ORIGINAL ARTICLE

# In vitro study on the color change of tooth enamel bleached with violet LED

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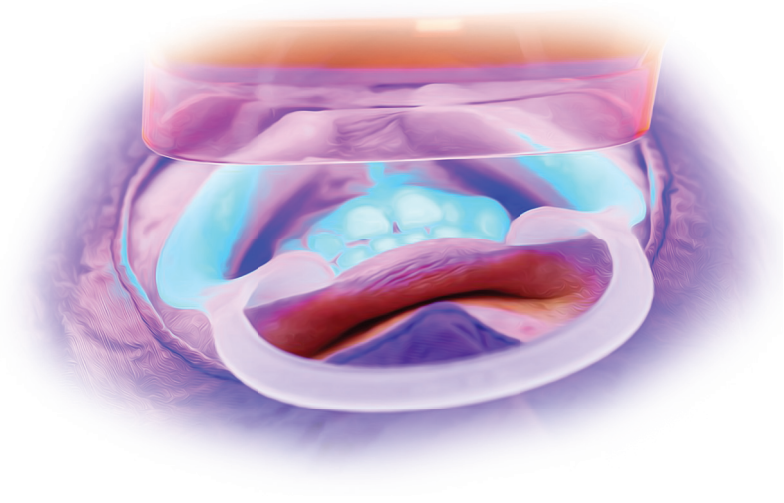

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## ABSTRACT

***Introduction:*** *Tooth bleaching is a procedure very requested in dental offices, stimulating constant research for new products and technologies. This study aimed to evaluated in vitro the color change of tooth enamel bleached with violet LED associated or not with hydrogen peroxide gel.* ***Methods:*** *48 fragments of bovine teeth (n=12), were sectioned (5x5x3 mm), flattened, and polished. The specimens were stained in 20ml of coffee, which was replaced every 24h for 7 days. The experimental groups were divided as follows: V-LED - Bleached with violet LED; HP - Bleached with 35% hydrogen peroxide (positive control); HP + V-LED - Bleached with 35% hydrogen peroxide + violet LED; and C - Not bleached (placebo gel) (negative control). The outcome variable was the analysis of color change, which was performed with a colorimetry spectrophotometer. Each specimen was read three times: 1. Before starting the immersion in staining solutions (baseline); 2. After coffee staining; and 3. After the treatments. The data were tabulated and analyzed statistically with analysis of variance (one-way ANOVA) and Tukey's test (*$\alpha$*=0.05).* ***Results:*** *The ΔE values showed significant difference between groups. HP + V-LED showed higher values of color change and C group lower. V-LED and HP were statistically similar to HP + V-LED and C.* ***Conclusion:*** *Bleaching with 35% hydrogen peroxide and violet LED might cause clinically visible color changes, regardless of the association of treatments, and the association of treatments enhances the effectiveness of the bleaching treatment.*

**Keywords:**

*Dental Esthetic. Hydrogen peroxide. Tooth bleaching.*

## INTRODUCTION

Among the several procedures aiming to improve self-image, tooth bleaching is one of the most requested in dental offices, which motivates the performance of studies with new products and technologies in the search for increasingly practical and safe procedures with excellent results.[1-6]

Currently, the most common forms of tooth bleaching involve at-home or in-office techniques with hydrogen peroxide or carbamide at different concentrations. Higher concentrations of the bleaching gel are indicated for in-office use (30 to 40% for hydrogen peroxide and 35 to 37% for carbamide peroxide). For at-home applications, lower concentrations are used aided by individual impression trays (3 to 9.5% for hydrogen peroxide and 10 to 22% for carbamide peroxide).

Different light sources (laser and LED) may be used in association with peroxides for in-office bleaching techniques,[1,4] aiming to aid the chemical reaction of hydrogen peroxide, increasing the release of oxygen and the formation of free radicals with higher kinetic energy, which increases the fragmentation of chromogenic molecules.[7]

Several studies confirm the effectiveness of tooth bleaching with the use of peroxide gels associated or not with light sources.[1,4,8,9] However, the use of violet LED has also been considered an alternative for bleaching techniques with a potentially good esthetic result,[3,5] minimizing the adverse effects of bleaching, such as roughness increase, bond strength reduction, and postoperative sensitivity, considering these factors are related to the use of gels (peroxides).

Different from the conventional method in which the techniques used require the application of peroxide gel, the violet LED would have sufficient energy to break the pigments through a physical process, in which the emission of violet light (405-410 nm) matches the absorption peak of the pigmented molecules.[2] Thus, this study aimed to assess in vitro the color change of tooth enamel bleached with violet LED associated or not with 35% hydrogen peroxide gel.

## MATERIALS AND METHODS

### Experimental design

The study was randomized and the sample included 48 fragments of bovine teeth (n=12). The experimental groups were divided as follows: V-LED - Bleached with violet LED; HP - Bleached with 35% hydrogen peroxide; HP + V-LED - Bleached with 35% hydrogen peroxide + violet LED; and C – Not bleached (placebo gel). The outcome variable was the analysis of color change, which was performed with the help of a colorimetry spectrophotometer.

### Sample collection

Forty-eight bovine incisors without any enamel change that could compromise analysis, such as cracks, hypoplasia, or hypomineralization, were selected. They were cleaned with the help of periodontal curettes and washed in deionized water. The sections were performed with diamond disc mounted in an electrical precision cutter (Isomet 1000; Buehler, Lake Bluff, IL, USA) in the middle third of each tooth, in order to obtain 5x5x3-mm fragments.

The fragments were flattened in a rotary polisher with water refrigeration (DP-9U2; Struers S/A, Copenhagen, Denmark) and abrasive files of #600 and #1200 granulations (Hermes Abrasives Ltd., VA, USA). They were polished with 0.3-µm alumina paste (Arotec S/A Ind. Com., São Paulo, Brazil) and polishing felt (ATM, Altenkirchen, Germany)[10]. After polishing, the specimens were cleaned in ultrasound for 5 minutes to remove surface debris.

### Staining of enamel fragments

The staining process of the enamel samples was performed with coffee solution (Melitta Extra Forte; Melitta do Brasil Ind. e Com. Ltda, São Paulo, Brazil) at the ratio of 300 mL of water to 6 g of powder. The samples were immersed individually in 20 mL of staining solution at 37°C, which was replaced every 24 h for 7 days. After enamel staining, the fragments were washed in deionized water for 1 minute and dried with absorbent paper.

### Bleaching of enamel fragments

Bleaching sessions were performed in each of the groups according to the following protocols:

**V-LED - Violet LED (Bright Max Whitening, MMO, São Carlos, SP, Brazil):** The light was applied 8 mm from the tooth surface for 60 seconds and turned off for a 30-second pause. This was repeated 20 times so that the total time of light delivery was 20 minutes and the total pause time was 10 minutes, resulting in a total time of the clinical session of 30 minutes. Eight sessions were performed (as recommended by the manufacturer) with a 7-day interval in between.

**HP - 35% hydrogen peroxide (Whiteness HP Blue, FGM, Joinville, SC, Brazil):** The hydrogen peroxide was applied covering the entire surface of enamel fragments for 40 minutes and the gel was moved every 10 minutes aided by a disposable applicator to release occasional oxygen bubbles produced and to restore the gel contact with the teeth. After bleaching, the gel was removed and the surface was washed with abundant deionized water. Three sessions were performed with a 7-day interval in between.

**HP + V-LED - 35% hydrogen peroxide (Whiteness HP Blue, FGM, Joinville, SC, Brazil) + violet LED (Bright Max Whitening, MMO, São Carlos, SP, Brazil):** The hydrogen peroxide was applied on the surface of enamel fragments as described for group HP, added by the association of violet LED application as described for group V-LED. Three sessions were performed with a 7-day interval in between.

**C - Placebo gel (control):** The placebo gel has the same composition of the bleaching gels, except for the addition of peroxides, and it was applied as described for the bleaching gels of group HP. Three sessions were performed with a 7-day interval in between.

Among the sessions and after finishing bleaching, the samples were maintained in deionized water immersion.

The performance of the bleaching treatments followed the safety rules required, such as the use of protective goggles for bleaching with violet LED.

## Color change analysis

The analyses were performed in a SP62S colorimetry spectrophotometer with the QA Master I software (X-Rite Incorporated-Neu-Isenburg, Germany), with focal aperture of 4 mm, spherical geometry measuring d/8° of illumination, observation angle of 10°, and wavelength between 400 and 700 nm.

For obtaining the color values, first the spectrophotometer was calibrated with the pure white and pure black standards. Next, each fragment was positioned on a white tile for standardizing the color readings. Each specimen was read three times: 1. Before starting the immersion in staining solutions and the bleaching procedures (baseline); 2. After coffee staining; and 3. After the treatments.

After assessing the color of each sample at the times determined, the device calculated ΔL*, Δa*, and Δb*, which are the variations of L*, a*, and b* values between baseline and the other analyses (staining and after bleaching). The ΔE* was calculated by the following formula:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

The L* refers to the luminosity coordinate (grey scale) with values from zero (black) to 100 (white). The values of a* and b* are the chromaticity coordinates in the red-green and yellow-blue axes, respectively. Positive a* values indicate deviation of chromaticity toward the red hue and negative values indicate deviation toward the green hue. Similarly, positive b* values indicate deviation toward the yellow hue and negative values indicate deviation toward the blue hue[11]. Thus, the a* and b* values are equal to zero at the intersection of axes, increasing toward red and yellow and decreasing toward blue and green[11]. The luminosity scale is located in the center, perpendicular to the a* and b* axes, to which zero corresponds to grey[12].

The data for color change analysis were assessed with the BioEstat 5.0 software. The Kolmogorov-Smirnov test was performed to verify data normality. Considering the data were parametric, one-way analysis of variance (ANOVA) was performed and the means were compared with Tukey's test (5%).

## RESULTS

In the analysis performed for group comparison at baseline and after staining, ANOVA showed that staining was similar for the specimens of all groups (ρ=0.2127) (Table 1).

**Table 1**: Mean (±SD) of ΔE of specimens that were initially analyzed (baseline) and later stained with coffee.

| GROUPS | ΔE | OVERALL MEAN |
|---|---|---|
| **V-LED** | 5.8130 (1.3530) | 5.5646 (ρ = 0.2127) |
| **HP** | 5.1976 (1.1304) | |
| **HP + V-LED** | 5.1848 (1.0655) | |
| **C** | 6.0628 (1.1455) | |

In the analysis for comparing the ΔE of specimens at the time of staining and after the treatments, Tukey's test showed higher values for HP + V-LED group and lower values for C (control), and the treated groups V-LED and HP were statistically similar to HP + V-LED and C groups (ρ=0.1385). HP + V-LED group differed significantly from the C group (p<0.01) (Table 2).

The comparison of ΔL among groups (difference between lighter and darker - luminosity) showed that all groups (regardless of bleaching treatment) differed significantly from the C group (Table 2).

The comparison of Δb among groups (difference between yellow and blue) showed that the C group presented values closest to yellow in the CIELab scale and that group HP + V-LED, which associated the treatments presented values farther from yellow (Table 2).

Table 2: Mean (±SD) of ΔE, ΔL and Δb of specimens that were stained and later received bleaching treatments.

| GROUPS | ΔE | ΔL | Δb |
|---|---|---|---|
| **V-LED** | 3.7870 (1.0265) $^{AB}$ | **1.6733 (2.6793)** $^{A}$ | -1.7567 (0.6009) $^{B}$ |
| **HP** | 3.1863 (1.1286) $^{AB}$ | **1.2533 (1.2333)** $^{A}$ | -2.3083 (1.0830) $^{AB}$ |
| **HP + V-LED** | 4.5296 (1.8569) $^{A}$ | **2.8933 (2.0072)** $^{A}$ | -3.0267 (1.3014) $^{A}$ |
| **C** | 2.6011 (0.9237) $^{B}$ | **-1.3500 (1.6416)** $^{B}$ | -0.3008 (1.0944) $^{C}$ |

Means followed by different letters represent significant differences in each column (ρ<0.05).

## DISCUSSION

This study assessed the effect of violet LED for tooth bleaching, associated or not with 35% hydrogen peroxide. The results obtained in this study showed that when comparing ΔE values (amount of color change of a sample), the group associating treatments (HP + V-LED) presented the greatest difference in color change when comparing the samples stained and later treated, followed by groups V-LED (violet LED) and HP (35% hydrogen peroxide). The smallest difference in color change was observed for C (Placebo gel - control).

Dentistry studies show controversies regarding the value of color difference (ΔE) that could be clinically visible, but DOZIC et al.[13] assessed the color of healthy upper anterior teeth and found perceptible differences, in clinical conditions, when ΔE is higher than 3.0 units. Therefore, all the techniques used in this study, such as the bleaching treatment either associated or not, caused clinically visible color changes.

The literature confirms the bleaching potential of the use of peroxides at high concentrations without the association of light sources.[8,10] Peroxides can dissociate into free radicals of oxygen and hydrogen, diffusing in the dental tissues, breaking the pigment molecules into simpler ones with lower rate of light absorption and consequently lighter than the original compounds,[14,15] thus promoting tooth bleaching. However, their use is often questioned due to the potential effects on tooth structure, especially related to tooth sensitivity and structural changes in enamel, whereas the violet light is a new alternative for bleaching treatments.[16]

Clinical studies have shown that violet LED can promote tooth bleaching associated with low-concentration gels[5] and even without the combination with peroxides,[3,17] corroborating the results of the present study. Due to the wavelength from 405 to 410 nm, the violet light interacts better with the pigmented molecules, weakening their ligations and transforming them into smaller molecules.[2]

When both techniques were associated (35% hydrogen peroxide + violet LED), the results showed the benefit of such association, potentially because of the chemical interaction of the hydrogen peroxide gel added to the physical change of the violet LED. Recent studies affirm that the amount of hydrogen peroxide diffused in the dental tissue increases, as its diffusion time decreases, in the presence of light.[18] However, Gallinari et al.[6] did not observed increase in efficiency when associated violet LED and 35% hydrogen peroxide. Possibly, these results may be related to the color of the gel used. Gel shades close to violet, used in this study, may have allowed a greater light absorption that gel of green color. The staining were also different, Gallinari et al.[6] used black tea and this study used coffee.

Although other studies also show positive results from the association of blue LED with peroxides,[4] another advantage of using violet LED would be the light penetration depth, whereas it is estimated that violet LED causes less adverse effects on enamel and dentin than blue LED because of the shorter wavelength.[19]

The ΔL analysis allowed verifying that, regardless of treatment with either 35% hydrogen peroxide or violet LED, isolated or not, all of them differed significantly from the control group for luminosity, thus showing the effectiveness of the protocols used. After ΔE confirmed the color change of the specimens, the ΔL showed that it also occurs for bleaching, which was not seen in the control group (C).

Another major aspect for consideration is that the analysis of Δb showed that the control group (C) presented values closest to yellow in the CIELab scale and that group HP + V-LED, which associated the treatments (violet LED + peroxide), presented values farther from yellow. The effectiveness of the bleaching treatment by the Δb parameter is verified by the decrease in values. Clinically, colder tones, that is, closer to blue than yellow, are expected for bleaching treatments.

This study showed that violet LED may cause clinically visible color changes and that its bleaching action is enhanced when administered concomitantly with

35% hydrogen peroxide, which makes the instrument promising for clinical application. Thus, further studies should be performed aiming to investigate the long-term effects caused on tooth structure and color stability.

## CONCLUSION

Bleaching with 35% hydrogen peroxide and violet LED might cause clinically visible color changes, regardless of the association of treatments. However, their association increased a color change. **Acknowledgements**: The authors would like to thank the Institutional Scholarship Program for Scientific Initiation and Research Support for the scholarship awarded, and University of São Paulo, for authorization to use the equipments.

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