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Effect of Adhesive Restoration and Bleaching Technique on the Concentration of Hydrogen Peroxide In the Pulp Chamber

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Clinical Relevance

Hydrogen peroxide is able to diffuse into the pulp chamber even in the absence of enamel restorations. Silorane adhesive restorations allow a higher amount of hydrogen peroxide into the pulp chamber, compared to non-restored teeth.

SUMMARY

This study aimed to quantify the concentration of hydrogen peroxide into the pulp chamber in the presence or absence of adhesive enamel restorations and to analyze the resin-dentin interface of bleached groups. Bovine incisors (120) were randomly divided into three groups according to enamel treatment (n=40 each): (1) enamel without restoration (control); (2) enamel cavities (3 mm diameter × 1.5 mm depth) restored with a silorane-based (SB) system; or (3) enamel cavities (3 mm diameter × 1.5 mm depth) restored with a dimethacrylate-based (DB) system. Restorations were thermocycled, and all groups were submitted to one application of 35% hydrogen peroxide (HP) agent for 45 minutes and subjected to four light activation methods (n=10 each): without light, light-emitting diode (LED), LED/diode laser, or halogen light. Acetate buffer solution was placed into the pulp chamber before bleaching, and this solution was collected to spectrophotometrically determine the concentration of HP that reached the pulp chamber after bleaching. Rhodamine B was added to the HP

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agent and applied on additional enamel samples of each group for 24 hours. Samples were sectioned mesiodistally, and the bleaching agent was traced using confocal microscopy. According to two-way analysis of variance and Tukey test (α=0.05), the HP concentration in the pulp chamber of the control group was significantly lower than that of the SB group ($p<0.05$), regardless of light activation. No differences were observed between DB and SB groups and between control and DB groups, except for the DB halogen light activated group, which exhibited higher HP intrapulpal concentration ($p<0.05$). Confocal microscopy exhibited HP diffusion through the interface of the SB and DB restored groups as well as enamel prisms in the control group. The SB restorative system increased the HP diffusion into the pulp chamber, but HP was able to diffuse even in the absence of enamel restorations.

INTRODUCTION

Vital bleaching is believed to be a safe procedure; however, in the past few years the adverse effects of hydrogen peroxide (HP) have been revealed, particularly on the enamel surfaces. Investigations have disclosed changes on enamel morphology, an increase of enamel roughness and porosity,[1-3] and a decrease of enamel microhardness,[4,5] enamel cohesive strength,[6] and bond strength of adhesive restorations to bleached enamel.[7] Although the findings can be conflicting,[8] a decrease of enamel mineral content has also been observed; however, it could be controlled by incorporating specific concentrations of calcium and fluoride in bleaching agents.[5,9]

The mechanism of tooth bleaching is not completely elucidated; however, it is assumed that HP, the active element of bleaching agents, can form a number of different active oxygen species depending on the reaction conditions (temperature, pH, light, and presence of transition metals). Free radicals, such as hydroxyl, attack organic compounds (chromophores) that possess extended conjugated chains of alternating single or double bonds. Bleaching of the chromophore can occur by cleavage or oxidation of the conjugated chain.[10]

The oxidative mechanism of HP and the release of free radicals, which are unstable and able to react with the organic molecules, could be associated with the enamel morphologic changes.[10] In addition, the low molecular weight and the ability to diffuse into the pulp chamber brings concerns over the possible effects of HP in the pulp tissues.[11] Some studies indicate that even low HP concentrations easily diffuse through enamel and dentin, reaching the pulp chamber[12-14] and leading to pulp cell toxicity.[12-14] It has also been observed that the cytotoxicity is dose dependent on the concentration of the HP that reaches the pulp chamber[7,14,15] and that high HP concentrations could cause more damage to pulp cells than low HP concentrations.[16,17]

Based on the thermocatalytic theory, high-concentration in-office bleaching agents can be associated with different light sources (light-emitting diode [LED], LED/diode laser, halogen light) in order to increase the efficiency and hasten the decomposition of HP.[18] The efficiency of these lights on the bleaching agents is still debatable, although it was demonstrated that LED or laser applied to 35% HP increases the final concentration of HP in the pulp chamber.[19]

Trans-enamel-dentin penetration can be intensified by dentin exposure as a result of gingival recession, abrasion, abfraction, and erosion lesions, or by the presence of adhesive restorations.[13] Previous studies confirmed that HP diffusion is dependent on the quality of the enamel surface and that the HP intra-pulp concentration is higher in the presence of adhesive restorations[20] and dependent on the restorative material used.[15]

Adhesive systems with different formulations have been proposed to increase the longevity of composite restorations.[21] The main goal of these systems is to decrease the clinical application steps and the errors inherent in the application technique and to increase the adhesive stability within the hybrid layer.[22] Low-shrinkage composite resins share the same philosophy, as the intention is to decrease the marginal failures. Silorane-based composites are classified as a low-shrinkage material with polymerization shrinkage close to 1% by volume, while traditional bisphenol A glycidyl methacrylate (bis-GMA)–based composites present 2% to 5% of volumetric polymerization shrinkage.[21,23] The silorane monomer is obtained by the reaction of oxirane and siloxane hydrophobic monomers, in which the oxirane and siloxane open up during polymerization to bond to other monomers. Polymerization of the silorane is promoted by the opening of the oxirane rings that cause volumetric expansion, which tends to compensate for polymerization shrinkage.[24] But even the possible advantages of this system may not prevent HP diffusion to

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the pulp chamber when a bleaching agent is applied to restored teeth.

Because more information is necessary to understand the adverse effects of HP penetration into the pulp chamber, the aim of this study was to evaluate the intra-pulp concentration of light-activated HP applied on enamel restored with different restorative systems. The null hypothesis tested was that there is no difference in the intra-pulp concentration of HP, light-activated or not, regardless of the HP application on enamel, with or without adhesive restorations.

METHODS AND MATERIALS

Experimental Design

The experimental units (120 bovine incisors) were submitted to the factors under study (n=10):

1. *Enamel treatment* (three levels): control (without enamel restoration), silorane-based (SB) restoration (Silorane System Adhesive/Filtek Silorane P90, 3M ESPE, St Paul, MN, USA), and dimethacrylate-based (DB) restoration (Scotchbond Universal/Filtek Supreme, 3M ESPE).
2. *Light-activation methods* (four levels): control (without light-activation), LED, LED/diode laser, and halogen quartz-tungsten light.

Samples were treated with a high-concentration bleaching agent (HP – 35% hydrogen peroxide, Whiteness HP Maxx, FGM, Dental Products, Joinville, Brazil). The optical density of the solution was determined spectrophotometrically and converted into micrograms equivalent to the HP. The adhesive interface was observed by confocal laser scanning microscopy.

Sample Preparation, Group Division, and Bleaching Treatment

One hundred twenty extracted bovine incisors with standardized crown dimensions were selected. After cleaning, the teeth were stored in a 0.1% thymol solution at 4°C for 30 days. In order to select those without surface defects, all teeth were examined under a stereomicroscope. The roots were cut with diamond discs (KG Sorensen, Barueri, Brazil) up to 2 mm below the cementoenamel junction and then the roots were discarded. The pulp tissue was removed using files (Hedstrom files, Maillefer Dentsply, Ballaigues, Switzerland) and then the pulp chamber was washed with distilled water. The cervical pulp orifice was widened with a round bur (No. 1016 HL, KG Sorensen) to allow for the placement of the acetate buffer solution into the pulp chamber. The total thickness (enamel and dentin) of the buccal side was measured with a caliper (Golgran, São Paulo, Brazil) and standardized to 3.5 mm. Teeth were randomly assigned to the experimental groups described above.

Enamel cavities (3 mm in diameter and 1.5 mm deep) were prepared in the buccal surface of the crowns with diamond burs (No. 3053 and No. 3017, KG Sorensen) in a standard cavity device. The adhesive systems and composites were applied according to the manufacturer's instructions (Table 1) and light-activated for 20 seconds (Valo LED, Ultradent Products Inc, South Jordan, UT, USA) with an irradiance of 800 mW/cm^2. Restorations were polished with a sequence of four sandpaper discs (Sof-Lex, 3M ESPE): coarse, 100 μm; medium, 29 μm; fine, 14 μm; and superfine, 5 μm. Each was used for 15 seconds in a single direction. At each disc exchange, the composite surface was washed and air-dried for 5 seconds; polishing discs were discarded after a single use.

Thermal Cycling

To age the bonded interface, samples of all groups were submitted to 5000 thermal cycles (MCT2 – AMM, São Paulo, Brazil) in deionized water baths at 5° to 55°C ± 1°C. For both the control and restored groups, 24 hours after the thermal-cycling procedure two coats of nail varnish (Revlon Inc, New York, NY, USA) were applied up to 2 mm around the bonded interface, leaving a standard exposed enamel area of 16.6 mm^2 for application of the bleaching agents. Teeth were fixed vertically in individual vials with the pulp chamber opening in the upper position to allow access to the acetate buffer inserted within the chamber.

Bleaching Procedure

The teeth, with or without restorations, were submitted to a single 45-minute bleaching application of a 35% HP agent (Whiteness HP Maxx, FGM; Table 1) and subjected to one of four light-activation methods (n = 10 each):

1. Enamel without restoration and bleaching without light activation
2. Enamel without restoration and bleaching combined with LED light
3. Enamel without restoration and bleaching combined with diode laser light
4. Enamel without restoration and bleaching combined with halogen light

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Table 1: *Commercial Name and Manufacturer for the Materials Used, Composition, and Manufacturer Directions*[a]

Commercial Names and Manufacturers	Composition	Manufacturer Directions
Bleaching agent		
Whiteness HP Maxx *(FGM Dental products, Joinville, SC, Brazil)*	35% HP (after mixture), thickener, pigment, neutralizing agents, glycol, distilled water	After mixture, the agent is applied on the surface for 15 minutes and stirred 3-4 times to promote oxygen release; repeat twice. Light sources can be used to accelerate bleaching.
Dental Restoratives		
Filtek Z350 XT (3M/ESPE, St Paul, MN, USA)	Bis-GMA, UDMA, TEGDMA, Bis-EMA particles of silica and zircônia/silane, BHT, photoiniciator system, and pigments.	Composite increments of 2 mm
Filtek Low Shrinkage Posterior P90 - Silorane (3M/ESPE)	Particles of quartz and silica/silane, yttrium fluoride, 3,4-epoxy cyclohexylethylcyclopolymethylsiloxane, bis-3,4-poxycyclohexylethyl phenylmethylsilane,initiator system: camphorquinone and iodonium salt (donator of electrons), stabilizers, and pigments	Composite increments of 2 mm
Silorane Adhesive system – Self-etch primer (3M/ESPE)	Phosphate methacrylates, copolymer of Vitrebond, Bis-GMA, HEMA, water, ethanol, particles of silica treated with silane, initiators, and stabilizers	Dry enamel and apply the self-etching primer with moderate friction for 10 seconds, air dry for 5 seconds, and light cure for 10 seconds. Apply the adhesive and light cure for 10 seconds.
Silorane Adhesive system – Bond (3M/ESPE)	Hydrophobic dimethacrylate, phosphate methacrylates, TEGDMA, particles of silica treated with silane, initiators, and stabilizers	
Scothbond Universal (3M/ESPE)	MDP phosphate monomer, dimethacrylate resins, HEMA, Vitrebond copolymer, filler, ethanol, water, initiators, and silante	Enamel acid etching for 15 seconds; wash and dry; apply the adhesive for 20 seconds, air-dry for 5 seconds, and light cure for 10 seconds.

[a] *Source: MSDS data sheet*
Abbreviations: BHT, butyl hydroxy toluene; Bis-EMA, bisphenol A ethoxylated dimethacrylate; Bis-GMA, bisphenol A glycidyl methacrylate; HEMA, hydroxyethylene glycol dimethacrylate; TEGDMA, triethylene glycol dimethacrylate;UDMA, urethane dimethacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate

5. Enamel restored with SB system and bleaching without light activation
6. Enamel restored with SB system and bleaching combined with LED light
7. Enamel restored with SB system and bleaching combined with diode laser light
8. Enamel restored with SB system and bleaching combined with halogen light
9. Enamel restored with DB system and bleaching without light activation
10. Enamel restored with DB system and bleaching combined with LED light
11. Enamel restored with DB system and bleaching combined with diode laser light
12. Enamel restored with DB system and bleaching combined with halogen light

The bleaching agent was weighed (0.01 g), applied on the exposed enamel area and then light-activated, according to the manufacturer's instructions (Table 2).

Concentration of Hydrogen Peroxide Into the Pulp Chamber

Before the bleaching treatment, the pulp chambers were individually dried and filled with 150 μL of 2 mol/L acetate buffer (pH 4.5). The acetate buffer solution was applied to stabilize the HP that penetrates into the pulp chamber throughout the bleaching. After bleaching, the acetate buffer was removed and the solution was transferred to a glass test tube. The pulp chamber of each tooth was filled for a second time with 150 μL acetate buffer for 1 minute, and this solution was placed in the same glass tube. In addition, distilled water (2650 μL), Leuco Crystal Violet (100 μL of 0.5 mg/mL; Sigma-Aldrich, St Louis, MO, USA), and horseradish peroxidase (50 μL of 1 mg/mL; Sigma-Aldrich) were also added to each tube, according to the method described by Berger and others.[25]

This solution produced a blue color, and the optical density obtained in the tubes was measured in a

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Table 2: *Light Sources, Product Features, and Application Protocol*[a]

Light Sources	Commercial Names	Characteristics	Application Protocol
LED light	Valo (Ultradent Products Inc, South Jordan, UT, USA)	Wavelength: 395-480 m Light intensity: 790 mW/cm^2	The bleaching gel remains 1 minute without agitation and is light irradiated for 2 minutes. The procedure is repeated three times with 1-minute interval among irradiations.
LED/laser diode	Whitening Laser Light Plus (DMC Equipment, São Carlos, Brazil)	LED wavelength: 470 ηm Infrared diode laser wave length (3): 810 ηm and power of 0.2 W	The bleaching gel remains 1 minute on the surface without agitation and is light irradiated for 2 minutes. The procedure is repeated three times with 1-minute interval among irradiations.
Halogen light	Optilux 501 (Demetron/Kerr, Danbury, CT, USA)	Wavelength: 560 ηm Light intensity: 600-800 mW/cm^2	The bleaching agent is applied on the surface for 2 minutes and irradiated for 30 seconds. The irradiation is repeated three times with a 2-minute interval.

[a] *Source: MSDS data sheet*
Abbreviation: LED, light-emitting diode.

spectrophotometer (DU 800, Beckman Coulter Inc, Brea, CA, USA) at a wavelength of 596 nm. A standard curve of known HP concentrations was obtained to convert the optical density values of each specimen into microgram (μg) equivalents of HP/mL of solution. The values were then converted into micrograms per milliliter. Due to the chemical instability of HP,[26] a second standard curve of known HP concentrations was obtained to determine the real concentration of the commercially available bleaching agent. This concentration was measured for the stoichiometric calculation.

Laser Scanning Confocal Fluorescence Microscopy (LSCFM)

To determine the integrity of the adhesive interface, three additional samples were prepared and observed using LSCFM. Rhodamine B (0.1 mM, Sigma-Aldrich) was added to the bleaching agents and the agents were placed in contact with the enamel surface at the recommended bleaching time. After bleaching, teeth were sectioned (with oil lubrication) at the central area of the restorations (Isomet 1000, Buehler, Lake Bluff, IL, USA) and the exposed inner interfaces were ground and polished (polishing machine, Ecomet 3000, Buehler) with abrasive paper (No. 400, No. 600, and No. 1200) and diamond pastes (0.3 and 0.1 μm, Metaldi Supreme, Buehler). The samples were sonically cleaned to remove polishing residues, and the interface area was analyzed using LSCFM (TCS SP5AOBS, Leica Microsystems CMS GmbH, Wetzlar, Germany), then scanned by an argon laser with a wavelength of 529 nm. The groups submitted to light-activation methods (LED, LED/diode laser, and halogen light) were not observed by LSCFM, as rhodamine B is sensitive to light variations, a factor that could compromise the results. Figure 1 schematically depicts the methodologic procedures.

Statistical Analyses

The exploratory data analysis of the results was submitted to the software Proc Lab (SAS 9.0, SAS Institute, Cary, NC, USA). The normal distribution and homoscedasticity of the values were verified and a parametric analysis was performed. The concentration of HP that reached the pulp chamber (μg/mL) was submitted to two-way analysis of variance (ANOVA) (enamel treatment × light activation) and Tukey test (pre-set alpha of 0.05).

RESULTS

A summary of the HP concentration in the pulp chamber (μg/mL) is shown in Table 3. Two-way ANOVA and Tukey test indicated that the HP concentration in the pulp chamber of the group without enamel restorations (control) was significantly lower than that of the groups restored with the SB system, regardless of the light activation method used ($p<0.00001$). The same analyses indicated that the intrachamber HP concentration of enamel restored with the DB system was significantly higher than that of the control group when HP was combined with halogen light activation

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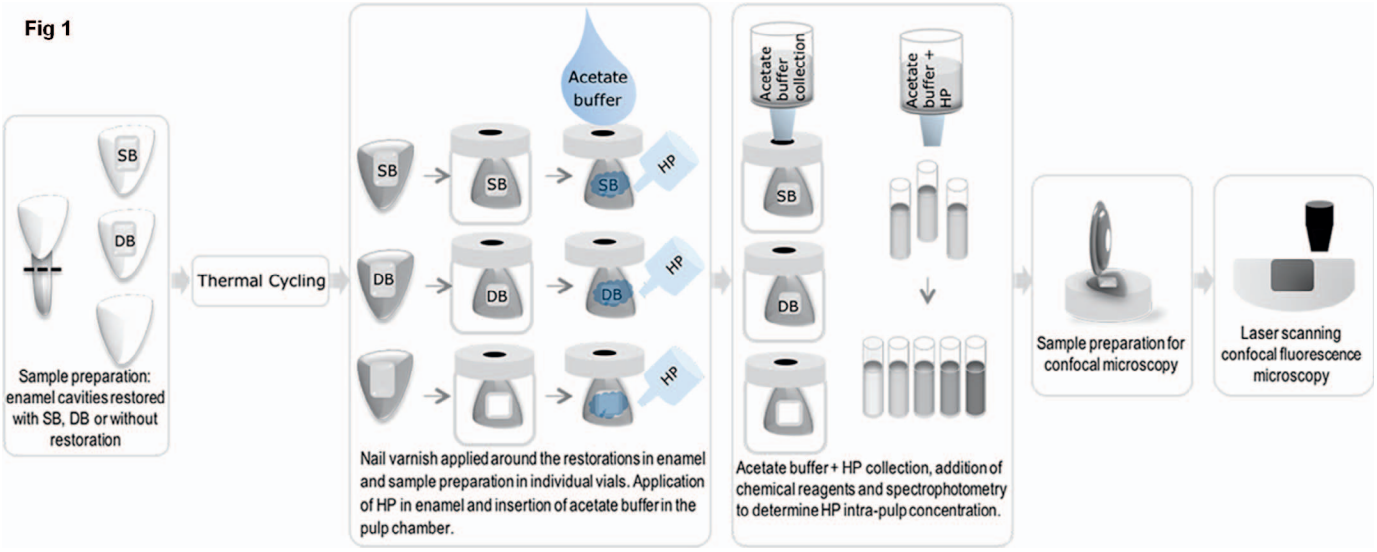

Figure 1. *Schematic representation of the methodological procedures.*

($p<0.05$). Finally, no differences were observed comparing the data from the two groups with enamel restorations (SB and DB, $p>0.05$), regardless of the light-activation method used.

The LSCFM images of the HP penetration in the enamel/dentin interface and/or at the adhesive interface are represented in Figure 2A-C. Figure 2A represents the control group (enamel without restoration). Figures 2B and C represent the enamel with SB and DB adhesive restorations, respectively. In Figure 2A, the greatest concentration of rhodamine B is observed in the aprismatic enamel, whereas in Figures 2B and C, the bleaching agent penetrated the interface, reaching the dentin surrounding the adhesive interface.

DISCUSSION

The results of this study indicate that there were no differences in the intra-pulp concentration of HP between the groups with adhesive restorations (silorane or dimethacrylate). However, the intra-pulp concentration of HP of enamel without a restoration (control) was significantly lower compared with that of the silorane-treated group. Therefore, the null hypothesis was rejected because the SB adhesive restorations exhibited higher HP intra-pulp concentration than the control group, regardless of the light activation method used.

The permeability of the dental structure associated with the ability of the transenamel/dentin diffusion of the bleaching agents has become the focus of a number of research studies that evaluated the cytotoxic effects of HP free radicals that reach the pulp. In general, the studies evaluate the effects of bleaching agents on cultured odontoblast-like MDPC-23 cells.[27,28] According to some of these evaluations, HP toxicity is dose dependent, but even the lowest peroxide concentrations could promote

Table 3: *Mean and Standard Deviation of Hydrogen Peroxide (HP) Concentration in the Pulp Chamber (μg/mL) After 35% HP Treatments (Light Activated or Not) on Enamel Without (Control) and With Dimethacrylate-Based (DB) and Silorane-Based (SB) Adhesive Restorations*[a]

Groups	Control (Without Restoration)	DB Restorative System	SB Restorative System
35% HP	4.1 (2.4) Aa	7.9 (4.5) Aab	8.3 (4.9) Ab
35% HP + light-emitting diode light	3.2 (2.5) Aa	7.4 (2.5) Aab	10.5 (4.7) Ab
35% HP + diode laser light	3.1 (2.3) Aa	7.5 (2.7) Aab	8.8 (4.5) Ab
35% HP + halogen light	2.9 (2.4) Aa	10.6 (4.3) Ab	11.0 (4.0) Ab

[a] *Means followed by distinct letters differ statistically at 5%, according to two-way analysis of variance and Tukey test ($p<0.05$; uppercase letters for columns, lowercase letters for rows).*

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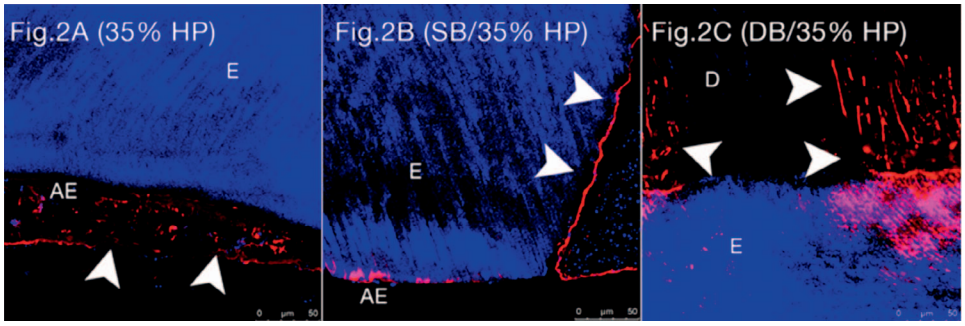

Figure 2. *Laser scanning confocal fluorescence microscopy of bleached enamel (with or without adhesive restoration). (A): In the control group (intact enamel), the arrows indicate the infiltration of rhodamine B dye (in red) and demonstrate the penetration of HP into the aprismatic enamel (AE). (B): The arrows indicate the infiltration of rhodamine B dye at the adhesive interface and dye deposition at the aprismatic enamel (AE). (C): The arrows indicate the infiltration of rhodamine B dye first in enamel (E) and, afterward, reaching dentin (D).*

pathological effects and reduce cell viability by approximately 77%.[13,14,27]

In previous *in vivo* evaluations, pulp damage was observed in human incisors after a 38% HP application on enamel for 45 minutes.[28] In another *in vivo* study, after the application of a calcium-free 35% HP agent applied three times for 15 minutes each or a single 45-minute application on enamel, both without light activation, the coronal pulp tissue exhibited partial necrosis associated with tertiary dentin deposition in five mandibular extracted incisors.[29]

The enamel/dentin permeability, time, application mode,[25] heating/light-activation,[30] quality and thickness of enamel,[15] and presence of adhesive restorations[20] are among the factors that influence HP penetration into the pulp chamber. The intra-pulp HP concentration was greater in the SB group compared with the control group; however, the DB group was similar to the control (except for halogen light activation) and the SB groups. Benetti and others[20] observed that the intra-pulp concentration of HP free radicals was greater in the presence of adhesive restorations, whereas Camargo and others[15] reported that among different restorative materials (composite resin, glass ionomer cement, and glass ionomer cement modified by resin) HP penetration into the pulp chamber was dependent on the material used and was lower for composite resin restorations. It is known that HP penetration occurs due to its low molecular weight and ability to denature proteins, which increases the ionic movement through the dental hard structure.[25] In addition, HP penetration can increase due to polymerization shrinkage that creates gaps at the enamel/resin interface and possible changes in the adhesive interface of enamel restorations promoted by the bleaching agents.[15,31,32]

In the current study, restorations were thermocycled before HP application, in order to age the interface and promote adhesive failure. Although two different adhesives were evaluated, it is noteworthy that after 5000 cycles, there were no perceptible failures in the enamel interface; however, when penetration of HP was assessed, the SB agent allowed greater HP penetration than the DB agent. The silorane adhesive is a self-etching adhesive, and according to its application technique, the manufacturer suggests excluding enamel etching. Therefore, it is possible that application of the silorane adhesive without etching of the cavosuperficial enamel contributed to the inferior performance of this system. It is postulated that a significant increase in enamel bond strength results from enamel etching before the application of this adhesive.[33,34]

In a recent study,[35] the performance of the SB restorative system was evaluated with respect to polymerization shrinkage in low (Class V) and high (Class I) C-factor cavities. According to that study and another investigation,[34] the silorane composite promoted higher polymerization shrinkage stress compared with the conventional dimethacrylate adhesive system, and the reason could be related to the monomeric composition.[35] The silorane adhesive system is composed of a hydrophilic acidic primer (SSA Primer) with a mild pH of 2.7, which promotes dentin demineralization of approximately a few hundred nanometers. The primer of the silorane system is composed of a dimethacrylate phosphate functional monomer, hydroxyethylene glycol dimethacrylate (HEMA), BIS-GMA, itaconic acid copolymer, silica, and camphorquinone dissolved in a water-alcohol solution.[34,35] The bonding resin is a hydrophobic high-viscosity agent (SSA Bond), basically composed of dimethacrylate, triethylene glycol dimethacrylate (TEGDMA), silica, camphorquinone, and function monomers.[33,35] The positive aspect of the high concentration of HEMA in the primer is that it prevents phase separation. On the other hand, HEMA increases the susceptibility of this adhesive to water sorption. Further, due to the highly hydrophobic nature of the adhesive, previous

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studies have observed the presence of water between the layers of the primer and the hydrophobic adhesive resin.[33,35] Therefore, the adhesive interface may be considered the problematic link of the SB system[21,33] and possibly the reason for the higher HP intra-pulp penetration.

Some studies have evaluated the microleakage of SB and DB materials. In an *in vitro* experiment, microleakage was observed in Class I restorations using different bonding techniques for both SB and DB materials, but the SB materials showed more microleakage compared with the DB composite systems.[36] Another evaluation observed that the SB material did not provide better marginal integrity than low shrinkage methacrylate-based composites.[37] According to the authors, this microleakage could be related to the formation of an oxygen inhibition layer during the curing of silorane primer before the application of the bonding agent.[37,38] This layer formed between the cured primer and the bond was observed in micro-Raman spectroscopy as an intermediate zone of approximately 1 μm and may be the weakest zone of silorane adhesives.[38]

Another *in vitro* study[39] evaluated the effect of 30% HP on marginal integrity of adhesive restorations. It observed that although bleaching did not significantly affect the marginal integrity of the restorations, the SB composite exhibited greater microleakage in gingival margins than the DB composites tested. The authors also suggested that the micrometer intermediate zone was the weakest link of the interface and proposed that the free radicals released by HP could lead to an increase in the microleakage of SB compostites.[39]

The DB restorative system achieved intermediate penetration values, which were not statistically different from those of the control group. It is important to observe that the application of this adhesive involved selective enamel etching, which was carried out as recommended by the manufacturer. In a preceding study, the good performance of this adhesive was noted when selective enamel etching was performed even after thermal cycling.[40] As rhodamine B was added to the bleaching gel, the dye penetration shown in the laser scanning confocal fluorescence microscopy was the pathway of HP in the enamel/dentin/resin-tooth interfaces. It can be noted that the dye penetration was more noted in the presence of the adhesive interface (either SB or DB restoratives). The interface aging with thermal cycling might have caused damage at the bonded interface, which enabled the infiltration of the peroxide along with the dye.

According to the thermocatalytic principle of HP decomposition, light application (LED, LED/diode laser, halogen light) combined with in-office bleaching agents aims to accelerate the decomposition of HP.[18] This principle states that the temperature increase accelerates the release of hydroxyl radicals, so when light energy strikes the bleaching agent, a fraction is absorbed and the acquired energy is converted into heat. To increase light absorption and, as a result, increase heat conversion into energy, some agents contain specific dyes, such as carotene, which has a reddish color and increases the absorption of blue light. To increase the absorption of red or infrared light, some agents contain silica nanoparticles, which provide a bluish color to the bleaching agents.[18] However, the effectiveness of light activation of bleaching agents is still controversial.[41] Hein and others[42] observed that the application of heat and light was unable to increase *in vitro* HP decomposition, but the temperature of the agents containing carotene increased it considerably, raising concerns about pulp injuries. It has also been observed that activation of 35% HP with an LED light or LED/diode laser increases the final concentration of peroxide into the pulp chamber,[19,41] which possibly promotes cell injuries.[29] Contrarily, it has been previously observed that the application of a laser or quartz tungsten halogen light was unable to increase bleached enamel permeability; however, bleaching promoted greater permeability than unbleached enamel without light irradiation.[43]

In the present study, no differences were noted in the pulp chamber concentration of HP between the control and DB groups when exposed to different light activation sources (LED, LED/diode laser, or halogen light), except for the DB group combined with halogen light, which exhibited a greater HP concentration compared with the control group. Although only one group (DB/halogen light) presented greater HP concentration compared with the control, the authors of this study indicate that light activation of any type could be dismissed since the decomposition of the agents occurs even in the absence of light.[26]

Previous studies have noted that the penetration of high-concentration HP agents was dependent on the application time, since the longer the contact of the agent with the enamel surface, the higher the intra-pulp concentration[44] and, possibly, the greater the risk of cytotoxicity. As a consequence, reducing the application time of 35% HP has been proposed as a way to reduce the cytotoxic effects.[45,46] According

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to these authors, reducing the application time would not compromise the desired whitening effect and would decrease tooth sensitivity and the adverse effects in pulp cells.[44,45]

Although a significant number of articles indicate toxicity effects in pulp cells, other studies attest that bleaching agents do not generate acute or minor toxic effects. Vongsavan and Matthews[47] maintain that *in vitro* studies typically show greater penetration of peroxides than those performed under *in vivo* conditions. These authors verified that dye penetration into the pulp chamber was lower in *in vivo* than *in vitro* conditions because pulpal pressure and the dentinal fluids (a mixture of albumin, transferrin, tenascin, and proteoglycans) that flow inside the tubules from the pulp to the outer dentin surface prevent the diffusion of fluids and other substances into the pulp chamber.[47] Therefore, the use of extracted teeth without pulpal pressure and dentinal fluids probably allows greater intra-pulp penetration than in clinical situations. Still, one should consider that the pulp tissue has a self-protection system against damages caused by HP by means of a production of enzymes, such as peroxidase and catalase, which promote the molecular breakdown of the products of the HP reaction.

The immediate and most common clinical consequence of pulp tissue alteration is dental hypersensitivity[45] that, although transient, still causes discomfort to the patient and uncertainties regarding the long-term consequences of bleaching. Thus, *in vivo* long-term studies evaluating HP pulp penetration would clarify the magnitude of undesirable clinical consequences.

CONCLUSION

The bleaching agent tested (35% HP) is able to reach the pulp chamber in the presence or absence of enamel adhesive restorations. Among the groups, it could be observed that the HP intra-pulp concentration was greater in the presence of an SB adhesive restoration compared with intact enamel (control, ie, without adhesive restoration). Different light sources did not influence the HP intra-pulp penetration for the control group and enamel with an SB restorative system.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the University of Campinas, Piracicaba Dental School.

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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REFERENCES

1. Pinto CF, Paes Leme AF, Cavalli V, & Giannini M (2009) Effect of 10% carbamide peroxide bleaching on sound and artificial enamel carious lesions *Brazilian Dental Journal* **20(1)** 48-53.
2. Berger SB, Cavalli V, Martin AA, Soares LE, Arruda MA, Brancalion ML, & Giannini M (2010) Effects of combined use of light irradiation and 35% hydrogen peroxide for dental bleaching on human enamel mineral content *Photomedicine and Laser Surgery* **28(4)** 533-538, http://dx.doi.org/10.1089/pho.2009.2506.
3. De Abreu DR, Sasaki RT, Amaral FL, Flório FM, & Basting RT (2011) Effect of home-use and in-office bleaching agents containing hydrogen peroxide associated with amorphous calcium phosphate on enamel microhardness and surface roughness *Journal of Esthetic and Restorative Dentistry* **23(3)** 158-168, http://dx.doi.org/10.1111/j.1708-8240.2010.00394.x.
4. Lia Mondelli RF, Garrido Gabriel TR, Piola Rizante FA, Magalhães AC, Soares Bombonatti JF, & Ishikiriama SK (2015) Do different bleaching protocols affect the enamel microhardness? *European Journal of Dentistry* **9(1)** 25-30, http://dx.doi.org/10.4103/1305-7456.149634.
5. Cavalli V, Rodrigues LK, Paes-Leme AF, Brancalion ML, Arruda MA, Berger SB, & Giannini M (2010) Effects of bleaching agents containing fluoride and calcium on human enamel *Quintessence International* **41(8)** e157-e165.
6. da Silva AP, de Oliveira R, Cavalli V, Arrais CA, Giannini M, & de Carvalho RM (2005) Effect of peroxide-based bleaching agents on enamel ultimate tensile strength *Operative Dentistry* **30(3)** 318-324.
7. Lima AF, Fonseca FM, Freitas MS, Palialol AR, Aguiar FH, & Marchi GM (2011) Effect of bleaching treatment and reduced application time of an antioxidant on bond strength to bleached enamel and subjacent dentin *Journal of Adhesive Dentistry* **13(6)** 537-542, http://dx.doi.org/10.3290/j.jad.a19813.
8. Smidt A, Feuerstein O, & Topel M (2011) Mechanical, morphologic, and chemical effects of carbamide peroxide bleaching agents on human enamel in situ *Quintessence International* **42(5)** 407-412.
9. Cavalli V, Rodrigues LK, Paes-Leme AF, Soares LE, Martin AA, Berger SB, & Giannini M (2011) Effects of the addition of fluoride and calcium to low-concentrated carbamide peroxide agents on the enamel surface and

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subsurface *Photomedicine and Laser Surgery* **29(5)** 319-325, http://dx.doi.org/10.1089/pho.2010.2797.

10. Joiner A (2006) The bleaching of teeth: a review of the literature *Journal of Dentistry* **34(7)** 412-419.
11. Kina JF, Huck C, Riehl H, Martinez TC, Sacono NT, Ribeiro AP, & Costa CA (2010) Response of human pulps after professionally applied vital tooth bleaching *International Endodontic Journal* **43(7)** 572-580, http://dx.doi.org/10.1111/j.1365-2591.2010.01713.x.
12. Soares DG, Ribeiro AP, Lima AF, Sacono NT, Hebling J, & de Souza Costa CA (2013) Effect of fluoride-treated enamel on indirect cytotoxicity of a 16% carbamide peroxide bleaching gel to pulp cells *Brazilian Dental Journal* **24(2)** 121-127, http://dx.doi.org/10.1590/0103-6440201302161.
13. Soares DG, Ribeiro AP, Sacono NT, Coldebella CR, Hebling J, & Costa CA (2011) Transenamel and transdentinal cytotoxicity of carbamide peroxide bleaching gels on odontoblast-like MDPC-23 cells *International Endodontic Journal* **44(2)** 116-125, http://dx.doi.org/10.1111/j.1365-2591.2010.01810.x.
14. Lima AF, Ribeiro AP, Soares DG, Sacono NT, Hebling J, & de Souza Costa CA (2013) Toxic effects of daily applications of 10% carbamide peroxide on odontoblast-like MDPC-23 cells *Acta Odontologica Scandinavica* **71(5)** 1319- 1325, http://dx.doi.org/10.3109/00016357.2012.762992.
15. Camargo SE, Valera MC, Camargo CH, Gasparoto Mancini MN, & Menezes MM (2007) Penetration of 38% hydrogen peroxide into the pulp chamber in bovine and human teeth submitted to office bleaching technique *Journal of Endodontics* **33(9)** 1074-1077.
16. Trindade FZ, Ribeiro AP, Sacono NT, Oliveira CF, Lessa FC, Hebling J, & Costa CA (2009) Trans-enamel and trans-dentinal cytotoxic effects of a 35% H_2O_2 bleaching gel on cultured odontoblast cell lines after consecutive applications *International Endodontic Journal* **42(6)** 516-524, http://dx.doi.org/10.1111/j.1365-2591.2009.01544.x.
17. Coldebella CR, Ribeiro AP, Sacono NT, Trindade FZ, Hebling J, & Costa CA (2009) Indirect cytotoxicity of a 35% hydrogen peroxide bleaching gel on cultured odontoblast-like cells *Brazilian Dental Journal* **20(4)** 267-274.
18. Buchalla W, & Attin T (2007) External bleaching therapy with activation by heat, light or laser—A systematic review *Dental Materials* **23(5)** 586-596.
19. Camargo SE, Cardoso PE, Valera MC, de Araújo MA, & Kojima AN (2009) Penetration of 35% hydrogen peroxide into the pulp chamber in bovine teeth after LED or Nd:YAG laser activation *European Journal of Esthetic Dentistry* **4(1)** 82-88.
20. Benetti AR, Valera MC, Mancini MN, Miranda CB, & Balducci I (2004) In vitro penetration of bleaching agents into the pulp chamber *International Endodontic Journal* **37(2)** 120-124.
21. Klautau EB, Carneiro KK, Lobato MF, Machado SM, & Silva e Souza MH Jr (2011) Low shrinkage composite resins: Influence on sealing ability in unfavorable C-factor cavities *Brazilian Oral Research* **25(1)** 5-12.
22. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, & De Stefano Dorigo E. (2008) Dental adhesion review: Aging and stability of the bonded interface *Dental Materials* **24(1)** 90-101.
23. Al-Boni R, & Raja OM (2010) Microleakage evaluation of silorane based composite versus methacrylate based composite *Journal of Conservative Dentistry* **13(3)** 152-155.
24. Weinmann W, Thalacker C, & Guggenberger R (2005) Siloranes in dental composites *Dental Materials* **21(1)** 68-74.
25. Berger SB, Tabchoury CP, Ambrosano GM, & Giannini M (2013) Hydrogen peroxide penetration into the pulp chamber and dental permeability after bleaching *General Dentistry* **61(3)** e21-e25.
26. Cavalli V, Marson FC, Berger SB, & Giannini M (2013) Color alteration and hydrogen peroxide decomposition of high-concentrated bleaching agents on enamel *Dental Materials* **29(supplement 1)** p e37, http://dx.doi.org/10.1016/j.dental.2013.08.078.
27. de Lima AF, Lessa FC, Gaspartoto Mancini MN, Hebling J, de Sousa Costa CA, & Marchi GM (2009) Cytotoxic effects of different concentrations of a carbamide peroxide bleaching gel on odontoblast-like cells MDPC-23 *Journal of Biomedical Materials Research. Part B, Applied Biomaterials* **90(2)** 907-912, http://dx.doi.org/10.1002/jbm.b.31362.
28. Costa CA, Riehl H, Kina JF, Sacono NT, & Hebling J (2010) Human pulp responses to in-office tooth bleaching *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **109(4)** e59-e64, http://dx.doi.org/10.1016/j.tripleo.2009.12.002.
29. Roderjan DA, Stanislawczuk R, Hebling J, Costa CA, Reis A, & Loguercio AD (2015) Response of human pulps to different in-office bleaching techniques: Preliminary findings *Brazilian Dental Journal* **26(3)** 242-248, http://dx.doi.org/10.1590/0103-6440201302282.
30. Dias Ribeiro AP, Sacono NT, Lessa FC, Nogueira I, Coldebella CR, Hebling J, & de Souza Costa CA (2009) Cytotoxic effects of a 35% hydrogen peroxide bleaching gel on odontoblast-like MDPC-23 cells *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **108(3)** 458-464, http://dx.doi.org/10.1016/j.tripleo.2009.05.006.
31. Cavalli V, de Carvalho RM, & Giannini M (2005) Influence of carbamide peroxide-based bleaching agents on the bond strength of resin-enamel/dentin interfaces *Brazilian Oral Research* **19(1)** 23-29.
32. Fronza BM, Rueggeberg FA, Braga RR, Moglievych B, Soares LES, Martin AA, Ambrosano GMB, & Giannini M (2015) Monomer conversion, microhardness, internal marginal adaptation and shrinkage stress of bulk-fill resin composites *Dental Materials* **31(12)** 1542-1551, http://dx.doi.org/10.1016/j.dental.2015.10.001.
33. Duarte S Jr, Phark JH, Varjão FM, & Sadan A (2009) Nanoleakage, ultramorphological characteristics, and microtensile bond strengths of a new low-shrinkage composite to dentin after artificial aging *Dental Materials* **25(5)** 589-600, http://dx.doi.org/10.1016/j.dental.2008.10.012.

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34. Mine A, De Munck J, Van Ende A, Cardoso MV, Kuboki T, Yoshida Y, & Van Meerbeek B (2010) TEM characterization of a silorane composite bonded to enamel/dentin *Dental Materials* **26(6)** 524-532, http://dx.doi.org/10.1016/j.dental.2010.01.010.

35. Isaac SZ, Bergamin AC, Turssi CP, Amaral FL, Basting RT, & França FM (2013) Evaluation of bond strength of silorane and methacrylate based restorative systems to dentin using different different cavity models *Journal of Applied Oral Sciences* **21(5)** 452-459, http://dx.doi.org/10.1590/1679-775720130120.

36. Alshetili MS, & Aldeyab SS (2005) Evaluation of microleakage of silorane and methacrylate based composite materials in Class I restorations by using two different bonding techniques *Journal of International Oral Health* **7(2)** 6-9.

37. Kermanshah H, Yasini R, & Hoseinifar R (2016) Effect of cyclic loading on microleakage of silorane based composite compared with low shrinkage methacrylate-based composites *Dental Research Journal* **13(3)** 264-271.

38. Santini A, & Miletic V (2008) Comparison of the hybrid layer formed by silorane adhesive, one-step self-etch and etch and rinse systems using confocal micro-Raman spectroscopy and SEM *Journal of Dentistry* **36(9)** 683-691, http://dx.doi.org/10.1016/j.jdent.2008.04.016.

39. Hashemikamangar SS, Ghavam M, Mahinfar N, & Kharazi Fard MJ (2014) Effect of 30% hydrogen peroxide on marginal integrity of silorane-based versus methacrylate-based composite restorations *Journal of Dentistry of Tehran University of Medical Sciences* **11(5)** 545-553.

40. Batra C, Nagpal R, Tyagi SP, Singh UP, & Manuja N (2013) In vitro bonding effectiveness of three different one-step self-etch adhesives with enamel etching *Journal of Investigative and Clinical Dentistry* **5(3)** 225-226, http://dx.doi.org/110.1111/jicd.12039.

41. Caviedes-Bucheli J, Ariza-García G, Restrepo-Méndez S, Ríos-Osorio N, Lombana N, & Muñoz HR (2008) The effect of tooth bleaching on substance P expression in human dental pulp *Journal of Endodontics* **34(12)** 1462-1465, http://dx.doi.org/10.1016/j.joen.2008.09.013.

42. Hein DK, Ploeger BJ, Hartup JK, Wagstaff RS, Palmer TM, & Hansen LD (2003) In-office vital tooth bleaching—What do lights add? *Compendium of Continuing Education in Dentistry* **24(4A)** 340–352.

43. Turssi CP, Shiavoni RJ, Serra MC, & Froner IC (2006) Permeability of enamel following light-activated power bleaching *General Dentistry* **54(5)** 323-326.

44. Marson F, Gonçalves R, Silva C, Cintra L, Pascotto R, Santos PD, & Briso A (2015) Penetration of hydrogen peroxide and degradation rate of different bleaching products *Operative Dentistry* **40(1) 72-79**, http://dx.doi.org/10.2341/13-270-L.

45. Soares DG, Basso FG, Hebling J, & de Souza Costa CA (2014) Concentrations of and application protocols for hydrogen peroxide bleaching gels: Effects on pulp cell viability and whitening efficacy *Journal of Dentistry* **42(2)** 185-198, http://dx.doi.org/10.1016/j.jdent.2013.10.021.

46. Cintra LT, Benetti F, da Silva Facundo AC, Ferreira LL, Gomes-Filho JE, Ervolino E, Rahal V, & Briso AL (2013) The number of bleaching sessions influences pulp tissue damage in rat teeth *Journal of Endodontics* **39(12)** 1576-1580, http://dx.doi.org/10.1016/j.joen.2013.08.007.

47. Vongsavan N, & Matthews B (1991) The permeability of cat dentin in vivo and in vitro *Archives of Oral Biology* **36(9)** 641-664.

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