

## RESEARCH ARTICLE

# Assessment of the effect of experimental bleaching agent with nano-bioactive material on postoperative sensitivity: A randomized, triple blind clinical trial

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## Abstract

**Objectives:** This clinical study aimed to evaluate the effect of incorporating bioactive nanoparticles (n-Bm) inside an in-office bleaching gel on the risk and intensity of tooth sensitivity (TS) and on bleaching effectiveness.

**Materials and methods:** Sixty-six participants were selected and randomly assigned into two groups: control-only in-office gel and experimental-in-office gel with n-Bm. Teeth were bleached in two sessions (3 × 15-min). TS was recorded using a VAS and NRS. The color change was evaluated by subjective (VITA Classical and VITA Bleachedguide) and objective (Easysshade spectrophotometer) methods at baseline and 30 days after the end of treatment. The TS was evaluated by McNemar, Wilcoxon Signed Rank, and paired *t* test. The color changes between groups were compared using paired *t* test ( $\alpha = 0.05$ ).

**Results:** No significant differences between the groups were observed in the risk (control = 27% [95%IC 18–39]; experimental = 21% [95%IC 13–32]) and intensity of TS, as well as in the color change ( $p > 0.05$ ) for any color measurement.

**Conclusion:** The inclusion of n-Bm into the bleaching agents did not affect the whitening effectiveness, as well as the risk and intensity of TS between groups. However, the results of the absolute risk of TS were low for both in-office gels used.

**Clinical significance:** Despite no significant differences between groups, both experimental bleaching agents present suitable results with low values for TS.

## KEYWORDS

bioactive agents, dentin sensitivity, hydrogen peroxide, tooth bleaching, tooth sensitivity

## 1 | INTRODUCTION

The clinical effectiveness of at-home and in-office bleaching procedures is widely reported and documented in the literature.<sup>1</sup> Bleaching procedures are considered the simplest, most cost-effective, and least

invasive method of treating colored teeth when compared with direct and indirect restorations.<sup>2,3</sup> While in the at-home procedures, low concentration peroxides are used, higher concentration peroxides are used during in-office treatments.<sup>4</sup> Therefore, the main difference between the two techniques is related to the time from the treatment

to the appearance of whitening effects, where for in-office bleaching, the time is shorter, conferring an expressive advantage in comparison to at-home whitening.<sup>5-7</sup> However, the in-office technique presents a higher risk of sensitivity when compared to the at-home technique.<sup>3,4</sup>

Several studies have shown that bleaching agents are at high risk for causing tooth sensitivity (TS); approximately 67–100% of patients who undergo in-office treatment with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) reported TS.<sup>1,8-12</sup> This is due to the fact that H<sub>2</sub>O<sub>2</sub> has the ability to diffuse freely through enamel and dentin, which is attributed to its low molecular weight,<sup>13</sup> and can easily reach the pulp tissue and result in different magnitudes of TS,<sup>12</sup> causing in some cases, the interruption of whitening by the patient, due to an unpleasant experience.<sup>14</sup>

However, the diffusion of H<sub>2</sub>O<sub>2</sub> can be increased through the presence of microscopic defects on the enamel surface of the tooth, such as porosity,<sup>15</sup> with a consequent reduction in the enamel micro-hardness.<sup>16</sup> These defects can be due to the acidic pH presented by the bleaching agents that would have the potential to promote such changes, mainly in the interprismatic region.<sup>17</sup> In addition, cracks and/or fissures can facilitate the diffusion of H<sub>2</sub>O<sub>2</sub> into the pulp chamber.<sup>18-20</sup> For instance, at least two clinical studies showed that the presence of cracks and/or fissures significantly increased patient reports of TS.<sup>21,22</sup>

Thus, products that allow the repair of these microscopic defects to reduce or minimize TS, due to the decrease in H<sub>2</sub>O<sub>2</sub> diffusion, have been studied. Clinically, the use of bioactive materials, such as ACP (amorphous calcium phosphate), CCP-ACP (ACP in combination with the phosphopeptide casein),<sup>23</sup> HA (hydroxyapatite),<sup>24</sup> and other bioactive glasses, have good results in reducing TS due to bleaching procedures. However, most clinical studies incorporate these compounds in pastes and/or mousses with prior application or after bleaching,<sup>23,25-28</sup> which means adding a clinical step and, consequently, increasing the operator's working time, making the procedure more complex. It is worth mentioning that only two studies<sup>24,29</sup> performed a mixture of bioactive materials in a commercial gel for in-office whitening. However, Vano et al.<sup>24</sup> evaluated a lower hydrogen peroxide concentration and Borges et al.<sup>29</sup> should be considered a case report, as only three patients were evaluated.

Among the bioactive materials, bioglass (BG) stands out as an amorphous ceramic material<sup>30</sup> and as a mechanism of action, the nucleation, and precipitation of calcium and phosphate ions, thus promoting deposition of HA on the mineral surface and providing repair,<sup>31</sup> remineralization,<sup>32</sup> and tooth surface repair.<sup>33</sup> In addition, when used in its nanoparticle form, BG can have high bioactivity.<sup>34</sup>

To the authors' knowledge, no clinical studies were found that incorporated bioactive materials inside bleaching agents for application during in-office treatment. Therefore, this randomized, triple-blind, split-mouth clinical study aimed to evaluate the effect of incorporating bioactive nanoparticles (n-Bm) inside a 40% H<sub>2</sub>O<sub>2</sub> bleaching gel on the risk and intensity of TS, as well as on the bleaching effectiveness when compared to the same in-office bleaching gel without n-Bm.

## 2 | MATERIALS AND METHODS

### 2.1 | Ethics approval and protocol registration

This clinical investigation was approved (protocol number 2.476.451) by the scientific review committee and by the committee for the protection of human participants of the State University of Ponta Grossa, PR, Brazil. It was registered in the Brazilian Clinical Trials Registry (REBEC, RBR-9zzg4d). We prepared this article using the protocol established by the Consolidated Standards of Reporting Trials statement within-person designs.

### 2.2 | Trial design, settings, and locations of data collection

This study was a randomized, split-mouth, and triple-blind clinical trial in which the patient, operator, and evaluator were blinded to the group assignment. All participants were informed about the nature and objectives of the study. The clinical study was performed from March, 2019 to October, 2019, and all bleaching procedures carried out within the Clinics of the Dental School of the State University of Ponta Grossa.

### 2.3 | Recruitment

Recruitment of participants was carried out through social media advertising. All the participants, who were participants signed an informed consent form before being enrolled in the clinical study.

### 2.4 | Eligibility criteria

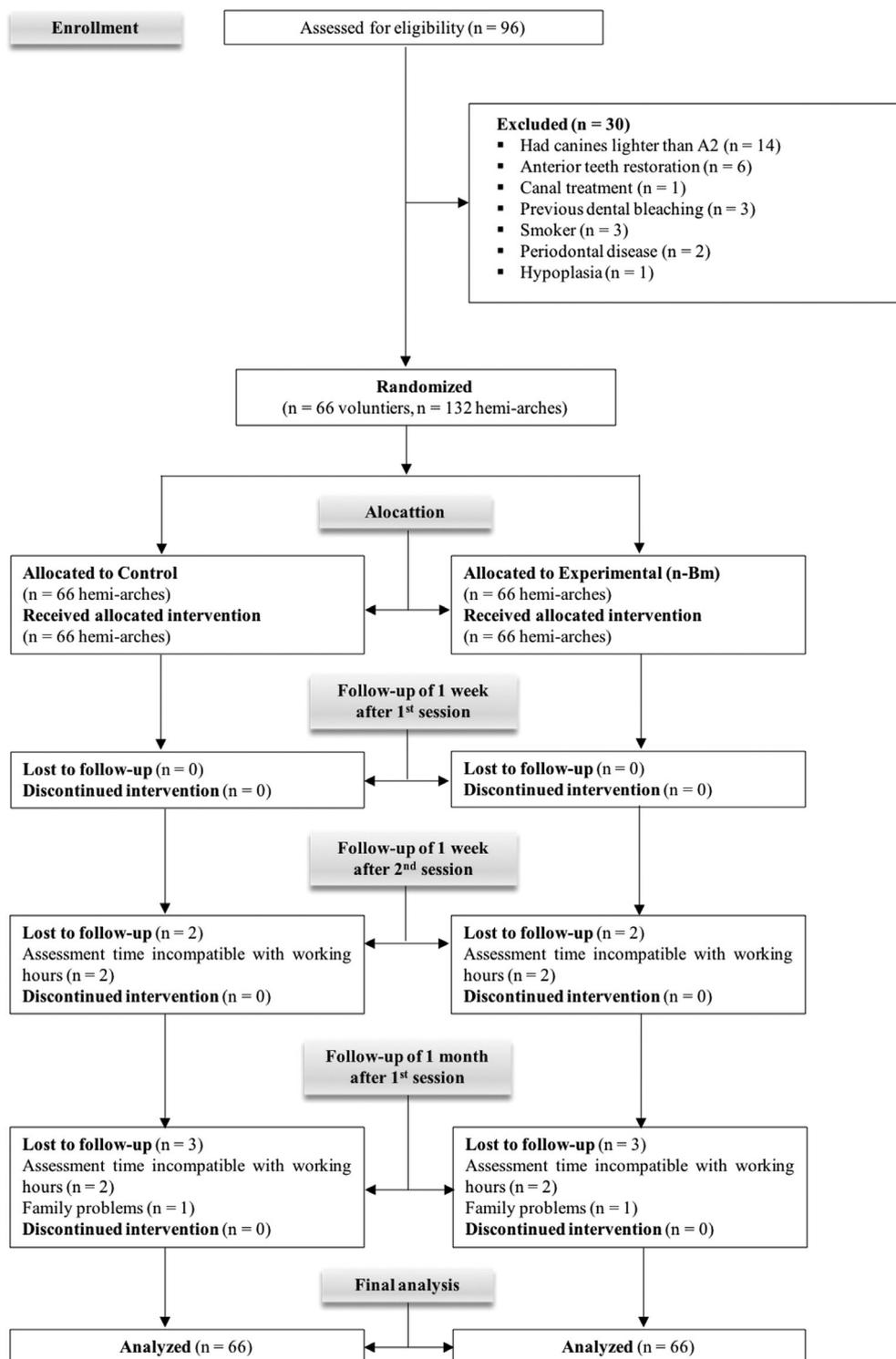
Based on pre-established criteria, we selected 66 subjects volunteered for this clinical study. The participants included in the present study should be a good general and oral health and at least 18 years old. The participants were required to have caries-free maxillary anterior teeth without restorations, periodontal disease, or endodontic treatment, with canine shade A2 or darker, as judged by comparison with a value-oriented shade guide (VITA Classical, VITA Zahnfabrik, Bad Säckingen, Germany; Figure 1).<sup>35</sup>

Participants with orthodontic apparatus, dental prosthesis, and several internal tooth discolorations as tetracycline stains, fluorosis, hypoplasia, or pulpless teeth, were not included. Additionally, pregnant/lactating women, participants with bruxism or another pathology that could cause sensitivity (such as recession dentinal exposure, presence cracks in teeth), anti-inflammatory, and/or analgesic drug taking, smokers, or participants who had previously tooth-whitening procedures were also excluded from this clinical study.

### 2.5 | Sample size calculation

The primary outcome of this study was the absolute risk of TS (the number of patients [percent] who reported pain at some point during

**FIGURE 1** Flow diagram of study design phases including enrollment and allocation criteria



and up to 48 h after the treatment). It was considered 87% of TS based on clinical trials evaluating in-office bleaching gels.<sup>36,37</sup> Therefore, the minimum sample size required in this superiority trial was 59 participants to have an 80% chance of detecting, as significant at the two-sided 5% level, a decrease in the primary outcome measure from 90% in the control group to 70% in the experimental group (which represents a difference of 20% in the absolute risk of TS). Sample size was increased by 10% to compensate for eventual loss of participants.

## 2.6 | Random sequence generation and allocation concealment

The randomization process was performed in the website ([www.sealedenvelope.com](http://www.sealedenvelope.com)) by a third person, who was not involved in implementation and evaluation steps. The distribution of the group to be first assigned were recorded on sequentially numbered cards and located in opaque and sealed envelopes. The information

contained inside of envelope determined the treatment to be assigned in the upper right maxillary arch, while the other arch received the alternate treatment. Once the participant was eligible for the procedure and all initial assessments were completed, the allocation assignment was revealed, opening the envelope immediately after implementation.

## 2.7 | Blinding

This study was a randomized, split-mouth, triple-blind clinical trial in which the patient, operator and evaluator were blinded to the group assignment. A third researcher, not involved in implementation and the evaluation process, was responsible for the randomization process.

## 2.8 | Bleaching gel preparation

The in-office bleaching gels were prepared with only 40% H<sub>2</sub>O<sub>2</sub> (control) or by the addition of synthesized 5% nano-bioactive material to 40% H<sub>2</sub>O<sub>2</sub> (n-Bm; experimental). Both were delivered to operators in identical white syringes coded as "A" and "B" and another white bottle with H<sub>2</sub>O<sub>2</sub> with a specific thickener, the sodium alginate. All procedures were done in the pharmaceutical laboratory of the local university.

For the manipulation of the gel used in the study, an H<sub>2</sub>O<sub>2</sub> with an initial concentration of 50% was used, adding to it a mass portion of 5% of n-Bm, so that the final concentration of H<sub>2</sub>O<sub>2</sub> was 40%. The concentration of 5% of n-Bm was used, because, it's the usually concentration of desensitizer added in the commercial products. For the gel without the nanoparticles, a proportional increase in mass of their respective components was made, so that both gels containing a final concentration of 40%. After being prepared, the gels were filled in identical white syringes, coded as "A" and "B," and the H<sub>2</sub>O<sub>2</sub> with a thickener, was kept in a dropper bottle. The experimental bleaching agents was compound by water, H<sub>2</sub>O<sub>2</sub>, sodium alginate, and methylparaben following the appropriate proportions of each one (Table 1).

## 2.9 | Study intervention

Four operators, with more than 4 years of clinical experience, performed the whitening protocol on the participants. All patients received the same tooth whitening treatment. After placing a lip retractor (Arcflex, FGM, Prod. Odont. Ltda, Joinville, SC, Brazil), a light-curing gingival barrier (Top Dam, FGM, Joinville, SC, Brazil) was placed on the tissue of the teeth to be whitened (from the left second premolar to the second right premolar of the upper and lower arch), and then it was light-cured (Radii-Cal, SDI, Bayswater, Australia), as recommended by the manufacturer. In addition, the light-cured gingival barrier was placed between the central incisors (upper and lower) to avoid contact between the different applied bleaching gels, since the study was split-mouth.

After applying this gingival barrier, the bleaching gels were handled in the proportion of two drops of H<sub>2</sub>O<sub>2</sub> to one drop of the gel contained in the syringe, mixed and applied to the buccal surface of the teeth to be whitened from both dental arches, simultaneously, with the aid of a micro applicator (Microbrush FGM, Prod. Odont. Ltda, Joinville, SC, Brazil). One different micro applicator was used for each group. Both gels were applied on the tooth surface for 15 min, repeating this procedure two more times, totaling three applications of 15 min each. At the end of the first 15 min exchange, the gels were aspirated with the aid of a disposable suction cup (SS Plus do Brasil, Ltda., Maringá, PR, Brazil) and the teeth cleaned with wet gauze. Care was taken that a saliva ejector and gauze were used for each hemi-arch. At the end of the last application, the same gel removal procedure was performed, followed by abundant washing with water, using the triple syringe. The same procedure was repeated 1 week later.

## 2.10 | In vivo pH analysis

A pH meter with a 6 mm circular and flat surface pH electrode (ExStik pH100 Meter; Exttech Instruments, Nashua, NH) was positioned directly on the middle tooth surfaces of canines and central incisors of the patient's maxillary and held in position until the pH was stabilized on the screen. As the pH electrode is very sensitive, it was possible to make

**TABLE 1** List of components used in the formulation of the bleaching agents

Bleaching agent	Base composition		Especific composition		Manufacturer
	Ounce (oz)	% wt	Ounce (oz)		
Control (CT)	<ul style="list-style-type: none"> <li>H<sub>2</sub>O—27.9</li> <li>H<sub>2</sub>O<sub>2</sub> 50%—67</li> <li>Sodium alginate—5</li> <li>Methylparaben—0.1</li> </ul>	—	—	—	<ul style="list-style-type: none"> <li>Sodium alginate—Biotecc—Commerce of products for laboratory Eireli, São José dos Pinhais, PR, Brazil.</li> </ul>
Experimental (n-Bm)	<ul style="list-style-type: none"> <li>H<sub>2</sub>O—22.9</li> <li>H<sub>2</sub>O<sub>2</sub> 50%—67</li> <li>Sodium alginate—5</li> <li>Methylparaben—0.1</li> </ul>	n-Bm 5%	5		<ul style="list-style-type: none"> <li>H<sub>2</sub>O<sub>2</sub>—Farmanil Quima, Curitiba, PR, Brazil.</li> <li>Methylparaben—LabSynth, Diadema, SP, Brazil.</li> </ul>

three measurements for each tooth. For each one of the three applications of session, the pH was registered. This assessment was conducted in a six participants, with to intention to know the behavior and stability of bleaching agent during de application.

## 2.11 | Outcomes

### 2.11.1 | TS evaluation

Participants were instructed to keep a daily record of whether they experienced sensitivity. The patient was asked to indicate the numerical value of degree of sensitivity using a 5-point numeric rating scale (NRS) where 0 = none, 1 = mild, 2 = moderate, 3 = considerable, and 4 = severe. Also, to express their pain intensity using a visual analog scale (VAS) was used too. This scale was a 10 cm horizontal line with scores of 0 and 10 at the ends, where 0 = no sensitivity and 10 = severe sensitivity. The participant marked, with a vertical line across the horizontal line of the scale, the region that best represented the TS that the participant had during each time assessment. Then, we measured the distance in millimeters from the 0 end with the aid of a millimeter ruler. For each side of arch was measured pain by these scales and we emphasized to the participants that we were interested in differences between the right and left sides.<sup>9,11,38,39</sup>

The worst score from the NRS and highest numeric value obtained in the VAS during all bleaching treatments were considered for statistical purposes, so that only a single value was taken from the 2-week treatment. The values were arranged into two categories: absolute risk of TS, which represented the percentage of participants who reported TS at least once during treatment, and the overall TS for each time assessment.

### 2.11.2 | Color evaluation

We recorded shade evaluation before, 1 week after the first bleaching session, 1 week after the second bleaching session, and 1 month after the end of the bleaching treatment. Color evaluation was never performed immediately after each bleaching session to avoid the effect of dehydration and demineralization on color measures. The measurement area of interest for shade matching was the middle one-third of the facial surface of the upper canines.<sup>11,38,40,41</sup> We performed the subjective color evaluation using shade guides VITA Classical and the VITA Bleachedguide 3D-MASTER. Additionally, for an objective color evaluation we used the spectrophotometer VITA Easyshade (VITA Zahnfabrik, Bad Säckingen, Germany). is worth to mentioning that the color evaluation was completed in a room under artificial lightning conditions, under a color corrected light (Rite Lite, AdDent, Inc., Danbury, CT).

For color evaluation with the Vita Classical scale, the 16 tabs of the shade guide were arranged from the highest (B1) to the lowest (C4) value. Although this scale is not linear in the truest sense, for the purpose of analysis, as already performed in others published studies,<sup>11,42</sup> the changes were treated as though they represented a continuous and approximately linear ranking. The VITA Bleachedguide

3D-MASTER contains lighter shade tabs and is already organized from the highest (0M1) to the lowest (5M3) value.<sup>9,43,44</sup>

One examiner, blinded to the allocation assignment scheduled these participants for bleaching and evaluated their teeth against the shade guide at the different time assessments. Color changes were calculated from the beginning of the active phase through to the after 1-month of the end of the treatment by calculating the change in the number of shade guide units ( $\Delta$ SGU), which occurred toward the lighter end of the value-oriented list of shade tabs, for both scales.

For the color evaluation with spectrophotometer VITA Easyshade, an impression of the maxillary arch was taken with dense silicone paste (Zetaplus and Oranwash Kit, Zhermack, Italy). The impression was extended to the maxillary first premolars and served as a standard color measurement guide to the spectrophotometer. For each dental component to be evaluated, a window was created on the labial surface of the molded silicone guide using a metal device with a radius of 6 mm and well-formed borders.<sup>45</sup> The tip of the device was then inserted into the silicone guide, and we obtained the  $L^*$ ,  $a^*$ , and  $b^*$  parameters of color from the spectrophotometer (CIE Lab color coordinates). The  $L^*$  value represents the lightness (value from 0 [black] to 100 [white]),  $a^*$  value represents the measurement along the red-green axis, and  $b^*$  value represents the measurement along the yellow-blue axis.

Several color measures were performed based on the spectrophotometer parameters. First, the classical CIE Lab color evaluation ( $\Delta E^*_{ab}$ ) was calculated by the change of color before (baseline) and after 1-month of the end of treatment, which is calculated using the formula:  $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$  (Comission Internationale de l'Eclairage).<sup>46</sup>

Second, it was used the color change evaluation based on the more recently CIEDE2000 ( $\Delta E_{00}$ ), according to the following equation<sup>47</sup>:  $\Delta E_{00} = [(\Delta L'/k_L S_L)^2 + (\Delta C'/k_C S_C)^2 + (\Delta H'/k_H S_H)^2 + R_T(\Delta C'/k_C S_C \cdot \Delta H'/k_H S_H)]^{1/2}$  where  $\Delta L'$ ,  $\Delta C'$ , and  $\Delta H'$  are the differences in lightness, chroma, and hue, respectively.  $S_L$ ,  $S_C$ , and  $S_H$  are considered weighting functions to adjust the total color difference for variation in the location of the color difference pair in  $L'$ ,  $a'$ , and  $b'$  coordinates.  $k_L$ ,  $k_C$ , and  $k_H$ , the parametric factors, are correction terms for experimental conditions. And finally,  $R_T$  is a rotation function that accounts for the interaction between chroma and hue differences in the blue region.<sup>47,48</sup>

Third, the whiteness index for dentistry ( $WI_D$ ), which is based on CIE Lab coordinates, was used too, and calculated as the following equation:  $WI_D = (0.511 \times L^*) - (2.324 \times a^*) - (1,100 \times b^*)$ .<sup>49</sup> Higher  $WI_D$  values indicate whiter teeth (whiteness), while lower  $WI_D$  values (including negative values) indicate darker teeth (darkness).

## 2.12 | Statistical analysis

The analysis followed to intention-to-treat protocol and involved all participants who were randomly assigned.<sup>35</sup> In case of missing data, the last observation was carried forward. The statistician was also blinded to the groups.

The absolute risk of TS of both groups was compared with the McNemar test ( $\alpha = 0.05$ ). The relative risk and confidence interval for the

	Control	Experimental (n-Bm)
Canine baseline color SGU (mean ± SD)	9.1 ± 2.7	9.3 ± 2.7
Age years (mean ± SD)	22.3 ± 5.7	
Gender (female %)		68.18
Race	Caucasian (%)	84.84
	Middle eastern (%)	7.57
	African (%)	7.57

**TABLE 2** Baseline demographics of the volunteers included in this clinical trial<sup>a</sup>

Abbreviation: SD, standard deviation.

<sup>a</sup>SGU shade guide unit measured by Vita Classical.

effect size were calculated. The comparison of the TS intensity (NRS and VAS data) of the bleaching groups at each assessment point was performed with the Wilcoxon Signed Rank test and paired *t* test, respectively. On the other side, comparisons between different times within each group were performed with the Friedman test and repeated measure one-way ANOVA and Tukey's test, respectively for NRS and VAS data. The color changes between groups ( $\Delta$ SGU for both scales,  $\Delta E_{ab}$ ,  $\Delta E_{00}$ , and  $WI_D$ ) between baseline versus 1 month after the end of treatment) were compared with a paired *t* test. In all statistical tests, the significance level was 5%. We performed all analyses by the software SigmaPlot Version 11.0 (Systat Software).

### 3 | RESULTS

#### 3.1 | Baseline data and characteristics of included participants

A total of 96 participants were examined in a dental chair to check whether they met the inclusion and exclusion criteria. Though, only 66 participants were recruited to this clinical trial (Figure 1). The baseline color of the participants and the distribution of the genders were described in Table 2.

#### 3.2 | Adherence to the protocol and dropouts

Two participants no attended the recall visits one week after the second bleaching session. The same two that no attended the anterior recall visit, no come back for this, for recall visit one month after treatment and other participant did not attend 1 month after treatment, totalizing three participants. Figure 1 depicts the participants flow diagram in the different phases of the study design.

#### 3.3 | In vivo pH analysis

For the pH values, there was a statistically significant difference ( $p < 0.006$ ) between the groups, as shown in Table 3. The presence of BG nanoparticle significantly increased the pH of the bleaching agent used.

**TABLE 3** Means and standard deviations of pH variation at the different assessment points for both groups

Assessment times	pH variation		<i>p</i> <sup>a</sup> value
	Control	Experimental (n-Bm)	
15 min	4.4 ± 0.5	5.3 ± 0.3	0.006
30 min	4.2 ± 0.5	5.3 ± 0.3	0.001
45 min	4.3 ± 0.4	5.2 ± 0.4	<0.001

<sup>a</sup>Paired *t*-test.

### 3.4 | Tooth sensitivity

#### 3.4.1 | Risk of TS

Regarding the absolute risk of TS, there was no statistically significant difference between the groups ( $p = 1.00$ ) as shown in Table 4. Although, the low absolute risk of TS in both groups, thirteen participants due to adverse effects, needed oral medication (anti-inflammatory or analgesic) and three needed local medication (application of a desensitizing agent), eight participants for each.

#### 3.4.2 | Intensity of TS

Regarding the intensity of TS, there was no statistically significant difference between the groups for both pain assessment scales (NRS; Table 5;  $p > 0.37$  and VAS; Table 6;  $p > 0.16$ ). The intensity of TS was significantly higher during bleaching and up to 24 h afterward, in both groups and scales (NRS; Table 5;  $p < 0.05$  and VAS; Table 6;  $< 0.05$ ). However, there was a decrease in TS in the periods up to 24 and 48 h (Tables 5 and 6). After 1 week, no patient reported spontaneous sensitivity (data not shown).

### 3.5 | Color evaluation

There was no difference for color change between groups in any of the evaluation methods (Table 7;  $p > 0.10$ ). At the end

**TABLE 4** Comparison of the number of patients who experienced TS during bleaching regimen in both groups with absolute and the risk ratio<sup>a</sup>

Treatment	Tooth sensitivity (number of participants)		Absolute risk (95% CI)	Risk ratio (95% CI)
	Yes	No		
Control	18	48	27 (18–39)	0.78 (0.42–1.43)
Experimental (n-Bm)	14	52	21 (13–32)	

<sup>a</sup>Mc Nemar test ( $p = 1.00$ ).

**TABLE 5** Medians and interquartile ranges of the TS intensity at different assessment points using the numeric rating scale (NRS)

Assessment times	Control*	Experimental (n-Bm)	$p$ value**
During bleaching	1 (0–2) a	1 (0–2) A	0.67
Up to 1 h	2 (1–3) b	2 (1–2.75) B	0.37
Up to 24 h	1 (0–2) a,b	1 (0–2) A,B	0.42
Up to 48 h	0 (0–1) c	0 (0–1) C	0.62

\*Friedman test for each column. Within each column, significant differences are represented by distinct uppercase or lower-case letters.

\*\*Wilcoxon Signed Rank test for each line.

of the tooth whitening protocol, a whitening of approximately 6–7 units was detected on the VITA Classical color scale in both groups, for  $\Delta E_{ab}$  varied by approximately 13 units, for  $\Delta E_{00}$  varied about 8 units and for  $WI_D$  the mean changed approximately 35–36 units (Table 7). The results of the subjective method (VITA Classical and VITA Bleachedguide 3D-MAS-TER) and the objective evaluation with the spectrophotometer, corresponded to the hypothesis of equality between the groups after tooth whitening.

## 4 | DISCUSSION

The aim of this study, which incorporated n-Bm in a bleaching gel, was to decrease TS through the deposition of n-Bm on the enamel surface, as well as on the enamel's microscopic defects. The n-Bm could also act through the occlusion of the enamel defects, consequently blocking the flow of fluids inside and guiding the principle of the hydrodynamic mechanism of dentin.<sup>50–52</sup>

From this principle, the incorporation of a nano-bioactive material in  $H_2O_2$  gels of high concentration appears to be a good alternative, mainly because this material was in nanoparticulate form. It will be expected that, with a reduced size, the contact with the surface is greater, and consequently, the capacity to penetrate into the tooth structure increases.

According to Matis et al.<sup>53</sup> and Pinheiro et al.<sup>54</sup> bioactive materials can facilitate the deposition of calcium ( $Ca^{2+}$ ) and phosphate ( $PO_4^{3-}$ ) ions that are lost during the bleaching treatment, in the form of ACP, forming a new layer crystallized by the reaction with hydroxyl, carbonate, and fluoride in the oral cavity. Furthermore, some in-office

bleaching agents are delivered with a low pH (around 2.0), to improve the product's shelf life.<sup>55–57</sup> However, this leads to modifications in the chemical composition, promoting the demineralization and accumulation of minerals on the enamel surface. When a remineralization agent is added, the pH value increases, similar to that was observed in this study, where the experimental bleaching agent with n-Bm had high pH values.

It is known that, in the degradation reaction that occurs during the bleaching procedure, the perhydroxyl radical ( $HO_2\bullet$ ) reacts with the  $H_2O_2$  present on the dental surface and forms hydroxyl ( $OH\bullet$ ) and perhydroxyl ( $HO_2\bullet$ ) free radicals. The free radicals are responsible for breaking the double bonds of organic compounds present inside tooth structures,<sup>58,59</sup> generating smaller molecules that make the tooth more opaque and whiter to the human eye. The formation of these free radicals from  $H_2O_2$  depends on the pH of the medium and can play an important role with regard to the development of TS sensitivity.<sup>60</sup> Although the pH values were higher, the addition of n-Bm in the composition of an experimental bleaching agent did not decrease the absolute risk and intensity of TS reported by patients. The deposition on the enamel surface by n-Bm seems to have not been enough to prevent the penetration of  $OH\bullet$  and  $HO_2\bullet$ , resulting in TS in patients.

In the previously published clinical studies that used some type of bioactive or bioceramic material (nanoparticles of hydroxyapatite (n-HA), ACP, bioactive glasses) as a desensitizing agent in bleaching gels or as a pre- or postbleaching procedure had good results in reducing TS.<sup>25,26,28,29</sup>

A closer view of these studies showed that approximately 80% of patients had TS. As already described, TS is a common side effect of patients who undergo in-office bleaching,<sup>9–11,36,37</sup> and surprisingly, the same percentages of TS in this study were quite low: control at 27% and with n-Bm at 21%. Some hypotheses may explain the results obtained, such as the new formulation of the bleaching agents playing a key role in reducing absolute risk and intensity rates of TS.

Thickener agents like carbopol (carboxypolyethylene polymer) cause detrimental effects on enamel over time.<sup>61</sup> Its ionic characteristics, low pH stability, and carboxylic acid derivation can contribute to enamel degradation,<sup>62</sup> consequently facilitating the diffusion of  $H_2O_2$  through the pulp chamber and providing an appearance of TS. For this reason, a thickener agent substitution was used in this study. Sodium alginate, a sodium salt of alginic acid and natural polysaccharide, that, when reacting with  $H_2O_2$ , formed a more reticulated network on the

**TABLE 6** Means and standard deviations of the TS intensity at the different assessment points using visual analog scale (VAS)

Assessment times	Control*	Experimental (n-Bm)	p value**	Mean difference (95% CI)
During bleaching	2.9 ± 3.3 a	2.5 ± 3.5 A	0.16	−0.4 (−1.49 to 0.69)
Up to 1 h	3.8 ± 3.0 b	3.6 ± 3.1 B	0.37	−0.2 (−1.25 to 0.85)
Up to 24 h	3.1 ± 2.9 a,b	2.9 ± 2.8 A,B	0.21	−0.2 (−1.18 to 0.78)
Up to 48 h	0.9 ± 1.4 c	0.8 ± 1.3 C	0.49	−0.1 (−0.57 to 0.37)

\*One-way ANOVA and Tukey's test. Within each column, significant differences are represented by distinct uppercase or lower-case letters.

\*\*Paired t-test for each line.

**TABLE 7** Means and standard deviations of  $\Delta$ SGU obtained with the Vita Classical and Vita Bleachedguide 3D-MASTER,  $\Delta E_{ab}$ ,  $\Delta E_{00}$ , and  $W_{ID}$  between baseline versus 1-month post bleaching along with p-value and the mean difference (95% confidence interval)

Color evaluation tool	Control	Experimental (n-Bm)	p-value <sup>a</sup>	Mean difference (95% CI)
$\Delta$ SGU (Vita Classical)	6.3 ± 2.0	6.6 ± 2.2	0.10	0.3 (−0.42 to 1.02)
$\Delta$ SGU (Vita Bleached)	7.2 ± 2.7	7.4 ± 3.0	0.19	0.2 (−0.78 to 1.18)
$\Delta E_{ab}$	12.9 ± 4.4	13.2 ± 6.7	0.77	0.3 (−1.65 to 2.25)
$\Delta E_{00}$	8.1 ± 3.1	8.4 ± 4.1	0.61	0.3 (−0.95 to 1.55)
$W_{ID}$	35.9 ± 6.9	36.3 ± 6.7	0.81	0.4 (−0.36 to 1.45)

<sup>a</sup>Paired t-test.

dental enamel surface. Thus, it prevented the diffusion of  $H_2O_2$  from being greater and afforded less permeability through the pulp chamber and consequent reduction in TS, for both groups.

Moreover, the initial binding of  $Ca^{2+}$  by alginate chains may be formed with a multicomplex formation. More specifically,  $Ca^{2+}$  ions are explicitly considered as linkers holding two chains together by a short-range attraction. This scenario comprises calcium ion-induced contact points, creating an aspect as tilted egg-box.<sup>63</sup> This is interesting because, as already mentioned, bioactive materials can facilitate the deposition of  $Ca^{2+}$  during the bleaching procedure, thus strengthening the bond with sodium alginate helped to create a more cross-linked structure and made diffusion of  $H_2O_2$  through the pulp chamber more difficult. Therefore, future studies need to be done to evaluate the effect of different thickener agents on TS associated with in-office bleaching.

Regarding clinical effectiveness, no significant difference was observed between both groups for objective and subjective measures. Many methods may be used to assess color changes. The Vita 3D-Master Bleachedguide scale has a greater number of shades (29 shades vs. 16 shades for the Vita Classical scale), which makes it adequate for use in bleaching studies because of greater uniformity between shades and the presence of lighter shades.<sup>43</sup> However, the Vita Classical scale is more often used than the Vita 3D Master is because it is easier to use. In this study, both scales were used, and the results agreed with other studies that measured the color in canines too.<sup>11,38</sup>

The objective method used in this study (spectrophotometer) evaluated the color changes by CIELab ( $\Delta E^*_{ab}$ ) and CIEDE2000 ( $\Delta E_{00}$ ), some dentistry-related studies have reported better performance of CIEDE2000 compared to CIELab formula.<sup>64–66</sup> The  $\Delta E^*_{ab}$  calculation considers the  $L^*$ ,  $a^*$ , and  $b^*$  coordinates, which do not detect small to

medium color differences.<sup>67</sup> However, the CIEDE2000 formula corrects the irregularity in the differences between colors in the  $L^*$ ,  $a^*$ , and  $b^*$  color spaces, improving the performance for blue and gray colors.<sup>66,67</sup> Thus, to complement the outcomes of this study,  $W_{ID}$  was used.  $W_{ID}$  has been studied in the context of the dental bleaching agents and some dentifrices,<sup>68,69</sup> and it was important to observe how the nano-bioactive material can interfere with the whiteness perception.

In the present study the split-mouth design, in which trial designs are randomly assigned to two body parts, was used. The carry across effect is the most worrying concern researchers raise for this type of design. Although, because the topical action of the experimental agent being tested and because patients can differentiate pain in both sides of the mouth,<sup>45,70–72</sup> this was not a problem for this study. Furthermore, this type of design is common for in-office bleaching studies, in which the products being applied by a clinician are not susceptible to interfering with the other mouth side.<sup>11,38</sup> This may be more challenging in clinical studies that evaluate at-home protocols because placement of materials is under the patients' control. And when the statistical analysis is considered, this type of study design removes the inter-individual variability from the estimates of the treatment effect<sup>73,74</sup> and also increases study power without the need of a high sample size, which justifies the choice.

## 5 | CONCLUSION

The incorporation of n-Bm in the bleaching agents did not interfere in the bleaching effectiveness. However, there was not an observed difference for the absolute risk and intensity of TS. The rates obtained were very low.

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## DISCLOSURE

The authors do not have any financial interest in the companies whose materials are included in this article.

## AUTHOR CONTRIBUTIONS

**Adrieli Burey:** Developed the project, submitted on ethics and research committee of the local university and registered in the Clinical Trials website. Responsible for the patient's recruitment, worked on the development of clinical procedures. Worked on the data's process and statistical analyses and worked on the paper writing. **Elisama Sutil:** Participated on the patient's recruitment, was responsible for random sequence generation and allocation concealment and helped on patient's instructions. **Maira Alejandra Nunez Aldaz:** Participated on the patient's recruitment, in the clinical procedures and helped on the patient's instructions. **María Luján Méndez Bauer:** Participated on the patient's recruitment, in the clinical procedures, and helped on the patient's instructions. **Márcia Rezende:** Supervised the project development, was one of the evaluators on the color evaluation, participated on the paper writing. **Alessandra Reis:** Conceived of the presented idea, contributing data and analysis tools, supervised the project. Revised the writing manuscript. **Osnara Maria Mongruel Gomes:** Conceived of the presented idea, was one of the evaluators on the color evaluation and revised the writing manuscript. **Paulo Vitor Farago:** Conceived of the presented idea, developed the experimental gel, and revised the writing manuscript. **Alessandro D. Loguercio:** Conceived and designed of the presented idea, supervised the entire project, worked on the data analysis and worked on the paper, and revised the writing manuscript.

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