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Effect of internal bleaching agents on dentinal permeability of non-vital teeth: quantitative assessment

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Abstract – The aim of this *in vitro* study was to assess quantitatively dentin permeability of pulpless teeth after intracoronal bleaching therapy with three different agents. Twenty-four maxillary central incisors were randomly assigned to four groups according to the bleaching agent used: I – non-bleached control; II – 37% carbamide peroxide; III – sodium perborate/20% hydrogen peroxide paste; IV – 27% carbamide peroxide. After standard access and root-canal preparation the access opening, biomechanical preparation and root-canal filling, a cervical glass ionomer plug was prepared and intracoronal bleaching procedures were carried out in a standardized fashion. The access cavities were opened and the teeth were externally sealed and immersed in a 10% copper sulfate aqueous solution for 30 min, in vacuum for the first 5 min. Then, samples were removed, dried with absorbing paper and immersed in a 1% rubianic acid alcohol solution, for the same above-mentioned period in solution and in vacuum. Copper ion penetration was indicated by the rubianic acid staining. Mean values and SD for the experimental groups were: I – 7.88% (±1.33), II – 16.94% (±5.72); III – 11.45% (±3.90) and IV – 8.98% (±4.19). Data were submitted to one-way ANOVA. The results showed that the 37% carbamide peroxide provided the highest increase in dentin permeability, followed by sodium perborate with 20% hydrogen peroxide. The 27% carbamide peroxide provided the lowest results and showed statistical similarity to the control group. On basis of these findings, it may be concluded that, among the tested intracoronal bleaching agents, 37% carbamide peroxide presented an optimized overall performance in increasing dentinal permeability.

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Key words: dental internal bleaching; dentin permeability; bleaching agents

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Materials used for bleaching pulpless teeth are evaluated for the speed and efficacy in re-establishing the natural color of the teeth and their potential (or lack thereof) of causing damage to surrounding structures.

One of the most important properties of a bleaching material is its ability to allow penetration of the bleaching agent through dentinal permeability (1, 2). The deeper the penetration, the more pigment that causes chromatic alteration of the dental tissues can be reversed by the oxidation reaction.

Several procedures have been reported to increase dentinal permeability, including phosphoric acid etching of tooth structure before placing bleaching agents (3), smear layer removal (4) and heat application (1, 5, 6). However, thermocatalytic bleaching techniques have recently been questioned due to the

deleterious effects that may be produced on dentinal structures (7, 8).

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 (9). The water-based sodium perborate paste has been reported to have less potential to harm dental tissues (10–12). Different types of sodium perborate (mono-, tri- or tetra-hydrated) may be used with similar results (10, 13).

The 10% carbamide peroxide gel has been used for internal bleaching of non-vital teeth simultaneously with external bleaching (14, 15). The 10% carbamide peroxide has provided similar performance to sodium perborate for internal bleaching (16).

Reports regarding the effect of these gels on vital teeth (17), enamel (18), cementum (19) and pulp tissue (20–22) already exist. However, little information is available focusing on the action of carbamide peroxide gels on dentinal permeability of pulpless teeth. Therefore, the aim of this *in vitro* investigation was to assess the effect of different internal bleaching agents on dentinal permeability of non-vital anterior teeth.

Materials and methods

Maxillary central incisors extracted within a 6-month period and stored in 0.4% sodium azide solution at 4°C, were scaled with ultrasonic scaler to remove calculus and remnants of periodontal ligament, polished with water/pumice slurry in dental prophylactic cups, thoroughly rinsed and dried. After careful visual inspection and tactile examination using the tip of an explorer, under a ×10 stereoscopic magnifying lens (Carl Zeiss-Jena), 24 sound teeth with no sign of cracks or structural anomalies were selected. Specimens were randomly assigned to four experimental groups of equal size ($n = 6$), according to the bleaching substance used: I – control, in which a dry, small cotton pellet was placed in the pulp chamber, instead of bleaching agent; II – 37% carbamide peroxide gel (Whiteness Super-Endo; FGM Prod. Odontológicos, Joinvile, Brazil); III – sodium perborate paste (Merck KGaA, Darmstadt, Germany) prepared with 20% hydrogen peroxide (Beira Alta Ind. e Com. de Prod. Químicos Ltda, Guarulhos, Brazil) and IV – 27% carbamide peroxide gel (prepared at Ribeirão Preto School of Pharmaceutical Sciences, USP, Ribeirão Preto, Brazil).

Conventional lingual access openings were prepared, pulp tissue was extirpated and working length was determined at 1 mm from apical foramen. 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 canal 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 were removed from root canal at the cervical third using a heated endodontic condenser. A cervical plug of glass ionomer cement (Vitremer, 3M Dental Products, St. Paul, USA) was placed 1 mm inside the pulp chamber and 2 mm below the amelocemental junction into root canal, with the specific role of protecting apical and periodontal tissues from contact with bleaching substances. The whitening agents were placed into coronal pulp chamber, except for control group, covered with a thin layer of cotton and the lingual access was temporarily sealed with Vitremer glass ionomer cement.

Specimens were submitted to three changes of bleaching agents, with a 72-h interval between each one. In the meantime, teeth were kept in artificial saliva at 37°C and 100% relative humidity until the next 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 (23).

The temporary glass-ionomer restorations were removed and access cavities were entirely cleaned and rinsed. 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 fore-mentioned technique: the first 5 min in vacuum and, after vacuum removal, teeth were kept for additional 25 min in the solution (24). Copper ions were revealed by the rubianic acid, resulting in 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 water-cooled 300-μm thick diamond saw (Minitom, Struers A/S, Copenhagen, Denmark). Roots were removed at the cemento-enamel junction and crowns were embedded in chemically activated acrylic resin blocks. Teeth were serially sectioned in a mesiodistal direction, thus providing three transverse cuts 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

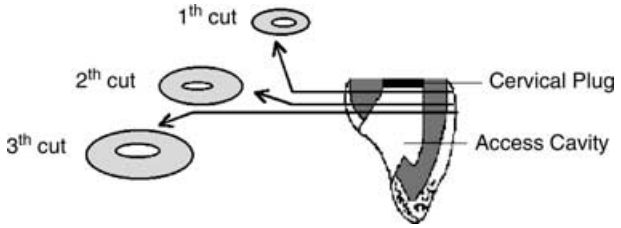

Fig. 1. Schematic representation of specimen sectioning. Lateral view.

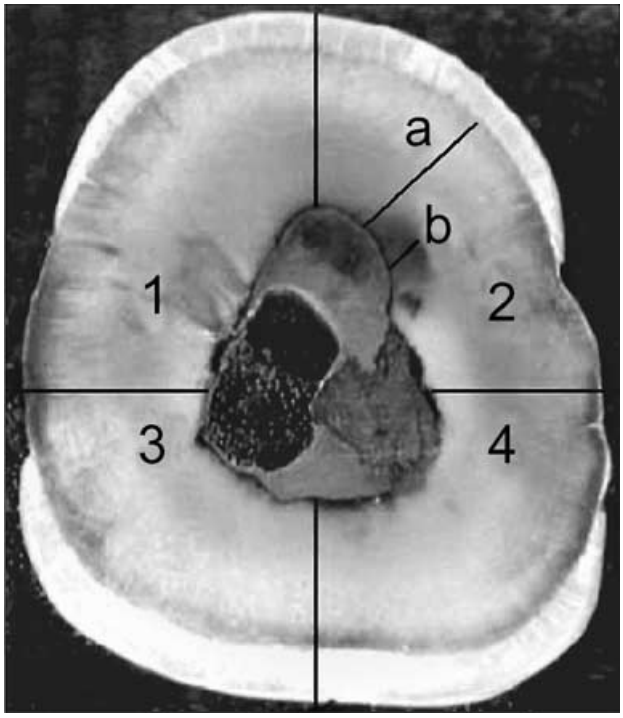

Fig. 2. Transverse section with schematic drawing illustrating quadrants (1–4) and the quantitative measureaments: (a) the total extent of dentin in the quadrant; (b) the largest extent of dye penetration in the quadrant.

grounded under water refrigeration using #400- to #600-grit silicon carbide paper to obtain a flattened, smooth surface and a final thickness of approximately 100 μm.

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 were analyzed using the *KS300-v2.0* software (Kontron Elektronik, GmbH, Eching bei München, Germany).

Each cut was divided into four areas of similar size (Fig. 2) and two quantitative measurements (in mm) were obtained in each quadrant: the total extent of dentin (Fig. 2a) and the largest extent of dye penetration (Fig. 2b). Then, the percentage of dye penetration and the mean value for each section were calculated. Final percentage of copper ion penetration in each tooth resulted from the mean of the three cuts. Data obtained were submitted to one-way ANOVA using a factorial design with material (bleaching agent) as independent variable. Means were compared to Tukey interval calculated at the 0.01 significance level.

Results

The results for the experimental groups are illustrated in Table 1. Statistical analysis of data showed significant difference ($P < 0.01$) among the studied bleaching agents.

It was noticed that all tested substances produced an increase of dentinal permeability as compared to the non-bleached control group. The best performance in increasing dentin permeability was provided by the 37% carbamide peroxide, followed by the sodium perborate/20% hydrogen peroxide paste. The 27% carbamide peroxide gel presented the lowest values and was not statistically different from the control group.

Discussion

Bleaching action is ascribed to the oxireduction reaction between the whitening agent and the darkened substrate. This reaction modifies the dyed molecule and alters some of its characteristics, including color. However, the success of bleaching therapy is directly related to the ability of the whitening substance to penetrate deep into dentinal tubules and reach the discolored molecules (1, 25). This is an important property of a bleaching agent in its ability to effect the dentinal permeability (1).

In the reported investigation, the penetration of copper ions into dentinal tubules clearly indicated

Table 1. Mean values (%) and standard deviation of dentinal permeability after intracoronal bleaching therapy

	Cotton pellet (control)	37% carbamide peroxide	Sodium perborate + hydrogen peroxide	27% carbamide peroxide
Copper ions penetration	7.88% (±1.33) b	16.94% (±5.72) a	11.45% (±3.90) c	8.98% (±4.19) b

Same letters (a–c) indicate statistical similarity.

that dentin permeability was altered when tooth structure was subjected to the action of bleaching agents. The greatest penetration was noticed with the use of 37% carbamide peroxide gel, followed by sodium perborate/hydrogen peroxide paste. In a recent clinical trial, Dutra et al. (26) also observed that the 37% carbamide peroxide gel (Whiteness Super-Endo) was effective in tooth bleaching, providing, within five sessions on the average, satisfactory color alteration with no reported adverse effects or toxic reactions.

The findings of our investigation showed that the tested agents increased dentinal permeability as compared to the non-bleached control group, thus illustrating their ability in penetrating dentinal canalicules and reaching the discolored molecules. The 37 and 27% carbamide peroxide gels showed different performance; apparently with higher concentration, improved dentinal permeability resulted.

The internal and external use of 10% carbamide peroxide for bleaching therapy of non-vital teeth has shown optimized results in clinical trials (14, 15). The findings of the reported study demonstrated that carbamide peroxide in lower concentration (27%) did not provide significant alteration of dentinal permeability when placed into pulp chamber for internal bleaching of endodontically treated teeth.

Sodium perborate combined with hydrogen peroxide in concentrations ranging from 3 to 35% has been reported to produce improved results in tooth bleaching (9, 27, 28). Although similar performances have been demonstrated with the use of either distilled water or hydrogen peroxide to prepare sodium perborate paste (12, 29), the water-based paste has shown less alteration in dentinal microhardness (30) and better effectiveness in preventing the occurrence of external resorption (11, 13). Conversely, the use of hydrogen peroxide in higher concentrations (30 and 35%) associated to sodium perborate resulted in a decrease in dentinal and enamel microhardness (18, 31, 32) and may lead to alterations of chemical structure of dentine and cementum (2).

In the present study sodium perborate paste was prepared with the addition of 20% hydrogen peroxide, which produced an intermediate increase of dentinal permeability between those provided by 37 and 27% carbamide peroxide gels.

Although the findings of the reported investigation showed that 37% carbamide peroxide led to a significant increase in dentinal permeability, one should not overlook the fact that increased penetration into the dentinal tubules may lead to increased external root resorption bleaching agents with great ability in penetrating into dentinal tubules are also more susceptible to produce external resorption (6, 19, 24, 33).

On the basis of these results, and within the limitations of an *in vitro* study, it may be concluded that the 37% carbamide peroxide provided the highest increase in dentinal permeability and the 27% carbamide peroxide produced the lowest results in altering dentinal permeability. The sodium perborate associated with 20% hydrogen peroxide was less effective than the 37% carbamide peroxide, but provided better results than the 27% carbamide peroxide.

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