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In vitro assessment of dentinal permeability after the use of ultrasonic-activated irrigants in the pulp chamber before internal dental bleaching

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Abstract – This *in vitro* study aimed to assess dentin permeability quantitatively after the use of different irrigants into the pulp chamber, with or without ultrasonic activation, before the application of an internal bleaching agent. Thirty maxillary anterior teeth, treated endodontically, were randomly assigned to six groups, according to the irrigant used: group I, distilled water; group II, 17% EDTA; group III, 1% sodium hypochlorite; for groups IV, V, and VI, respectively, the same solutions were used, but were ultrasonicated. In groups I, II, and III, the irrigant that filled the pulp chamber was left undisturbed for 15 s and was then aspirated; in groups IV, V, and IV, the irrigants were placed into the pulp chamber, ultrasonic-activated for 15 s, and were then aspirated. This sequence was repeated three times for all groups. Afterwards, for all groups, the pulp chamber was dried, filled with a bleaching agent, and sealed with glass ionomer cement. At each change of the whitening agent, these procedures were repeated. Then, the temporary restorations were removed, access cavities were cleaned, and teeth were immersed in a 10% copper sulfate aqueous solution, submitted to vacuum and immersed in a 1% rubianic acid alcohol solution. Copper ion penetration was revealed by the rubianic acid. After staining, roots were removed at the cemento-enamel junction (CEJ) and sectioned in a mesiodistal direction starting from the cervical plug level. The sections were thinned, observed under an optical microscope, the images were digitized, and copper ion penetration was measured in each section using a specific software. Means and SD were: group I, 2.41 (±1.45); group II, 5.22 (±1.79); group III, 8.32 (±2.55); group IV, 3.73 (±0.89); group V, 14.83 (±4.99); and group VI, 10.51 (±2.65). Statistical analysis using two-way ANOVA and Tukey test showed that, regardless of the irrigant, ultrasonication increased dentinal permeability ($P < 0.01$). Comparing the overall effectiveness of the tested solutions, EDTA yielded the greatest increase in dentinal permeability ($P < 0.01$). Based on these results, it may be concluded that use of ultrasonic-activated irrigants in the pulp chamber, before the accomplishment of internal dental bleaching procedures, may result in a remarkable increase of dentin permeability, which may enhance the efficiency of the whitening agent.

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Key words: dentin permeability; irrigating solutions; dental bleaching; ultrasonic activation

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The color of a person's teeth has a strong influence on his/her appearance and may interfere with the individual's social and emotional behavior.

Dental bleaching is a conservative treatment that provides good results at relatively low costs, as compared to composite resin or porcelain veneers and total crowns. Furthermore, if dental whitening procedures are accurately performed, the occurrence of side-effects is considerably minimized. For such reasons, dental bleaching has become an essential component of esthetic dental treatments, recently becoming the single most requested esthetic procedure for adults (1).

Over the 20th century, different techniques and chemical products have been proposed for whitening teeth with chromatic alterations. To date, the development of techniques and materials for dental bleaching relies not only on their efficiency and quick recovery of tooth natural color, but also on their ability not to cause damage to dental structures, oral mucosa, and the patient's health. Nevertheless, there are still some questions raised on dental bleaching of non-vital teeth concerning external cervical reabsorption (2, 3), effects on dentin microhardness (4, 5), recurrent staining (6–8), and dentin permeability alterations produced by different whitening agents (9, 10).

The action of a dental bleaching agent is related to its ability in penetrating into dentinal tubules and modifying, by means of an oxide-reduction reaction, the pigments that caused the chromatic alteration of dental substrate. Therefore, 32 or 50% ortho-phosphoric acids are reported to increase dentinal permeability (11). Likewise, the removal of smear layer from dentin surface is also known to considerably increase the permeability of dentinal substrate on both vital and non-vital teeth (12, 13).

In 1957, Richiman (14) pioneered the use of ultrasonic activation for endodontic purposes, but because of the unfavorable results reached at that time, its use for root canal instrumentation was reintroduced solely in the late 1970s (15). If compared to other endodontic instrumentation techniques, activation by an ultrasonic system acts equally or even better in the removal of smear layer from root canal walls (16–18) and in the alteration of root canal dentin permeability (19, 20).

The association of ultrasound with EDTA or sodium hypochlorite at different concentrations (from 0.5% up to 5.25%) provides better cleansing of root canal walls, as well as of areas not reached by endodontic instruments, such as isthmus, deltas, and lateral canals (21–25).

In dental bleaching technique, the ultrasonic devices are employed to activate endodontic instruments or periodontal tips inside the pulp chamber in order to remove smear layer or debris from that site, leading to the increase of dentinal permeability and hence to an enhanced performance of the whitening agent (26, 27).

Therefore, in view of the aforementioned features, the aim of this study was to assess quantitatively the alteration in dentin permeability after the use of different irrigating solutions inside the pulp chamber, with or without ultrasonic activation, before the accomplishment of an internal bleaching technique.

Materials and method

Thirty human maxillary central incisors, stored in 0.4% sodium azide solution at the Laboratory of Endodontic Research (Ribeirão Preto School of Dentistry, Brazil), were selected after careful examination, using the tip of an explorer under a 10× stereoscopic magnifying lens (Carl Zeiss-Jena), to discard teeth with cracks or structural anomalies.

Conventional lingual access openings were prepared, pulp tissue was extirpated, and the working length was determined at 1 mm from apical foramen. For all teeth, biomechanical preparation was performed using a 1% sodium hypochlorite solution with an initial file followed by four instruments of higher caliber, and cervical and middle-thirds of root canals were enlarged to a #3 Gates-Glidden bur (Dentsply/Maillefer, Ballaigues, Switzerland) at low speed. Root canal was filled with guta-percha points and Grossman endodontic cement (Endofill, Dentsply Ind. e Com. Ltda., Petrópolis, Brazil).

Starting from the predetermined working length, 3 mm of filling material was removed from root canal at cervical third using a heated endodontic condenser. A cervical glass-ionomer cement plug (Vidrion, S.S. White Artigos Dentários Ltda., Rio de Janeiro, Brazil) was placed 1 mm inside the pulp chamber and 2 mm below the amelocemental junction on the buccal side into the root canal, with the specific role of protecting apical and periodontal tissues from contact with the bleaching substance.

After preparation of the cervical plug, the pulp chamber was filled with the evaluated irrigating solutions. Specimens were randomly assigned to six experimental groups of equal size ($n = 5$) as follows: group I (negative control), distilled deionized water; group II, 17% EDTA; group III, 1% sodium hypochlorite; and in groups IV (positive control), V, and VI, the same solutions were used, respectively, but were ultrasound-activated. For groups I, II, and III, the pulp chamber was filled with the irrigating solution, which was left undisturbed in the cavity for 15 s and was then aspirated; these procedures were repeated three times. For groups IV, V, and VI, the pulp chamber was filled with the irrigating solution and the #37 tip of the ultrasound apparatus (Profi Bios, Dabi Atlante, Ribeirão Preto, Brazil) was introduced inside the chamber without touching the cavity

Fig. 1. Schematic representation of the pulp chamber is filled with the irrigating solution and the tip of the ultrasound is activated.

walls, and was then activated for 15 s at peak power and no refrigeration (Fig. 1). Afterwards, the irrigating solution was aspirated. This procedure was also repeated three times. In all groups, this irrigating regimen was accomplished before the exchanges of the bleaching agent.

The bleaching agent, a sodium perborate (Merck KGaA, Darmstadt, Germany) and distilled water paste at a 3:1 ratio, was prepared. The paste was placed into the coronal pulp chamber, covered with a cotton pellet, and the lingual access was temporarily sealed with Vidrion® glass ionomer cement.

Specimens were submitted to three exchanges of the bleaching agent, with a 5-day interval between each one. In the meantime, teeth were kept in artificial saliva at 37°C and 100% relative humidity until the following application of the whitening substance. After the last change, the coronal chamber was filled with a neutralizing paste prepared with calcium hydroxide (Merck KGaA, Darmstadt, Germany) and distilled water for 15 days (28).

Next, the temporary glass-ionomer restorations were removed and the access cavities were entirely cleaned and rinsed in running water. Teeth were externally waterproofed with two layers of etil cianoacrylate (Super-Bonder®; Henkel Loctite Adesivos Ltda., Itapevi, Brazil) and immersed in a 10% copper sulfate aqueous solution (Merck KGaA, Darmstadt, Germany) for 30 min in vacuum for the first 5 min. Then, specimens were removed from the copper sulfate solution, dried with absorbing paper, and immersed in a 1% rubianic acid alcohol solution (Merck KGaA, Darmstadt, Germany), following the aforementioned technique: the first 5 min in vacuum, and, after vacuum removal, teeth were kept for additional 25 min in the solution (29). Copper ions were revealed by the rubianic acid, resulting in a specific coloration that ranged from dark blue to black, depending on the amount of copper ion penetration.

Afterwards, specimens were individually positioned in a sectioning machine with a water-cooled 300-μm thick diamond saw (Minitom, Struers A/S, Copenhagen, Denmark). Roots were removed at the amelocemental junction and crowns were embedded in chemically activated acrylic resin blocks. Teeth were serially sectioned in a mesiodistal direction, thus providing three transverse cuts that were 500 μm thick. The first sectioning was accomplished at the level of the cervical plug and the subsequent cuts were obtained incisally from that point (Fig. 1). The sections were ground under water-cooling using #400- to #600-grit silicon carbide paper to obtain a flat, smooth surface and a final thickness of approximately 200 μm.

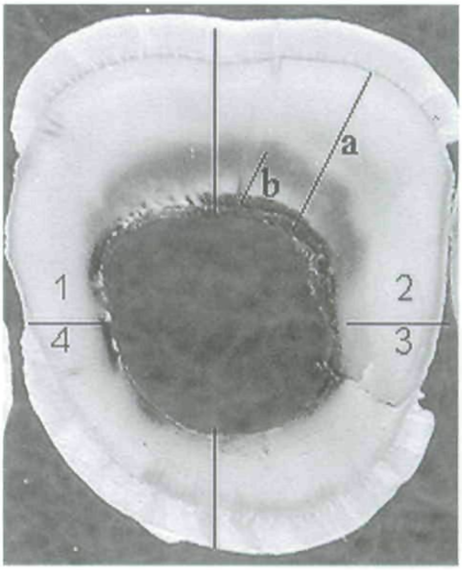

Fig. 2. Transverse section with schematic drawing illustrating quadrants (1–4) and the quantitative measurements: (a) the total extent of dentin in the quadrant; (b) the largest extent of dye penetration in the quadrant.

The cuts were identified, carefully fixed on microscopic slides, and observed under a 2.5× magnification optical microscope (Axioskope- Zeiss, Jena, D-07740, Germany) connected to a color video camera with 10× lens (TK-1270, JVC, Tokyo, 55473, Japan). The images obtained were transmitted to a personal computer and, after digitization, they were analyzed using the KS300 v.2.0 software (Kontron Elektronik, GmbH, Eching bei München, Germany), which enables a quantitative measurement (in millimeters) of dye penetration extent.

Each cut was divided into four areas of similar size and two measurements were obtained in each quadrant: the total extent of dentin (Fig. 2a) and the largest extent of dye penetration (Fig. 2b). The percentage of dye penetration in each quadrant was calculated by the dentin's thickness on the same quadrant. Then, the percentage of dye penetration and the mean value for each section were calculated. Final percentage of copper ion infiltration in each tooth resulted from the average of the three cuts. Then, the final average of dye penetration in each group was calculated from the means obtained in each tooth.

Data obtained were submitted to one-way ANOVA using a factorial design with material as independent

Table 1. Final average (X) and standard deviation (SD) for the experimental groups

	Groups					
	I	II	III	IV	V	VI
$X \pm$ SD	2.41 $\pm$1.45	5.22 $\pm$ 1.79	8.3 $\pm$ 2.55	3.73 $\pm$ 0.89	14.83 $\pm$ 4.99	10.51 $\pm$2.65

variable. Means were compared to Tukey interval calculated at the 0.01 % significance level.

Results

The results reached in the experimental groups are shown on Table 1. Statistical analysis of data showed that, for normal curve adherence test of logarithm-transposed data, the Ho (Null Hypothesis) probability was 23.76% for normal sample distribution for 4 df. Table 2 exhibits the average of the variation factors technique and irrigants.

Discussion

During the procedures for internal bleaching of endodontically treated teeth, efforts should be focused on selecting materials and techniques that effectively re-establish the natural color of the dental element without causing any damage to tooth substrate and/or surrounding tissues and structures.

The use of bleaching agents must be carefully monitored as they may induce morphological alterations, mainly in the chemical structure of dentin and cementum, making these substrates prone to degradation (10, 30), and also in the microhardness of dentin and enamel (4, 5).

The mixture of sodium perborate with water has been advised for preventing (or at least minimizing) the risk of external root reabsorptions that may occur when oxygen peroxide is employed instead of water (31), and also for producing less alteration in dentinal microhardness (5). The use of acid etching prior to the application of a bleaching agent (11) coupled with the removal of smear layer (13) are procedures that aim to increase dentinal permeability and therefore to enhance the effectiveness of bleaching agents. Although the thermo-catalytic techniques increase dentine permeability considerably (9), they are contraindicated for their deleterious effects on dentinal structures (32, 33).

Table 2. Variation factor (technique and irrigants)

	Variation factor
Technique	
No ultrasonic	5.32*
Ultrasonic	9.69†
Irrigants	
Water	3.07‡
Sodium hypochlorite	9.42§
EDTA	10.03¶

Different symbols indicate statistically different results.

The association of ultrasonic activation with different irrigating solutions is reported to provide good results as regards to the removal of smear layer from root canals (16–18, 21–25), as well as the increase of dentinal permeability (19, 20).

The ultrasonic activation of 1 % sodium hypochlorite in pulp chamber, prior to the application of sodium perborate plus 3% hydrogen peroxide for dental bleaching, was claimed to provide good results in clinical practice (26). The use of 0.5% sodium hypochlorite and Endo-PTC cream activated by an ultrasound device in the pulp chamber also resulted in alteration of dentinal permeability (27).

The findings of the reported research disclosed that ultrasonic activation increased dentinal permeability, regardless of the irrigating solution evaluated. In the non-ultrasonicated groups, sodium hypochlorite provided higher increase of dentinal permeability than 17% EDTA and distilled water. On the other hand, ultrasonic-activated 17% EDTA provided a significant increase of dentinal permeability, as compared to the other evaluated solutions.

The use of ultrasonicated endodontic irrigating solutions inside the pulp chamber prior to the accomplishment of dental bleaching procedures aims to improve the cleansing of the coronary dentinal walls by removing the smear layer, thereby allowing a better performance of the whitening agent. In the conducted study, sodium hypochlorite was equally used for all groups during the root canal instrumentation, with the main purpose of simulating clinical conditions. Therefore, it did not influence the outcomes.

The ultrasonic activation into the root canal for 30 s can raise the external root temperature to 32–37°C when a continuous flow of irrigant is used, while a temperature of 40°C is reached with an intermittent irrigant flow (34). During the *in vitro* ultrasonication of the irrigant inside the pulp chamber for 15 s, no significant change on the external crown temperature was observed. However, further studies have yet to investigate such condition.

The ultrasonic activation of endodontic irrigating solutions in the pulp chamber prior to the application of the bleaching agents provides optimal cleansing and increases dentinal permeability, thereby allowing the use of whitening agents that are less toxic and less aggressive to dental and periodontal tissues, with promising results in the re-establishment of the esthetics of endodontically treated teeth with chromatic alterations.

On the basis of these results, and within the limitations of the employed methodology, it may be concluded that the use of ultrasonic-activated irrigating solutions – 17% EDTA, 1% sodium hypochlorite, and distilled water – in the pulp chamber produced a remarkable increase in dentinal permeability. Among the irrigating regimen proposed in this study, the ultrasound-activated 17% EDTA promoted greater increase of dentinal permeability, as compared to 1% sodium hypochlorite and distilled water. The increase of dentinal permeability provided by the ultrasonic activation of irrigants inside the pulp chamber may allow the choice for whitening agents less aggressive to dental tissues when dental bleaching is performed.

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