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Quantification of Peroxide Ion Passage in Dentin, Enamel, and Cementum After Internal Bleaching With Hydrogen Peroxide

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Clinical Relevance

It is not possible to extrapolate the results directly to the clinical setting; however, hydrogen peroxide when placed in the pulp chamber passes through dental hard tissues and reaches the external surface and periodontal tissues.

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SUMMARY

The aim of this study was to evaluate the amount of peroxide passage from the pulp chamber to the external enamel surface during the internal bleaching technique. Fifty bovine teeth were sectioned transversally 5 mm below the cemento-enamel junction (CEJ), and the remaining part of the root was sealed with a 2-mm layer of glass ionomer cement. The external surface of the samples was coated with nail varnish, with the exception of standardized circular areas (6-mm diameter) located on the enamel, exposed dentin, or cementum surface of the tooth. The teeth were divided into three

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experimental groups according to exposed areas close to the CEJ and into two control groups (n=10/group), as follows: GE, enamel exposure area; GC, cementum exposed area; GD, dentin exposed area; Negative control, no presence of internal bleaching agent and uncoated surface; and Positive control, pulp chamber filled with bleaching agent and external surface totally coated with nail varnish. The pulp chamber was filled with 35% hydrogen peroxide (Opalescence Endo, Ultradent). Each sample was placed inside of individual flasks with 1000 μL of acetate buffer solution, 2 M (pH 4.5). After seven days, the buffer solution was transferred to a glass tube, in which 100 μL of leuco-crystal violet and 50 μL of horseradish peroxidase were added, producing a blue solution. The optical density of the blue solution was determined by spectrophotometer and converted into microgram equivalents of hydrogen peroxide. Data were submitted to Kruskal-Wallis and Dunn-Bonferroni tests (α=0.05). All experimental groups presented passage of peroxide to the external surface that was statistically different from that observed in the control groups. It was verified that the passage of peroxide was higher in GD than in GE ($p<0.01$). The GC group presented a significantly lower peroxide passage than did GD and GE ($p<0.01$). It can be concluded that the hydrogen peroxide placed into the pulp chamber passed through the dental hard tissues, reaching the external surface and the periodontal tissue. The cementum surface was less permeable than were the dentin and enamel surfaces.

INTRODUCTION

The use of tooth whitening techniques has become a common treatment modality aimed at achieving esthetically desirable appearance. This procedure preserves tooth structure and avoids invasive restorative interventions to correct color anomalies.[1]

In order for intrinsic stains to be removed by the action of bleaching agents, the oxidizing ions must penetrate enamel and dentin,[2–5] leading to an oxidation reaction. Hydrogen peroxide (HP) is a strong oxidizing agent that can act on dentin, modifying its mechanical and chemical properties. The bleaching mechanism of HP has not yet been fully established, and some controversy exists.[6,7]

The HP dissociates into free radicals with unpaired electrons and is being reduced by releasing these electrons, while as a consequence the substances that accept the electrons are being bleached by oxidation. This results in a molecular rupture and in a change in the energy absorption at a molecular level within the pigmented dental organic matrix, forming simpler and smaller molecules that reflect light in a different way, creating a successful bleaching effect.[1,8,9]

The permeability of teeth increases after penetration of oxidative ions,[3,4,7] allowing the passage of these ions to the external root surface and consequently to the periodontal ligament. Another potential consequence of the bleaching action can be the development of cervical root resorption. This can occur as a result of the fact that HP and other oxygen radicals penetrate the periodontal membrane, causing tissue and cellular destruction.[4,10] Nevertheless, use of 30% HP and sodium perborate (SP) as bleaching agents[3,11,12] has been extensively practiced by those in the dental profession.

In order to quantify the peroxide ions that are released by a bleaching agent from the pulp chamber through exposed dentin, dentin that is covered by enamel or by cementum during internal bleaching procedures, this study tested the following null hypothesis: (H0) the bleaching agent will not pass through the dental hard tissues.

MATERIALS AND METHODS

This project was developed in accordance with the Research Ethics Code (approved under No. 002451/2008-PH/CEP). Fifty freshly extracted bovine lateral incisors from early calves of approximately the same age were cleaned, immersed in physiological saline, and kept in a freezer (−18°C) until use. Access openings were prepared on the lingual surface with a high-speed hand piece and diamond bur under copious water-cooling. Pulp tissue was extirpated and the pulp chamber irrigated with saline.

The roots were sectioned perpendicular to the long axis, 5 mm below the cemento-enamel junction (CEJ), using a carbide disc in a low-speed hand piece. A glass ionomer cement (Vidrion R, SS White, Rio de Janeiro, Brazil) plug was placed 2 mm apical from the CEJ, serving as a barrier to the root canal. The root below the glass ionomer barrier was etched with 35% phosphoric acid gel, rinsed, and sealed with a light-curing bonding agent (Single Bond Adhesive, 3M ESPE, Minneapolis, MN, USA) and a light-curing resin composite (TPH, Dentsply/Caulk Div., Milford, DE, USA).

Passage of HP was evaluated by application to dimensionally standardized areas on the external

surface of the tooth. The external surface of the samples was coated with nail varnish, with the exception of standardized circular areas (6-mm diameter) located on the enamel, dentin-exposed, or cementum surface of the tooth. The circular areas of exposed dentin with 6-mm width and 2-mm depth were created using cylindrical diamond burs (No. 2094, KG Sorensen Ind. Ltda, Barueri, SP, Brazil).

In order to make the specimens waterproof, two layers of transparent nail varnish were applied. After removal of the labels dimensionally standardized areas were available for measurements.

The teeth were divided into three experimental groups according to exposed areas close to the CEJ and into two control groups (n=10/group), as follows: GE, enamel exposure area; GC, cementum exposed area; GD, dentin exposed area; Negative control, no presence of internal bleaching agent and uncoated surface; and Positive control, pulp chamber filled with bleaching agent and external surface totally coated with nail varnish.

The pulp chamber was filled with 35% HP (Opalescence Endo, Ultradent Products Inc, South Jordan, UT, USA), followed by a resin composite filling (TPH, Dentsply/Caulk Div.). After light-curing the remainder of the access opening was sealed after etching with 37% phosphoric acid (Alpha Etch gel, DFL, Rio de Janeiro, Brazil) for 15 seconds, rinsing, lightly air-drying, and application of a bonding agent (Single Bond Adhesive, 3M ESPE). After light-curing for 20 seconds (780 mW/mm^2), a resin composite (TPH, Dentsply/Caulk Div.) was placed and light-cured for 20 seconds.

The specimens were placed in a reservoir containing 1000 μL of acetate buffer solution, 2M (pH=4.5), making sure that the exposed areas were submerged. The acetate buffer was necessary to stabilize the HP that might pass from the pulp chamber to the external surface. Specimens were then stored in an incubator at 37°C at 100% relative humidity. After seven days, the acetate buffer solution was removed from the reservoirs using micropipettes and transferred to a glass tube. The reservoirs were rinsed twice with deionized water. One hundred microliters of 0.5 mg/mL leuco-crystal violet (Sigma Chemical Co, Sigma-Aldrich, São Paulo, Brazil) and 50 μL of 1 mg/mL enzyme horseradish peroxidase (Sigma Chemical Co, Sigma-Aldrich) were also added to each tube, and the solution was diluted to 3 mL with distilled water. The optical density of the resulting blue color in the tubes was measured with a spectrophotometer (UV Spectrophotometer, UV-1203, Shimadzu, Kyoto, Japan) at a wavelength of 596 nm.

Table 1: *Amount of Peroxide (μg/mL) That Penetrated From the Pulp Chamber to the External Root Surface During Internal Bleaching (SD = Standard Deviation)*

Groups	Mean, μg/mL (±SD)
Group enamel	0.957 (1.366±0.766)
Group cementum	0.886 (1.630±0.582)
Group dentin	0.965 (2.234±0.758)
Negative control	0.033 (0.099±0.000)
Positive control	0.024 (0.043±0.003)

A standard curve, in which eight points of HP were associated at intervals of 0.25 μg/mL until 2.0 μg/mL, according to their corresponding absorbance, was used to convert the optical density values obtained from the samples into microgram equivalents of HP. The results were statistically analyzed using Kruskal-Wallis and Dunn-Bonferroni tests ($p<0.05$).

RESULTS

The amount of peroxide (μg/mL; mean ± SD) that penetrated from the pulp chamber through the tooth structure is shown in Table 1. All experimental groups presented passage of peroxide bleaching agent to the root surface and were statistically different from the negative and positive control groups ($p<0.05$). It was verified that the ion penetration was higher in GD (0.965 μg/mL; 2.234 ± 0.758), followed by GE (0.957 μg/mL; 1.366 ± 0.766). GC demonstrated passage of peroxide that was lower, at 0.886 μg/mL (1.630 ± 0.582), than that of GD and GE ($p<0.05$).

The data were submitted to the Dunn-Bonferroni statistical test and it was verified that control and experimental groups showed statistically significant differences ($p<0.05$). The Kruskal-Wallis test showed no statistically significant difference among the experimental groups ($p>0.05$).

DISCUSSION

External and internal bleaching agents are able to penetrate into dentin.[12–14] These bleaching agents reduce the calcium and phosphate ions of the dental

hard tissues, promoting a higher permeability in these tissues. Carrasco and others[13] showed that different bleaching agents can increase the permeability of the coronal dentin of endodontically treated teeth.

The passage of HP occurs mainly as a result of its low molecular weight and ability to denature proteins, which increases the ion movement through the enamel, dentin, and cementum. Dental hard tissues present an organic content; the HP penetrates through these structures, promoting increased porosity and loss of substances of the protein matrix as a result of free radical oxidation.

The present study verified that the penetration of the peroxide from the pulp chamber to the external root surface occurred in all experimental groups. This was demonstrated by measuring ions from the bleaching agents passing through dentin, enamel, and cementum. These results are not in accordance with those of Kehoe,[15] who reported that cementum presented a barrier for ion passage.

The amount of bleaching agent that passages through the tooth structure is influenced by enamel, dentin, and cementum thickness. It is known that the higher the enamel, dentin, or cementum thickness, the less will be the passage of bleaching agents. In our study we observed a lower passage of HP in the cementum that can be correlated with the different thicknesses of the dental hard tissues.

Fuss and others[16] evaluated the diffusion of a mixture of SP plus HP and calcium hydroxide. They determined that the whitening materials, in contrast to calcium hydroxide, offered an easier and faster diffusion through dentin. This faster diffusion is probably due to the small size molecules of the bleaching agents.[5,16,17] Additionally, the cervical region is more permeable compared to other areas of the root,[18] even when the bleaching agent is sealed within the pulp chamber. Pressure builds up inside the pulp chamber, because of the oxygen liberation caused by HP and SP decomposition. This pressure forces the bleaching agents into the dentinal tubules.[7,19] HP passage as well as other oxygen radicals are not desirable and must be minimized. When in contact with tissues, these ions may cause cellular and tissue destruction.[10]

In the present study, it is of interest to note that oxidative ions were able to pass through exposed dentin, dentin covered by enamel, and dentin covered by cementum. However, it is known that this junction may exhibit flaws that expose the dentin to the periodontal tissue in this area. According to Neuvald and Consolaro,[20] this is the case for almost 10% of teeth. Under these conditions, ions coming from the bleaching agents can reach these tissues easily and in high quantities, thus initiating an inflammatory reaction.

A significant aspect that must be taken into consideration is that dentin autoimmunity presents specific proteins, which often are not accessible to the human immunologic recognition cells. Despite its organization, the direct exposure of noncollagen proteins to the bone maintains the dentin proteins incorporated in a mineralized matrix and acts as a sequestrated antigen.[12]

Nevertheless, with exposure, in the case of an inflammatory process at the cervical region, the cells are not recognized as their own by the human immune system. A specific response will start, represented by cellular intent on eliminating antigens, in which macrophages will act as the main executors. However, if the CEJ presents an irregular anatomical form, the initiator mechanism of resorptive processes requires the presence of local factors, such as cytokine liberation that activate clastic cells Other factors can cause external cervical resorptions, such as bleaching agents, trauma, and induced dental movement.[21]

In the present study, we used bovine teeth as they presented similar size and age, promoting a better standardization of the specimens. The diameter of dentin tubules in bovine teeth is smaller and the intertubular dentin area is larger than in human teeth.[22] On the other hand, the human tooth and its structural and morphologic characteristics can influence the passage of bleaching agents. A previous study[22] showed that human teeth are more permeable to bleaching agents than bovine teeth.

Based on the results of this study, the null hypothesis (H0) was rejected. Thus, this study demonstrated that the oxidative power of the HP was able to pass through all hard dental tissues. It could be concluded that when placed into the pulp chamber, the HP passes through dental hard tissues, reaching the external surface and periodontal tissues.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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