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ORIGINAL ARTICLE

Catalysis-based approaches with biopolymers and violet LED to improve in-office dental bleaching

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Abstract

This in vitro experimental investigation aimed to evaluate the impact of the combined application of a nanofiber scaffold (NS), a polymeric catalyst primer (PCP) containing 10 mg/mL of heme peroxidase enzyme, and violet LED (LEDv) on the esthetic efficacy (EE), trans-amelodentinal cytotoxicity (TC), and procedural duration of conventional in-office bleaching therapy. To achieve this, 96 standardized enamel/dentin discs were individually placed in artificial pulp chambers. A 35% hydrogen peroxide (H_2O_2) bleaching gel was administered for 45, 30, or 15 min to the enamel, either previously coated with NS + PCP or left uncoated, followed by irradiation with LEDv for 15 min or no irradiation. The established groups were as follows: G1, negative control (no treatment); G2, 35% H_2O_2/45 min; G3, NS + PCP + LEDv; G4, NS + PCP + 35%H_2O_2/45 min + LEDv; G5, NS + PCP + 35%H_2O_2/30 min + LEDv; and G6, NS + PCP + 35%H_2O_2/15 min + LEDv. Extracts (culture medium + gel components diffused through the discs) were collected and applied to odontoblast-like MDPC-23 cells. EE (ΔE_{00} and ΔWI) and TC were assessed using ANOVA/Tukey analysis ($p < 0.05$). The EE analysis revealed no statistical differences between G6 and G2 ($p > 0.05$). Cells in G6 exhibited higher viability and lower oxidative stress compared to other bleached groups ($p < 0.05$). In conclusion, employing NS + PCP + LEDv to catalyze a 35%H_2O_2 bleaching gel applied for 15 min to the enamel resulted in successful esthetic improvements and reduced the cytotoxicity commonly linked with traditional in-office bleaching procedures.

Keywords Tooth bleaching · Cytotoxicity · Odontoblasts · Violet LED

Introduction

The demand for a whiter smile has increased worldwide, and even people with esthetically pleasing smiles started to feel dissatisfied with the color of their teeth [1]. Dental bleaching stands out among the esthetic procedures for smile improvement as a conservative, safe, and minimally invasive technique [2]. Moreover, several studies have shown that changing the color of teeth using different dental bleaching protocols increases smile satisfaction and improves patients' quality of life [3–5].

Bleaching gels with high hydrogen peroxide (H_2O_2) concentrations have been extensively used for conventional in-office tooth bleaching (CIOTB) [6]. The main issue regarding this professional esthetic therapy is associated with the high risk of post-bleaching sensitivity (BS) [7, 8]. Previous studies showed that 50–93% of patients subjected to CIOTB have complained of BS [8, 9]. The number of individuals who have experienced this adverse effect at least once during treatment is up to 61%, and on a pain scale from 0 to 4, the reported mean pain intensity is 2.8 [10]. Bleaching sensitivity occurs partly due to the ability of H_2O_2 in diffusing through enamel and dentin to damage pulp tissues, and this side effect directly relates to the concentration of this highly reactive molecule in gels [11]. Researchers have shown that

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the higher the amount of H_2O_2 in the pulp, the higher the cellular oxidative stress level [12–15], injuring pulp cells and inducing inflammation in this specialized connective tissue [15, 16].

Researchers showed that reducing H_2O_2 concentration in bleaching gels and/or the time of product application to the enamel harms the esthetic outcome of the therapy [12, 17]. These strategies required more tooth bleaching sessions, potentially limiting their use in clinical practice [18, 19]. Therefore, studies have been actually carried out to assess dental bleaching protocols based on the degradation of H_2O_2 in bleaching gels; this approach presented appealing results because H_2O_2 degradation generates molecules with high oxidative potential and shorter half-life time [13, 20, 21]. In this context, a number of in vitro investigations showed that the hydroxypropyl methylcellulose (HPMC) polymer carries catalyst molecules that increase the rate of H_2O_2 degradation, and its association with polycaprolactone (PCL)-based electrospun scaffolds produces even more significant results [13, 22, 23]. These authors demonstrated that combining these biopolymers for in-office tooth bleaching may reduce cytotoxicity and improve the esthetic outcome of this professional therapy.

Another recent study determined that the photocatalysis of H_2O_2 with violet LED (LEDv) also favors the esthetic results of bleaching gels [24, 25] without increasing cytotoxicity [26]. It has been theorized that the wavelength range of V-LED (405–410 nm) aligns with the absorption peak of pigment molecules within the dental structure, particularly in superficial stains. This alignment is believed to result in the disruption of bonds, initiating a physical process that promotes tooth bleaching effect [22, 24–26]. However, the esthetic impact of using V-LED without combination with bleaching gels has been considered as limited [24, 25]. Previous studies demonstrated that when associated with gels containing hydrogen peroxide or carbamide, V-LED has the potential to enhance the efficacy of tooth bleaching therapy through a photocatalysis phenomenon [22, 24, 25]. Therefore, the combined use of photocatalysis (LEDv) with catalyst biopolymers seems further beneficial to the esthetic outcomes and biocompatibility of in-office dental bleaching [22].

Considering the relevant advances to date regarding the use of H_2O_2 degradation approach to perform dental bleaching protocols, this study aimed to assess the influence of the combined use of a nanofiber scaffold (NS), a polymeric catalyst primer (PCP) with 10 mg/mL of heme peroxidase enzyme, and violet LED (LEDv) on esthetic efficacy (EE), trans-amelodentinal cytotoxicity (TC), and potential time reduction to perform CIOTB. The null hypothesis was that the combined use of NS + PCP + LEDv for in-office dental bleaching does not interfere with color changes, cytotoxicity, and application time of an in-office bleaching gel.

Materials and methods

Determination of sample size

Utilizing G*Power software (version 3.1; University Dusseldorf, Dusseldorf, Germany), the number of biological replicates required for the "esthetic efficacy" and "cytotoxicity" response variables was computed. The test power (1-β error probability) and α error probability were set at 0.80 and 0.05, respectively. The effect size for esthetic efficacy was determined to be 4.03, while trans-amelodentinal cytotoxicity had an effect size of 3.35. A sample size of eight specimens per group was estimated for all response variables. To enhance the reliability of observations, each protocol was executed in two separate experiments (duplicates) to minimize systematic errors and ensure reproducibility of observations.

Preparation of the nanofiber scaffold (NS; *n* = 8)

A solution comprising 12.5% polycaprolactone (PCL; Sigma-Aldrich) in chloroform/dimethylformamide (80:20) (Labsynth Produtos para Laboratório, São Paulo, SP, Brazil) was meticulously formulated through continuous magnetic agitation overnight [13]. Subsequently, the resulting solution was loaded into a 5-mL syringe (Descarpak, São Paulo, SP, Brazil) equipped with an 18-gauge stainless steel needle (Becton Dickinson, Franklin Lakes, NJ, USA) connected to an automatic injection device (KDScientific, Holliston, MA, USA), dispensed at a rate of 1 mL/h. The high voltage source was set at 12 kV, and a consistent 15 cm separation was maintained between the needle tip and the flat collector. Carefully, the nanofiber biopolymer adhered to the collector was detached, and circular samples measuring 6 mm in diameter were acquired using a skin punch (Koplast, Barra do Rio, Jaraguá do Sul, SC, Brazil) [13, 22, 23].

Development of the polymeric catalyst primer (PCP; *n* = 8)

A flow-resistant PCP, designed to form a consistent film on enamel, was synthesized [13]. The primary polymer in this formulation was hydroxypropyl methylcellulose (HPMC; Sheffcel, Kerry; Beloit, WI, USA). To create a 10% solution, 1 g of HPMC powder with a viscosity of 100 mPa s was combined with 10 mL of H_2O. The resulting gel, prepared under magnetic agitation for 6 h at room temperature, was then refrigerated for 24 h. The heme peroxidase enzyme (HPR; Sigma-Aldrich) was introduced at

a concentration of 10 mg/mL into the HPMC solution to act as a catalyzing agent, giving rise to the PCP.

Standardization of enamel/dentin discs

Preparation and standardization of enamel/dentin discs involved the acquisition of 96 uniform discs (diameter, 5.6 mm) from whole bovine incisors using 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 underwent correction using 400- and 600-granulation sandpapers (T469-SF- Noton, Saint-Gobam Abrasivos Ltda, Jundiaí, SP, Brazil). Manual rotary movements for each granulation were employed until a standardized thickness of 2.3 mm for both enamel and dentin, mirroring the thickness of lower incisors [19–23].

Esthetic efficacy assessment (*n*= 8)

The standardized enamel/dentin discs were stained with black tea [20–23], and the following groups were established: G1, negative control (no treatment); G2, 35% H_2O_2/45 min; G3, NS + PCP + LEDv; G4, NS + PCP + 35% H_2O_2/45 min + LEDv; G5, NS + PCP + 35% H_2O_2/30 min + LEDv; and G6, NS + PCP + 35% H_2O_2/15 min + LEDv (Table 1). In negative control (G1), no treatment was performed to enamel. In positive control (G2), only the bleaching gel was applied to enamel for 45 min in order to simulate the traditional in-office bleaching therapy. In G3, the enamel surface of the discs was covered with NS and an aliquot of 10 µL of PCP, and then submitted LEDv irradiation for 15 min (Fig. 1). In G4, G5, and G6, after coating enamel surface of the discs with NS and PCP, the bleaching gel was manipulated, immediately applied to the biopolymers, and the LEDv irradiation was performed

Table 1 Distribution of the control and experimental groups

Groups	Bleaching gel	NS + PCP + LEDv	Protocol
G1	-	-	No treatment
G2	X	-	Commercial bleaching gel (Whiteness HP, FGM) applied for 45 min
G3	-	X	Enamel coated with NS + PCP, followed by LEDv irradiation for 15 min
G4	X	X	Enamel coated with NS + PCP, followed by bleaching gel (Whiteness HP, FGM) application for 45 min and LEDv irradiation for 15 min
G5	X	X	Enamel coated with NS + PCP, followed by bleaching gel (Whiteness HP, FGM) application for 30 min and LEDv irradiation for 15 min
G6	X	X	Enamel coated with NS + PCP, followed by bleaching gel (Whiteness HP, FGM) application for 15 min and LEDv irradiation for 15 min

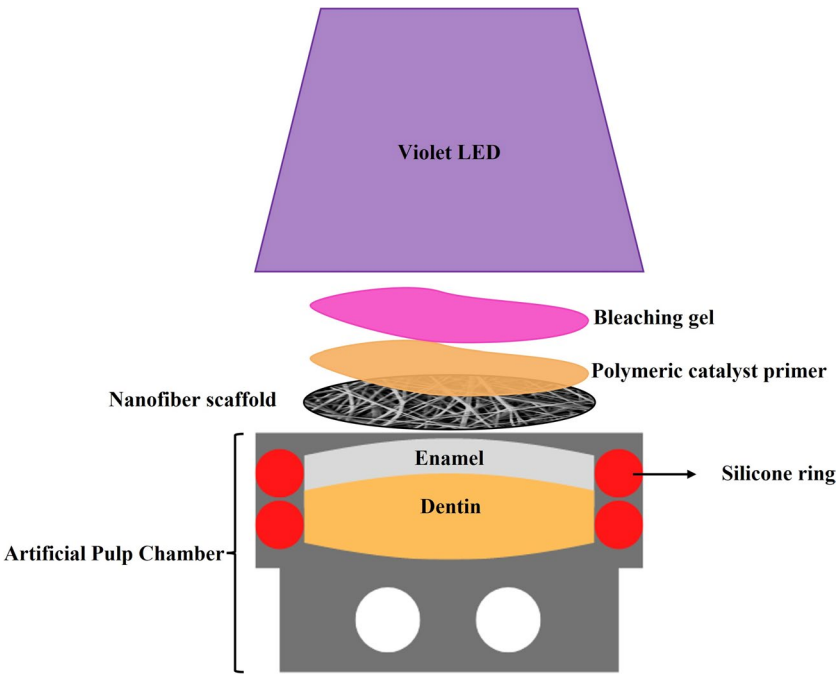

Fig. 1 Schematic representation of the experimental protocols evaluated in the present study

for 15 min. The device used in this study (MMOptics, São Carlos, SP, Brazil) features 4 LED diodes emitting within the 405 ± 10 nm spectral range, with an output power of 350 mW for each super LED, summing up to a total optical power of 1.5 W. This equipment incorporates a delivery system utilizing an anatomically designed tip made of polished acrylic. The tip effectively homogenizes and collimates the light beams from each LED diode, ensuring their organized availability in the corrected area of 10.7 cm^2. This meticulous design results in an irradiance of 140.2 mW/cm^2. All protocols were carried out in dark environment to avoid interference of different light source with the violet LED irradiation parameters established for this in vitro study.

Following the placement of the discs within a white silicone matrix, the bleaching effectiveness was evaluated using a UV-reflective spectrophotometer (Reflection Spectrophotometer ML Color Guide, BYK Gardner GmbH Gerestsried, Germany). Color changes were assessed by comparing the values obtained before (baseline) and 72 h after the completion of the bleaching procedures, employing the CIEDE 2000 (ΔE_{00}) method and the Whitening Index (ΔWI), using the following equations:

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

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

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

Subsequently, the color parameters (ΔE_{00} and ΔWI) acquired for all groups underwent statistical analysis according to the following description. The perceptibility (PT) and acceptability (AT) threshold values were set at 50:50%, with ΔE_{00} thresholds at 0.8 (PT) and 2.7 (AT) and ΔWI thresholds at 0.72 (PT) and 2.60 (AT) [27].

Trans-amelodentinal cytotoxicity

MDPC-23 cell culture

Immortalized odontoblast-like MDPC-23 cells, sourced from rats' dental papillae, were provided by Professor Carl T. Hanks (University of Michigan, Ann Arbor, MI, USA). Since 2001, this cell line has been stored at the Laboratory of Experimental Pathology and Biomaterials of the Department of Physiology and Pathology, School of Dentistry, São Paulo State University (UNESP), Araraquara, Brazil. MDPC-23 cells (passage #65) were defrosted and grown in 75 cm^2 sterilized plates (KASVI Imp., Curitiba, PR, Brazil) using Dulbecco's Modified Eagle's medium (DMEM; GIBCO. Grand Island, NY, USA). The medium included 10% fetal bovine serum (FBS; GIBCO), 100 IU/mL and 100 g/mL of penicillin and streptomycin, respectively, and 2 mmol/L of glutamine (GIBCO). The cultivation took place in a humid environment at 37 °C with 5% CO_2 and 95% air. Subsequent passages were performed to reach an optimal cell quantity for experimentation.

Experimental procedure

Enamel/dentin discs were inserted into artificial pulp chambers (APCs) and subjected to sterilization using ethylene oxide (Acecil, Central de Esterilização Comércio e Indústria LTDA, Campinas, SP, Brazil). Subsequently, each disc/APC set was individually placed in 24-well sterilized acrylic plates (KASVI Imp.) containing 1 mL of DMEM [19–24]. Following the enamel bleaching procedure, the extracts (DMEM + components from the bleaching gel diffused through enamel and dentin) were promptly collected for use in cytotoxicity tests.

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

One hundred microliter aliquots of extracts from each group were dispensed into 5-mL plastic tubes (KASVI Imp.) containing 900 µL of acetate buffer solution (2 mol; pH 4.5), which stabilizes H_2O_2. Subsequently, 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). After vortex agitation, the tubes received 50 µL of a 1 mg/mL horseradish peroxidase enzyme solution (Sigma-Aldrich). The absorbance of the solutions was measured using a spectrophotometer (Synergy H1) at a wavelength of 596 nm. The optical density values obtained in the samples were converted into µg of H_2O_2 per mL of the extract using the standard curve generated from known amounts of H_2O_2 [19–23].

Measurement of hydrogen peroxide (H_2O_2) in the extracts ($n = 8$)

To quantify H_2O_2 levels in the extracts, 100 µL aliquots from each experimental group were dispensed into 5-mL plastic tubes (KASVI Imp.) containing 900 µL of acetate buffer solution (2 mol; pH 4.5), which serves to stabilize H_2O_2. Subsequently, 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). Following vortex agitation, the tubes were supplemented with 50 µL of a 1 mg/mL horseradish peroxidase enzyme solution (Sigma-Aldrich). The absorbance of the resulting solutions was measured using a spectrophotometer (Synergy H1) at a wavelength of 596 nm. Optical density values obtained from

the samples were then converted into µg of H_2O_2 per mL of the extract using a standard curve generated from known quantities of H_2O_2 [19–23].

Evaluation of cell viability (Alamar Blue assay; *n* = 8)

To assess cell viability, 100 µL aliquots of each extract were applied to MDPC-23 cells previously cultured at a density of 10,000 cells in 96-well sterilized acrylic plates (KASVI Imp.). Following a 1-h incubation period, the extracts were removed, leaving the cells adhered to the bottom of the plate. Subsequently, these cells underwent a 4-h incubation with a solution consisting of 90 µL of DMEM and 10 µL of Alamar Blue (Life Technologies; Grand Island, NY, USA). The resulting solution was then collected and transferred to a 96-well plate (KASVI Imp.), and the absorbance was measured at 570 nm using a spectrophotometer (Synergy H1, Biotek, Winooski, USA) [19–23].

Cellular oxidative stress analysis (*n* = 8)

The evaluation of cellular oxidative stress involved estimating the production of reactive oxygen species (ROS) by cells immediately following the bleaching procedure. To achieve this, MDPC-23 cells, previously cultured in 96-well sterilized plates, were exposed to the carboxy-H_2DCFDA probe (Invitrogen, San Francisco, CA, USA) at a concentration of 10 µg/mL for 30 min. This probe, permeable to the cell membrane, emits fluorescence upon contact with ROS. Subsequently, 100 µL aliquots of extracts from each group were applied to the cells for 1 h, and the fluorescence intensity was measured at 592-nm excitation and 517-nm emission (Synergy H1) after exposure to the extracts. Data normalization was performed using the negative control group, as described previously [19–23].

Cell morphology assessment (*n* = 4)

In this analysis, MDPC-23 cells were cultured on glass coverslips positioned at the base of 24-well plates, and the previously outlined bleaching procedures were carried out. Following the incubation of cells with the extracts, the cells were removed, and the MDPC-23 cells that adhered to the glass coverslips were fixed and processed for subsequent microscopic examination [13]. After mounting on metallic stubs, the coverslips with the cells underwent gold metallization (Sistevac, Rolândia, PR, Brazil). Subsequently, the morphology of MDPC-23 cells was evaluated using a scanning electron microscope (SEM, JEOL JSM 6610; JEOL Ltda, Akishima, Tokyo, Japan) to generate photomicrographs representing each experimental group.

Statistical analyses

The collected data were subjected to the Shapiro–Wilk test to evaluate normality and the Levene test to examine homoscedasticity. For the assessments of esthetic efficacy, cell viability, oxidative stress, and H_2O_2 diffusion, a one-way analysis of variance (one-way ANOVA), followed by Tukey's post-test was used. All statistical analyses were performed with a significance level set at 5% using IBM SPSS Statistics for Windows, Version 25.0, Armonk, NY.

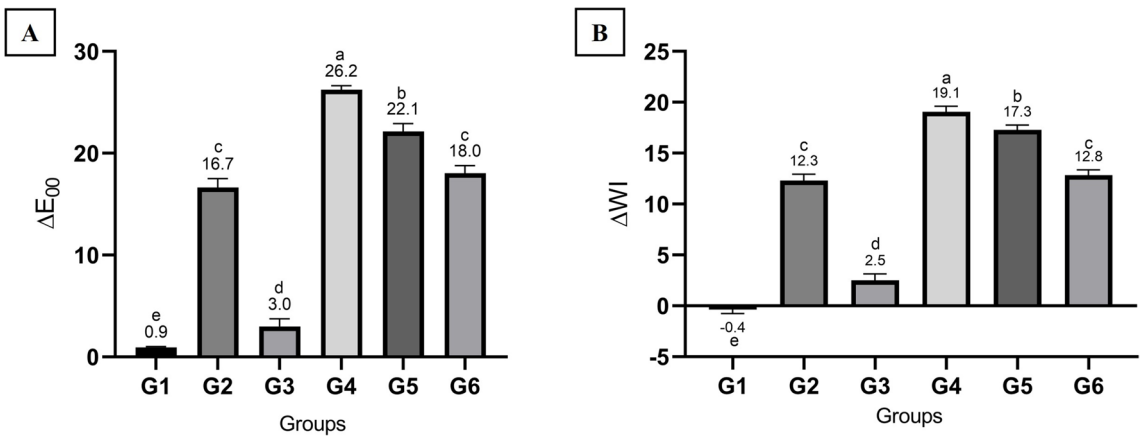

Fig. 2 **A** CIEDE2000 color change analysis (ΔE_{00}); bars represent mean and standard deviation values. Numbers show the means of each group. Different letters indicate statistically significant differences ($n = 8$; ANOVA/Tukey = 5%). **B** Whitening Index (ΔWI); bars represent mean and standard deviation values. Numbers show the means of each group. Different letters indicate statistically significant differences ($n = 8$; ANOVA/Tukey = 5%)

Results

Esthetic efficacy assessment

The ΔE_{00} equation showed a mild bleaching potential in G3 compared to G1 (negative control) ($p < 0.05$) (Fig. 2A). Using NS + PCP + LEDv significantly increased color changes in G4 and G5, in which the gel remained on the enamel for 45 and 30 min, respectively, compared to G2 (positive control) ($p < 0.05$) (Fig. 2A). The bleaching potential in G6 was similar to G2 ($p > 0.05$) that represented the CIOTB (Fig. 2A). Similar results occurred for ΔWI analysis (Fig. 2B). The use of NS + PCP + LEDv enhanced color changes in G4 and G5 compared to G2 ($p < 0.05$), without statistical differences between G6 and G2 ($p > 0.05$).

Quantification of H_2O_2 in the extracts

A higher H_2O_2 trans-amelodentinal diffusion occurred in G2 compared to G4, G5, and G6 (Fig. 3A; $p < 0.05$). No statistical difference was observed between G4 and G5, in which NS + PCP + LEDv was used and the bleaching was applied to the enamel for 45 or 30 min, respectively ($p > 0.05$). G6 presented a lower H_2O_2 trans-amelodentinal diffusion value than the other groups ($p < 0.05$).

Cell viability assessment

Cell viability decreased in all groups that received the bleaching gel, regardless of using NS + PCP + LEDv or not and the time of bleaching gel application, compared to G1 (negative control) and G3 (NS + PSP + LEDv) (Fig. 3B; $p < 0.05$). The highest MDPC-23 cell viability among the bleached groups occurred in G6 ($p < 0.05$). When compared to the other groups, the lowest cell viability occurred in G2 that represented the CIOTB ($p < 0.05$).

Cellular oxidative stress analysis

Significant oxidative stress increase was observed in all groups that used bleaching gels associated with NS + PCP + LEDv or not, compared to G1 and G3 (Fig. 3C; $p < 0.05$). The highest oxidative stress value occurred in G2 ($p < 0.05$). The combined use of NS + PCP + LEDv and the bleaching gel application for only 15 min to the enamel (G6) significantly decreased oxidative stress compared to the other bleached groups ($p < 0.05$).

Cell morphology assessment

The obtained SEM images (Fig. 4) determined that the morphology and number of cells adhered to the glass substrate were similar among the groups that did not receive bleaching gels on the enamel (G1 to G3). In these groups, cells exhibited wide cytoplasm from which several thin cytoplasmic extensions were originated. The size and number of cells that remained adhered to the glass substrate decreased in all bleached groups (G2, G4, G5, and G6) compared to G1 and G3. Coating the enamel with NS + PCP and using LEDv to irradiate the bleaching gel was decisive in maintaining more cells adhered to the substrate, regardless of product application time. However, this effect was even more significant in G6, in which the catalyst strategy was used and the bleaching gel was applied to enamel for only 15 min.

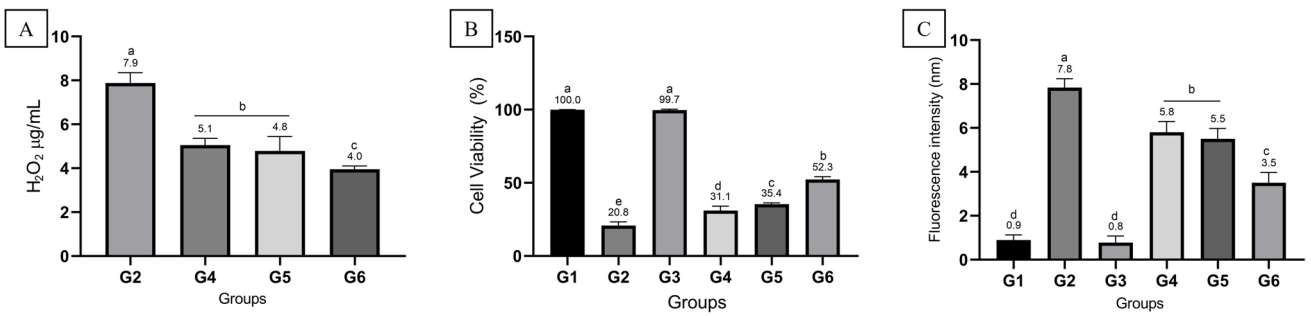

Fig. 3 **A** H_2O_2 diffusion analysis (H_2O_2 µg/mL); bars represent mean and standard deviation values. Numbers show the means of each group. Different letters indicate statistically significant differences ($n = 8$; ANOVA/Tukey = 5%). **B** Cellular cytotoxicity analysis (cell viability%); bars represent mean and standard deviation values. Numbers show the means of each group. Different letters indicate statistically significant differences ($n = 8$; ANOVA/Tukey = 5%). **C** Oxidative stress analysis (fluorescence intensity); bars represent mean and standard deviation values. Numbers show the means of each group. Different letters indicate statistically significant differences ($n = 8$; ANOVA/Tukey = 5%)

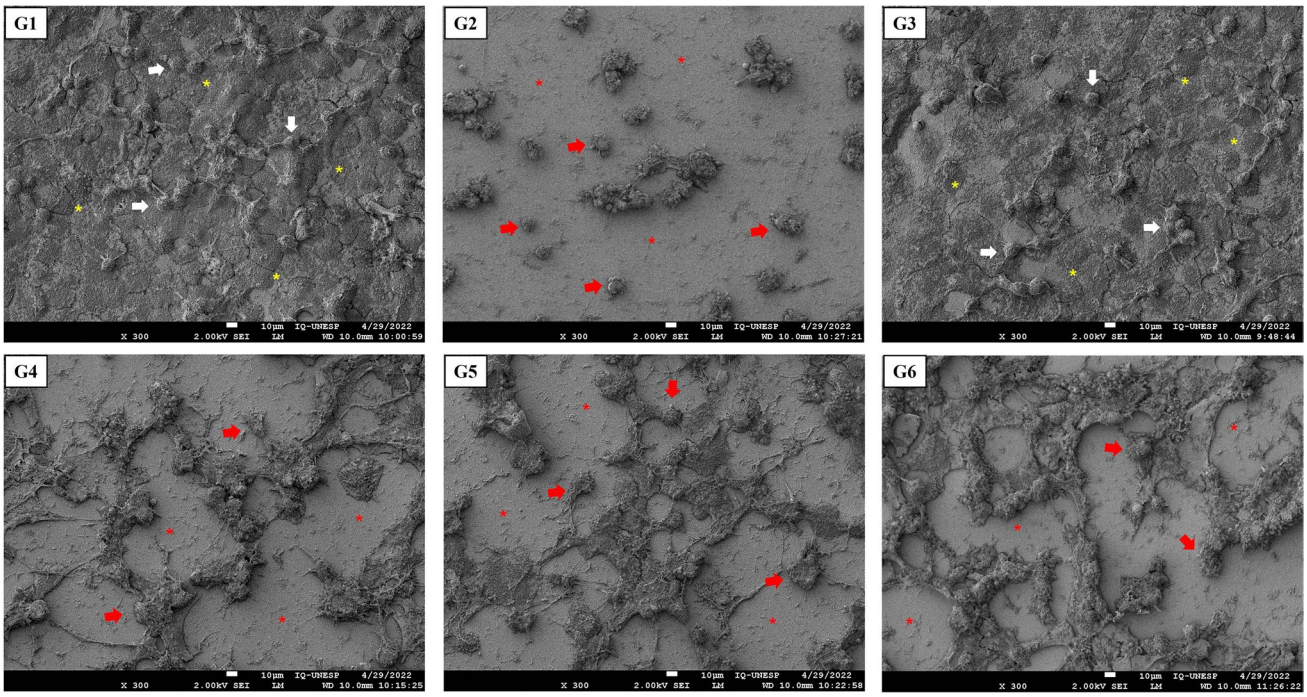

Fig. 4 Cell morphology analysis. SEM, ×300. G1 and G3 showed cells with extensive cytoplasm, polygonal morphology (white arrow), and elongations from the membrane and cytoplasm (yellow asterisk) on the glass substrate. The bleached groups (G2, G4, G5, and G6) presented large glass substrate areas (red asterisk), considering that a few cells exposed to the extracts remained adhered. These cells exhibit relevant morphological changes (red arrows). G6 presented the most adhered cells and morphology closest to normality among the bleached groups

Discussion

In vivo studies have shown severe and irreversible damage to the human dental pulp caused by the trans-amelodentinal diffusion of H_2O_2 released from bleaching gels [16, 28]. These adverse pulpal effects seem directly related to the H_2O_2 concentration in bleaching gels and their application time to enamel [12, 17]. However, a recent study demonstrated that reducing H_2O_2 concentration from 35 to 20% in an in-office bleaching gel did not prevent the partial necrosis of the coronal pulp and root pulp inflammation [28]. Toxic concentrations of H_2O_2 reaching the pulp tissue have been related to a lower capacity of this molecule to interact and degrade organic pigments located in mineralized dental tissues. Thus, the excess of unreacted H_2O_2 ($H_2O_{2\text{-free}}$) diffused through enamel and dentin to reach the pulp produces oxidative stress in local cells and lipid peroxidation, resulting in irreversible cell damage [29]. However, researchers have shown that combining strategies that increase the degradation kinetics of the H_2O_2 molecule in bleaching gels reduces the amount of $H_2O_{2\text{-free}}$, decreasing the cytotoxicity of in-office bleaching therapy [13, 19–23]. The new molecules produced from H_2O_2 degradation have a shorter half-life time and high oxidative potential that may favor the esthetic outcome and reduce treatment toxicity [21–23]. The present investigation demonstrated that using the NS + PCP + LEDv catalyst strategy accelerated the color changes of darkened dental tissues, allowing them to reach esthetic efficacy in only 15 min. This catalyst strategy also reduced H_2O_2 trans-amelodentinal diffusion, minimizing the toxic effects of the procedure on pulp cells. Hence, the null hypothesis of the present study was rejected.

The benefits of using enzyme catalysts to degrade H_2O_2 in bleaching gels have been confirmed by the significant enhance in hydroxyl radical ($HO^{\bullet}$) production [13, 20, 22, 30]. When in contact with H_2O_2, the peroxidase enzyme reduces the kinetic energy required to start a molecule reaction, increasing the rate of H_2O_2 degradation. Consequently, there is a high production of $HO^{\bullet}$ and other reactive molecules, capable of decomposing rapidly organic pigments present in hard dental structures [31, 32]. Horseradish peroxidase (HRP), the most currently investigated enzyme chemical catalysts, is obtained from a vegetable source of *Armoracia rusticana* roots; this enzyme consists of a Fe^{3+} metallic core, which favors the homolytic scission of H_2O_2 in a process similar to the Fenton reaction [32–34]. Additionally, LEDv seems to mainly benefit the esthetic efficacy of the in-office bleaching therapy, potentially representing an appealing coadjuvant for this treatment [24–26]. Catalyst biopolymers, such as NS and PCP, favor H_2O_2 degradation,

increasing esthetic efficacy and reducing the cytotoxicity of the bleaching therapy [13, 22, 23]. Thus, developing innovative in-office tooth bleaching protocols with the potential to reduce treatment time and the adverse biological effects of this therapy is imperative at this moment. The present study showed significant changes in color (ΔE_{00}) and the Whitening Index (ΔWI) in the groups that used the NS + PCP + LEDv-combined strategy. G4 and G5, which respectively applied the gel to the enamel for 45 and 30 min, showed approximate increases of 57% and 32% for ΔE_{00} and 55% and 40% for ΔWI, respectively, compared to the conventional in-office dental bleaching protocol (G2). Although the bleaching gel application time to the enamel decreased by only 15 min in G6, the achieved esthetic efficacy with the combined use of NS + PCP + LEDv was statistically similar to G2.

The analysis of $H_2O_{2\text{-free}}$ diffused through the enamel/dentin discs indirectly determined the degree of H_2O_2 degradation in the bleaching gel. The generated by-products, such as hydroxyl radicals ($HO^{\bullet}$), peri-hydroxyl ($HO_2^{\bullet}$), and superoxide ($O_2^{\bullet}$), are simple and highly reactive molecules with a short half-life time [35, 36]. Therefore, these molecules can be eliminated from the medium immediately after interacting with the pigments located in mineralized dental tissues [37–39], preventing trans-amelodentinal diffusion. Conversely, the absent degradation of the H_2O_2 molecules, which has limited reactivity and a long half-life time, allows their diffusion at toxic concentrations up to the pulp space. Thus, the higher the H_2O_2 degradation in bleaching gels, the lower the product cytotoxicity, and vice versa. The highest H_2O_2 diffusion value of the present study occurred in G2, which represented conventional in-office dental bleaching. Coating the enamel with NS + PCP and using LEDv to irradiate the bleaching gel applied for 45 (G4), 30 (G5), or 15 (G6) min, respectively, reduced around 49%, 39%, and 36% of cell viability compared to G2. Using the NS + PCP + LEDv catalyst strategy did not prevent complete cell damage compared to G1 (100% of cell metabolism), allowing to suggest that the high H_2O_2 concentration in the in-office bleaching gel of this study (35%H_2O_2) prevented a complete degradation of this molecule. Consequently, despite the excellent esthetic outcome, G6 showed a moderate cytotoxic effect, in which MDPC-23 cell viability decreased due to higher oxidative stress. Thus, further studies are required to determine the potential biological side effects of bleaching gels with lower H_2O_2 concentrations when combined with the catalyst strategy (NS + PCP + LEDv) assessed in this investigation.

In the present study, the strategy of employing two polymers (NS + PCP) in combination with LEDv irradiation of the bleaching gel was assessed in vitro. Clinically, NS would be initially used as a polymeric strip to cover enamel of the buccal surface of teeth. Thus, the PCP would be prepared and immediately applied on the NS. Finally, after applying the bleaching gel on the PCP, the product would be submitted to LEDv irradiation for 15 min. However, it is important to emphasize that the formulation of the two experimental polymeric materials (NS and PCP) requires technical-scientific knowledge, as well as specific equipment for their production, which may be considered as limitations of the present study. Furthermore, for the catalyst approach of LEDv irradiation of the bleaching gel, it is necessary to acquire the LEDv device, which has not been frequently used in dentistry nowadays. However, one can consider that strips of NS may be produced by the dental company and then included in the commercial dental bleaching kit. In this way, two bottles (solution of 10% HPMC and solution of HPR) may also be provided for professional hand-mixing preparation during the in-office tooth bleaching procedure.

Considering the compelling and promising scientific data obtained in this study, which may drive the development of innovative bleaching therapies that are more compatible and faster, it is worth noting that the in vitro results must not be immediately extrapolated to clinical situations [13, 21–23]. That is because vital teeth present factors that may limit the H_2O_2 trans-amelodentinal diffusion, such as exudation pressure of the dentinal fluid and the intratubular presence of collagen and cytoplasmic process from odontoblasts. Therefore, besides assessing the use of the NS + PCP + LEDv catalyst strategy associated with bleaching gels at lower concentrations, it would be worth performing in vivo studies to determine the esthetic effectiveness and safety of the clinical use of this professional therapy.

Conclusion

In spite of requiring some additional clinical steps (NS + PCP + LEDv), the catalysis-based strategy assessed in this study reduces by 30 min the time spent to perform a session of the conventional in-office tooth bleaching and significantly diminishes the pulp cells toxicity commonly caused by this professional esthetic therapy.

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Declarations

Conflict of interest The authors declare no competing interests.

References

1. Maghaireh GA, Alzraikat H, Taha NA (2016) Satisfaction with dental appearance and attitude toward improving dental esthetics among patients attending a dental teaching center. J Contemp Dent Pract 17(1):16–21. https://doi.org/10.5005/jp-journals-10024-1796
2. Dourado Pinto AV, Carlos NR, Amaral FLBD, França FMG, Turssi CP, Basting RT (2019) At-home, in-office and combined dental bleaching techniques using hydrogen peroxide: randomized clinical trial evaluation of effectiveness, clinical parameters and enamel mineral content. Am J Dent 32(3):124–132
3. Pereira R, Silveira J, Dias S, Cardoso A, Mata A, Marques D (2022) Bleaching efficacy and quality of life of different bleaching techniques—randomized controlled trial. Clin Oral Investig 1–11. https://doi.org/10.1007/s00784-022-04678-5
4. Meireles SS, Goettems ML, Dantas RVF, Della Bona Á, Santos IS, Demarco FF (2014) Changes in oral health related quality of life after dental bleaching in a double-blind randomized clinical trial. J Dent 42(2):114–121. https://doi.org/10.1016/j.jdent.2013.11.022
5. Cavalcante L, Bomgiovanni C, Antunes K, Cardoso M, Loureiro E, Tannure P, Schneider LF (2022) Color change, tooth sensitivity, aesthetic self-perceptions and quality of life after at-home dental bleaching. Braz Dent Sci 25(1). https://doi.org/10.4322/bds.2022.e2739
6. Alqahtani MQ (2014) Tooth-bleaching procedures and their controversial effects: a literature review. Saudi Dent J 26(2):33–46. https://doi.org/10.1016/j.sdentj.2014.02.002
7. Maran BM, de Paris Matos T, de Castro ADS, Vochikovski L, Amadori AL, Loguercio AD, Reis A, Berger SB (2020) In-office bleaching with low/medium vs. high concentrate hydrogen peroxide: a systematic review and meta-analysis. J Dent 103:103499. https://doi.org/10.1016/j.jdent.2020.103499
8. Kielbassa AM, Maier M, Gieren AK, Eliav E (2015) Tooth sensitivity during and after vital tooth bleaching: a systematic review on an unsolved problem. Quintessence Int 46(10). https://doi.org/10.3290/j.qi.a34700
9. Meireles SS, Santos ME, Lustosa ÍMC, Leite ELL (2021) Effects of a reduced in-office bleaching protocol with 37.5% hydrogen peroxide on effectiveness and tooth sensitivity: a double-blind randomized clinical trial. J Esthet Restor Dent 33(5):824–831. https://doi.org/10.1111/jerd.12744
10. Rezende M, da Silva KL, Miguel TC, Farago PV, Loguercio AD, Martins LD, Reis A (2020) Prior application of 10% potassium nitrate to reduce postbleaching sensitivity: a randomized triple-blind clinical trial. J Evid Based Dent Pract 20(2):101406. https://doi.org/10.1016/j.jebdp.2020.101406
11. Rezende M, Loguercio AD, Kossatz S, Reis A (2016) Predictive factors on the efficacy and risk/intensity of tooth sensitivity of dental bleaching: a multi regression and logistic analysis. J Dent 45:1–6. https://doi.org/10.1016/j.jdent.2015.11.003
12. Soares DG, Basso FG, Hebling J, de Souza Costa CA (2014) Concentrations of and application protocols for hydrogen peroxide bleaching gels: effects on pulp cell viability and whitening efficacy. J Dent 42(2):185–198. https://doi.org/10.1016/j.jdent.2013.10.021
13. Ortecho-Zuta U, de Oliveira Duque CC, de Oliveira Ribeiro RA, Leite ML, Soares DG, Hebling J, Briso ALF, de Souza Costa CA (2021) Polymeric biomaterials maintained the esthetic efficacy and reduced the cytotoxicity of in-office dental bleaching. J Esthet Restor Dent 33:1139–1149. https://doi.org/10.1111/jerd.12805
14. Soares DG, Marcomini N, Duque CCDO, Bordini EAF, Zuta UO, Basso FG, Hebling J, Costa CADS (2019) Increased whitening efficacy and reduced cytotoxicity are achieved by the chemical activation of a highly concentrated hydrogen peroxide bleaching gel. J Appl Oral Sci 27:e20180453. https://doi.org/10.1590/1678-7757-2018-0453
15. Soares DG, Basso FG, Hebling J, de Souza Costa CA (2015) Effect of hydrogen-peroxide-mediated oxidative stress on human dental pulp cells. J Dent 43(6):750–756. https://doi.org/10.1016/j.jdent.2014.12.006
16. de Souza Costa CA, Riehl H, Kina JF, Sacono NT, Hebling J (2010) Human pulp responses to in-office tooth bleaching. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 109(4):e59–e64. https://doi.org/10.1016/j.tripleo.2009.12.002
17. Soares DG, Basso FG, Hebling J, de Souza Costa CA (2015) Immediate and late analysis of dental pulp stem cells viability after indirect exposition to alternative in-office bleaching strategies. Clin Oral Investig 19:1013–1020. https://doi.org/10.1007/s00784-014-1321-3
18. Soares DG, Basso FG, Pontes EC, da Garcia FRL, Hebling J, de SouzaCosta CA (2014) Effective tooth-bleaching protocols capable of reducing H2O2 diffusion through enamel and dentine. J Dent 42:351–358. https://doi.org/10.1016/j.jdent.2013.09.001
19. de Oliveira Duque CC, Soares DG, Basso FG, Hebling J, de Souza Costa CA (2017) Influence of enamel/dentin thickness on the toxic and esthetic effects of experimental in-office bleaching protocols. Clin Oral Investig 21:2509–2520. https://doi.org/10.1007/s00784-017-2049-7
20. Ribeiro RADO, de Oliveira Duque CC, Ortecho-Zuta U, Leite ML, Hebling J, Soares DG, de Souza Costa CA (2022) Influence of manganese oxide on the esthetic efficacy and toxicity caused by conventional in-office tooth bleaching therapy. Oper Dent 47(4):425–436. https://doi.org/10.2341/20-257-L
21. de Oliveira Ribeiro RA, Zuta UO, Soares IPM, Anselmi C, Soares DG, Briso ALF, Hebling J, de Souza Costa CA (2022) Manganese oxide increases bleaching efficacy and reduces the cytotoxicity of a 10% hydrogen peroxide bleaching gel. Clin Oral Investig 1–10. https://doi.org/10.1007/s00784-022-04688-3
22. Martins BV, Dias MF, de Oliveira Ribeiro RA, e Silva MLDA, Hebling J, de Souza Costa CA (2022) Innovative strategy for in-office tooth bleaching using violet LED and biopolymers as H2O2 catalysts. Photodiagn Photodyn Ther 102886. https://doi.org/10.1016/j.pdpdt.2022.102886
23. Ortecho-Zuta U, de Oliveira Duque CC, de Oliveira Ribeiro RA, Leite ML, Soares DG, Hebling J, Briso ALF, de Souza Costa CA (2022) Strategy for reducing cytotoxicity and obtaining esthetic efficacy with 15 min of in-office dental bleaching. Clin Oral Investig 26(5):4099–4108. https://doi.org/10.1007/s00784-022-04379-z
24. Kury M, Wada EE, da Silva PS, Picolo MZD, Giannini M, Cavalli V (2022) Colorimetric evaluation after in-office tooth bleaching with violet LED: 6- and 12-month follow-ups of a randomized clinical trial. Clin Oral Invest 26:837–847. https://doi.org/10.1007/s00784-021-04062-9
25. Kury M, Wada EE, Silva DPD, Tabchoury CPM, Giannini M, Cavalli V (2020) Effect of violet LED light on in-office bleaching protocols: a randomized controlled clinical trial. J Appl Oral Sci 28:e20190720. https://doi.org/10.1590/1678-7757-2019-0720
26. de Oliveira Ribeiro RA, Peruchi V, de Oliveira Fernandes L, Anselmi C, Soares IPM, Hebling J, de Souza Costa CA (2022) The influence of violet LED application time on the esthetic efficacy and cytotoxicity of a 35% H2O2 bleaching gel. Photodiagn Photodyn Ther 103069. https://doi.org/10.1016/j.pdpdt.2022.103069
27. Paravina RD, Ghinea R, Herrera LJ, Bona AD, Igiel C, Linninger M, Sakai M, Takahashi H, Tashkandi E, Mar Perez MD (2015) Color difference thresholds in dentistry. J Esthet Restor Dent 27:S1–S9. https://doi.org/10.1111/jerd.12149

28. Roderjan DA, Stanislawczuk R, Soares DG, de Souza Costa CA, Favoreto MW, Reis A, Loguercio AD (2023) Effect of medium or high concentrations of in-office dental bleaching gel on the human pulp response in the mandibular incisors. Restor Dent Endod 48. https://doi.org/10.5395/rde.2023.48.e12
29. Marson FC, Gonçalves RS, Silva CO, Cintra LTA, Pascotto RC, Santos PD, Briso ALF (2015) Penetration of hydrogen peroxide and degradation rate of different bleaching products. Oper Dent 40(1):72–79. https://doi.org/10.2341/13-270-L
30. Gopinath S, James V, Vidhya S, Karthikeyan K, Kavitha S, Mahalaxmi S (2013) Effect of bleaching with two different concentrations of hydrogen peroxide containing sweet potato extract as an additive on human enamel: an in vitro spectrophotometric and scanning electron microscopy analysis. J Conserv Dent 16:45–49. https://doi.org/10.1590/1678-7757-2018-0453
31. Travassos AC, Torres CRG, Borges AB, Barcellos DC (2010) In vitro assessment of chemical activation efficiency during in-office dental bleaching. Oper Dent 35:8. https://doi.org/10.2341/09-256-L
32. Berglund GI, Carlsson GH, Smith AT, Szöke H, Henriksen A, Hajdu J (2002) The catalytic pathway of horseradish peroxidase at high resolution. Nature 417(6887):463–468. https://doi.org/10.1038/417463a
33. Veitch NC (2004) Horseradish peroxidase: a modern view of a classic enzyme. Phytochemistry 65(3):249–259. https://doi.org/10.1016/j.phytochem.2003.10.022
34. Sato S, Nakamura K, Nakamura H (2017) Horseradish-peroxidase-catalyzed tyrosine click reaction. ChemBioChem 18(5):475–478. https://doi.org/10.1002/cbic.201600649
35. Khanmohammadi M, Dastjerdi MB, Ai A, Ahmadi A, Godarzi A, Rahimi A, Ai J (2018) Horseradish peroxidase-catalyzed hydrogelation for biomedical applications. Biomater Sci 6(6):1–43. https://doi.org/10.1039/C8BM00056E
36. Gantumur E, Sakai S, Nakahata M, Taya M (2019) Horseradish peroxidase-catalyzed hydrogelation consuming enzyme-produced hydrogen peroxide in the presence of reducing sugars. Soft Matter 15(10):2163–2169. https://doi.org/10.1039/C8SM01839A
37. Kawamoto K, Tsujimoto Y (2004) Effects of the hydroxyl radical and hydrogen peroxide on tooth bleaching. J Endod 30(1):45–50. https://doi.org/10.1097/00004770-200401000-00010
38 Goldberg M, Grootveld M, Lynch E (2010) Undesirable and adverse effects of tooth-whitening products: a review. Clin Oral Investig 14(1):1–10. https://doi.org/10.1007/s00784-009-0302-4
39. Carey CM (2014) Tooth whitening: what we now know. J Evid Based Dent Pract 14:70–76. https://doi.org/10.1016/j.jebdp.2014.02.006