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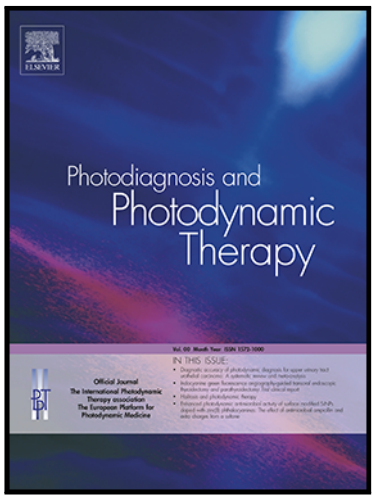

Direct dentin bleaching: would it be possible?

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Running title: Violet LED in dentin bleaching

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Clinical Relevance:

Violet LED promoted significant chromatic change in dentin tissue pigmented with black tea and coffee, providing greater temperature rise in teeth pigmented with blood.

Highlights

- Blood pigmented teeth less bleaching effect.
- Violet LED light has clinically detectable bleaching potential on dentin pigmented by different substances.
- The violet LED promoted a greater temperature rise in blood-pigmented teeth.
- The control group showed highly satisfactory results in all parameters analyzed, it immediately contributes to obtain better esthetic results associated with a non-compromising adhesion of materials.

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ABSTRACT

This study aims to evaluate *in vitro* the effect of violet LED when applied directly to dentin tissue pigmented by different substances. We analyzed the chromatic alteration, the bleaching effect and the temperature variation. Hence, 60 bovine dentin tissue discs were divided into five groups: N-Natural Pigmentation; T-Black Tea; C-Soluble Coffee; W-Red Wine; B-Equine Blood. Individualized pigmentation protocols were performed and all groups reached the same chromatic change value. Subsequently, we simultaneously performed a bleaching session and measured temperature variation using a K-type thermocouple device. Data on chromatic change (ΔE, ΔE00, Δa, Δb and ΔL), whitening effect (WID) and temperature variation were subjected to one-way Anova and Tukey's post-test at a 5% significance level. The C group showed the most relevant chromatic change values, similar to the N group, responding

positively to the treatment. However, the B group differed from the control group, which showed difficulty to respond to the treatment. Regarding the whitening index, only the W group showed lower results than the others. The B group showed the greatest temperature changes. Concluded that the violet LED offered chromatic change, which generated a bleaching effect, and the pigmentations with red wine and blood showed the greatest difficulty to respond to the treatment, besides promoting a higher temperature rise in teeth pigmented with blood.

Keywords: Dental bleaching. Dentin. Violet LED. Pigmentation.

Introduction

The chromatic alterations that affect the tooth structure can be of intrinsic or extrinsic etiology. Intrinsic pigmentations can occur during the formation of the tooth element (congenital) or be acquired during life (trauma, filling materials or internalization of pigments). On the other hand, extrinsic changes are superficial stains formed on dental enamel, caused by intense consumption of foods containing dyes, such as coffee, black tea, red wine, among others [1-3].

In general, professional cleaning and polishing of the enamel surface can be used to treat superficial stains [4]. However, the presence of diffusion channels and superficial changes in the substrate such as the presence of cracks, crevices in restorative interfaces and exposed dentin, can enable pigment molecules to reach the dentin tissue and, consequently, cause deep chromatic changes, transforming pigments normally related to superficial changes into intrinsic discolorations [5].

In this clinical condition, the professional may indicate a restorative procedure or choose to try to oxidize these pigments by whitening techniques. However, restorative procedures will always be a challenge due to the reproduction of the dental contour, texture and optical characteristics, especially in anterior teeth [6,7]. Thus, the chromatic harmony will be more challenging to achieve with the dentin pigmented with more intense colors due to the greater restorative procedure to correct the chromatic alteration, becoming an obstacle to the professional [8-11].

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Therefore, the performance of bleaching procedures before restorations has been common in esthetic rehabilitation planning, since acting on lighter substrates helps to achieve successful results in the completion of these cases [12].

Tooth whitening negatively influences the adhesion of resin materials to the tooth structure, requiring a waiting period between 7 and 21 days to eliminate the oxygen stored in the tissues and to reestablish the bond strength values [13-15]. Thus, performing tooth whitening with peroxides makes an immediate restorative resolution unfeasible in cases in which the dentin tissue still remains chromatically altered [16,17].

Besides, the bleaching treatment is characterized by being a non-selective oxidation process that also acts on other structures of the dental element, which leads to changes in the hard tissue and in the pulp tissue [18-24].

Thus, the ideal treatment would be a technique able to chromatically change the substrate without the side effects related to the presence of peroxides in the pulp-dentin complex and the need to wait such a long period to perform restorative procedures [13,14,25]. In this context, we propose the use of violet light emitting diodes (LEDs) alone or associated with whitening gels in low concentrations. Violet LED has a wavelength of 405-410 nm, which coincides with the peak of absorption of chromophore molecules, allowing its selective breakdown into smaller molecules [26].

In vitro models were the first to analyze this technology, associating it with different gel concentrations. These studies observed an association between violet LED and gels with low peroxide concentration, which offered a significant gain in chromatic change [27,28]. Later, Gallinari et al. (2020) [29,30] conducted a series of clinical cases with the same experimental groups, which validated the results from the *in vitro* experiment [27]. The violet LED's wavelength is expected to limit its action in the layers closer to the light source, and its penetration power in deep areas is restricted [29]. In this context, the eventual efficacy of using violet LED directly on a pigmented dentin would neither produce the aforementioned oxidative stress, nor interfere with the adhesion of restorative materials, which would represent a new treatment protocol.

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However, the greatest concern when using light sources in the bleaching treatment is related to the production and propagation of heat into the pulp space [27-31], and it is essential to measure this variation during its use.

Therefore, this study aims to evaluate *in vitro* the action of violet LED in the chromatic alteration and intrapulpal temperature in dentin tissue, having as null hypotheses: (1) the pigmenting substances would not influence the chromatic alteration in the dentin tissue; (2) violet LED light would not have a whitening effect when directly applied on the dentin; (3) pigmenting substances would not influence the intrapulpal temperature of the dentin tissue.

Materials and methods

1. Experimental design

The project showed one factor under study: (1) pigmenting substances at five levels: (N-naturally darkened teeth; T-black tea; C-soluble coffee; W-red wine and B-equine blood). Three response variables were considered: chromatic alteration, whitening index and temperature variation.

2. Obtaining and preparing samples

Before the experiment, the research project was sent and approved by the Ethics Committee on the Use of Animals – CEUA (protocol: 426/2020). A total of 300 bovine incisors obtained from slaughtered animals aged between 24 and 36 months were used. The teeth were cleaned with periodontal curettes and then subjected to prophylaxis with pumice stone and water, with the aid of a Robinson brush (KG Sorensen Ind. E Com. Ltd, São Paulo, Brazil). To prevent bacterial proliferation, clean teeth were stored in a 0.1% thymol solution and kept in a refrigerator at an approximate temperature of 4°C until the beginning of the experimental phase [27].

After cleaning, the teeth had their roots separated from the crown at the cementoenamel junction. Then, the crowns were fixed in a device coupled to the platform of a bench drill (model FGC-16, Ferrari, São Paulo, SP, Brazil), with the aid of a diamond tip for cutting glass (8 mm in diameter, Dinser Diamond Tools Ltda, Sacomã, SP, Brazil), and under constant irrigation. A total of 5.7 mm

diameter enamel/dentin discs were obtained from the middle third of the buccal surface of each tooth.

After obtaining the discs, the dentin surface was regulated by manual rotating movements on 400 and 600 granulated aluminum oxide sandpaper (T469-SF-Noton, Saint-Gobam Abrasives Ltda, Jundiai, SP, Brazil). Then, all the dental enamel was removed using the same sandpaper, mounted on an Aropol E polisher (Arotec, Cotia, SP, Brazil), resulting in dentin discs approximately 2 ± 0.2 mm thick, measured in a digital caliper (model 500-144B, Mitutoyo Sul América Ltda, SP, Brazil). To remove the smear layer, a 0.5M EDTA solution, pH 7.2 was applied for 15 seconds, followed by washing with deionized water.

A total of 120 discs that showed values closer to the general mean were selected (65.32 ± 0.5). The measurement was made using a Visible Ultraviolet Reflection Spectrophotometer, Model UV-2450 (Shimadzu, Kyoto, Japan), with the specimens positioned on a black silicon matrix.

3. Sample pigmentation protocol

The 120 discs were divided to allocate 12 specimens with the lowest E values of the entire sample in the naturally darkened dentin (N) group. The remaining samples were divided and received different staining protocols.

N Group – Composed of naturally darker discs, no pigmentation protocol was performed.

T Group – The discs were stored in tubes containing one mL of black tea infusion at room temperature. The infusion was made using 1.6 g of black tea (Tea Matte Leão, Curitiba, PR, Brazil) for each 200 mL of distilled water. The pigmentation process was monitored for two days, and the solution was changed daily.

C Group – The samples were stored in polypropylene tubes (Eppendorf, Hamburg, Germany) containing one mL of Original *Nescafé* soluble coffee infusion at room temperature. The infusion was performed with one g of powder and 100 ml of boiling distilled water. The samples remained immersed for three days after cooling the solution, being changed daily.

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W Group – The samples were stored in polypropylene tubes (Eppendorf, Hamburg, Germany) containing one mL of red wine Cabernet Sauvignon (Concha y Toro, Santiago de Chile, Chile) at room temperature for one day.

B Group – Pigmentation with equine blood was performed using the modified technique of Freccia and Peters [32]. A centrifuge 5702 R (Eppendorf AG, Hamburg, Germany) was used to hemolyze the red blood cells and obtain the degradation products inside the dentinal tubules, which operated at 4,400 rpm for 10 minutes, at 37ºC, twice a day (24 h).

Once the pigmentation processes were completed, the specimens from all groups were immersed in distilled water to leach the excesses not incorporated into the dentin structure. Then, they received prophylaxis with pumice stone and water to remove surface stains.

After pigmentation, the dentin discs were analyzed in the Visible Ultraviolet Reflection* spectrophotometer Model UV-2450 (Shimadzu, Kyoto, Japan). The specimens that showed extreme values were discarded, thus, 12 specimens were selected per group.

4. Bleaching session and analysis of intrapulpal temperature change

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A silicone matrix was made before the bleaching treatment, perfectly adapted to the acrylic tip of the violet LED emitting equipment (Bright Maxx Whitening Photobleaching, MMOptics Ltda., São Carlos, SP, Brazil). This matrix had four larger perforations through which the specimens were positioned to keep the superficial dentin five mm away from the light source. Other smaller perforations were facing the inner face of the samples, and the free ends of a type K thermocouple (Risepro, Kowloon, Hong Kong, China) were positioned, which allowed the simultaneous temperature analysis in all samples, accurate to 0.05°C. The set formed by the inner face of the dentinal disc and the thermocouple terminal were covered with a thermal paste (Implastec, Votorantim Ind. Brasileira, São Paulo, SP, Brazil). The entire apparatus was kept inside a greenhouse at 36°C (Figure 1).

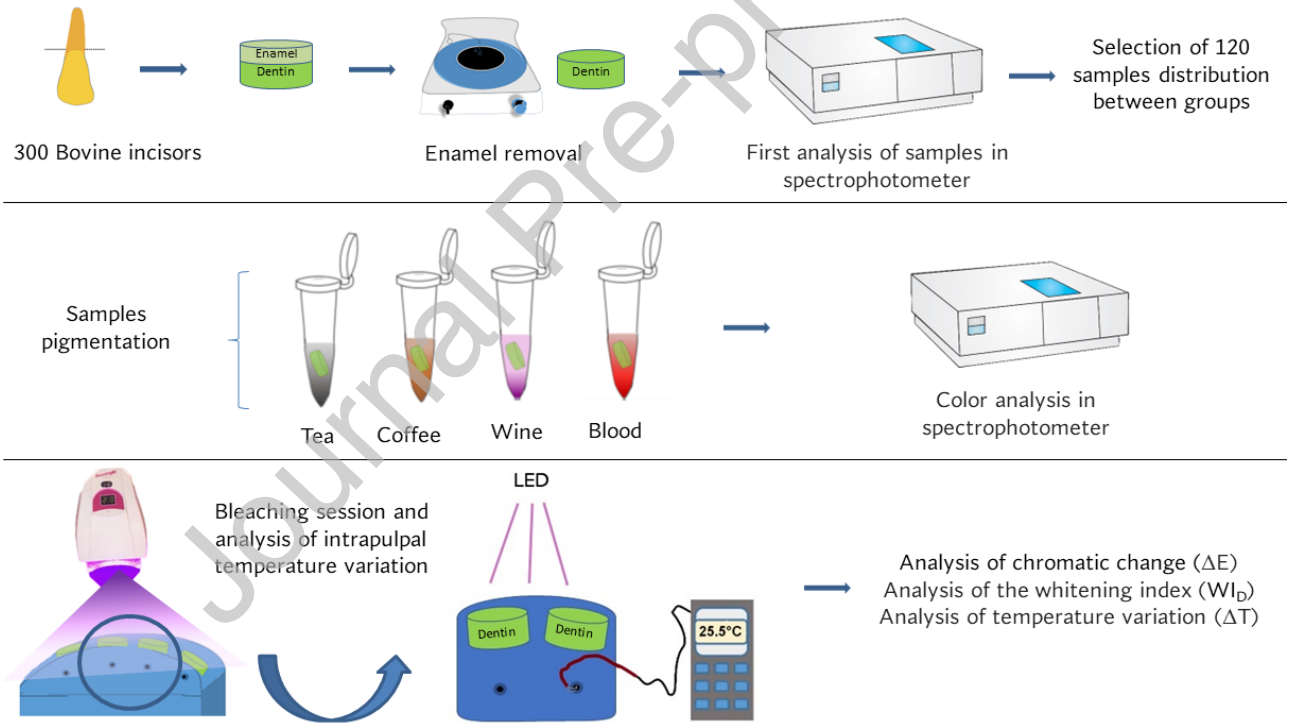

Figure 1- Experimental steps flowchart.

The violet LED device (Bright Photo Brightener Maxx Whitening, MMOptics Ltd., São Carlos, SP, Brazil), used with 4 LED emitting diodes in the spectral range of 405+-10nm, the output power of 350mW each LED, totaling 1.5W of optical power delivered. This equipment has a delivery system through an anatomically designed tip in polished acrylic that homogenizes and collimates

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the light beams of each LED diode, to be made available, in an organized way, in the corrected area of 10.7 cm2, resulting in irradiance of 140.2mW/cm2. Thus, the treatment was performed in a single clinical session, with three irradiations of 20 minutes and 10 minutes apart, following the manufacturer's instructions.

This protocol was adopted to simulate a clinical condition in which the professional needs to lighten a darkened dentin substrate to restore it in the same session.

5. Analysis of color change and whitening index

The chromatic change of the discs was measured in a spectrophotometer, comparing the values obtained after the pigment treatment with the reading after the bleaching session, using the CIE L*a*b* color model.

The axial “L” extends from black to white and is known as luminosity. The “a” coordinate represents the amount of red (positive values) and green (negative values), and “b” represents the amount of yellow (positive values) and blue (negative values). For color analysis, the CIE L*a*b* system estimates the color distance between two points using the formula:

$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)2]^{1/2}$ and

$\Delta E00 = [(\Delta L´/KLSL)^2 + (\Delta C´/KCSC)^2 + (\Delta H´/K\ HSH)^2 + RT*(\Delta C´/KCSC)*(\Delta H´/K\ HSH)]^{1/2}$

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The color analysis was complemented by the whitening index (WID) based on the axes evaluated in the CIELab system, which allowed to emphasize the bleaching effect of the treatment [33]. The positive values of the WID index indicate a greater bleaching effect of the sample and the low and/or negative values indicate less sample whitening effect, which is a simple linear formulation obtained by the following formula:

$WID = (0.511 \times L^*) – (2.324 \times a^*) – (1.100 \times b^*)$.

Group	ΔE	ΔE00	ΔWI_D
N	10,21 (5,61) A	8,66 (4,99) A	4,53 (2,02) A
T	6,20 (2,66) B	5,30 (2,54) B	4,12 (1,44) A
C	7,74 (4,03) AB	6,81 (3,65) AB	3,01 (2,86) A
W	4,28 (3,08) B	3,80 (2,70) B	-1,36 (1,40) B
B	3,99 (2,23) B	3,80 (1,95) B	1,91 (1,76) A

6.

Statistical analysis

Sigma Plot 14.0 software (Systat, San Jose, CA, USA) was used for statistical analysis. After data collection, they were subjected to the Shapiro-Wilk normality test and the one-way ANOVA test. Subsequently, the data were subjected to the Tukey's post-test considering a 5% significance level.

Results

Analysis of ΔE, ΔE00 e ΔWI_D

By assessing only the effect of violet LED in different groups, compared the changes in readings performed after pigmentation with those observed after the use of LED. Table 1 shows that ΔE and ΔE00 had similar responses and that the N group had the highest values, differing statistically from W and B groups, which presented the lowest values ($p<0.05$). The T, C, W and B groups were statistically similar ($p<0,05$). When comparing the WID variation, LED was similar in N, T and C groups ($p>0.05$), while teeth pigmented with red wine (W) or blood (B showed no positive response to the action of light (Table 1).

Table 1- Mean (standard deviation) of ΔE, ΔE000 values occurring after pigmentation of samples (T1) with data observed after bleaching (T2)

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Different letters (capital letters in the lines) indicate a statistically significant difference (p<0.05)

Analyses of the ΔL, Δa e Δb

Regarding luminosity, W and B groups showed similar performance (p>0.05), with lower values of ΔL. On the other hand, N, C and T groups showed the highest values; however, T and C were also similar to W group (p>0.05). The analysis of the a* coordinate showed that the B group had the lowest values, differing statistically from the other groups, which were similar. In the b* coordinate, W and B groups had the highest Δb values (p>0.05). The N, T and C groups showed similar negative values (Table 2).

Table 2 – Mean (standard deviation) of the values of ΔL, Δa and Δb occurred after the pigmentation of the samples (T1) with the data observed after bleaching (T2).

Different letters (capital letters in the lines) indicate a statistically significant difference (p<0.05)

Analysis of temperature variation (ΔT)

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Group	ΔL	Δa	Δb
N	9,12 (6,80) A	0,81 (0,34) A	-1,61 (2,15) B
T	5,11 (3,70) AB	0,37 (0,35) A	-2,15 (1,22) B
C	7,20 (4,40) AB	0,44 (0,59) A	-0,32 (2,27) AB
W	3,71 (2,93) BC	0,58 (0,20) A	1,74 (1,48) A
B	0,74 (4,14) C	-1,07 (1,01) B	0,88 (1,20) A

Table 3 shows that the temperature variation depended on the pigment substance used. The N, T and W groups showed the smallest changes, whereas B generated the greatest changes ($p<0.05$).

Table 3 – Mean (standard deviation) of ΔT (ºC) detected during bleaching treatment

Group	ΔT (ºC)
N	3,75 (0,59) C
T	3,88 (1,05) BC
C	4,36 (0,59) B
W	4,14 (0,59) BC
B	6,16 (0,86) A

Different letters (capital letters in the lines) indicate a statistically significant difference ($p<0.05$)

Discussion

Routinely, the clinician is exposed to challenging conditions to obtain a favorable pattern of tooth color, especially in cases where the dentinal tissue is pigmented, resulting from extrinsic and intrinsic stains. Thus, the design proposed in this study aimed at the individual analysis of these conditions, so that the results obtained could suggest more accurate prognoses through the use of the proposed treatment. In this way, the possibility of using a whitening protocol without the side effects of therapies based on the application of peroxides, by the isolated use of violet LED, maybe a protocol change in whitening dentistry, offering paths for biologically safer treatments.

The efficacy of this technique could represent a significant gain in clinical time because the professional could perform LED bleaching on teeth with intrinsic chromatic changes and, in the same session, would perform the restorative procedure, since the violet LED does not affect the adhesion of adhesive materials to dentin tissue [34].

Thus, this study aimed to evaluate an alternative to the conventional bleaching treatment by using a source of violet LED to degrade the chromophore molecules and offer an immediate bleaching effect on the dentin tissue, without

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using oxidizing agents [26]. Comparative analysis of bleaching teeth pigmented by substances routinely consumed can contribute to better understand the bleaching therapy and adopt specific dosages for each type of chromatic change.

In the initial experimental stage, red wine and equine blood showed high pigmentation capacity, which required shorter immersion time compared to other substances. Liporine et al. (2010) [35] had already demonstrated that red wine had a high pigmentation capacity in a short period of time, which is corroborated by other authors [36-39]. Blood, by the oxidation of hemoglobin molecules, quickly sediments chromophore agents, producing the characteristic dark color [40].

When analyzing the results of chromatic alteration (ΔE and $\Delta E00$) after pigmentation, although all groups showed significant chromatic alteration after the use of LED, the type of pigmentation influenced the results obtained, thus denying our first null hypothesis. The fastest groups to pigment were the ones that obtained less chromatic alteration due to the use of violet LED, which showed that the greater the pigmenting potential, the lower the efficacy of the bleaching treatment.

These results suggest that photoreception may have occurred differently for each pigment substance tested, which made the violet LED promote distinct chromatic effects for each pigment, being less effective in those with absorption peaks outside its wavelength (405-410 nm).

Regarding the WID whitening index, Gallinari et al. (2019) [27] observed that the isolated use of the violet LED caused a chromatic alteration and a discrete but significant bleaching effect [41,42]. This study evidences that the group pigmented by red wine was the least affected despite all experimental groups showed bleaching effect, thus refusing our second null hypothesis. However, despite the limitation in the bleaching effect promoted by violet LED, the results obtained in dentinal tissue agree with the literature that evaluates the entire tooth (enamel + dentin) [27,29,30].

We found changes in the L* and b* coordinates when separately analyzing the chromatic axes, except for the groups pigmented by wine and blood, which showed reduced effects. Possibly because both substances have a high capacity

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to chemically interact with the tooth structure, a hypothesis already defended by Berger et al. (2008) [38] and Marin et al. (1997) [40], regarding wine and blood, respectively. This condition supposedly makes both substances less reactive to the effects of light.

We also observed that the b* values tended to decrease (reduction in yellow and increase in blue) in teeth pigmented with black tea. Gallinari et al. [27] observed similar data using the same pigmenting agent, obtaining a considerable reduction in the b* values. Light source may have acted directly on the yellowish pigments from the tea, destabilizing and breaking double carbon bonds present in the chromophores.

The control group showed highly satisfactory results in all parameters analyzed, emphasizing the results obtained in the E* and L* axes, which underwent the most significant changes. Therefore, this treatment would help teeth indicated for esthetic rehabilitation on naturally darkened teeth, since it immediately contributes to obtain better esthetic results associated with a non-compromising adhesion of materials.

The study of the thermal change from the prolonged emission of light is relevant whenever treatments with light sources are adopted. In the experimental model used (characterized by long exposure to LED, small thickness of the dental tissue and proximity to the pulp chamber), it was observed that the treatment with violet LED excessively increased the temperature in the groups pigmented with equine blood, with a value of 6.16 °C, which would lead to a possible compromise of pulpal health [43], thus rejecting our third null hypothesis.

Possibly, the Fe-containing heme radical in the blood absorbs more violet light than the organic pigments, generating greater heating. Molecules with Fe atoms are grouped so that they have a smaller distance between them, being less permeable to the passage of photons (with kinetic energy) like those of violet light. As a result, they collide in greater quantity with Fe atoms (inorganic dye), generating agitation (vibration in Hertz) and heat. Organic dyes with carbon (70 pm -atomic radius), hydrogen (53pm) and oxygen (60pm) in relation to Fe (126pm) have a smaller collision area (less absorption) of photons and, therefore, less agitation of these atoms and greater permeability in the light of water. It is

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important to highlight that the specimens used in the study were very thin and obtained only from dentin tissue, without protection from dental enamel. Thus, the data obtained so far suggest that the violet LED should not be used in vital teeth that present hemorrhagic chromatic alterations [44,45].

Although therapies based on the use of peroxides are still the first option to obtain effective bleaching results, we observed that violet LED can be developed to achieve better results when used directly on chromatically affected substrates, during the restorative transoperative period, without oxidative stress or interference in the adhesion process.

Led-Violet therapy has been showing promise in the field of health sciences. This light is rated 3B under international biosafety regulations, which means it poses an eye hazard. Thus, the manufacturer itself already indicates and provides protective glasses (dark green or orange) for the patient and the operator, informing the need for the correct protection. In addition, during clinical use, the gingival tissues are protected with light-curing barriers and the lips are separated by cotton rolls and mouth openers. It is also recommended that the distance between the equipment and the dental elements does not exceed two centimeters, as greater distances can dissipate the emitted light, in addition to unnecessarily exposing the lips to irradiation.

Despite the mandatory use of glasses and this clinical management, some interesting results are already found in the literature with the use of the equipment in living tissues, the literature being rich in works that contemplate significant antimicrobial properties against a wide range of bacterial and fungal agents [45-49].

Regarding tooth whitening, recently, studies have been developed to evaluate the effectiveness of the technique as well as its possible side effects. Ribeiro et.al. (2022), stated that Led-Violeta improved the aesthetic results of in-office tooth whitening and did not affect cytotoxicity, even with increasing exposure time to violet light. Menezes et.al. (2022), also found “in vitro” that the use of this technology produced significant changes in color, preserving the integrity of dental enamel, without causing genotoxic effects on vital cells. Silva et al. (2022), found “in vivo” that violet LED therapy did not induce pulp tissue

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inflammation or fibrosis in rats, accelerating the maturation of dentinal collagen fibers. Finally, when analyzing recently published clinical research, Panhóça, et. al. (2022) observed that the Violet LED was able to desensitize teeth during treatment, while Gallinari et al. (2019) observed that the violet LED potentiated the whitening effect when used with 10%PC gels, causing a change in sensitivity caused by thermal stimuli.

Thus, considering the recent commercialization of this technology, our results may contribute to further clarify the behavior of this light source as a new lightening therapy.

Conclusion

1 The pigmenting substances influenced the bleaching efficacy promoted by the violet LED on the dentin tissue, with red wine and blood showing greater difficulty to positively respond to the treatment.

2 The violet LED promoted a greater temperature rise in blood-pigmented teeth.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper

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