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ORIGINAL ARTICLE

# Effects of experimental in-office bleaching gels incorporated with co-doped titanium dioxide nanoparticles on dental enamel physical properties

**Matheus Kury[1,2] · Fernando Luís Esteban Florez[3] · Cínthia Pereira Machado Tabchoury[4] · Vanessa Cavalli[1]**

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## Abstract

To evaluate the physical properties of enamel submitted to hydrogen peroxide (HP) incorporated with titanium dioxide nanoparticles (NP) co-doped with nitrogen and fluorine and irradiated with violet LED light (LT). Enamel–dentin disks were randomly allocated ($T_0$) into groups, according to HP (HP6, HP15, or HP35) and NP (no NP, 5NP, or 10NP) concentrations, and irradiated or not with LT. A negative control (NC) group was set. After three bleaching sessions ($T_1$, $T_2$, and $T_3$), specimens were stored in saliva for 14 days ($T_4$). Enamel surface microhardness number (KHN), surface roughness (*Ra*), cross-sectional microhardness ($\Delta S$), energy-dispersive spectroscopy (EDS), scanning electron (SEM), and polarized light (PLM) microscopies were performed. Surface KHN was significantly influenced by NP over time, independently of LT irradiation. At $T_3$ and $T_4$, gels with 5NP and 10NP exhibited no KHN differences compared to NC and baseline values, which were not observed under the absence of NP. NP incorporation did not statistically interfere with the $\Delta S$ and *Ra*. PLM images exhibited surface/subsurface darkening areas suggestive of demineralizing regions. SEM demonstrated some intraprismatic affection in the groups without NP. EDS reported a higher enamel calcium to phosphorus ratio following 10NP gels applications. Gels with NP maintained the enamel surface microhardness levels and seemed to control surface morphology, upholding the mineral content. None of the proposed experimental protocols have negatively influenced the enamel surface roughness and the cross-sectional microhardness.

**Keywords** Tooth bleaching · Hydrogen peroxide · Light · Nanoparticles · Dental enamel

## Introduction

In-office tooth bleaching holds the advantage of immediately resolving tooth discoloration by using high-concentrated hydrogen peroxide (HP) gels [1]. Nonetheless, there are several reports in the literature showing that application of these bleaching gels onto the enamel surface could cause adverse effects on the physical properties of enamel's surface and its underlying subsurface. For instance, 35–40% HP decreased the enamel surface and cross-sectional microhardness [2–4], increased the surface roughness [5, 6], and negatively influenced the enamel surface morphology and mineral content [7, 8]. From the clinical standpoint, these events could be translated into weakened enamel and increase in tooth sensitivity levels [1, 3, 6].

Such conditions could be a result of the oxi-reductive action of hydrogen peroxide, which penetrates into the dentin through the interprismatic spaces of enamel [9]. The pH of the gels is also a feasible cause of the enamel surface

✉ Matheus Kury
matheus.rodrigues123@docente.unip.br

1 Operative Dentistry Division, Department of Restorative Dentistry, Piracicaba School of Dentistry, University of Campinas, 901 Limeira Av., Areião, Piracicaba, SP 13414-903, Brazil

2 Dental Research Division, Paulista University, 1212 Doutor Bacelar St., Indianópolis, São Paulo, SP 04026-002, Brazil

3 Dental Materials Division, Department of Restorative Sciences, Oklahoma University Health Sciences Center College of Dentistry, 1201 N Stonewall Ave, Oklahoma City, OK 73117, USA

4 Biochemistry Division, Department of Physiological Sciences, Piracicaba School of Dentistry, University of Campinas, 901 Limeira Av., Areião, Piracicaba, SP 13414-903, Brazil

alterations, possibly due to an ion-exchange mechanism. Efforts have been made to overcome these drawbacks, such as the incorporation of calcium and fluoride ions [10, 11] into experimental and commercial bleaching gels and the increase in the gel's pH [12]. Indeed, some studies have attested that such ions incorporation and increase in the pH's gel were capable of minimizing the microhardness loss and tooth sensitivity, respectively [12–14].

The reduction of HP concentration also could be considered an interesting approach because it could attenuate the oxidative reactions occurring on the enamel surface as well as directly influence the amount of reactive oxygen species (ROS) or unreacted HP reaching the pulp chamber and, consequently, reduce the risk for tooth sensitivity [15, 16]. However, this approach should be accompanied by alternatives to maintain the optimal immediate esthetic outcomes from high-concentrated bleaching. In this direction, the incorporation of catalysts, i.e., titanium dioxide, ferrous sulfate, catalase and manganese oxide, into the gels could accelerate or increase the generation of ROS that would interact with the so-called chromophores molecules in dentin and turn teeth whiter at the same level reported for high concentrations of HP [17–20].

Recently, the in vitro incorporation of titanium dioxide nanoparticles co-doped with nitrogen and fluorine into experimental in-office bleaching gel allowed up to five times reduction in the HP concentration (6% and 15%) with the maintenance of the optimal immediate color and whiteness index changes levels [21]. However, this was only possible under the light irradiation with a violet LED light. It was previously shown that the doping of this titanium dioxide nanoparticle rendered the absorption spectrum in the violet light range ($\lambda = 390$–$420$ nm) twice as much in comparison to a commercially available nanoparticle (P25, Degussa) [22]. Briefly, when the nanoparticle interacts with an appropriate light wavelength, the electrons from the valence band (fundamental state) are promoted into the conduction band (excited state). Then, the electron vacancy, in the valence band is positively charged and will recombine with the conduction's free electrons, releasing heat or light [23]. In case these electrons do not recombine, generation of positive and negative electrons may occur and participate in various oxi-reduction reactions on the surface of the nanoparticle, including the generation of longer-living reactive oxygen species [24].

Interestingly, the low-concentrated experimental gels incorporated with the co-doped nanoparticles also preserved the shape and absorbance values of carbonate and phosphate peaks of dental enamel under an FTIR evaluation [21], presenting an upregulation trend of the enamel mineral ratio, contrarily to the gels without the nanoparticles. However, the evaluation of other enamel physical properties are paramount to determine if these gels would overcome the aforementioned adverse effects caused by in-office bleaching. Therefore, the objective of this study was to evaluate the effects of light-irradiated experimental in-office bleaching gels incorporated with titanium dioxide nanoparticles (NP) co-doped with nitrogen and fluorine on enamel surface microhardness, cross-sectional microhardness, roughness, morphology, and calcium/phosphorus content. The null hypotheses tested were that the incorporation of the NP into the experimental gels would not affect the enamel (i) microhardness or the (ii) surface roughness.

## Materials and methods

### Experimental design

The specimens ($n = 190$) were prepared and randomly allocated into groups ($n = 10$/group), according to the study factors:

- *Hydrogen peroxide concentration (HP):*
- HP6: experimental bleaching gel with hydrogen peroxide at 6%.
- HP15: experimental bleaching gel with hydrogen peroxide at 15%.
- HP35: experimental bleaching gel with hydrogen peroxide at 35%.
- *Nanoparticles concentration (NP):*
- Without NP.
- 5NP: experimental bleaching gel incorporated with 5% of NP.
- 10NP: experimental bleaching gel with 10% of NP.
- *Light irradiation (LT):*
- Dark conditions.
- Light irradiation (LT).
- *Time (T)—depending on the variable response:*
- Baseline *($T_0$).*
- After the first bleaching session *($T_1$).*
- After the second bleaching session *($T_2$).*
- After the third and last bleaching session *($T_3$).*
- After 14 days maintained in artificial saliva *($T_4$).*

A group was maintained only in artificial saliva for the duration of the study (negative control). The specimens were submitted to enamel surface (from $T_0$ to $T_4$) and cross-sectional ($T_4$) microhardness analyses, surface roughness evaluation ($T_0$ and $T_4$), polarized light and scanning electron microscopies ($T_4$), and energy-dispersive X-ray spectroscopy ($T_4$).

## Specimen's preparation

Two-hundred and fifty bovine incisor teeth were collected, cleaned, and stored at $4^0$C for no longer than 30 days. These teeth were then positioned in a water-cooled holder coupled to a bench drill (Pratika FSB16P, Schultz, Joinville, SC, Brazil). A diamond bur for glass (⌀8 mm, Di Martino Brocas Diamantadas Ltda, Campinas, SP, Brazil) was used to obtain enamel–dentin disks (diameter = 5.6 mm) from the middle third of the incisor's buccal surface. The dentin end of the disks was abraded with 600-grit sandpaper (Norton Saint-Gobain, Guarulhos, SP, Brazil) using a rotary polisher (Arotec, São Paulo, SP, Brazil), and the outer enamel surface was abraded with 400- and 1200-grit sandpapers (Norton Saint-Gobain) and polished using polishing cloths (3 M Brazil, Sumaré, SP, Brazil) with diamond suspensions (1 µm, 0.50 µm and 0.25 µm, Erios, São Paulo, SP, Brazil). The final diameter of the disks, checked with a digital caliper (Cisel, São Paulo, SP, Brazil), was equal to 2.5 mm (enamel = 1 mm, dentin = 1.5 mm). Immediately after the specimens' preparation and before any bleaching procedures or immersion in artificial saliva ($T_0$), the specimens were tested to obtain the initial Knoop surface microhardness number (KHN). Taking into consideration that the entire set of specimens presented a 350.0 ± 41.0 KHN mean, those specimens with KHN 10% higher or lower than the mean were excluded from the research.

## Nanoparticles' synthesis and experimental gel's preparation

The synthesis of NP has been previously described [21, 22]. The reagents – 1.7 g of Ti $(OBu)_4$ (97%, Sigma-Aldrich, St. Louis, MO, USA), 4.6 g $C_2H_5OH$ (200-proof Decon Labs, King of Prussia, PA, USA), 6.8 g $C_{18}H_{35}NH_2$ (Sigma-Aldrich, 70%), 7.1 g $C_{18}H_{34}O_2$ (Sigma-Aldrich, 90%), and 5% of $NH_4F$ (based on Ti content; crystalline, ACS, Alfa Aesar, Haverhill, MA, USA)—were mixed with an ethanol–water solution into a high-pressure reaction vessel (Borosilicate Glass-lined; Paar Series 4593, Bench Top Reactor System, Moline, Il, USA). This vessel reacted at 180 °C (15 psi) and stirred at 280 rpm for 24 h. The solution was then dispensed into a Falcon tube containing ethanol (200-proof, Decon Labs, King of Prussia, PA, USA) and centrifuged for 15 min at 8,000 rpm three times. The preparation of the experimental gels has also been previously reported in detail [21]. A commercial hydrophilic polymer (Carbomer 940 NF, Spectrum, Gardena, CA) was diluted into ultrapure water by means of a planetary and orbital stand-alone mixer (Speed Mixer, DAC 400.1 FVZ, FlackTek Inc, Landrum, SC, USA). The pH of this experimental gel was around 6.0, before being mixed with HP solutions. Aliquots of the co-doped nanoparticles (1 and 2 mL of NP, ~ 40 mg/mL) suspended in ethanol were poured into separated Falcon tubes and centrifuged at 8,000 rpm for 5 min. The ethanol was removed from the tube and the NPs were incorporated into 20 g of the experimental gels, which were mixed at 2450 rpm for 20 s (Speed Mixer, DAC Iso.1 FVZ, FlackTek Inc, Laudrum, SC, USA). As a result, the resulting gels contained 5 and 10% of NP (v/w), respectively.

## Groups division and bleaching procedures

The 190 specimens selected were then randomly distributed into 19 groups ($n = 10$), according to the bleaching treatments with the experimental gels assigned to each one:

- NC: negative control, stored in artificial saliva throughout the study.
- HP6: 6% hydrogen peroxide gel.
- HP6 + LT: 6% hydrogen peroxide gel, light irradiated.
- HP15: 15% hydrogen peroxide gel.
- HP15 + LT: 15% hydrogen peroxide gel, light irradiated.
- HP35: 35% hydrogen peroxide gel.
- HP35 + LT: 35% hydrogen peroxide gel, light irradiated.
- HP6 + 5NP: 6% hydrogen peroxide gel incorporated with 5% of NP.
- HP6 + 5NP + LT: 6% hydrogen peroxide gel incorporated with 5% of NP and light irradiated.
- HP15 + 5NP: 15% hydrogen peroxide gel incorporated with 5% of NP.
- HP15 + 5NP + LT: 15% hydrogen peroxide gel incorporated with 5% of NP and light irradiated.
- HP35 + 5NP: 35% hydrogen peroxide gel incorporated with 5% of NP.
- HP35 + 5NP + LT: 35% hydrogen peroxide gel incorporated with 5% of NP and light irradiated.
- HP6 + 10NP: 6% hydrogen peroxide gel incorporated with 10% of NP.
- HP6 + 10NP + LT: 6% hydrogen peroxide gel incorporated with 10% of NP and light irradiated.
- HP15 + 10NP: 15% hydrogen peroxide gel incorporated with 10% of NP.
- HP15 + 10NP + LT: 15% hydrogen peroxide gel incorporated with 10% of NP and light irradiated.
- HP35 + 10NP: 35% hydrogen peroxide gel incorporated with 10% of NP
- HP35 + 10NP + LT: 35% hydrogen peroxide gel incorporated with 10% of NP and light irradiated.

The experimental gels were mixed with HP6, HP15, or HP35 solutions as previously reported [21]. The specimens were submitted to three 30-min bleaching sessions ($T_1$, $T_2$ and $T_3$) at 7-day intervals. The specimens were stored in artificial saliva [25] among the intervals and during 14 days

after the last bleaching session. The LT groups were light irradiated based on a protocol described elsewhere [26, 27].

### Enamel surface microhardness

Enamel surface microhardness was determined using a Knoop microhardness device (Future Tech-FM-1e, Tokyo, Japan). Three indentations were performed in the central area of each specimen, 100-µm apart from each other under a static load of 50 g for 5 s [28]. The mean values were obtained (in Kgf/mm$^2$) at baseline ($T_0$) as described in the "*Specimen preparation*", also 24 h after each bleaching session ($T_1$, $T_2$, and $T_3$), and 14 days after the last bleaching session and storage of the specimens in artificial saliva ($T_4$).

### Enamel surface roughness

Surface roughness (Ra, in µm) was determined by a surface tester (Mitutoyo Surftest SJ-410, Kawasaki, Japan) at $T_0$ and $T_4$. Three measurements were performed in each specimen by rotating the specimen 45°, with a cutoff set at 0.25 mm and speed of 0.2 mm/s [29].

### Enamel cross-sectional microhardness (CSMH)

After the final surface microhardness measurement at $T_4$, all the enamel–dentin disks were cross-sectionally cut into two halves. One half of each specimen was embedded in polystyrene resin (Future Tech-FM-1e, Tokyo, Japan), leaving the inner face exposed. The inner face of all specimens was polished with 400-, 600-, and 1200-grit sandpapers (Norton Saint-Gobain) during 5 min for each grit. CSMH analysis was performed using the same microhardness tester aforementioned, using a Knoop indenter with a 25 g load for 5 s. The indentations were made 20, 30, 40, 50, 60, 80, 100, 120, 160, and 200 µm from the outer enamel surface [10]. The values were averaged, and the mean was expressed in KHN. $\Delta S$ was calculated for each specimen, representing the area of hardness loss calculated by numerical integration which uses a trapezoidal rule based on the difference between the area under the curve of sound enamel (kgF/mm$^2$ × µm) subtracted from the area of the demineralized one [30].

### Polarized light microscopy (PLM)

Some of the other halves were used for PLM, and the inner surfaces of the samples ($n = 2$/group) were polished to a thickness of 100 µm (±0.1 µm) and mounted on glass plates with deionized water. The images were obtained under 200× magnification [10] using a polarized light microscope (DM LSP, Leica Microsystems) to qualitatively evaluate the demineralization depth.

### Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS)

Some of the other remaining halves ($n = 2$/group) were prepared to perform SEM and EDS analyses. The surface morphology was observed under SEM, and enamel calcium/phosphorus content were assessed by EDS. The specimens were dry incubated (37 °C, 24 h), placed on an acrylic stub, and carbon coated in preparation for analysis in an EDS (Noran Instruments, Middleton, WI, USA) coupled to an SEM (JSM 5600 LV, JEOL, Tokyo, Japan). The SEM images were acquired at 500× magnification. Five different locations of each specimen were selected in EDS to determine the calcium (Ca) and phosphorus (P) atomic weight (15 kVp, working distance = 20 mm, spot size = 55) [31], and the Ca/P ratio was then calculated.

### Statistical analyses

Data were submitted to exploratory analyses of normal distribution (Shapiro–Wilk) and homoscedasticity (Levene). The Ra values were transformed in $\text{Log}_{10}$ to meet the assumptions for normal distribution and homoscedasticity ($p > 0.05$). Enamel surface microhardness (KHN) and roughness (Ra) data were submitted to four-way repeated measures ANOVA. Since KHN exhibited statistical differences in some factors and interactions, pairwise comparisons were performed by post hoc Bonferroni test. The negative control group was tested against all the other experimental groups using the Dunnet test. $\Delta S$ data were analyzed by three-way ANOVA and Tukey test. All the statistical analyses were performed in the SPSS software (IBM, Chicago, Il, USA), at a significance level of 5%.

## Results

### KHN

Table 1 depicts the mean and standard deviation values of KHN (kgF/mm$^2$) over time. The NPs ($p < 0.001$) significantly influenced the KHN values. The factor time ($p < 0.001$) and the interactions time*HP ($p = 0.010$) and time*NPs ($p < 0.001$) significantly influenced the results.

At baseline, no differences in KHN were noted among groups ($T_0$; $p > 0.05$). At $T_1$, a significant drop was detected in some groups in comparison to $T_0$. At $T_1$, HP6 and HP6 + LT exhibited lower KHN than the negative control ($p < 0.05$), HP6 presented KHN lower than HP35, and HP6 + LT lower than HP15 + LT and HP35 + LT ($p < 0.05$). After the second bleaching session ($T_2$), most

**Table 1** Means and standard deviation KHN values taking into consideration the enamel surface tested before bleaching ($T_0$), 24 h after each one of the three bleaching sessions ($T_1$, $T_2$ and $T_3$) and 14 days of storage in artificial saliva ($T_4$)

| | HP6 | | | HP15 | | | HP35 | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0NP | 5NP | 10NP | 0NP | 5NP | 10NP | 0NP | 5NP | 10NP |
| NO LT | | | | | | | | | |
| $T_0$ | 357.2(28.2) **A** **Aa** | 357.0(27.8) **Aa** **A** | 356.9(30.5) **A** **Aa** | 354.4(27.4) **A** **Aa** | 355.6(27.2) **A** **Aa** | 357.5(25.5) **A** **Aa** | 358.9(27.8) **A** **Aa** | 356.5(27.3) **A** **Aa** | 355.9(27.1) **A** **ABa** |
| $T_1$ | 286.5(39.9) **B** **Cb*** | 339.7(22.4) **Aa** **A** | 347.8(32.0) **A** **Aa** | 312.5(38.4) **AB** **BCb** | 351.6(36.2) **A** **ABa** | 361.5(34.5) **A** **Aa** | 321.4(46.3) **A** **Ba** | 358.9(37.6) **A** **Ab** | 351.0(31.9) **A** **ABb** |
| $T_2$ | 300.4(36.2) **A** **Cb*** | 351.7(32.7) **Aa** **A** | 361.6(29.3) **A** **Aa** | 301.2(17.1) **A** **Cb*** | 354.2(34.5) **A** **ABa** | 350.3(25.9) **A** **Aa** | 307.7(50.6) **A** **Bb*** | 336.8(42.7) **A** **Aab** | 369.3(33.5) **A** **Ab** |
| $T_3$ | 282.6(31.6) **B** **Cb*** | 348.2(26.0) **Aa** **A** | 351.8(20.7) **A** **Aa** | 315.8(28.5) **A** **BCb*** | 337.1(24.8) **A** **Bab** | 348.9(20.7) **A** **Ab** | 317.5(40.8) **A** **Bb*** | 358.7(22.2) **A** **Aa** | 344.6(25.4) **A** **Ba** |
| $T_4$ | 313.8(28.2) **A** **Bb*** | 358.2(17.4) **Aa** **A** | 350.2(17.4) **A** **Aa** | 328.5(27.1) **A** **Bb*** | 377.0(21.7) **A** **Aa** | 355.0(17.4) **A** **Aa** | 326.6(45.4) **A** **Bb*** | 351.1(37.9) **A** **Aab** | 360.3(26.1) **A** **ABa** |
| LT | | | | | | | | | |
| $T_0$ | 356.4(27.8) **A** **Aa** | 358.2(26.6) **Aa** **A** | 357.3(26.9) **A** **Aa** | 357.1(28.6) **A** **Aa** | 355.8(29.9) **A** **Aa** | 357.7(30.1) **A** **Aa** | 355.5(31.3) **A** **Aa** | 356.3(27.7) **A** **Aa** | 358.1(30.2) **A** **Aa** |
| $T_1$ | 280.5(43.5) **B** **Bb*** | 339.7(22.1) **A** **ABa** | 328.2(36.2) **A** **Ba** | 320.7(28.4) **A** **BCb** | 342.9(47.0) **A** **Aab** | 355.7(25.2) **A** **Aa** | 328.8(26.1) **A** **Ba** | 340.6(40.3) **A** **ABa** | 332.2(31.3) **A** **Ba** |
| $T_2$ | 302.6(39.7) **A** **Bb*** | 331.2(27.7) **A** **Bab*** | 344.9(25.9) **A** **ABa** | 308.1(15.7) **A** **Cb*** | 334.6(52.5) **A** **Aab** | 351.1(37.9) **A** **Aa** | 310.0(27.6) **A** **Bb*** | 323.4(33.9) **A** **Bab*** | 366.9(35.6) **A** **Ab** |
| $T_3$ | 292.0(16.6) **A** **Bb*** | 353.7(32.6) **A** **ABa** | 346.9(23.4) **A** **ABa** | 306.0(18.7) **A** **Cb*** | 344.4(22.7) **A** **Aab** | 363.3(25.7) **A** **Ab** | 311.0(41.8) **A** **Bb*** | 347.2(22.6) **A** **Aa** | 354.8(32.6) **A** **ABa** |
| $T_4$ | 335.5(40.0) **A** **Ab*** | 350.1(28.2) **A** **ABab** | 362.8(20.1) **A** **Aa** | 332.5(22.7) **A** **Bb** | 351.1(22.7) **A** **Aab** | 365.2(27.5) **A** **Aa** | 326.7(28.3) **A** **Bb*** | 335.9(15.9) **A** **ABab** | 354.5(20.3) **A** **ABa** |

NC: $T_0$: 356.9(31.7)—$T_1$: 355.6(33.0)—$T_2$: 379.5(23.3)—$T_3$: 360.5(36.4)—$T_4$: 370.9(43.8)

Distinct uppercase letters compare the means among the different time points and within the same experimental gel. Distinct lowercase letters compare the means among different % of NP (0, 5 and 10) and within the same % of HP and time point. Distinct and isolated uppercase letters (in the middle columns) indicate significant difference among the % of HP (6, 15 and 35%) within the same % of NP and time point. Asterisks indicate significant differences with the negative control (NC) within each time point separately. All the statistical tests were conducted taking into consideration a 5% level of significance

NP-containing groups still exhibited comparable KHN to their baseline values and to NC, except HP6 + 5NP + LT and HP35 + 5NP + LT.

Immediately after the last bleaching session ($T_3$), groups without NPs presented KHN statistically inferior to their corresponding baseline values and to NC, regardless of HP concentration and LT irradiation. Except for HP15 + 5NP (with or without LT), all groups without NPs promoted lower KHN than treatments performed with gels incorporated with NPs. Fourteen days after artificial saliva immersion ($T_4$), only the NP-containing groups maintained KHN compatible with baseline and NC values. HP6 + LT kept KHN similar to baseline, but lower than NC.

**Table 3** Means and standard deviation of $\Delta S$ values calculated from the cross-sectional KHN values from 20 to 200 µm distance from the enamel surface

| | HP6 | HP15 | HP35 |
|---|---|---|---|
| NO LT | | | |
| 0NP | 9.597,6 (3.690,0) | 10.197,4 (3.702,4) | 10.288,8 (4.105,5) |
| 5NP | 10.707,7 (1.559,5) | 8.955,4 (3.935,5) | 11.793,9 (3.255,7) |
| 10NP | 10.857,5 (2.496,5) | 11.438,0 (4.071,3) | 12.274,8 (3.759,3) |
| LT | | | |
| 0NP | 11.320,3 (3.746,6) | 11.694,9 (3.219,8) | 10.906,0 (4.059,5) |
| 5NP | 12.136,2 (3.554,9) | 11.860,0 (4.087,1) | 10.452,5 (2.878,1) |
| 10NP | 10.235,3 (2.173,5) | 12.471,4 (2.791,9) | 11.828,2 (4.695,4) |

*NC* 9.756,0 (2.570,6)

## Ra

Table 2 illustrates the *Ra* values obtained before ($T_0$) and 14 days after bleaching ($T_4$). No significance was detected in any of the factors and their interactions (ANOVA; $p > 0.05$). At both time points, no differences were noted among groups or NC (Dunnet; $p > 0.05$), or $T_0$ and $T_4$ ($p > 0.05$).

## ΔS and demineralization depth

No statistically significant differences among factors or interactions were noted for $\Delta S$ ($p > 0.05$, Table 3). Also, none of the groups presented statistical differences with the negative control ($p > 0.05$).

Figure 1 depicts the mean cross-sectional KHN values. Overall, the non-containing NP groups (red lines) exhibited a KHN similar to the NC (black lines). In most occurrences, the 5NP (blue lines) and 10NP (green lines) groups presented curves higher than NC, especially for 10NP-containing gels. It is possible to observe that the plateau of the curves occurred mostly at 60-µm distance from the outer enamel surface.

Figure 2 reveals that the enamel surface and subsurface were sound in the negative control group, with no apparent enamel diffraction. Some groups displayed darkened areas, represented by asterisks, which could be suggestive of enamel demineralization. Such regions can be observed in enamel treated with HP35, independently of the presence of NPs, and also with HP6 without NPs. All groups exhibited a surface free of major irregularities such as valleys caused by surface disruption.

## Enamel surface morphology and Ca/P

Figures 3, 4, and 5 display representative SEM images of enamel and the EDS elemental mapping for each group at $T_4$. NC exhibited a flat and polished surface, whereas the non-containing NP groups presented at least some extent of irregularities comparable to the removal of intraprismatic core and presence of deep porosities and pits (white arrows—Fig. 2). The groups bleached with experimental gels incorporated with 5NP (Fig. 3) and 10NP (Fig. 4) exhibited surfaces more compatible with the NC, with the continuity of the polished enamel. The semi-quantitative analysis of EDS revealed that the calcium to phosphorus ratio in the NC was equal to $1.91 \pm 0.09$. The Ca/P ranged from $1.86 \pm 0.03$ to $1.90 \pm 0.04$ in the non-containing NP groups. This ratio varies from $1.85 \pm 0.02$ to $1.91 \pm 0.06$

**Table 2** Means and standard deviation *Ra* values obtained taking into consideration the enamel surface tested before bleaching ($T_0$) and 14 days of storage in artificial saliva ($T_4$)

| | HP6 | | | HP15 | | | HP35 | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0NP | 5NP | 10NP | 0NP | 5NP | 10NP | 0NP | 5NP | 10NP |
| NO LT | | | | | | | | | |
| $T_0$ | 0.029 (0.005) | 0.033 (0.009) | 0.027 (0.005) | 0.028 (0.008) | 0.025 (0.007) | 0.029 (0.008) | 0.033 (0.009) | 0.032 (0.006) | 0.028 (0.012) |
| $T_4$ | 0.030 (0.007) | 0.032 (0.008) | 0.028 (0.006) | 0.030 (0.010) | 0.027 (0.007) | 0.029 (0.009) | 0.030 (0.008) | 0.031 (0.010) | 0.027 (0.007) |
| LT | | | | | | | | | |
| $T_0$ | 0.033 (0.009) | 0.031 (0.013) | 0.027 (0.004) | 0.032 (0.010) | 0.026 (0.010) | 0.030 (0.010) | 0.032 (0.006) | 0.026 (0.004) | 0.030 (0.007) |
| $T_4$ | 0.032 (0.005) | 0.030 (0.006) | 0.027 (0.006) | 0.025 (0.006) | 0.028 (0.004) | 0.032 (0.012) | 0.027 (0.007) | 0.032 (0.007) | 0.033 (0.006) |

*NC* $T_0$: 0.029 (0.007)–$T_4$: 0.031 (0.006)

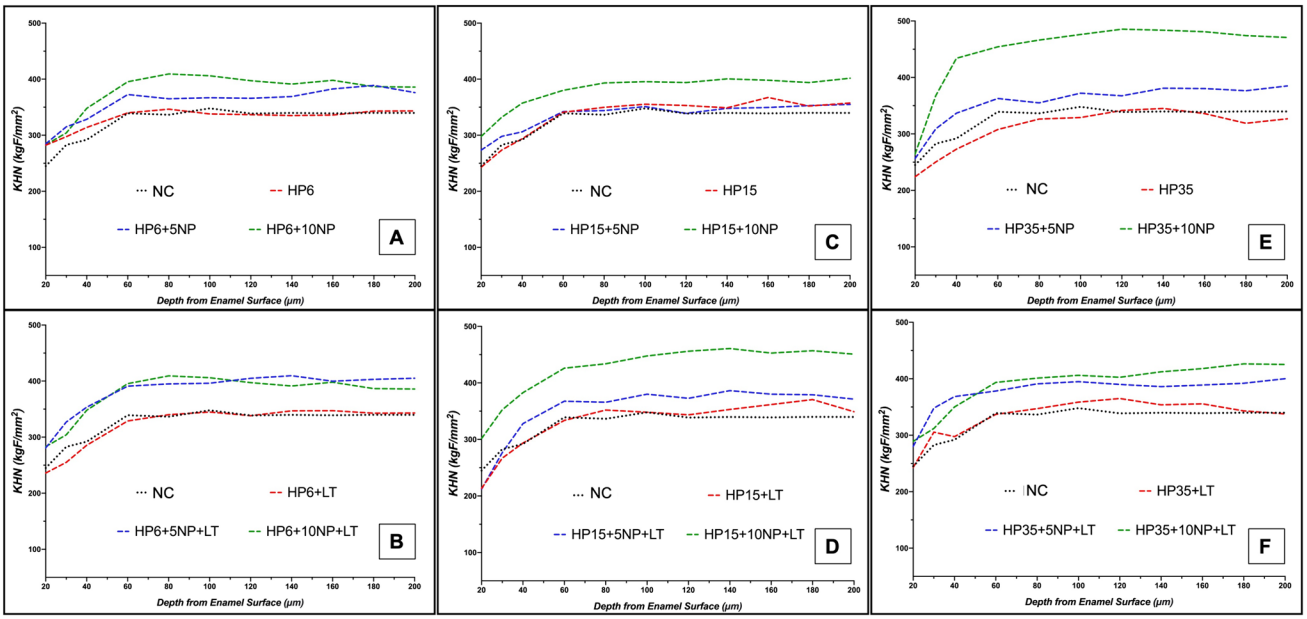


**Fig. 1** Graphs representing the KHN x µm progression for each group as compared to the negative control (NC). The black dotted line represents the NC group, repeated over all the graphs (**A**–**F**). The red dashed lines indicate the groups treated with non-containing NP gels, while blue and red indicate the 5NP and 10NP ones, respectively. HP6 gels without (**A**) and with (**B**) light (LT) are illustrated on the left side, HP15 gels without (**C**) and with (**D**) LT at the center, and HP35 gels without (**E**) and with (**F**) LT on the right side

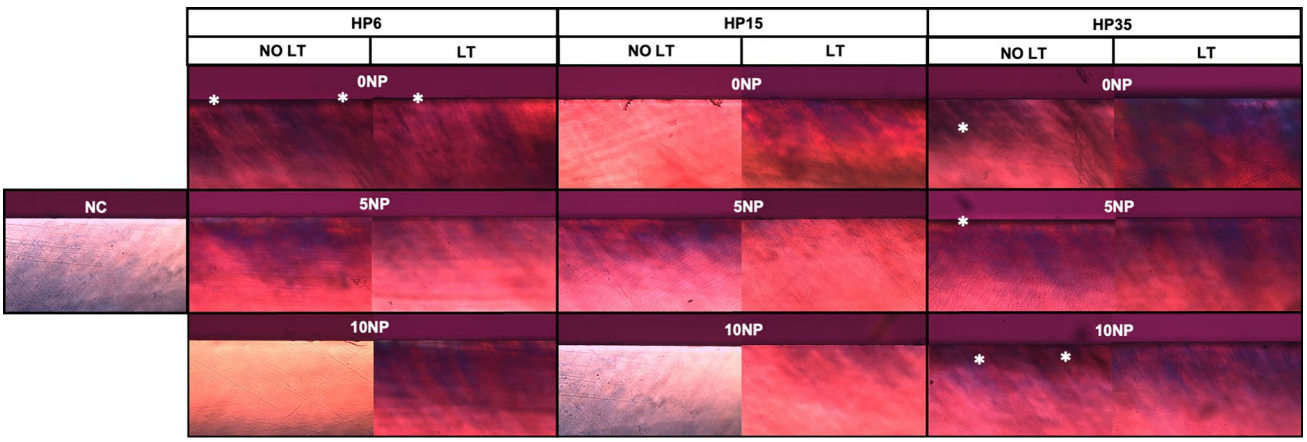


**Fig. 2** Representative polarized microscopy images that were acquired 14 days after the last bleaching session ($T_4$). Most of the experimental groups exhibited regular surface and subsurface characteristics comparable to the negative control (NC), free of major irregularities and steps caused by surface disruption. The asterisks point to darkened surface areas (HP6, HP6 + LT and HP35 + 5NP) and on the subsurface (HP35, HP35 + 10NP) of enamel

and from 2.02 ± 0.12 to 2.06 ± 0.14 in the 5NP and 10 NP groups, respectively.

## Discussion

The microhardness assessment was used by several other investigations to indirectly indicate whether the proposed treatments resulted in enamel demineralization or not [13, 14, 25, 28, 30]. Based on the results, it could be assumed that the experimental HP6, HP15, and HP35 gels without the NP (light irradiated or not) were not capable of upholding the enamel mineralization levels, 24 h elapsed from the first bleaching session to the end of the study. Even though most of these groups partially recovered the reduction in KHN after 14 days immersion in artificial saliva immersion, all of them were still significantly inferior to the negative control. On the other hand, all the NP-incorporated gels

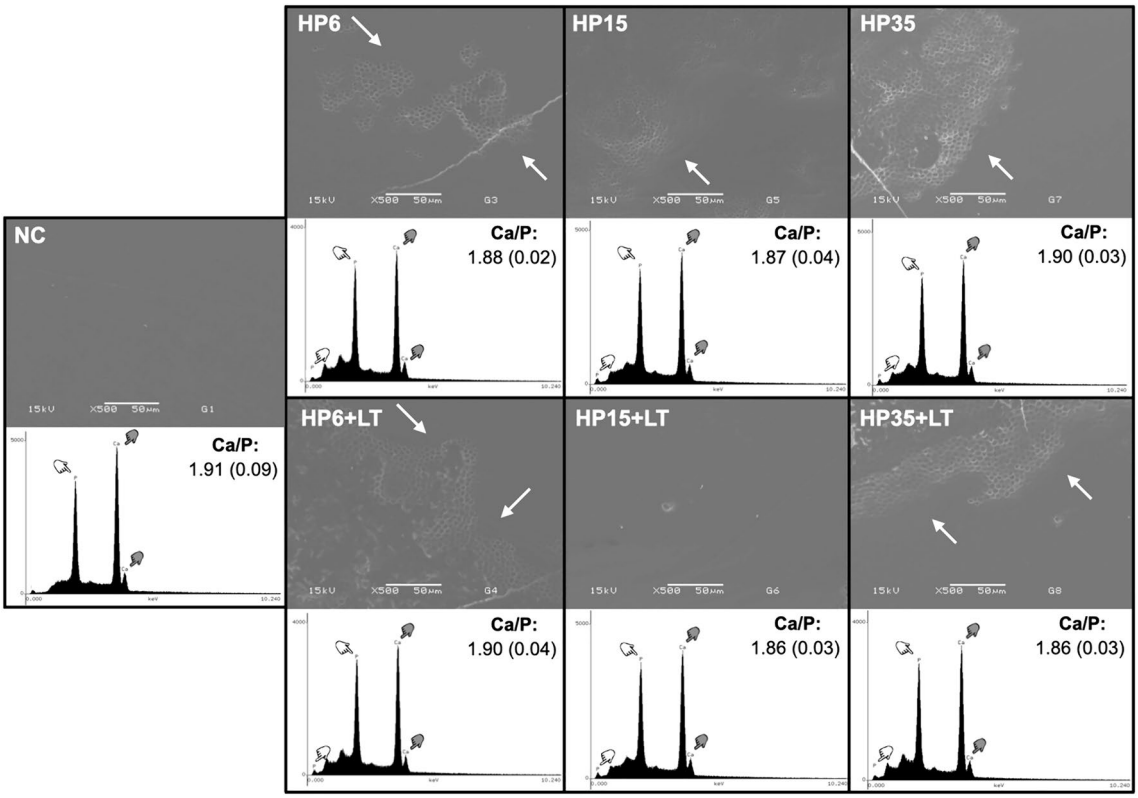


**Fig. 3** Representative SEM images and EDS elemental mapping of negative control and non-containing NP groups that were obtained 14 days after the last bleaching session ($T_4$). Negative control (NC) exhibited a flat and polished surface area. Most of the bleached groups herein illustrated presented discontinuity of the polished enamel surface, as shown by the white arrows pointing to affected intraprismatic areas. The gray finger points to the peaks of the Ca element, and the white finger corresponds to the P peaks in the elemental mapping images

(with or without LT) remained similar to the NC. Therefore, the first null hypothesis was accepted, since the co-doped titanium dioxide nanoparticles did not affect the enamel microhardness.

Others have shown that commercial and experimental gels with acidic and even neutral pH significantly reduce enamel surface microhardness immediately after the gel's application [6, 32–34]. Contrarily to these findings, Parreiras et al. (2014) [32] reported a significant KHN recovery after storage in artificial saliva for 7 days, reaching the baseline values, which was not the case in this study. The pH of the experimental HP gels herein tested was previously reported [21], indicating that the pH of carbomer-based polymer ranged from 5.1 (HP6) to 5.5 (HP15 and HP35) when mixed with HP solutions.

One might say that the acidic pH of the resulting gels could have played the main role in this scenario, but in a recent study, Torres et al. (2022) observed that an experimental carbomer-based bleaching gel adjusted to pH 6.5 promoted a significant enamel microhardness reduction, leading to the highest decrease detected among all the tested thickeners (carbomer, modified sulfonic acid, alkali swellable emulsion, semi-synthetic polysaccharide, and particulate colloids) [35]. Also, an in situ study demonstrated that a neutral carbomer gel without HP downgraded enamel surface microhardness [36], and there is evidence that polyacrylate-based polymers inhibited the growth of hydroxyapatite crystals [37]. According to Torres et al. (2022), a feasible explanation for this phenomenon relies on the $Ca^{++}$ ions released from the enamel permeating the gel and complexing with the anions in the carbomer, making the polymer undersaturated [35].

Nonetheless, the pH of the polyacrylate-based gels should not be ruled out as an important protective factor for the enamel surface microhardness, once the co-doped titanium dioxide incorporation into these gels increased their pH up to 6.0, showing a slightly higher increase after the 10NP addition [21]. In the present study, the experimental gels incorporated with 5% and 10% nanoparticles prevented a significant decrease in surface microhardness. It is also fundamental to bear in mind that the NP composition could have influenced this scenario. The fluorine content in the crystal lattice of the co-doped nanoparticle could have potentially favored the formation of fluorapatite based on

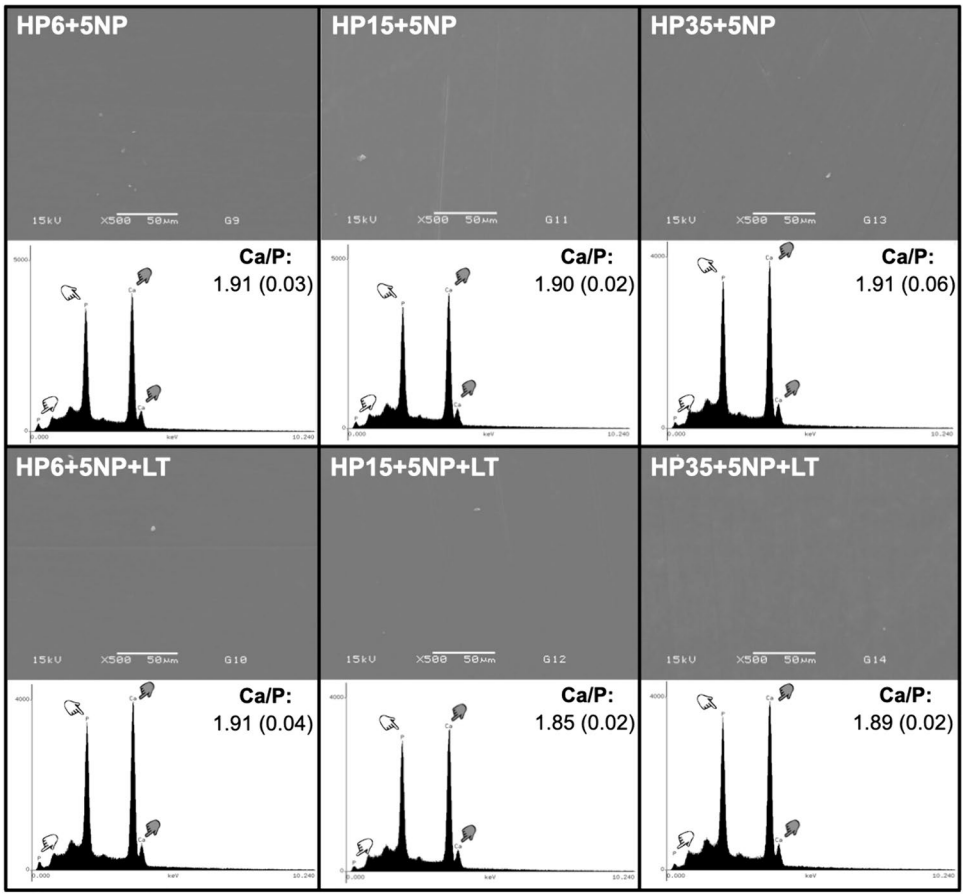


**Fig. 4** Representative SEM images and EDS elemental mapping of negative control and 5NP groups that were obtained 14 days after the last bleaching session ($T_4$). The 5NP groups maintained the continuity of the enamel surface, exhibiting a flat and homogenous area. Some marks on the surface (HP15 and HP6 + 5NP + LT) could be a result of the polishing procedures. The surface characteristics are similar to those found in the NC groups seen in Fig. 2. The gray finger points to the peaks of the Ca element, and the white finger corresponds to the P peaks in the elemental mapping images

an ion-exchange mechanism [38], turning the enamel surface more resistant to eventual pH drops [39] and undersaturation of the polymeric network in the gels. Indeed, other studies have already attested that incorporation of fluoride ions into bleaching gels upheld the microhardness, which was not observed in fluoride-free gels [10, 11, 34]. However, this aspect should be further investigated, as the concentration of fluorine in the experimental gel's environment could not suffice for the event described above to happen, and some studies have also indicated that incorporation of the calcium ion could offer increased microhardness protection when compared to fluorine [40, 41].

A previous FTIR analysis of dental enamel submitted to the gels tested herein has demonstrated a protection trend favored by NP on the mineral ratio of carbonate to phosphate peaks, and a preservation of these peaks' shape and absorbance values was also detected in comparison to the expressive changes caused by the carbomer-based gels without NP [21]. The decrease in the enamel mineral ratio has already been demonstrated along with a significant reduction in the enamel surface microhardness [42], which was corroborated by the present findings. Moreover, the present energy-dispersive X-ray spectroscopy revealed a numerically higher Ca/P ratio for groups bleached with 10NP. Yet, the EDS analysis is a semi-quantitative and subsurface analysis [31, 43] and that should be inferred with caution, most importantly because it was performed in a small number of specimens ($n = 2$/group).

Despite the surface microhardness loss detected in groups bleached without the NP, the in-depth microhardness analysis concluded that none of the bleaching protocols negatively affected the cross-sectional microhardness in comparison to the negative control. The mean $\Delta S$ detected in the groups are in accordance with those reported for sound dental enamel [30, 44]. The lower values of KHN up to 60 µm in depth were detected in previous studies after different types of surface treatments, even when the surface was left untreated [30, 34, 45]. Thus, it could be inferred that the outermost layers of the subsurface hold lower microhardness, thereby justifying the $\Delta S$ results herein found. In the present study, the conversion of microhardness to mineral concentration was not performed because of the formula's discrepancy in the literature [46, 47]. Nevertheless, both studies [46, 47] concluded that microhardness could be used as a measure of alterations in mineral content in in vitro studies, after a significant and direct co-relation with transversal microradiographical data. It is noteworthy that the in-depth KHN profiles for the groups bleached with NP, especially those

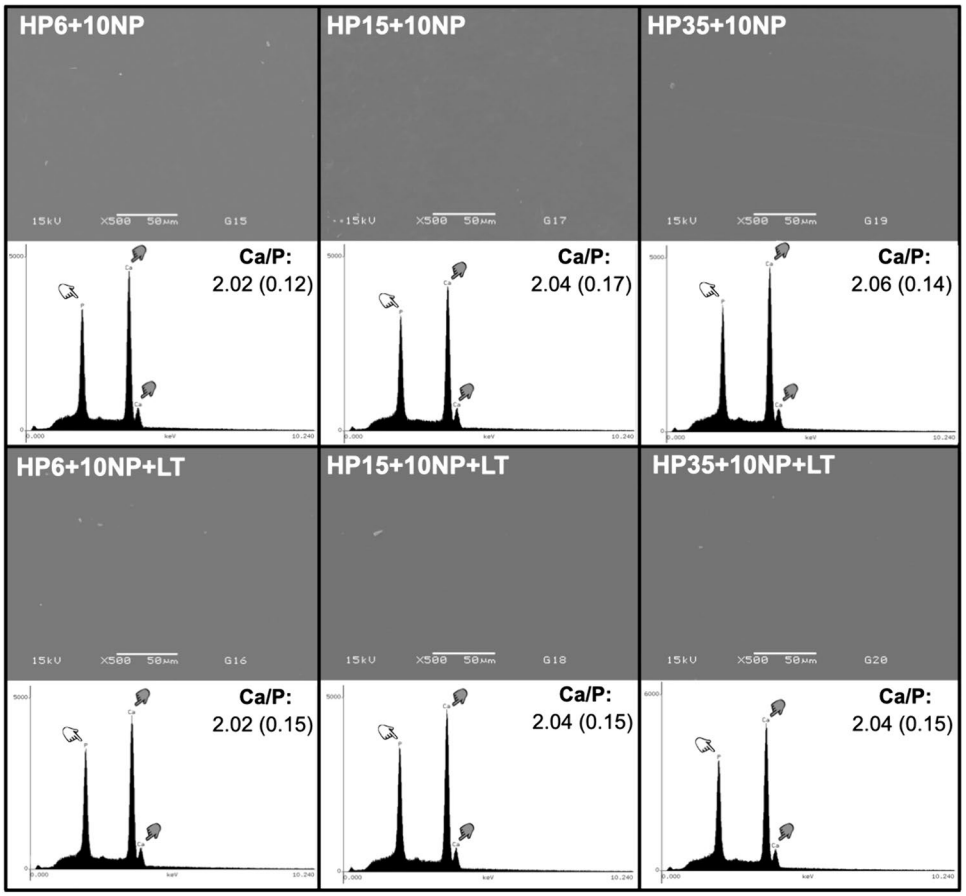


**Fig. 5** Representative SEM images and EDS elemental mapping of negative control and 10NP groups that were obtained 14 days after the last bleaching session ($T_4$). The 10NP groups exhibited a flat and homogenous area, with the continuity of the enamel surface maintained. As for the 5NP groups, the surface characteristics detected in the NC group (Fig. 2) were maintained. The gray finger points to the peaks of the Ca element, and the white finger corresponds to the P peaks in the elemental mapping images

with 10NP, were higher than those of their corresponding NP-free gels and NC, even in the outermost layers of the subsurface (up to 60 µm). Likewise, Cavalli et al. (2012) have found a similar pattern for the enamel mineral volume concentration in function of the lesion depth when comparing carbomer-based gels with or without sodium fluoride incorporation [11]. Therefore, the fluorine present in the crystal lattice of the NP could have also influenced the enamel cross-sectional microhardness.

Interestingly, polarized light microscopy indicated some darkened areas in the subsurface of some groups, which could emphasize that the surface of the enamel is dynamic with some areas presenting higher mineral content than others. Previous studies also found some darkened areas indicative of minor demineralization in groups with significantly higher maintenance of the mineral content than others [10, 11, 48]. Based on the low number of specimens tested under PLM (two 100-µm-thick enamel slice), these images are only representative. Further studies should increase the sample size and/or amplify the regions evaluated within the same specimens to successfully correlate these outcomes to those found under cross-sectional microhardness.

Furthermore, the enamel surface roughness was maintained similarly after the bleaching performances, compliant with the second and last null hypothesis. A previous observation of the *Ra* values under atomic force microscopy [21] indicated that some specimens treated with NP gels had higher *Ra* than others, but the statistical results from the present profilometry method pointed out that the experimental gels would not affect the roughness profile of enamel, as stated for other available in-office bleaching protocols [49]. It is important to highlight that the isolated affected intraprismatic areas detected under SEM observation did not influence the *Ra* values of the groups bleached without NP. These findings are different from those reported by Wijetunga et al. (2021), who showed that an acidic carboxymethyl cellulose-based bleaching gel negatively influences both the *Ra* and the surface morphology [50]. Either way, previous studies have also illustrated some changes in the surface morphology when treated with commercial carbomer-based hydrogen peroxide bleaching gels, but the alterations (fissures, pits and deep valleys) seemed to be more homogenous and to cover the entire surface under the SEM [26, 31, 51, 52] in contrast to the punctual ones observed in the present study.

Hence, the evaluation of the various enamel surface aspects herein performed strongly indicates that the use of the experimental gels would not harm the dental enamel,

but the incorporation of the NP could prevent the surface from microhardness loss and morphology alterations. The evaluation of three different HP concentrations under dark and light conditions took place because the incorporation of NP was shown to increase the HP6 and HP15 efficacy comparable to HP35 [21]. However, the incorporation of 5NP into HP6, followed by LT irradiation, was sufficient to overcome the bleaching outcomes of HP35. The incorporation of other chemical photocatalysts into bleaching gels is also accountable for the increase in their efficacy [18, 19, 53], which highlights the increasing search for safer in-office bleaching alternatives.

The present follow-on assessments, then, suggest that co-doped titanium dioxide nanoparticles not only allows the reduction of HP's concentration up to five times, as stated previously [21], but also protects the enamel surface when incorporated into a carbomer-based polymer. The dramatic reduction in the HP concentration is likely to be directly related to the lower diffusion of reactive oxygen species into the pulp chamber, thereby lowering the chances of cell damage and oxidative stress. Consequently, this approach holds an in-office bleaching protocol promise with reduced risk of tooth sensitivity and highly satisfactory esthetic outcomes, not affecting the dental enamel physical properties.

## Conclusion

Within the limitation of the present study, and based on the null hypotheses postulated, the following conclusions could be drawn:

- The lack of NP in the experimental gels significantly reduced the surface microhardness. However, the experimental carbomer-based gels incorporated with the co-doped $TiO_2$ nanoparticles did not alter the enamel surface microhardness throughout the entire in-office bleaching protocol, regardless of the HP concentration and LT irradiation.
- None of the experimental bleaching gels significantly affected the enamel cross-sectional microhardness and its surface roughness.

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**Data availability** Data will be provided upon request.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** Not applicable.

**Consent to participate** Not applicable.

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