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Influence of Tooth Pigmentation on H_2O_2 Diffusion and Its Cytotoxicity After In-office Tooth Bleaching

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Clinical Relevance

Pigments in tooth structures affect the diffusion of H_2O_2 through enamel and dentin. The bleaching methodology can be impacted.

SUMMARY

Objective: The aim of this study was to evaluate the influence of the presence of pigments in tooth structures on the trans-enamel and trans-dentin diffusion of hydrogen peroxide (H_2O_2) and its cytotoxicity after carrying out an in-office bleaching therapy.

Methods and Materials: A bleaching gel with 35% H_2O_2 was applied for 45 minutes (three times for 15 minutes) on enamel and dentin discs (n=6), either previously submitted to the intrinsic pigmentation protocol with a concentrated solution of black tea, or not, defining the following groups: G1, unbleached untreated discs (control 1); G2, unbleached pigmented discs (control 2); G3, bleached untreated discs; G4, bleached pigmented discs. The discs were adapted to artificial pulp chambers, which were placed in wells of 24-well plates containing 1 mL culture medium (Dulbecco's modified Eagle's medium [DMEM]). After applying the bleaching gel on enamel, the extracts (DMEM + components of bleaching gel that diffused through the discs) were collected and then applied on the cultured MDPC-23 odontoblast-

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like cells. Cell viability (methyl tetrazolium assay and Live & Dead, Calcein AM, and ethidium homodimer-1 [EthD-1] probes), the amount of H_2O_2 that diffused through enamel and dentin (leuco-crystal violet product), and the H_2O_2-mediated oxidative cell stress (SOx) and components of degradation were assessed (analysis of variance/Tukey; α=0.05).

Results: There was no significant difference between the groups G1 and G2 for all the parameters tested ($p>0.05$). Reduction in the trans-enamel and trans-dentin diffusion of H_2O_2 occurred for G4 in comparison with G3. Significantly lower cell viability associated with greater oxidative stress was observed for G3 ($p<0.05$). Therefore, in-office tooth bleaching therapy performed in pigmented samples caused lower cytotoxic effects compared with untreated samples submitted to the same esthetic procedure ($p<0.05$).

Conclusion: According to the methodology used in this investigation, the authors concluded that the presence of pigments in hard tooth structures decreases the trans-enamel and trans-dentin diffusion of H_2O_2 and the toxicity to pulp cells of an in-office bleaching gel with 35% H_2O_2.

INTRODUCTION

Tooth bleaching is a clinical procedure with great impact on esthetic dentistry and is the first alternative recommended for the treatment of chromatic changes originating in dental tissue, changes which result in alteration of light reflection and compromised beauty of the smile.

Briso and others[1] reported that the ease of performing the technique, preservation of enamel structure, and obtaining relatively fast clinical results are some of the benefits that have made the use of the in-office bleaching technique the procedure that is still preferred by many patients and professionals. However, the option to use highly concentrated bleaching gels has generated innumerable controversies, and reports of pulp tissue compromise in vital teeth submitted to treatments based on the use of in-office gels containing high concentrations of peroxide have been reported.[2,3] In this context, hypersensitivity is the main adverse effect.[1,4-6] Recent clinical studies have demonstrated that postbleaching effects may be associated with the trans-dentin diffusion of the hydrogen peroxide molecule (H_2O_2), the main active component of bleaching gels, and the byproducts of its degradation. These effects may range from inflammation of different intensities to the formation of areas of necrosis in the pulp.[2,3,7]

Bleaching of the tooth structure is known to occur due to the high oxidant power of H_2O_2 and its reaction products, which have the potential to break polypetide chains and organic components, including the chromophore agents present in the hard tissues of the tooth.[8-11] However, when bleaching gels containing high concentrations of H_2O_2 are used, unreacted (with chromophores) molecules can diffuse through the dentin tubules into the pulp chamber. Unreacted residual H_2O_2, being a toxic reactive species, may decrease pulp cell viability and cause oxidative stress and damage to the membranes of these cells.[2,3,12-15] Recent studies demonstrated that the intensity of the toxic effects of H_2O_2 is inversely proportional to the thickness of tooth enamel and dentin.[11] It is also directly related to the peroxide concentration and time of application used in professional in-office bleaching procedures. This means that the higher the concentration of H_2O_2 in the bleaching gel and the longer the time of contact of the product with enamel, the more intense will be the adverse cytotoxic effects.[13,15] Based on the mechanism of action, the hypothesis arose that, in dental tissues with a high level of pigment saturation, the pigments might react with a larger quantity of H_2O_2 coming from bleaching products, thereby reducing the trans-enamel and trans-dentin diffusion of this toxic molecule. Confirmation of this hypothesis seems to be relevant and would allow individualized and safe bleaching therapies to be established, preventing pulp damage and the occurrence of painful postbleaching symptoms.[6,16]

Thus, the stated hypothesis of this study was that elevated dentin pigment decreases H_2O_2 diffusion into the pulp to reduce cell cytotoxicity. For this purpose, the present *in vitro* study assessed the influence of intrinsic pigmentation induced in dentin on the trans-enamel and trans-dentin diffusion of H_2O_2, as well as the toxic effects of this reactive oxygen species (ROS) to odontoblast-like MDPC-23 pulp cells.

METHODS AND MATERIALS

Experimental Design

This investigation presented the following factors associate with the study: dental pigmentation at two levels (natural pigmentation and natural pigmentation + tea) and surface treatment at two levels

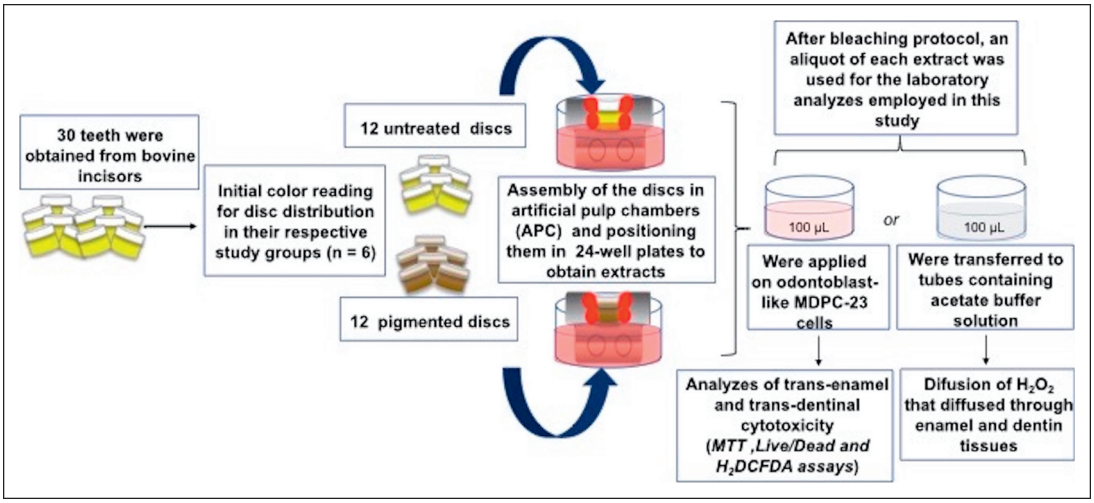


Figure 1. *Diagram of experimental design of this research.*

(bleaching gel with 35% H_2O_2 and unbleached substrate).

The response variables were trans-enamel and trans-dentin diffusion of H_2O_2 and the cytotoxicity of the treatment evaluated by cell viability tests (methyl tetrazolium assay, MTT), oxidative stress (carboxy-H_2DCFDA probe), and lesion of the cellular membrane (Calcein AM and ethidium homodimer-1 [EthD-1] probes). Twenty-four dental discs were used to perform the protocols (n=6 for each study group) as shown in Figure 1.

Preparation of Samples

Preparation of Enamel and Dentin Discs—A total of 30 enamel and dentin discs were obtained from the vestibular surface of bovine incisors. The teeth were cut with a diamond-coated trephine bur (Dinser Brocas Diamantadas LTDA, São Paulo, SP, Brazil), with an internal diameter of 5.6 mm, coupled to a bench drill (FSB 16 Pratika, Schultz, Joinville, SC, Brazil), in an environment submersed in water at 4°C. To standardize the thickness of the discs at 3.5 mm, the dentin surface was abraded with 400- and 600-grit water abrasive papers (T469-SF-Norton, Saint-Gobam Abrasivos Ltda, Jundiaí, SP, Brazil). The enamel and dentin sample thickness average was 0.91 ± 0.21 and 2.59 ± 0.13 mm, respectively. Measurement of the amount of enamel and dentin was performed using photographs taken by a DSLR camera (Canon T6i, F 22, ISO 200, speed 180, Otã, Tokyo, Japan). The images were analyzed using Image J software (National Institute of Health, Bethesda, MD, USA). After this, all the enamel surfaces of the discs were submitted to prophylaxis with pumice stone and water. Subsequently, the dentin surface was treated with ethylenediamine tetra-acetic acid (EDTA 0.5 N, Sigma-Aldrich Corp, St Louis, MO, USA) for 30 seconds to remove the smear layer.[12] Then, the discs were submitted to a colorimetric analysis to perform the first selection of 24 standardized samples, such as described in detail below.

First Selection of Discs—Before the experimental protocol, the first selection of discs was made based on initial color. For this purpose, the discs were positioned in an ultraviolet-visible (UV-Vis) reflectance spectrophotometer (Color Guide 45/0, BYK-Gardner GmbH, Geretsried, Germany) with a wavelength ranging from 400 to 700 nm. The color model of the CIE L*a*b* system was used as established by the Commission Internationale de l'Eclairage (CIE; International Commission on Illumination), which allows specification of the perception of colors of the tridimensional model. Three readouts were taken on the vestibular surface of discs.[12,15] After obtaining the L* and b* values, the mean value of the entire sample was calculated, and at this time, 24 discs that had L* and b* values closest to the mean values were selected. The mean of the L* values among the selected specimens was 60.87 (range, 58.46-61.71), whereas the mean of b* was 5.09 (range, 0.83-8.55). It is worth emphasizing that the axes L* and b* were considered because the chromatic changes generated by the bleaching treatment occurred preponderantly in these axes.

Pigmentation and Second Selection of Discs—At this time, half of the samples were submitted to an artificial pigmentation protocol,[17] and the other half of the samples remained with their natural color. Thus, as previously described by Moreira and

Table 1: *Experimental Groups According to Bleaching Gel Concentration (n=6)*

Group	Sample	Treatment
G1	Untreated unbleached discs	Without treatment
G2	Pigmented unbleached discs	Without treatment
G3	Untreated bleached discs	Gel with 35% H_2O_2 applied three times for 15 min
G4	Pigmented bleached discs	Gel with 35% H_2O_2 applied three times for 15 min

others,[19] 12 discs were incubated in a black tea infusion (0.16 g/mL) at 37°C for six days, with the solution changed every 48 hours. After the staining period, prophylaxis with pumice stone was performed on the enamel surface, and afterward, the samples were incubated in deionized water for 24 hours to eliminate the pigments not absorbed by dentin. The pigmented discs were again positioned in the UV-Vis reflectance spectrophotometer, and a chromatic change of $\Delta E = 8.77 \pm 1.25$ was found. Also obtained was the mean of L* (53.50; range, 51.81 to 59.33) and b* (9.00; range, 1.53 to 11.97).

Cell Culture

Immortalized MDPC-23 cells were obtained from the stock of cells of the Laboratory of Experimental Pathology and Biomaterials, Araraquara School of Dentistry, UNESP, SP, Brazil. These cells were seeded in 96-well plates (Corning Inc, New York, NY, USA) and incubated in Dulbecco's modified Eagle's culture medium (DMEM; GIBCO, Grand Island, NY, USA), containing 10% fetal bovine serum (FBS; GIBCO), 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L glutamine (GIBCO) in an atmosphere humidified at 37°C, with 5% CO_2 and 95% air, to obtain a standard of 80% of confluence.[12]

Experimental Procedure

The enamel/dentin discs were placed in artificial pulp chambers (APCs), kept in place between two silicone rings, and peripherally sealed with utility wax (Polidental, Cotia, SP, Brazil). Then, the disc/APC sets were sterilized in ethylene oxide (Acecil, Central de Esterilização Comércio e Indústria Ltda, Campinas, SP, Brazil). Afterward the sets were individually distributed in 24-well plates (Corning Inc) containing 1 mL DMEM without FBS. The culture medium remained in contact with the dentin, wheras the enamel was kept exposed either to receive bleaching gel or not, as demonstrated in Table 1. The discs were distributed into the following groups (n=6): G1, unpigmented and unbleached discs; G2, pigmented and unbleached discs; G3, unpigmented and bleached discs; G4, pigmented and bleached discs. For the bleaching procedure, the product Whiteness HP 35% (FGM Produtos Odontológicos, Joinville, SC, Brazil) was manipulated by adding three drops of the liquid phase containing H_2O_2 to one drop of thickener (3:1). After this, 20 μL of the resulting gel was applied to the enamel for 15 minutes, and the residual gel was aspirated using a sterilized cannula connected to a vacuum pump. This procedure was repeated two more times to yield a total of 45 minutes of exposure; this is consistent with bleaching protocols used in prior studies.[6,12-15,21] At the conclusion of the bleaching procedure, the extract (DMEM + bleaching gel components that diffused through the enamel and dentin) was collected, homogenized, and distributed into aliquots of 100 μL, which were applied on MDPC-23 cells seeded in 96-well plates (Corning Inc).[12] The cells were incubated for one hour in contact with the extracts to perform the cytotoxicity analyses, by means of the cellular viability tests (MTT), oxidative stress (carboxy-H_2DCFDA probe), and cellular membrane lesion (Calcein AM and EthD-1 probes). For quantification of the trans-enamel and trans-dentin diffusion of the H_2O_2 molecule, an aliquot of 100 μL of the extracts was transferred to a 24-well plate containing acetate buffer solution for stabilization of the H_2O_2 molecule.

Cell Viability (MTT Assay; n=6)—After having been exposed to the extracts for one hour, the cells were incubated at 37°C and 5% CO_2 for four hours in 90 μL DMEM culture medium, to which 10 μL of the MTT solution (3-4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide, Sigma-Aldrich Corp) was added, as previously described.[12] After this period, the formazan crystals resulting from the reduction of the tetrazolium salt by the succinate dehydrogenase present in the mitochondria of viable cells were dissolved in 100 μL isopropanol solution acidified in HCl 0.04 N, and the absorbance was measured at 570 nm (Synergy H1, Biotek, Winooski, VT, USA). The mean absorbance value obtained in group G1 was considered 100% cellular viability, and the percentage viability of the other experimental groups was calculated from this parameter.[12-15]

Oxidative Stress (Carboxy-H_2DCFDA Probe; n=6)—As previously described,[12,15] the cells seeded in 96-well plates (Corning Inc) were pretreated with the fluorescent probe carboxy-H_2DCFDA (Invitrogen, San Francisco, CA, USA) in a concentration of 5 μM at 37°C for 30 minutes and were then exposed to the extracts. Immediately afterward, fluorescence

Table 2: *Power of the Statistical Analysis of Cell Viability (MTT Assay)*

	p	Observed Power
Enamel pigmentation	<0.001	1.00
Enamel bleaching	0.008	0.888
Interaction	0.100	0.375

was evaluated at 492-nm wavelength excitation and 517-nm wavelength emission (Synergy H1, Biotek), and the values were normalized by the mean values of group G1.

Cell Membrane Disruption (Calcein AM and EthD-1 Probes; n=6)—For this qualitative analysis, the Live/Dead kit (Invitrogen) was used,[12] which is based on the use of two fluorescent markers. After exposure to the extracts, the cells seeded in 96-well plates (Corning Inc) were washed in PBS and incubated with culture medium supplemented with Calcein AM and EthD-1, in a concentration of 1:1000 for 45 minutes. Subsequently, the samples were evaluated under a fluorescence microscope (Leica DM 5500B, Nussloch GmbH, Nussloch, Germany) to obtain representative images of each group.

Quantification of Hydrogen Peroxide Diffusion (H_2O_2 μg/mL)

The quantity of H_2O_2 that diffused through the enamel and dentin was determined by the reaction of H_2O_2 with leuco-crystal violet dye (0.5 mg/mL, Sigma Chemical Co, St Louis, MO, USA) and with the horseradish peroxidase enzyme (1 mg/mL, Sigma Chemical Co), according to the methodology described by Mottola and others.[22] As previously mentioned,[12,15] an aliquot of 100 μL of the extract of each experimental group (n=6) was transferred to a plate with 900 μL acetate buffer solution for stabilizing the H_2O_2. Afterward, 500 μL of this solution (buffer solution plus extract) was transferred to test tubes containing water and leuco-crystal violet dye (0.5 mg/mL, Sigma Chemical Co). The tubes were shacked, and 50 μL of the solution at 1 mg/mL horseradish peroxidase enzyme (Sigma Chemical Co) was added. The final volume of the reaction was adjusted to 3 mL with distilled water. An aliquot of 100 μL of each solution was transferred to wells of 96-well plates (Corning Inc). The absorbance of the solutions was measured in a spectrophotometer at a wavelength of 596 nm (Synergy H1, Biotek). The standard curve of known quantities of H_2O_2 was used for converting the optic density values of the samples obtained in micrograms of H_2O_2 per milliliter of extract.

Table 3: *Power of the Statistical Analysis of Oxidative Stress (H_2DCFDA Probe)*

	p	Observed Power
Enamel pigmentation	<0.0001	1.00
Enamel bleaching	0.008	0.807
Interaction	0.020	0.674

Table 4: *Power of the Statistical Analysis of Quantity of H_2O_2 (μg/mL)*

	p	Observed Power
Enamel pigmentation	<0.0001	1.00

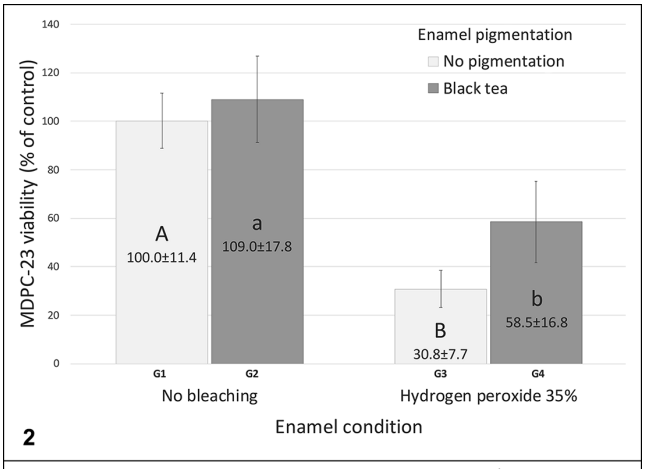
Statistical Analysis

Sample size was based on previous publications[12-15] (n=6). The cellular viability and oxidative stress data were normally distributed (Shapiro-Wilk, $p>0.05$); however, only cell viability data were homoscedastic (Levene, $p=0.304$). Therefore, these data were submitted to a two-way analysis of variance (ANOVA) considering the factors enamel bleaching and enamel pigmentation, complemented by pairwise comparisons with Tukey's test. The Games-Howell *post hoc* test was applied to oxidative stress data. Student's t-test was used for analyzing the quantity of H_2O_2 in the extracts of groups G3 and G4. Statistical inferences were taken at the 5% level of significance. The power of the statistical test is shown in Tables 2-4.

RESULTS

Trans-enamel and Trans-dentin Cytotoxicity

For groups G3 and G4, postbleaching cellular viability was reduced by approximately 69.2% and 50.5%, respectively, in comparison with their control groups, G1 and G2 ($p<0.05$). In addition, the presence of black tea pigments in dentin (G2) was found to cause no cytotoxicity to the MDPC-23 odontoblast cells (Figure 2). The cells belonging to groups G3 and G4 underwent significant oxidative stress compared to those of G1 and G2 ($p<0.05$; Figure 3). However, for the pigmented and bleached discs (G3), there was a reduction of approximately 24% in cellular oxidative stress in comparison to the unpigmented and unbleached discs (G4) ($p<0.05$). These data were correlated by means of analysis of the images obtained from the live/dead test, in which a large quantity of cells were marked positively with EthD-1 in G3 and G4 in comparison to G1 and G2. A larger quantity of cells marked positively with EthD-1 was detected in G3 compared to G4 (Figure 4).



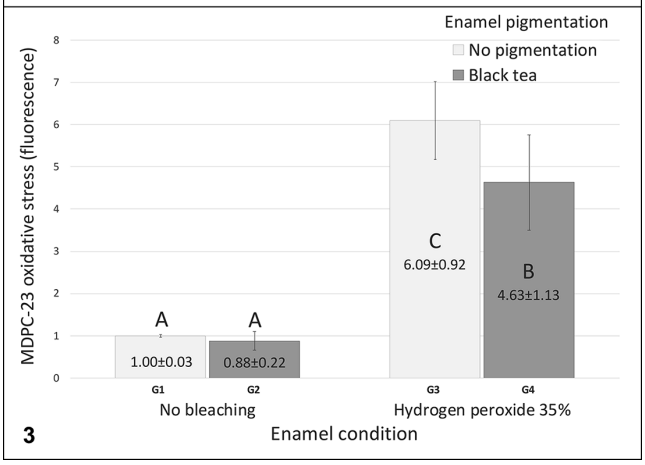


Figure 2. *MDPC-23 viability after contact with extracts produced by the application of 35% hydrogen peroxide gel on unaltered or black tea pigmented enamel. Unbleached enamel was used as control. Columns represent means, and error bars indicate standard-deviations (n=6). There was no interaction between the factors (*p*=0.100), but individually each factor had a significant effect on cell viability (enamel condition and enamel pigmentation,* p*<0.001). Therefore, columns connected by brackets within the same enamel condition were statistically different. Within the same enamel pigmentation, different uppercase letters allow comparison between the groups without pigmentation (G1 versus G3) and different lowercase letters allow comparison between the groups pigmented with black tea (G2 versus G4). Distinct letters indicate statistical difference between the groups.*

Figure 3. *Oxidative stress detected in MDPC-23 after contact with extracts produced by the application of 35% hydrogen peroxidase gel on unaltered or pigmented enamel. Unbleached enamel was used as control. Columns represent means, and error bars indicate standard deviations (n=6). There was interaction between the factors (*p*=0.020); therefore, columns identified with distinct letters are statistically different.*

Quantification of Hydrogen Peroxide Diffusion (H_2O_2 μg/mL)

A high level of trans-enamel and trans-dentin diffusion of H_2O_2 occurred in both groups in which the discs were bleached with gel containing 35% H_2O_2 (G3 and G4). However, this diffusion of H_2O_2 through the hard-dental tissues was significantly lower for the discs that were pigmented and then bleached (G4) in comparison with the untreated bleached discs (G3) ($p<0.05$). The volume of H_2O_2 that reached the pulp space was 6.68 and 4.74 μg/mL for G3 and G4, respectively (Figure 5).

DISCUSSION

The chromophores that may accumulate in tooth hard tissues are organic structures formed by aromatic compounds with the presence of amino acids.[23] Among them, tyrosine, tryptophan, and phenylalanine are more expressive with regard to the excitation of fluorophores present in the tooth.[23] In this research, we sought to assess the influence of black tea–induced tooth pigmentation on the trans-enamel and trans-dentin diffusion and cytotoxicity of the components of a commercial bleaching gel with a high concentration of H_2O_2 (35%). For this purpose, enamel and dentin discs were submitted to a protocol of intrinsic pigmentation with black tea before the bleaching procedure. Previous studies demonstrated that performing a professional bleaching protocol (35% H_2O_2 gel, three times for 15 minutes) onto enamel/dentin discs with 3.5-mm thickness, simulating maxillary central incisors, dramatically reduced the dental human pulp cell viability and caused intense changes in cell morphology.[12,13,15,19] However, for cytotoxicity analyses, these *in vitro* methodologies did not use pigmented samples. To understand the influence of the presence of staining agents on H_2O_2-mediated trans-enamel and trans-dentin cytotoxicity, the enamel/dentin discs were stained with black tea before carrying out the bleaching procedure. This dye was chosen because it is widely used in the literature to evaluate in-office bleaching therapies.[12,13,15,19] In addition, organic pigments present polyphenolic compounds in their structural formulation that are responsible for color sedimentation when in contact with organic substrate.[11,24-28] Despite the limitations of the present investigation, in which only black tea was used for enamel/dentin pigmentation, one should be aware that this pigment is quite representative. This is because it has a similar constituent chemical nature characterized by the presence of polyphenolic compounds, which differ among each other according to the number of phenol units present in the molecule structure.[28]

The test used to evaluate the trans-enamel and trans-dentin diffusion of H_2O_2 demonstrated that, for the pigmented and unbleached teeth (G4), a reduction of approximately 29% in the quantity of

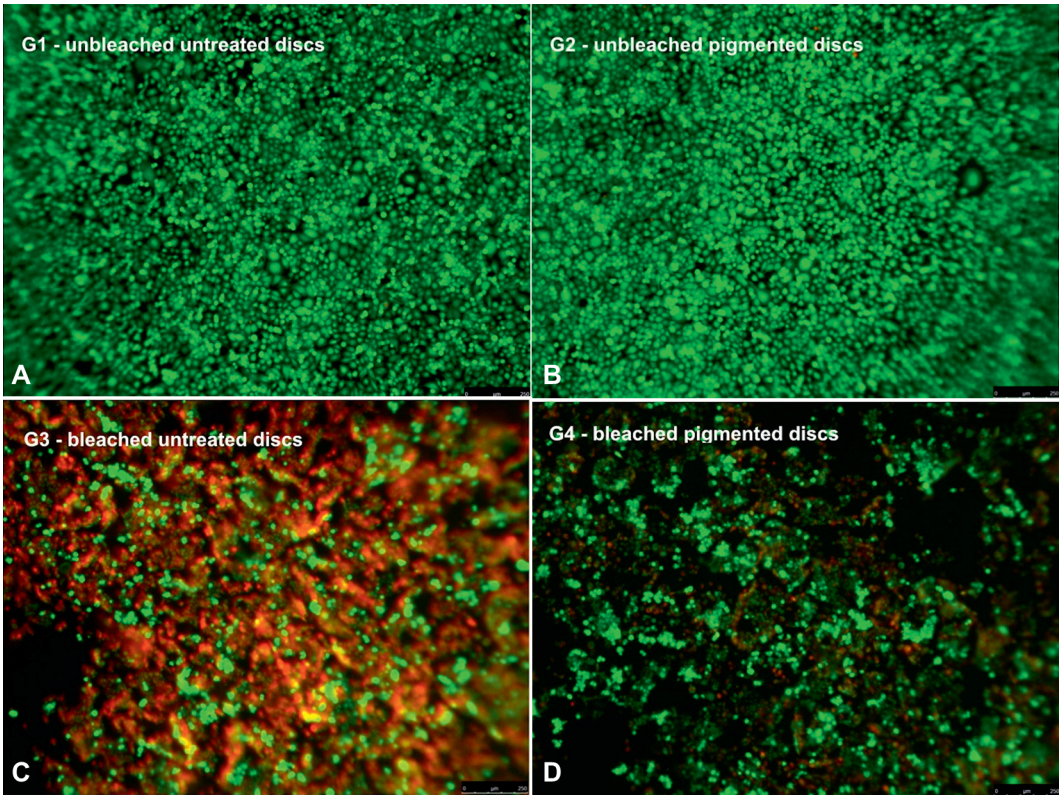


Figure 4. *Representative images of live/dead test for each group (A-D). When the cell membrane is ruptured, the EthD-1 marker penetrates into the cell and binds to the nucleotides, emitting fluorescence (red). This marker is incapable of crossing the whole cell membrane. The Calcein AM marker is capable of emitting fluorescence in cells with cytoplasmic esterase activity, irrespective of the cellular membrane condition (green).*

these diffused molecules occurred compared with values in the untreated bleached teeth (G3). These data corroborated the findings of the research of Moreira and others,[19] in which the authors observed

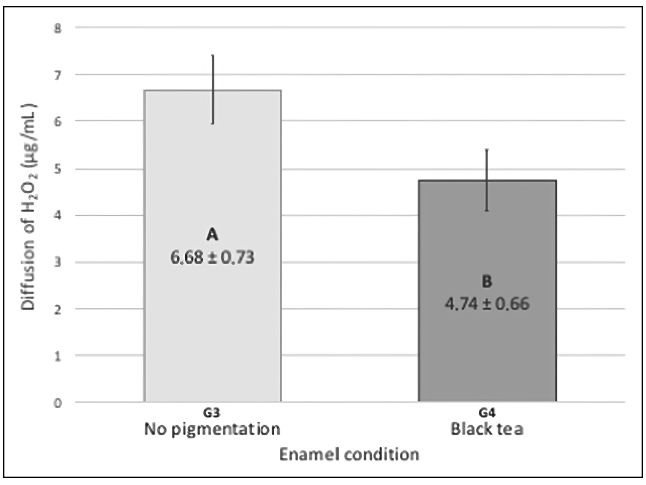


Figure 5. *Analysis of residual trans-enamel and trans-dentin H_2O_2: diffusion. Bar graph of mean values (numbers) and error bars indicate standard deviations of H_2O_2 (µg/mL) present on the extracts (n=6). Different letters show statistically significant difference among groups (Student's t-test;* $p<0.0001$).

that the presence of H_2O_2 that did not react with the pigments (residual H_2O_2) in dentin was approximately 6.25 µg/mL for untreated bleached discs and approximately 5.23 µg/mL for the pigmented bleached discs. In the study of Moreira and others,[19] the authors used bleaching gel with 38% H_2O_2 and observed trans-enamel and trans-dentin diffusion of this toxic molecule into dentin substrates that presented different intensities of staining. However, after the third bleaching session, the H_2O_2 diffusion values were statistically similar for all the groups. These data agree with the results obtained in the present research, because it was possible to demonstrate that the presence of pigmentation in the dental substrate significantly minimized the diffusion of residual H_2O_2, thus considerably reducing the cytotoxicity caused by the in-office bleaching procedure tested. Therefore, the stated hypothesis of this study was accepted.

The decrease in cellular cytotoxicity is directly related to the reduction in the rate of penetration of residual H_2O_2 diffused through enamel and dentin.[19] Therefore, we believe that the presence of chromophores of pigmentation in tooth substrates and their

interaction with H_2O_2 could limit the quantity of this molecule that would reach the pulp cells. However, the toxic effect of H_2O_2 may be of higher or lower intensity, varying according to the organic structure of the chromophore on which it is applied. In this research, the pigmentation agent used was black tea, which has antioxidant properties that make it highly reactive to peroxides, consuming a larger quantity of these molecules in its transit to the pulp space. This coloring agent has been widely used in laboratory research to evaluate the bleaching efficacy and cytotoxicity of gels containing H_2O_2.[12,14,15,19] Breakdown of the molecular structure of chromophores derived from black tea occurs gradually, so that part of the unreacted H_2O_2 remains free to diffuse through tooth substrates, as previously observed in colorimetric analyses.[12,14,15,19] This fact may explain the reduction in immediate cytotoxicity observed in this study, after we performed the bleaching procedure in pigmented discs (G4) in comparison with untreated bleached discs (G4). As reported by Sulieman,[30] the inorganic matrix present in many of the chromophores leads to them being trapped within the crystalline structure of the interprismatic region of enamel, which demands the local action of molecules with elevated oxidation potential to cause their fragmentation. As demonstrated in the literature, the decomposition of H_2O_2 into different ROS with higher oxidation potential is relatively slow when H_2O_2 is not catalyzed.[31,32] This makes reduction of the chroma (scale that refers to the quantity of saturation of the hue) occur in a gradual manner.[12,15,21] These data may perhaps explain, even if only partially, the fact that the in-office bleaching procedure assessed in this study had also caused an important cytotoxic effect, even when the procedure was used in teeth pigmented with black tea. We may suggest that the excess H_2O_2 and its derivatives that did not interact with the pigmentation chromophores present in dental hard tissues ended up diffusing through this tubular substrate to cause damage to the pulp cells, such as we observed in the live/dead test.

In a recent study, Guo and others,[23] validated the hypothesis that the rupture of organic chains, particularly those formed of aromatic compounds, arise from the process of oxidation promoted by H_2O_2 and its decomposition byproducts. These oxidant agents, which are in a state of electronic instability, act directly on aromatic rings or in unsaturated linear carbon chains, reducing the rate of light absorption.[33] It is important to point out that change in the color of the tooth structure results from changes in the properties of light reflection, which are directly related to the incident light.[34] For this reason, it has been defined that the perception of light teeth after bleaching therapy is the result of the association of local chemical and physical phenomena.[9] As the tooth may present different bands of frequency in the emission of fluorescence within the visible light spectrum,[23] it is thus suggested that the behavior of H_2O_2 may be muted by the chromophore on which this molecule acts. However, the aspects involving dental staining are complex because they may have intrinsic or extrinsic origin or even a combination of both,[30] and this is a factor to be considered during bleaching therapies. Furthermore, there is a point of saturation in the dental structure that limits the bleaching potential of H_2O_2 and consequently the tooth bleaching efficacy.[14-16] Therefore, one may suggest that, for each level of chromatic saturation of tooth, there is an ideal concentration of H_2O_2 in the gel and an adequate time of application of the product on enamel to get the best esthetic outcome and to prevent the diffusion of high concentrations of this molecule capable of damaging pulp cells. In this way, dental bleaching appears to be a clinical procedure that should be increasingly individualized, so that the diffusion of residual H_2O_2 through dentin can be limited, preventing the negative side effects of this esthetic therapy.

As previously demonstrated in the literature,[35] excess intracellular H_2O_2 interferes in the transcription factor peroxisome proliferator-activated receptor γ (PPARγ), which acts in the removal of ROS during the inflammatory process, limiting the regenerative potential of tissues.[35] In these conditions, the expression of odontoblast differentiation markers, such as alkaline phosphatase (ALP), dentin sialophospho-protein (DSPP), dentin matrix phosphoprotein-1 (DMP-1), and deposition of mineralization nodules are intensely affected, which may harm the regeneration of injured tissues.[13-16] In the present research, we demonstrated the occurrence of pulp cell death due to necrosis caused by the intense cellular oxidative stress. We observed that the MDPC-23 cells were positively stained with the Eth-1 probe, which binds to the DNA of cells with disrupted membranes. Elevated concentrations of toxic reactive species result in direct cell death by necrosis[36]; in live tissues such as the pulp, this may cause the release of large quantities of intracellular components, triggering an acute local inflammatory reaction either associated with the formation of broad areas of necrosis or not.[2,37-39]

In general, in the present laboratory study, it was possible to demonstrate that the presence of pigmentation in the tooth could reduce the diffusion of residual H_2O_2, and consequently, the trans-enamel and trans-dentin cytotoxicity of this reactive molecule. The enamel/dentin discs stained with black tea that were used in the present investigation to simulate human maxillary central incisors were obtained from bovine incisors. Because the bovine teeth have been considered adequate to replace human teeth,[40-45] a number of researchers have used this model to perform laboratory studies.[12-16] However, based on the fact that human teeth are more permeable to H_2O_2 than bovine teeth,[40] one may suggest that the diffusion of this reactive oxygen species (ROS) through enamel/dentin and its cytotoxicity to pulp cells might be more intense for human teeth.

However, the data obtained in this investigation must be interpreted with caution, because the reduction in cytotoxicity did not occur with sufficient intensity to consider the tested bleaching treatment harmless to odontoblast cells. On the other hand, it could be inferred that the bleaching treatment in teeth with little chromatic saturation must be performed with mild doses, using products with low concentrations of peroxides. Furthermore, we emphasize that, for this study, only an experimental bleaching protocol was used, and this was the case for the intrinsic pigmentation protocol as well. Therefore, future studies must be conducted to investigate the different types of chromophores present in the dental substrate, as well as other bleaching therapies. Nevertheless, this study allowed for a greater biological understanding of the interrelations between pigmentation chromophores and H_2O_2, in a manner to allow collaboration with studies in the area of bleaching, particularly for refining laboratory studies in this field of esthetic dentistry.

CONCLUSION

It was concluded that, according to the methodology used in this investigation, the presence of pigments in hard tooth structures decreases the trans-enamel and trans-dentin diffusion of H_2O_2, as well as the toxicity to pulp cells of an in-office bleaching gel with 35% H_2O_2.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the research ethics oversight committee guidelines and policies of the Univ Estadual Paulista–UNESP, Araraquara School of Dentistry.

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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