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ORIGINAL ARTICLE

Improved esthetic efficacy and reduced cytotoxicity are achieved with a violet LED irradiation of manganese oxide-enriched bleaching gels

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Abstract

Gels with high concentrations of hydrogen peroxide (H_2O_2) have been associated with cytotoxicity and consequent post-bleaching tooth sensitivity. This study assessed the bleaching efficacy (BE) and cytotoxicity (CT) of bleaching gels with low concentrations of H_2O_2 containing manganese oxide (MnO_2) and photocatalyzed with violet LED (LEDv). The following groups were established: G1: no treatment (negative control, NC); G2: 35% H_2O_2 (positive control, PC); G3: LEDv; G4: 10% H_2O_2; G5: 6% H_2O_2; G6: 10% H_2O_2 + MnO_2 + LEDv; G7: 6% H_2O_2 + MnO_2 + LEDv. To analyze BE, standardized enamel/dentin discs (E/DDs) were subjected to the bleaching procedures for 45 min (1 session). The color change was determined before and after performing the bleaching protocols (ΔE_{00}; ΔWI). To analyze CT, the E/DDs were adapted to artificial pulp chambers, and the extracts (culture medium + diffused gel components) were applied to cultured odontoblast-like MDPC-23 cells. Then, the cells were assessed concerning their viability (VB), oxidative stress (OxS), and Live/Dead. The amount of H_2O_2 diffused was also determined (ANOVA/Tukey; $p < 0.05$). Cell viability decreased in all bleached groups compared to G1 (NC; $p < 0.05$). The cells in G6 and G7 presented higher viability than in G2, G4, and G5 ($p < 0.05$). The BE in G7 was similar to G2 (PC; $p < 0.05$). The lowest OxS and H_2O_2 diffusion values were found in G6 and G7, compared to the other bleached groups (G2, G4, and G5; $p < 0.05$). The 6% H_2O_2 bleaching gel (G7) submitted to both methods of catalysis (MnO_2 + LEDv) caused only a mild cytotoxicity and maintained the excellent esthetic outcome promoted by in-office conventional tooth bleaching.

Keywords Tooth bleaching · Dental materials · Cell culture · Toxicity

Introduction

Tooth bleaching, one of the esthetic procedures most performed in the dental office, has been effective in reducing color pigments responsible for tooth darkening [1]. For this professional therapy, bleaching gels with high concentrations of hydrogen peroxide (H_2O_2) have been used to achieve fast and satisfactory clinical outcomes [2, 3]. However, a number of studies has shown that the in-office dental bleaching results in a high amount of H_2O_2 that does not react with the chromophores present in teeth [4, 5]. The authors showed that the residual H_2O_2, termed as free-H_2O_2 can diffuse through dentinal tubules to damage pulp cells. Hypothetically, the intense toxic effects caused by free-H_2O_2 to pulp cells would explain, at least partially, the post-bleaching tooth sensitivity reported by most patients subjected to conventional in-office tooth bleaching [6, 7]. Previous in vitro investigations showed that the intensity of toxic effect of such professional treatment to pulp cells is directly proportional to the time of contact of the bleaching gel with the enamel and concentration of H_2O_2 in the product [5]. Thus, performing in-office dental bleaching with low-concentrated gels seems to be an interesting strategy

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to ensure a safer and painless professional esthetic therapy for patients [8, 9]. A clinical trial performed by Bersezio et al. (2019) [10] showed that bleaching gels with low concentration of H_2O_2 may provide a satisfactory esthetic outcome. The authors reported that the bleaching effect achieved when using a gel with 6% H_2O_2 remained for longer periods of time compared to gels containing 35% H_2O_2.

The decomposition of the H_2O_2 molecules and consequent production of other reactive oxygen species (ROS), such as the hydroxyl ions ($OH^{\bullet}$), favor the esthetic efficacy of tooth bleaching and reduce the amount of free-H_2O_2 that can reach the pulp space [4, 11–13]. Thus, the chemical catalysis and/or photocatalysis of H_2O_2 in bleaching gels seem to be promising strategies for in-office tooth bleaching [14–16]. The current literature has shown the effective H_2O_2 decomposition in other ROS through different transition metals, such as catalyst agents derived from manganese [11, 13, 14]. However, among the chemical catalysts used for H_2O_2 decomposition, manganese oxide (MnO_2) stands out because of its low cost [17] as well as because its chemical reaction does not produce by-products in the oxidation process, contrary to the chemical reaction mediated by iron derivatives [14]. A recent study by Chiam et al. (2020) [18] showed the catalyst potential of MnO_2 when associated with visible light. Therefore, this oxide would work as a chemical catalyst and a photocatalyst of organic pigments and could be employed promisingly in bleaching therapies associated with the use of light sources.

The photocatalysis of H_2O_2 with violet LED has been investigated as a strategy to optimize the tooth bleaching outcome [19]. The violet LED wavelength can penetrate the enamel and dentin to react with local chromophores, causing tooth bleaching by photolysis [20]. Thus, violet LED would potentially cause chromatic changes in dental tissues even when not used in association with bleaching gels containing H_2O_2 [20, 21].

Based on the potential benefits of the chemical catalysis and photocatalysis of H_2O_2 to professional tooth bleaching, this study aimed to assess the influence of the association of manganese oxide (MnO_2) and violet LED on the esthetic efficacy and trans-amelodentinal cytotoxicity of bleaching gels with 6%- and 10% H_2O_2. The null hypothesis of this study was that reducing H_2O_2 concentrations in the gels, associated with different types of catalysis of this ROS, does not affect the esthetic efficacy and cytotoxicity of bleaching treatments.

Materials and methods

Sample size calculation

The number of biological replicates for the response variables "esthetic efficacy" and "cytotoxicity" was calculated using the G*Power software (version 3.1; University Dusseldorf, Dusseldorf, Germany). Power (1-β error probability) and α error probability were 0.80 and 0.05, respectively. The effect size for esthetic efficacy was 4.03 and the trans-amelodentinal cytotoxicity was 3.35. The estimated sample size of 8 specimen/group was defined for all response variables. Each protocol was conducted on two separate experimental occasions (duplicates) to minimize systematic errors and allow the observation of reproducibility.

Formulation of the experimental bleaching gels ($n = 8$)

The Carbopol thickener (polyacrylic acid Mv ~ 3,000,000, Sigma-Aldrich, St. Louis, MO, USA) was obtained by preparing a solution at 1% (v/m). The liquid phase containing 6%, 10%, or 35% H_2O_2 was prepared by diluting a stock solution with 35% H_2O_2 (35% hydrogen peroxide P.A.; Neon, Suzano, SP, Brazil). Next, 10 mg/mL of MnO_2 (Sigma-Aldrich, St. Louis, MO, USA) was incorporated in the bleaching gel aliquots corresponding to the groups that received the catalyst agent (MnO_2) associated with H_2O_2.

Achievement and standardization of enamel/dentin discs (E/DDs; $n = 8$)

One hundred and twelve standardized E/DDs (5.6 mm of diameter) were obtained from intact bovine incisors with a trephine diamond bur (Dinser Brocas Diamantadas Ltda., São Paulo, SP, Brazil) attached to a bench drill (FSB 16 Pratika, Schµltz, Joinville, SC, Brazil). The dentin surface was corrected by wear with sandpapers of 400 and 600-granulations (T469-SF-Noton, Saint-Gobam Abrasivos Ltda, Jundiaí, SP, Brazil) and manual rotary movements for each granulation until obtaining a standardized thickness of 2.3 mm of E/DD, simulating the thickness of lower human incisors [5, 22].

Assessment of bleaching efficacy ($n = 8$)

The E/DDs were subjected to laboratory staining with black tea, as previously described by de Oliveira Duque et al. (2020) [26], and distributed into groups (Table 1). The bleaching agents were manipulated immediately before their application to enamel, and the bleaching protocol was standardized in one session of 45 min. For the groups that used violet LED (Bright Max Whitening, MMOptics, São Carlos, SP, Brazil), irradiation was applied for 20 min without an interval during the bleaching session [15]; the device has 4 LED diodes emitting in the spectral range of 405 ± 10 nm, output power of 350

Table 1 Distribution of groups according to the experimental protocols

Groups	Treatments	Time
G1	No treatment (negative control)	45 min
G2	35% H_2O_2 (positive control)	45 min
G3	LED	45 min
G4	10% H_2O_2	45 min
G5	6% H_2O_2	45 min
G6	10% H_2O_2 + MnO + LED	45 min
G7	6% H_2O_2 + MnO + LED	45 min

mW each LED, totaling 1.5 W of optical power and irradiance of 140.2 mW/cm^2. After placing the discs in a white silicone matrix, bleaching efficacy was analyzed with a UV-reflective spectrophotometer (Reflection Spectrophotometer ML Color Guide, BYK Gardner GmbH Gerestsried, Germany). The values of L*a*b* obtained before (baseline) and 72 h after the bleaching procedures were used to assess color changes (CIEDE 2000, ΔE_{00}). The whitening index (ΔWI) was determined according to the following equations:

$$\sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_c S_c}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T \frac{\Delta C'}{k_c S_c}\frac{\Delta H'}{k_H S_H}} \quad (1)$$

$$\mathrm{WI} = 0.511\mathrm{L}^* - 2.3424\mathrm{a}^* - 1.100\mathrm{b}^* \quad (2)$$

$$\Delta\mathrm{WI} = \mathrm{WI} = \mathrm{WI}_{\mathrm{baseline}} \quad (3)$$

Next, the color parameters (L*, a*, b*, ΔE_{00}, and ΔWI) obtained for all groups were analyzed statistically (two-way ANOVA; Tukey's test). The values of perceptibility threshold (PT) and acceptability threshold (AT) were used as 50:50%, ΔE_{00} values were 0.8 (PT) and 2.7 (AT), and ΔWI values were 0.72 (PT) and 2.60 (AT) [24].

MDPC-23 cell culture

Immortalized odontoblast-like MDPC-23 cells maintained in the Laboratory of Experimental Pathology and Biomaterials of the São Paulo State University (UNESP), School of Dentistry, Araraquara, Brazil, were cultivated in sterilized plates of 75 cm^2 (KASVI Imp., Curitiba, PR, Brazil) with Dulbecco's Modified Eagle's medium (DMEM; GIBCO. Grand Island, NY, USA) containing 10% fetal bovine serum (FBS; GIBCO); 100 and 100 µg/mL, respectively, of penicillin and streptomycin; and 2 mmol/L of glutamine (GIBCO), in a wet atmosphere at 37 °C, 5% CO_2, and 95% air. The cells were passed until obtaining an adequate number for experimenting.

Experimental procedure

The E/DDs were individually adapted to artificial pulp chambers (APCs), and the disc/APC sets were sterilized in ethylene oxide (Acecil, Central de Esterilização Comércio e Indústria LTDA, Campinas, SP, Brazil). Then, each set was individually placed in the compartment of 24-well sterilized acrylic plates (KASVI Imp.) containing 1 mL of DMEM [5–7]. Immediately after concluding the bleaching procedures, the extracts (DMEM + bleaching gel components diffused across the E/DDs) were collected and stored in aliquots of 100 µL in cryogenic tubes for later use in cytotoxicity tests [22]

Trans-amelodentinal cytotoxicity assessment

To determine the indirect cytotoxic effects caused by the bleaching protocols, the MDPC-23 cells were subjected to three specific analyses:

Cell viability (Alamar Blue assay; *n* = 8)

Aliquots of 100 µL of each extract were applied to the MDPC-23 cells previously cultivated (10,000 cells) in compartments of 96-well sterilized acrylic plates (KASVI Imp.). One hour after incubation, the extracts were aspirated and the MDPC-23 cells that remained adhered to the bottom of the compartments were incubated for 4 h in contact with a solution composed of 90 µL of DMEM and 10 µL of Alamar Blue solution (Life Technologies; Grand Island, NY, USA). Then, the solution was collected and transferred to a 96-well plate (KASVI Imp.), and fluorescence was measured at 540 nm excitation and 590 nm emission with a spectrophotometer (Synergy H1).

Oxidative stress (*n* = 8)

Cellular oxidative stress was assessed by estimating the reactive oxygen species (ROS) produced by the MDPC-23 cells, immediately after bleaching. For this purpose, cells previously cultivated in compartments of 96-well sterilized plates were exposed for 30 min to the carboxy-H2DCFDA probe (Invitrogen, San Francisco, CA, USA) at a concentration of 10 µg/mL. This probe is permeable to the cell membrane and emits fluorescence when in contact with ROS. Next, the aliquots of 100 µL of the extracts from each group were applied to the cells for 1 h, and fluorescence intensity was assessed at 592 nm excitation and 517 nm emission (Synergy H1) after the time of exposure to the extracts. The data were normalized by the negative control group [22].

Live/Dead ($n=4$)

One hour after exposure to the extracts, the MDPC-23 cells were assessed with the Live/Dead assay (Live/Dead cell viability/cytotoxicity kit; Invitrogen, Carlsbad, CA, USA). Therefore, the cells were incubated ($n=8$) for 30 min in contact with 100 µL of DMEM, supplemented with Calcein AM and Ethidium Homodimer markers (Invitrogen) at the concentration of 1:1000. Next, the cells were assessed in a fluorescence microscope (Leica DM 5500B, Nussloch GmbH, Nussloch, Germany) to obtain representative images of each group.

Quantification of H_2O_2 in the extracts ($n=8$)

Aliquots of 100 µL of the extracts obtained from each group were transferred to 5-Ml-plastic tubes (KASVI Imp.) containing 900 µL of acetate buffer solution (2 mol; pH 4.5), which allows stabilizing H_2O_2. Then, 500 µL of this solution was transferred to new 5-mL-plastic tubes (KASVI Imp.) containing water and leucocrystal violet dye (0.5 mg/mL, Sigma-Aldrich). The tubes were agitated in a vortex (Vortex Mixer VM300, Vixar; Plymouth, MN, USA), and 50 µL of a solution of 1 mg/mL of horseradish peroxidase enzyme (Sigma-Aldrich) was added. The absorbance of solutions was measured in a spectrophotometer (Synergy H1) at a 596-nm wavelength. The standard curve of the known amounts of H_2O_2 was used for converting the optical density values obtained in the samples into µg of H_2O_2 per mL of extract [4, 5, 22].

Statistical analysis

Data were subjected to the Shapiro–Wilk test to assess normality, the Levene test to assess homoscedasticity, followed by a two-way analysis of variance (two-way ANOVA), considering the independent variables "concentration of H_2O_2" and "presence or absence of MnO+LED," complemented by Tukey's post-test. The analyses were performed using the SPSS (version 26.0; IBM, Chicago, IL, USA) and GraphPad Prism (version 9.0; GraphPad, San Diego, CA, USA) and statistical inferences were based on a 5% significance level.

Results

Bleaching efficacy

According to the results presented in Fig. 1, the ΔE_{00} and ΔWI values significantly increased in G2, G3, G4, G5, G6, and G7, compared to the negative control (G1) ($p<0.05$). The highest ΔE_{00} and ΔWI values were found in G6 (10% H_2O_2 + MnO_2 + LEDv) and G7 (6% H_2O_2 + MnO_2 + LEDv) compared to G2 (35% H_2O_2), G3 (LEDv), G4 (10% H_2O_2), and G5 (6% H_2O_2) ($p<0.05$). The G4 and G5 presented statistically similar ΔE_{00} and ΔWI values ($p>0.05$).

Trans-amelodentinal cytotoxicity

Cell viability (VB) significantly decreased in all bleached groups, compared to G1 (negative control) and G3 (LEDv) ($p<0.05$). The VB in G6 and G7 was higher than in G2, G3, G4, and G5 ($p<0.05$). Overall, the groups that used MnO_2 + LEDv to catalyze the bleaching gels (G6 and G7) presented higher MDPC-23 cell viability (Fig. 2A). Higher oxidative stress (OxS) occurred in G2, G4, G5, G6, and G6 than in G1 and G3 (Fig. 2B; $p<0.05$). However, the H_2O_2 catalysis performed with the association of MnO_2 + LEDv (G6 and G7) caused lower OxS than in G4, G5, and G2 ($p<0.05$) (Fig. 2B). Figure 3 shows the photomicrographs obtained with the Live/Dead assay. There was a lower number of living cells (marked with Calcein AM — green) and a higher number of dead cells (marked with EthD-1 — red) in all experimental groups

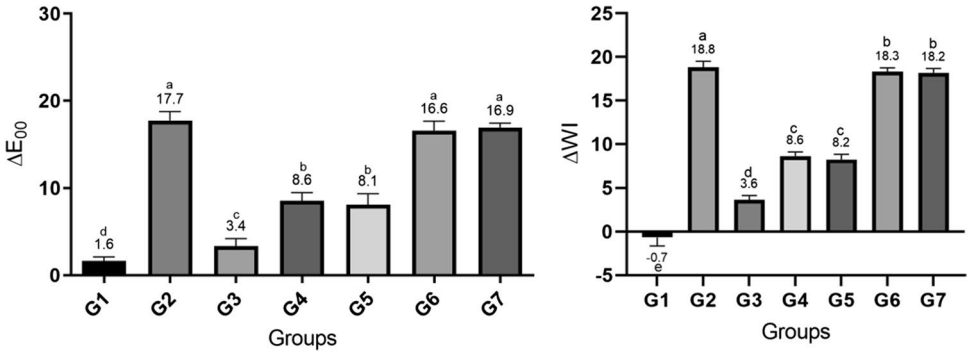

Fig. 1 Statistical analysis of CIEDE 2000 (ΔE_{00}) and ΔWI values for each group at different H_2O_2 concentrations, associated or not with MnO_2 + LED. The numbers are mean values of CIEDE 200 (ΔE_{00}) and ΔWI corresponding to each H_2O_2 concentration used. Lower-case letters allow comparing H_2O_2 concentrations for each group (two-way ANOVA; Tukey's test). Different letters show statistically significant differences ($p<0.05$)

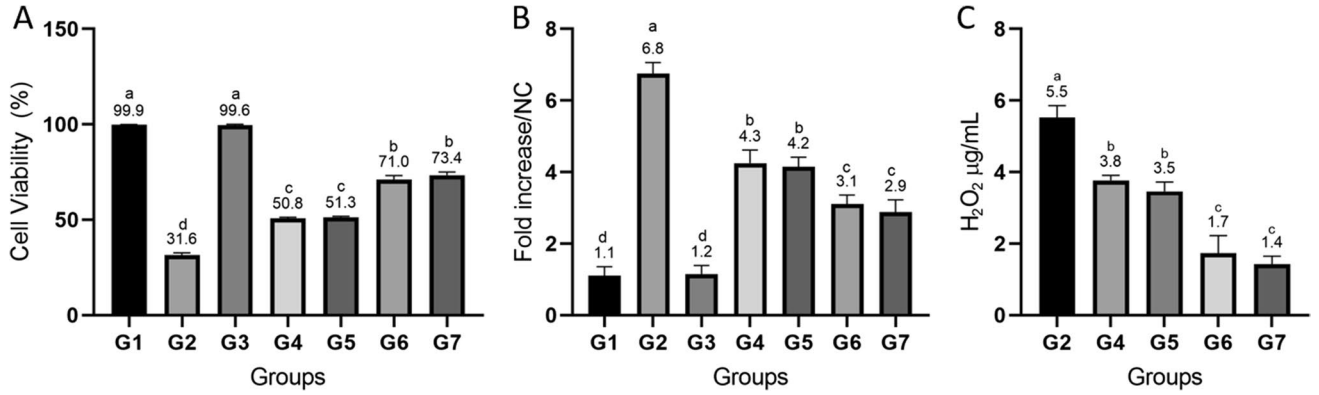

Fig. 2 Bar graph of the mean values (numeric) and standard deviation of the analyses of cell viability, oxidative stress, and diffusion of bleaching gel by-products. Different letters show statistically significant differences among the groups (two-way ANOVA; Tukey's test, $p < 0.05$)

(G2 to G7) than in G1 (negative control). However, there was a higher number of living cells adhered to the acrylic substrate in G6 and G7 than in G2, G3, G4, and G5.

Lower trans-amelodentinal diffusion of H_2O_2 occurred in G6 and G7 than in G2, G4, and G5, in which gels with 35, 10, and 6% H_2O_2 were applied directly to the enamel ($p < 0.05$; Fig. 2C). In G6 and G7, in which 10 and 6% H_2O_2 bleaching gels were associated with MnO_2 + LED, the trans-amelodentinal diffusion of H_2O_2 decreased by around 42% and 65%, respectively, compared to G2 (positive control).

Discussion

Researchers have demonstrated the severe pulpal damage caused by conventional in-office tooth bleaching therapies [7, 25]. This adverse effect has been related to the high amount of H_2O_2 that do not react with the chromophores of teeth (free-H_2O_2), and thus diffuse through the enamel and dentin to reach the pulpal space. When in contact with pulp cells, free-H_2O_2 triggers oxidative stress associated with lipid peroxidation, which invariably cause in irreversible cell damage [26]. As aforementioned, the intensity of pulp cell damage is directly related to the H_2O_2 concentration in gels and the application time on teeth [4]. To prevent or at least minimize the adverse effects caused by gels with high concentrations of H_2O_2, which are widely used for in-office tooth bleaching, innovative strategies have been proposed [4, 13–16, 22]. The present study assessed the influence of the photocatalysis (LEDv) and chemical catalysis (MnO_2) of H_2O_2 in gels containing 6 and 10% of this reactive molecule on the esthetic efficacy and cytotoxicity of the treatment. Overall, associating LEDv + MnO_2 in bleaching procedures with gels

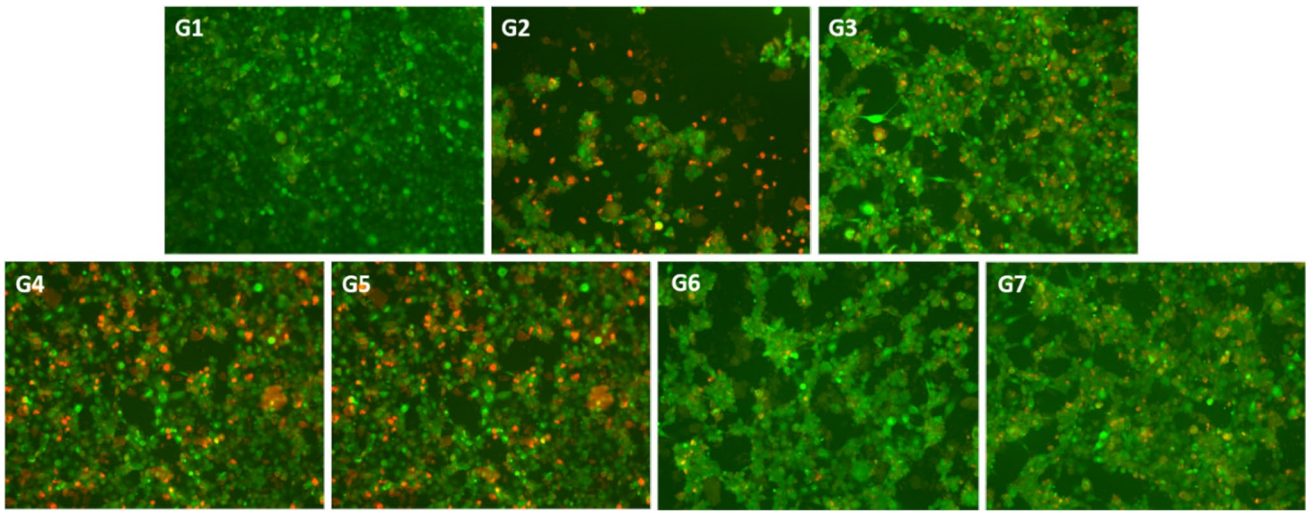

Fig. 3 Photomicrographs representing the Live/Dead assay. Fluorescence microscopy (10×). The green (calcein AM) and red (EthD-1) fluorescences indicate the presence of living and dead cells, respectively

containing low H_2O_2 concentrations (G6 and G7) reduced cell viability by around 30%. However, the 35% H_2O_2 gel (G2 — positive control) reduced MDPC-23 cell viability by around 70%. Besides presenting the lowest indices of H_2O_2 diffusion and cellular OxS, G6, and G7 also showed ΔE_{00} and ΔWI values similar statistically to G2, which represented conventional in-office tooth bleaching. Based on these data, the null hypothesis of the present study was rejected.

Bovine teeth have been extensively employed in dental research, especially due to the ethical questions of using human teeth [27]. Moreover, these teeth exhibit structure and chemical composition similar to human teeth [28, 29]. In this way, the present study used bovine teeth to obtain enamel/dentin discs (E/DDs) standardized for thickness (2.3 mm) to simulate human lower incisors, which allow a higher trans-amelodentinal diffusion of free-H_2O_2 [4, 5] Therefore, the methodology selected for this in vitro study characterizes the ultimate challenge of indirect cytotoxicity tests of experimental gels and new bleaching therapy proposals [4, 5, 25]. To simulate clinical conditions, the present study used artificial pulp chambers (APCs) to analyze trans-amelodentinal cytotoxicity and quantify free-H_2O_2 that can reach the pulpal space [30]. Bovine E/DDs adapted to APCs represent a well-established laboratory methodology that has been used for almost a decade in studies seeking to develop and analyze more effective and safer bleaching strategies for patients [4, 5, 14–16, 22, 30].

Using enzymes (peroxidase) and transition metals (ferrous sulfate and manganese gluconate) as chemical activators of the bleaching gel accelerates H_2O_2 decomposition, reducing the amount of free-H_2O_2 that can diffuse through hard tissues and increasing the bleaching efficacy of the treatment [11, 13, 14]. In a study by Soares et al. (2019) [14], the heme peroxidase duplicated the bleaching potential of a bleaching gel after a single 45-min session of applying the product to the enamel. However, the high cost of the enzyme and the specific temperature and pH conditions required for peroxidase to catalyze H_2O_2 have turned this bleaching strategy inadequate for clinical application. Manganese oxide (MnO_2) is abundantly found in nature and inexpensive, compared to enzymes [17]. Based on Fenton reactions, the mechanism of interaction of MnO_2 with H_2O_2 might occur through the process of manganese reduction by the reactive molecule [32]: $MnO_2 + H_2O_2 \rightarrow MnO$ (or Mn_2O_3) $+ H_2O$ [18, 33]. Considering these factors and based on previous studies that showed the positive effects of associating manganese-derived agents with in-office bleaching therapy, this oxide was selected for the present study.

To optimize the bleaching procedure and reduce the adverse effects of professional bleaching, the photoactivation of bleaching gels with different light sources was initially proposed some years ago [34]. Among the most recently studied light sources, violet LED stands out due to its ability to minimize the harmful effects commonly caused by in-office tooth bleaching [20]. This is because the violet LED wavelength promotes chromatic changes in dental tissues even without associating it with H_2O_2 gels. However, the isolated use of violet LED provided a mild bleaching efficacy, which characterizes the limitation of this specific esthetic therapy [20, 21]. Hence, researchers have attempted to use violet LED associated with bleaching gels at different H_2O_2 concentrations, and the results have been as promising [19–21]. The present study analyzed bleaching efficacy with a UV-reflective spectrophotometer. This first phase of the study determined the color parameters according to methods established in the literature, in which ΔE_{00} (CIEDE 2000) and ΔWI (whitening index) values are considered to assess the final color result after bleaching. Different from G2 (35% H_2O_2 — positive control), G4 (10% H_2O_2), and G5 (6% H_2O_2), only G6 and G7 subjected the bleaching gels to the chemical catalysis (MnO_2) and photocatalysis (LEDv) of H_2O_2. Overall, the 10% (G4) and 6% (G5) H_2O_2 gels reduced by approximately 50% the bleaching efficacy and whitening index, compared to G2. However, associating both catalysis methods applied to the 10% (G6) and 6% (G7) H_2O_2 gels determined an esthetic result similar to G2. These interesting scientific data indicate that catalyzing the H_2O_2 in bleaching gels with low concentrations of this toxic molecule may be an effective strategy to optimize tooth bleaching, potentially reducing pulp cell damage.

The trans-amelodentinal cytotoxicity of tooth bleaching protocols assessed in the present study was initially determined with the Alamar Blue assay. This analysis showed the lowest cell viability values in all groups bleached with H_2O_2 gels (G2, G4, G5, G6, and G7), compared to the negative control group (G1). However, among the bleached groups, the highest cell viability values were found in G6 and G7, which catalyzed H_2O_2 with MnO_2 + LEDv. Therefore, besides achieving the same esthetic efficacy as conventional in-office bleaching (G2), the association of H_2O_2 catalysis strategy reduced the cytotoxic effects of the treatment by around 40%. A recent study by Gallinari et al. (2020) [35] reported that violet LED favored the chromatic change of teeth when combined with a 10% carbamide peroxide gel. However, this bleaching protocol contributed to post-treatment tooth sensitivity. Perhaps the warming of dental tissues caused by the light source determined this negative clinical outcome presented by Gallinari et al. (2020) [35], considering that the increased dental pulp temperature may result in harmful effects on cells of this specialized connective tissue [36]. High temperatures in the pulp caused by light devices such as violet LED [37] may potentiate tooth sensitivity, which is an adverse effect reported by most patients subjected to tooth bleaching [36]. However, the present study showed that the isolated application of violet LED to the

enamel (G3) did not change MDPC-23 cell viability, which remained morphologically and functionally similar to G1 (control), the group characterized as 100% of cell viability. These interesting results determined that violet LED applied for 20 min to the enamel does not indirectly damage pulp cells. Therefore, considering the limitations of this laboratory research, using violet LED might not damage the pulp or induce tooth sensitivity. However, further investigations are required to explain and clarify this issue.

This study analyzed cellular oxidative stress (OxS) to complement the results of cytotoxicity caused by bleaching gels with low H_2O_2 concentrations, subjected or not to catalysis with MnO_2 + LEDv. In this type of test, the amount of free radicals produced by cells is directly proportional to the cellular OxS promoted by the toxic molecule [22]. Overall, lower OxS values were observed in those groups in which gels with reduced H_2O_2 concentrations (G4 to G7) were used in comparison with G2 (positive control). It is worth noting that the cells in G6 and G7 presented the lowest OxS index, compared to G2, G4, and G5. According to previous studies, the data on cellular OxS explain, at least partially, the higher cell viability values when subjecting the H_2O_2 in the bleaching gels to different types of catalysis [14, 22]. Certainly, the low cell damages in groups G6 and G7 occurred because of the decreased trans-amelodentinal diffusion of free-H_2O_2, as shown in previous studies [4, 5, 14]. This is usually due to H_2O_2 decomposition by chemical [4, 11, 13, 14] and enzyme [22] catalysts, or even by the photocatalysis promoted by violet LED [15, 19].

Despite the interesting findings obtained in this in vitro study, which guide the establishment of bleaching strategies that are more effective and biocompatible with the dentin-pulp complex, further in vivo investigations and detailed clinical trials are still needed. As previously reported, the scientific data of laboratory research should be carefully interpreted and not extrapolated immediately to clinical conditions [30, 38].

Conclusion

Chemical catalysis (MnO_2) associated with the photocatalysis (LEDv) of H_2O_2 present in a bleaching gel containing 6% of this reactive molecule maintains the bleaching efficacy achieved with conventional in-office tooth bleaching but significantly reduces trans-amelodentinal cytotoxicity usually caused by this professional esthetic therapy.

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Data Availability All procedures that involved the use of bovine teeth for scientific research purposes are in compliance with the precepts of Law No.11.794, of October 8, 2008, of Decree No 6.899, of July 15, and with the rules edited by the National Council of Control of Animal Experimentation ("Conselho Nacional de Controle da Experimentação Animal") (CONCEA).

Declarations

Compliance with ethical standards Not applicable.

Conflict of interest The authors declare no competing interests.

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