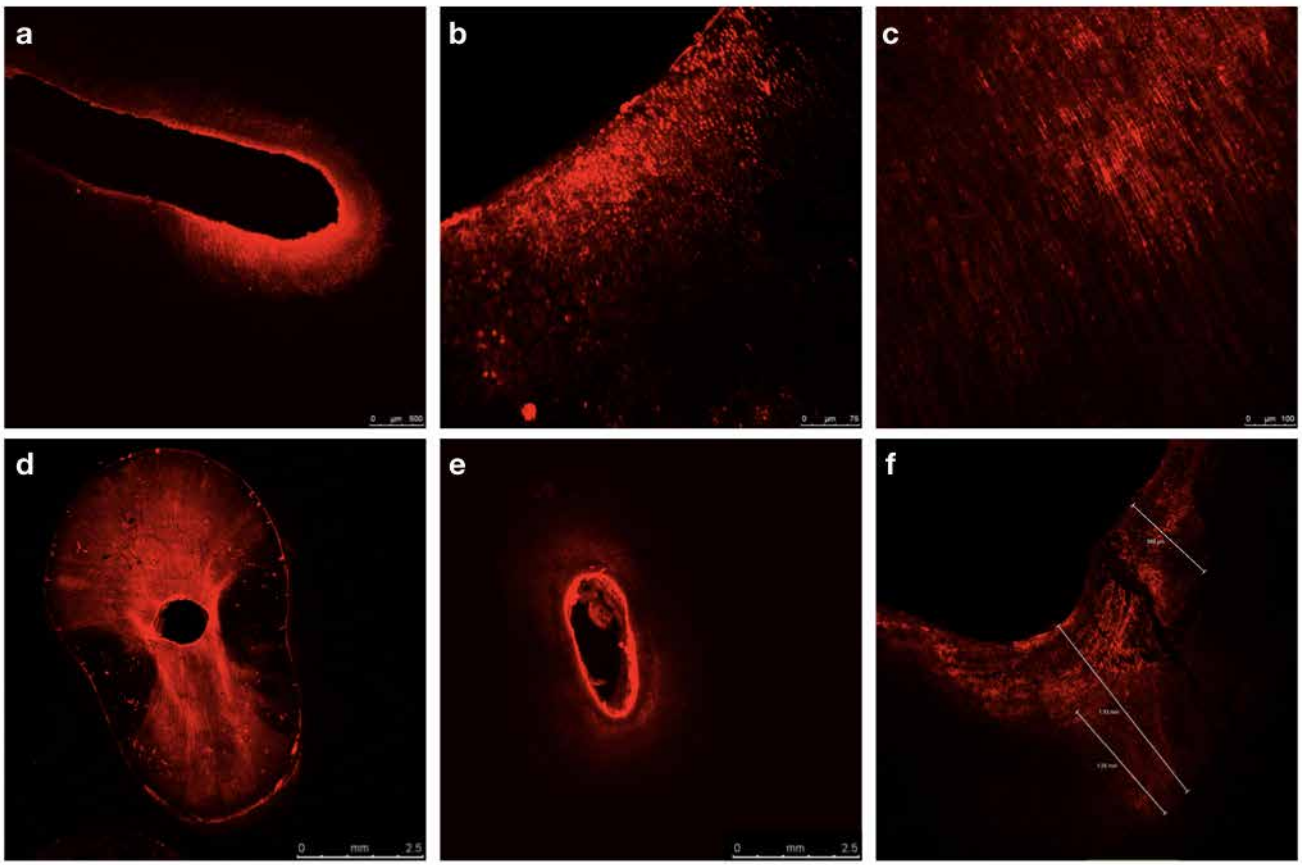


Table 1: Presents peroxide penetration depths in um into the dentinal tubules for the different groups. The minimal and maximal penetration depths into the dentinal tubules were 324 and 3045 µm, respectively, with a mean of 1607µm. The extent areas were significantly larger in the buccal and lingual directions compared to the mesial and distal directions in all groups.

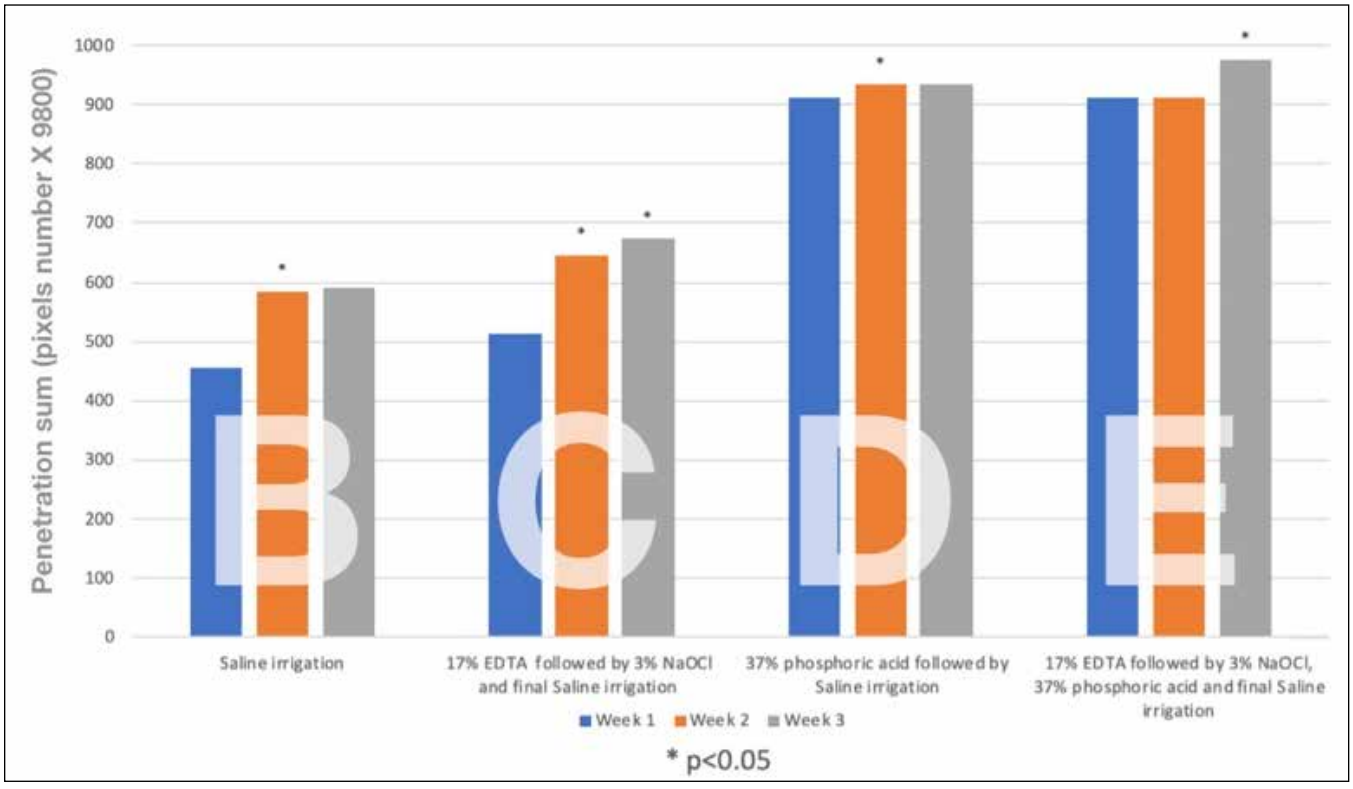
	Minimun Penetration	Maximum Penetration	Mean	Std. Deviation	P-value
Group B: Saline					
Week 1	433	2660	1492	481.9	
Week 2	655	2709	1503	789.6	0.003
Week 3	655	2709	1503	789.6	0.04
Group C: 17% EDTA followed by 3% NaOCl and final Saline irrigation					
Week 1	324	2700	1366.4	841	
Week 2	450	2700	1427	844	0.022
Week 3	380	2700	1332	860	0.169
Group D: 37% phosphoric acid followed by Saline irrigation					
Week 1	567	3000	1682	1015.6	
Week 2	789	3045	1964	836	0.027
Week 3	789	3045	1964	836	
Group E: 17% EDTA followed by 3% NaOCl, 37% phosphoric acid					
Week 1	567	3000	1684	1013	
Week 2	567	3000	1684	1013	0.00
Week 3	567	3000	1684	1013	0.00

models, in the current study a novel model was used, which histologically traces the actual routes of penetration *in-situ* [31,36]. In addition, negative histological controls were used to confirm the adequacy of the experimental model. No fluorescence was observed in the negative control groups, confirming the reliability of the experimental model.

Maximal penetration depth into the dentinal tubules was 3045 µm. Intracoronal bleaching requires healthy periodontal tissues and a root canal that is properly obturated to prevent the bleaching agent from reaching the periapical tissues[37] Previous studies have shown peroxide from bleaching agents to penetrate from the pulp chamber to the cervical region[38–40] during bleaching procedures. Radicular peroxide penetration should be as limited as possible, because the biological threshold of peroxide compounds causing irreversible damage to dental hard and soft tissue is unknown[21]. An adverse effect that has been reported following internal tooth bleaching is cervical root resorption[9]. It is important to note that there are a large number of cases of cervical root resorption after NVB in teeth that previously experienced dental trauma[41] The underlying mechanism for this effect is unclear, but it has been suggested that the bleaching agent reaches the periodontal tissues through the dentinal tubules and initiates an inflammatory reaction[42]. In vitro studies using extracted teeth showed that hydrogen peroxide placed in the pulp chamber penetrated the dentine [7,24,21,43,44], and that the penetration is greater in teeth with cervical cemental defects[38].

The fact that the fluorescence staining penetrated deeper in the bucco-lingual direction may be related to an anatomical-physiological phenomenon known as the “Butterfly Effect”, a butterfly-like appearance seen on root cross sections, that results

Figure 2: Fluorescence Penetration amount for the different groups (presents as the number of pixel X 9800). Groups B and C showed significantly larger penetration in weeks 2 and 3 compared to week 1 (P<0.05). Group D and E showed significantly larger penetration compared to groups B and C at all times (P<0.05).



from increased sclerosis along the dentinal tubules located on the mesial and distal sides of the canal lumen[45]. This effect is common in the single-rooted teeth of humans in a wide range of ages[46].

Prior to the application of the bleaching agent, preparation of the pulp cavity is required in order to remove remnants of restorative materials, root-filling materials, and necrotic pulp tissue[1] Additional cleaning of the pulp cavity with sodium hypochlorite is also recommended[47]. In some reports, conditioning of the dentin surface of the access cavity with 37% phosphoric acid is suggested in order to remove the smear layer and in order to open the dentinal tubules. This promotes the penetration of the bleaching agent deep into the tubules and increases its effectiveness[48]. Dentin permeability and cementum integrity play a key role in determining radicular penetration. The goal of the clinician is to increase the dentin permeability in order to facilitate the bleaching procedure and in order to achieve better esthetic results. However, this may lead to more diffusion of bleaching agents to the outer surrounding tissues [22]. In our study etching treated groups (D and E) showed significantly higher penetration of peroxide. In recent years it has been recommended to add the acid etching during the preparation of the pulp chamber prior to bleaching procedures [22,49]. This step was introduced after the recognition that dentinal surfaces prepared by rotary instruments are covered by a so-called "smear layer" consisting of enamel and dentin particles, mineralized collagen matrix, blood products and bacteria (6-8). Smear layer removal has been recommended in dentistry before composite restoration and prior to root canal canal filling[11,50–52] Walton *et al*.[53] advocated a 50% solution of phosphoric acid application for 60 seconds for smear layer removal[53]. The removal of the smear layer by acid etching has been shown to increase significantly the permeability of dentin in both vital and endodontically treated teeth, by opening up the orifices of the dentinal tubules[54,55] One might then expect improved penetration of bleaching agents into the stained dentin, thus enhancing their effectiveness[56,57]. Although an improvement in bleaching is hypothesized, the technique has not been universally accepted [3-5,9]. In addition, whether or not this extra etching step significantly improves the quality of an endodontic bleaching procedure has not been established [58,59]. Casey *et al*.[48] were unable to distinguish a significant difference between the effectiveness of the bleaching procedures using etching. Group E showed lesser penetration compared to group D, even though both groups included etching. A possible explanation for the differences in penetration may be attributed to the prior preparation of dentin using EDTA and NaOCl solutions that were incompletely rinsed off and were left inside the dentinal tubules, resulting in dilution of phosphoric acid concentration.

Bleaching success seems largely dependent on the application duration of the bleaching agent [60]. In our study, in groups B and C, where no etching had been used, a significant higher peroxide penetration was observed on the second week compared to the first week. However, in groups D and E, where etching conditioning was involved, penetration did not increase over time. Other studies [1,22] have stated that bleaching agents should be changed every 3-7 days and up to 4 times, while some showed preferable results after 3 weeks[61].

CONCLUSIONS

Bleaching agents penetrate to the extra-radicular region of teeth; however, the level of peroxide penetration is significantly higher when the irrigation sequence consists of phosphoric acid prior the bleaching agent placement. This fact may carry more risk of post-bleaching external root resorption, especially in traumatized dentition.

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