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Effect of Different Light Sources and Enamel Preconditioning on Color Change, H_2O_2 Penetration, and Cytotoxicity in Bleached Teeth

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Clinical Relevance

The use of a light activation source or enamel preconditioning did not affect the outcome of in-office bleaching with 35% hydrogen peroxide. However, the bleaching gel was capable of diffusing through enamel and dentin, causing toxic effects to the pulp cells.

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SUMMARY

This study evaluated the effects of acid etching of the enamel and the combination of different light sources (halogen light, light-emitting diodes [LEDs], and LED/Laser) and the bleaching product on color change, penetration of hydrogen peroxide (H_2O_2), and cytotoxicity over time. The color change (ΔE) and the amount of H_2O_2 that permeated the tooth tissue were analyzed using a spectrophotometer. Cell metabolism and morphology were evaluated using the methylthiazol tetrazolium assay and scanning electron microscopy, respectively. The ΔE values and H_2O_2 permeation were not significantly different under any of the experimental conditions. Tooth whitening significantly reduced cell metabolism, regardless of whether a light source was used. Preconditioning the enamel did not influence the cellular metabolism in any group. In conclusion, combining the bleaching product with different light sources and/or preconditioning the enamel resulted in few significant changes in color, transenamel and transdentinal pene-

tration of H_2O_2, or cytotoxicity and cell morphology.

INTRODUCTION

In order to accelerate and increase the effectiveness of whitening treatment, products with high concentrations of hydrogen peroxide (H_2O_2) have been developed. These products are used with different light sources, such as halogen lights, light-emitting diodes (LEDs), and lasers.[1,2]

The inclusion of these light sources is based on the assumption that the light projected on a whitening product is absorbed and is partially converted into heat, which increases the release of reactive oxygen species and thereby the effectiveness of the technique. Thus, the heat generated by the light source acts as a catalyst in the degradation of the bleaching product, facilitating its diffusion through the dental structure.[3,4]

However, some authors[5-8] have questioned whether these light sources actually catalyze the oxidation-reduction reactions and consequently improve these techniques or whether these claims are simply a marketing strategy designed to sell equipment.

Recently another clinical procedure has been developed to increase the effectiveness of the whitening treatment, and this procedure has yielded promising results. Acid etching of the enamel was initially recommended in the early 1970s[9] for teeth with stains caused by tetracycline, and it is based on the superficial histological changes caused by the action of phosphoric acid. Removal of the aprismatic layer and the increase in surface porosities could theoretically increase the permeability of the substrate for the application of the bleaching agent, thereby optimizing the clinical results.[9-11]

However, despite the esthetic success of this technique, some aspects related to the biological safety of bleaching have been questioned. Several clinical studies have shown that human teeth treated with H_2O_2 exhibit high rates of sensitivity[12-17] and show evidence of pulpal damage.[18,19]

Thus, it can be inferred that although the success of the treatment is directly related to the ability of the bleaching substance to penetrate into the dentinal tubules and react with the darkened molecules,[1] the excessive penetration of the peroxide into the complex pulp-dentin tissue is concerning because it can cause injury in the pulpal tissue.[20]

In this context, the aim of this study was to evaluate the effect of different light sources and acid preconditioning of the enamel on color change and transenamel and transdentinal penetration of H_2O_2 and the response of the pulp cells over time when enamel/dentin discs were subjected to a bleaching procedure with a 35% H_2O_2-based product.

Table 1: *Samples Distribution According to the Treatments*

Groups	Treatments
G1	HP
G2	HP + halogen light
G3	HP + LED
G4	HP + LED/Laser
G5	Ac. + HP
G6	Ac. + HP + halogen light
G7	Ac. + HP + LED
G8	Ac. + HP + LED/Laser

Abbreviations: Ac., 37% phosphoric acid gel; HP, 35% hydrogen peroxide (H_2O_2)-based product; LED, light-emitting diode.

METHODS AND MATERIALS

Specimen Collection and Standardization

The experimental units (enamel/dentin discs) were obtained from bovine incisors.[21] The 5.7 mm-diameter discs were obtained from the middle third of the buccal surface of the teeth.

The dentin surfaces of the discs were regularized using manual rotary motion with aluminum oxide sandpapers of different grades (400 and 600 grit) (T469-SF-Noton, Saint-Gobam Abrasives Ltda, Jundiai, SP, Brazil) until they had a thickness of 3.5 mm (1.3 mm of enamel and 2.2 mm of dentin, ±0.2 mm). To remove the smear layer, a 0.5 M ethylenediamine tetraacetic acid solution, pH 7.2, was applied for 30 seconds; this step was followed by washing with deionized water.

The selected enamel/dentin discs were divided into eight groups as shown in Table 1.

Treatments

Bleaching Treatment—Bleaching was performed with a H_2O_2-based product (35% Whiteness HP Maxx, FGM Dental Products, Joinville, SC, Brazil). The procedure was performed according to the manufacturer's recommendations, and the final product was inserted into a disposable, graduated, 1 mL syringe. Each specimen was treated with 0.04 mL of the bleaching product, which remained in contact with the dental tissue for 45 minutes (three applications of 15 minutes each) during each whitening session. The procedure was repeated

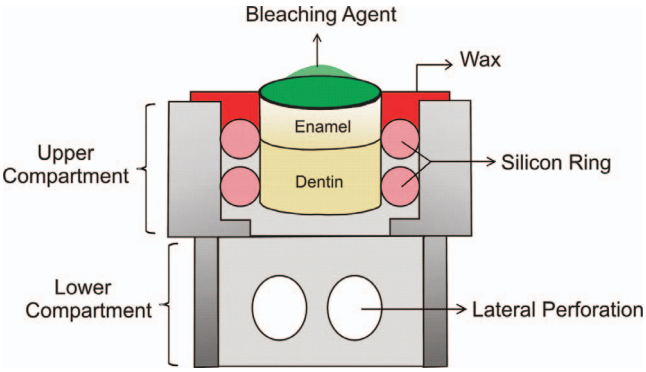

Figure 1. *Artificial pulp chamber.*

three times, with a one-week interval between sessions. The specimens were stored in artificial saliva at 37°C between bleaching sessions.

Light Sources and Acid Conditioning—Irradiation with a halogen lamp was conducted for 20 seconds at each application at a distance of 5 mm from the surface. The Ultralux lamp (Dabi Atlante, Ribeirão Preto, SP, Brazil), which has a light intensity of 500 mW/cm^2 and a wavelength between 450 and 500 nm, was used for this purpose.

LED irradiation was performed using the DB 686 Wireless (Dabi Atlante), which has a light intensity of 500 mW/cm^2 and a wavelength between 440 and 480 nm. Irradiation was conducted for 30 seconds (three applications of 30 s/session) at 5 mm from the enamel upon each reapplication of bleaching gel.

For irradiation of the whitening gel using a LED/Laser, the Whitening Lase II (DMC Equipment Ltda, São Carlos, SP, Brazil) was used. This equipment consists of six LEDs with an intensity of 120 mW/cm^2 and a wavelength of 470 nm and three infrared lasers with a wavelength of 808 nm and a power of 0.2 W. The product was irradiated for three minutes upon each application of bleaching gel (three applications of 3 min/session).

For the groups in which the enamel was conditioned before the whitening procedures, a 37% phosphoric acid gel (Condac 37, MGF Joinville, SC, Brazil) was applied for 30 seconds. The sample was subsequently washed with distilled water and was then dried.

Color Analysis—The selected 96 enamel/dentin discs were divided into eight groups (n=12) and the color analyzed using a reflection ultraviolet-visible spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan) before the first bleaching treatment, six days after each bleaching session, and 14 days after the end of the treatment period. Color evaluation was performed three times for each specimen at each analysis period and the mean values obtained for the evaluated area were subjected to statistical analysis. The CIE L*a*b* color model established by the Comission Internationale l'Eclairage (International Commission on Illumination), which allows the specification of color perception in three-dimensional models, was used. Analyses were performed on the buccal surface of the discs, and these values were compared to the initial reading. The data were submitted to analysis of variance (ANOVA) for repeated analysis (three-way repeated-measures ANOVA) with Scheffe test, with significance accepted at 5%.

Transenamel and Transdentinal Penetration of H_2O_2—To quantitate the amount of H_2O_2 that permeated the dental tissues, 96 enamel/dentin discs (n=12) were assembled in artificial pulp chambers (APCs)[22-27] (Figure 1). Acetate buffer solution (2 mL) was placed inside the APCs. From this moment on, the dentin surface remained in contact with this solution, and the H_2O_2 that diffused through the tooth structure reached the acetate buffer solution and had become part of it. After bleaching, leucocrystal violet dye (0.5 mg/mL; Sigma Chemical Co, St Louis, MO, USA) and peroxidase (1 mg/mL; Sigma) were mixed with 1 mL of the solution (acetate buffer).

This method, which was recommended by Mottola and others,[28] is based on the reaction of H_2O_2 with leucocrystal violet catalyzed by peroxidase. The color intensity of this mixture changes according to the amount of peroxide present. Thus, since the absorbance signal is proportional to the concentration of H_2O_2, it is possible to assess the amount of peroxide that permeated from the enamel surface into the solution contained in the APC. A standard curve of known H_2O_2 concentrations was used for conversion of the optical density obtained in the samples into micrograms of H_2O_2 per milliliter of acetate buffer solution, which represents the total H_2O_2 capable of diffusing through enamel and dentin discs.

The solutions were collected at four different times, as follows: T0, before applying the bleaching gel; T1, 30 minutes after the first bleaching session; T2, 30 minutes after the second bleaching session; and T3, 30 minutes after the third bleaching session.

The Friedman test was used at 5% to analyze the performance of each group over time, and the Kruskal-Wallis test at 5% was used to analyze the performances of the different light sources and pre-etching.

Culture of MDPC-23 Cells—The immortalized odontoblast-like cell line, MDPC-23, was cultured

in plastic bottles (75 cm^2; Costar Corp, Cambridge, MA, USA) in Dulbecco's Modified Eagle's Medium (DMEM, Sigma) supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY, USA), 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L glutamine (GIBCO). The cells were maintained in a humidified atmosphere at 37°C with 5% CO_2 and 95% air. To perform the cytotoxicity assay, 30,000 cells/cm^2 were seeded in each well of sterile 24-well plates (Costar Corp), and the cells were maintained in a humidified incubator with 5% CO_2 and 95% air at 37°C for 48 hours.

Cytotoxicity Evaluation—To evaluate cell toxicity, APCs with the discs in position were sterilized and were placed individually into 24-well plates containing 1 mL of culture medium without fetal bovine serum. Only the dentin surface remained in contact with the culture medium, and the enamel surface received the bleaching gel, as described above. After the specified time had elapsed, the bleaching gel was aspirated and the enamel surface was washed thoroughly with deionized water with concomitant aspiration. After 30 minutes, 500 μL of the extract (culture medium + the components of the bleaching gel that had permeated through the enamel/dentin disc) were collected and were applied to previously cultured odontoblast-like cells (MDPC-23). The cells were maintained in a humidified incubator at 5% CO_2 and 95% air at 37°C for one hour. Next, cell metabolism was analyzed using the methylthiazol tetrazolium (MTT) assay according to ISO 10993-5.[29]

Cell Metabolism Analysis (MTT Assay)—In each group, 10 out of 12 wells were used to analyze cell metabolism (n=10) by cytochemical demonstration of succinic dehydrogenase, which represents the mitochondrial respiration rate of the cells.[30] The cellular metabolic activity was analyzed using the MTT assay.[29] For this protocol, two additional groups were used as controls. In one of them, APC/disc sets were placed in contact with 1 mL of DMEM and no treatment was performed on the enamel surface. Next, the culture medium adjacent to the dentin surface was applied to the cultured MDPC-23 cells; the main absorbance of this group was considered to be 100% of cell metabolism, and the percentage for all samples was calculated based on this parameter. APC/disc sets subjected only to enamel acid conditioning were also used as a control group in order to evaluate if this procedure would interfere with cell metabolism parameters.

For this step, the extracts were aspirated and the cells were washed with 1 mL of phosphate buffered saline (PBS). Then, 900 μL of culture medium (DMEM) containing 100 mL of MTT solution (5 mg/mL; Sigma) was added to the cells. After four hours of contact with the cells, the MTT solution and the DMEM were carefully aspirated and were replaced with 600 μL of acidified isopropanol solution (0.04 N HCl) in each well. Three 100-μL aliquots of each well were transferred to 96-well plates (Costar Corp). Cell viability was evaluated using spectrophotometry at 570 nm wavelength with an enzyme-linked immunosorbent assay microplate reader (model 3550-UV, Bio-Rad Laboratories, Hercules, CA, USA). Two-way ANOVA with Tukey test at 5% was used to evaluate the influence of different light sources as well as the influence of acid etching on cellular metabolism.

Cell Morphology Analysis—Two samples from each group (n=2) were prepared to assess cell morphology using scanning electron microscopy (SEM). For this purpose, sterilized 12-mm-diameter cover glasses (Fisher Scientific) were placed at the bottom of the wells of sterile 24-well plates immediately before seeding of MDPC-23 cells.[31] After the incubation period, the extracts that were in contact with the cells were aspirated, and the MDPC-23 cells that remained adhered to the glass substrate (GS) were fixed in 1 mL of 2.5% buffered glutaraldehyde for one hour. These cells were subsequently rinsed three times with 1 mL PBS (five minutes per rinse), postfixed in 1% osmium tetroxide for 60 minutes, and processed for examination with SEM (JEOL-JMS-T33A Scanning Microscope, Peabody, MA, USA). The samples obtained in bleached groups were analyzed for cell density, cytoplasm shrinkage, and the presence of cell fragments on glass slides in order to determine the toxicity of the bleaching protocols to the MDPC-23 cells. These parameters were compared to those observed in the samples of the negative control group (APC/disc set with no treatment on enamel).[22-26,32]

RESULTS

Color Analysis

The average ΔE values are shown in Table 2. In all groups, a progressive and continuous change in color change (ΔE) values was observed with the bleaching treatment (the capital letters in the columns). The use or lack of use of different light sources did not influence the color change obtained at the different analysis periods ($p>0.05$) (lowercase in the lines). No statistical difference was observed when the techniques were compared under conditions "with" and "without" acid etching ($p>0.05$) (^{lowercase}overwritten).

Table 2: *The Average Values (Standard Deviation [SD]) of Color Change (ΔE) in Bleached Teeth with the Use of Different Light Sources and Under Conditions "With" and "Without" Acid Etching on the Enamel*[a]

Time	Without Light	Halogen Light	LED	LED/Laser
Without acid etching				
T0	0.00 (0.00) C a[a]	0.00 (0.00) D a[a]	0.00 (0.00) D a[a]	0.00 (0.00) C a[a]
T1	5.08 (2.61) B a[a]	3.92 (1.43) C a[a]	3.52 (1.47) C a[a]	3.98 (1.34) B a[a]
T2	6.29 (2.83) A a[a]	5.32 (1.62) B a[a]	5.33 (1.57) B a[a]	5.87 (1.23) A a[a]
T3	6.73 (2.97) A a[a]	5.96 (1.50) A a[a]	6.80 (1.73) A a[a]	6.07 (1.03) A a[a]
With acid etching				
T0	0.00 (0.00) D a[a]	0.00 (0.00) D a[a]	0.00 (0.00) D a[a]	0.00 (0.00) D a[a]
T1	5.26 (1.87) C a[a]	4.16 (1.95) C a[a]	4.81 (3.47) C a[a]	4.31 (1.98) C a[a]
T2	7.19 (2.51) B a[a]	6.22 (2.42) B a[a]	6.68 (3.94) B a[a]	5.79 (2.41) B a[a]
T3	7.98 (2.30) A a[a]	7.09 (2.07) A a[a]	8.01 (3.45) A a[a]	7.79 (2.22) A a[a]

Abbreviations: LED, light-emitting diode; T0, before applying the bleaching gel; T1, 30 minutes after the first bleaching session; T2, 30 minutes after the second bleaching session; and T3, 30 minutes after the third bleaching session.
[a] The different letters (capital in the columns, lowercase in lines, and superscript for the comparison between sessions of the groups with or without the use of acid) indicate statistically significant differences according to Scheffe test (p<0.05).

Transenamel and Transdentinal Penetration of H_2O_2

The mean values of H_2O_2 that permeated through the dental tissue are shown in Table 3. In all groups, similar penetration occurred at T1, T2, and T3, and these values were statistically different from those observed at T0 (uppercase in the columns).

The analysis of the influence of different light sources (lowercase in the lines) on the permeation of H_2O_2 revealed that between the groups that did not undergo pre-etching, the group in which the bleaching gel was combined with the LED/Laser showed the highest values at T1 ($p<0.05$); similar results were observed for the other groups ($p>0.05$). At T2, the lowest average was observed in the group in which the halogen light was used ($p<0.05$). In the groups in which the enamel had been previously etched with 37% phosphoric acid, only the association with the LED and LED/Laser light increased the penetration of peroxide at T1 with respect to those without light.

When comparing the conditions "with" and "without" acid etching ($^{\text{superscript}}$overwritten letters), it was observed that pretreatment of the substrate with phosphoric acid favored the penetration of peroxide only in T1 (bleaching associated with LED) and in T2 (bleaching associated with halogen light). No statistically significant difference was observed for the other times and groups ($p>0.05$).

Table 3: *The Mean (Standard Deviation [SD]) Peroxide Concentration (μg/mL) that Permeated Through the Dental Tissues After Bleaching Treatment, According to the Different Times and Treatments*[a]

Time	Without Light	Halogen Light	LED	LED/Laser
Without acid etching				
T0	−0.42 (0.02) B a[a]	−0.42 (0.01) B a[a]	−0.41 (0.04) B a[a]	−0.41 (0.01) B a[a]
T1	4.66 (0.52) A b[a]	4.51 (0.66) A b[a]	4.65 (0.36) A b[b]	5.43 (0.50) A a[a]
T2	5.41 (0.15) A a[a]	5.11 (0.18) A b[b]	5.44 (0.15) A a[a]	5.38 (0.27) A a[a]
T3	5.09 (0.31) A a[a]	5.11 (0.15) A a[a]	5.10 (0.18) A a[a]	4.94 (0.22) A a[a]
With acid etching				
T0	−0.41 (0.03) B a[a]	−0.42 (0.05) B a[a]	−0.42 (0.02) B a[a]	−0.43 (0.03) B a[a]
T1	4.56 (0.46) A b[a]	4.36 (0.38) A b[a]	5.24 (0.49) A a[a]	5.48 (0.46) A a[a]
T2	5.40 (0.32) A ab[a]	5.42 (0.13) A ab[a]	5.35 (0.29) A b[a]	5.60 (0.21) A a[a]
T3	5.14 (0.34) A a[a]	5.14 (0.15) A a[a]	5.11 (0.20) A a[a]	5.21 (0.14) A a[a]

Abbreviations: LED, light-emitting diode; T0, before applying the bleaching gel; T1, 30 minutes after the first bleaching session; T2, 30 minutes after the second bleaching session; and T3, 30 minutes after the third bleaching session.
[a] The different letters (uppercase in the columns [Friedman test], lowercase in the lines, and superscript for comparing the conditions "with" and "without" acid etching on enamel [Kruskal-Wallis]) indicate statistically significant differences (p<0.05).

Table 4: *Mean (Standard Deviation [SD]) of the Metabolism of Odontoblast Cells MDPC-23 (%) Subjected to the Bleaching Treatment Combined with the Use of Different Light Sources and Prior Acid Etching of the Enamel*[a]

Control	Without Light	Halogen Light	LED	LED/Laser
Without acid etching				
100.29 (3.13) Aa	63.72 (1.95) Ab	57.22 (4.55) Ab	61.43 (6.04) Ab	59.72 (7.17) Ab
With acid etching				
101.21 (4.68) Aa	57.11 (13.84) Abc	49.87 (9.03) Ac	62.91 (6.94) Ab	45.98 (3.15) Bc

Abbreviation: LED, light-emitting diode.
[a] *The different letters indicate difference statistically significant differences (uppercase in the columns and lowercase in the lines [Tukey test],* $p<0.05$).

Cell Metabolism (MTT Assay)

The cell metabolism results obtained from the MTT assay are presented in Table 4. When the conditions "with" and "without" acid etching were compared (uppercase in the columns), a statistically significant difference was observed only when the whitening gel was combined with the LED/Laser ($p<0.05$), whereas pretreating the enamel surface led to a decrease in odontoblast cell metabolism. It is also observed that APC/disc sets subjected only to enamel acid conditioning (control group) did not interfere with cell metabolism parameters. In the comparison among the groups (lowercase in the lines), no significant difference was observed between those that received or did not receive the different light sources; however, a significant reduction of cell metabolism (compared with the control group) was found in all cases ($p<0.05$).

Cell Morphology (SEM Analysis)

In the control group, which lacked bleaching treatment, a large number of cells covered the entire surface of the GS. These pulp cells were fully confluent, with regions of apparent mitosis (asterisk) (Figure 2A). Conversely, regardless of whether light was utilized or pre-etching of the enamel was performed, the bleaching treatment resulted in significant cellular changes (Figure 2B,C). These changes were characterized by cell death, followed by detachment from the GS. The few remaining cells showed significant morphological changes, presenting rounded, thin, and short cytoplasmic extensions (arrow). The remnants of the cytoplasmic membranes of cells that underwent cell death were apparent on the GS (circle).

DISCUSSION

Although tooth whitening is considered a conservative treatment,[33,34] concerns exist regarding its biological safety, especially when highly concentrated products are employed.[18,19,26,35-37] The use of peroxide in high concentrations (35%-38%) produces visible results after the first bleaching session, and this fact has been the main source of appeal for the increase in the use of this technique.[38-40]

In order to accelerate and increase the effectiveness of bleaching, these products have been combined with different light sources[1,3,8] as well as with pre-etching of the enamel.[11,41] The light sources are recommended as "peroxide activators," whereupon part of the energy that results when the light is focused on the bleaching gel is presumably absorbed and is converted into heat;[4] thus, the use of the light would accelerate the decomposition of peroxide hydrogen. Further, in order to increase light absorption, some bleaching products are mixed with specific dyes such as carotene (photosensitive). The orange-red color of the carotene increases the absorption of light,[4] thereby apparently increasing the effectiveness of the bleaching treatment.[41]

In the present study, the ΔE values obtained ranged between 3.52 and 8.01, indicating that the different protocols that were utilized resulted in a high degree of color change in the enamel/dentin discs. These data show that the bleaching treatment with 35% H_2O_2 was effective for tooth whitening regardless of the use of light. Similar observations were reported in several laboratory and clinical studies.[8,42,43]

On the other hand, the results of this study contradict those obtained by Calatayud and others,[44] who reported that the use of light sources favors the achievement of greater color changes. However, it is noteworthy that Calatayud and others performed the color analysis immediately after the bleaching procedure; therefore, this analysis was influenced by the dehydration caused by surgical isolation, the osmotic power of the bleaching gels, and the heat created by the lights.[42,45] To minimize these influences, the color analysis was performed six days after each bleaching session in this study.

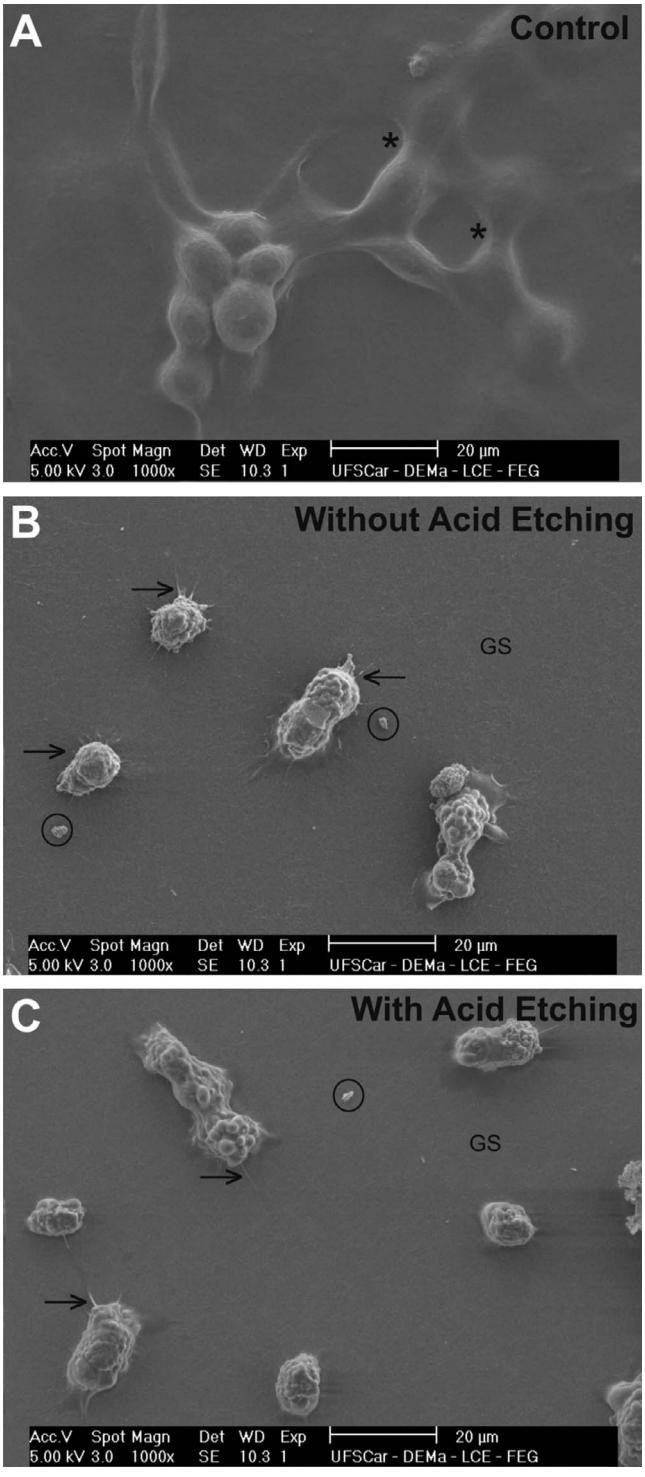

Figure 2. *Representative photomicrographs of cell morphology (1000×). (A) Control; a large number of MDPC-23 cells cover the entire surface of the GS, with apparent cell mitosis (asterisk). (B) Representative image of the groups that were not pretreated with acid etching on the enamel. (C) Representative image of the groups that were pretreated with acid etching on the enamel.*

Similar to the findings of Kanematsu and others,[41] it was observed that pre-etching the enamel using 37% phosphoric acid did not accelerate or increase the effectiveness of the bleaching techniques studied. It is known that phosphoric acid decreases the amounts of calcium and phosphate present in the enamel surface, and this decrease is even greater when the enamel is exposed to bleaching products shortly thereafter.[46] Thus, according to Li and others,[47] the high initial luminance (L*) values are directly related to dehydration and mineral loss, and the regression of these values is related to the process of rehydration and remineralization of this tissue.

Despite these considerations, given the recent questions about the safety of bleaching treatment, a complete study of these techniques should not be limited to the analysis of the color changes that occur in the teeth. Currently, the safety of this treatment is disputed because of the high levels of H_2O_2 detected in the pulp chamber. To address this issue, this study quantified the permeation of H_2O_2 toward the pulp chamber as well as the metabolic rate and morphology of the odontoblast-like cell line (MDPC-23) after different protocols were performed.

In this context, it is determined that H_2O_2 can penetrate the tooth structure and reach the pulp chamber, as observed when the later times were compared to time T0. In general, a pronounced increase in the amount of peroxide was not observed in the pulp chamber when pre-etching of the enamel was performed and different light sources were applied. These results are similar to those of Kwon and others,[3] who did not observe an increase in the amount of peroxide in the groups when bleaching was combined with a light source.

Regarding cytotoxicity, the components of the bleaching agent caused significant toxic effects to the pulp cells (MDPC-23). Nevertheless, in general, the use of different light sources as well as the use of pre-etching on enamel did not influence the cellular metabolism, except in the group in which the whitening gel was combined with the LED/Laser. In this group, a greater reduction of cell metabolism was observed when pre-etching was performed on the enamel. In this context, although the difference was not statistically significant when peroxide penetration was compared between the techniques "with" or "without" pre-etching of enamel, an increase in peroxide penetration was detected at all measured times, and this may have resulted in the reduction in cell metabolism.

Trindade and others[26] demonstrated severe damage to odontoblast cells after 3 × 15-minute applications of 35% H_2O_2, with a 92% reduction in MDPC-23 cell metabolism. In contrast, the mean general reduction of cell metabolism was approximately 43.33% in the present study. This lower percentage of cellular metabolism reduction can be attributed to the reduced contact time with the cell extract (one hour) when compared to that of the previous study (24 hours). The results of this study are similar to those reported by Soares and others.[32] The SEM images revealed numerous dead cells or cells displaced from the GS in all experimental groups that received the bleaching agent. The few cells that remained showed significant morphological changes, suggesting that the whitening procedure should be studied so that the best posology can be indicated for each individual case.

The results of this study cannot be directly extrapolated to a clinical situation because this is an *in vitro* study and it is known that human teeth have higher permeability compared to bovine teeth.[48] Furthermore, the presence of saliva, intrapulpal pressure, and odontoblast extensions can reduce the permeation of H_2O_2.[49-53] Moreover, the lymphatic system and antioxidants that are present in the pulp tissue protect pulp cells against external aggressions.[52,54,55]

However, the data obtained in this study suggest that the bleaching product can induce toxic effects in cells and pulp tissue. In this context, the concerns regarding the combination of bleaching products with different light sources, as well as acid etching of the enamel, appear to be negligible for both color change and peroxide penetration because the weakness of the technique is the biological safety of the bleaching procedure and not the resources employed to enhance its effectiveness. However, future studies should be conducted to elucidate the cellular mechanisms involved in the biological response to tooth whitening, which would eliminate or minimize these effects.

CONCLUSIONS

Under the experimental conditions of this study, the following conclusions can be made:

- The use of different light sources and prior acid etching of the enamel do not influence the change in tooth color obtained by bleaching;
- In general, the use of different light sources or acid etching prior to application of the whitening gel to the enamel does not influence H_2O_2 permeation through the dental tissues at the end of the treatment; and
- The application of 35% H_2O_2 to enamel/dentin discs promoted moderate toxic effects on pulp cells (MDPC-23); the presence of light or preconditioning of the enamel did not influence the cytotoxic effect.

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Regulatory Statement

This study was conducted at Aracatuba Dental School, UNESP–University Estadual Paulista in Brazil.

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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