Influence of Bleaching Agent Containing Bioactive Glass on Color and Microhardness of Bovine Enamel

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ABSTRACT: To try to reduce the deleterious effects of tooth whitening, bioactive materials have been used. Forty enamel/dentin blocks were stained on dark tea and randomly assigned into four groups (n=10): control group (unbleached), HP35 % (35 % hydrogen peroxide), BG45S5 (Bioglass 45S5® incorporated into HP35 %), and BIO (Biosilicate® incorporated into HP35 %). Colorimetric analysis and microhardness evaluation was performed at baseline, 24 hours and 7 days after the final whitening session. Two-way ANOVA for repeated measures and Bonferroni test was used at a significance level of 5 %. All the coordinates (ΔL*, Δa*, Δb*, ΔE00 and WID) showed a difference between the control group and the experimental ones (p<0.05). ΔE00 values indicated that all the groups did not show changes between the evaluation times (p>0.05), which suggest a color stability over a week. In contrast, after 7 days, the WID showed that control and PH35 % were different than the other groups (p < 0.023), although no differences were observed between BG45S5 and BIO groups (p = 1.000). No differences in enamel microhardness were found between the groups within the same evaluation time (p>0.05). The microhardness did not change over time (p>0.05), except for 35 % HP. In conclusion Bioglass 45S5® and Biosilicate® prevented enamel damage without negatively affect the whitening efficacy.

KEY WORDS: bioactive glass, enamel, hydrogen peroxide, tooth whitening, microhardness, tooth color.

INTRODUCTION

Tooth whitening is the first treatment option for discolored teeth. Currently, the most used bleaching agent is hydrogen peroxide (HP) and its action consists in degrading molecules with long carbon chains, impregnated in the dental structure, by an oxy-reduction process. The molecular rearrangement after that process changes the interaction between the light and the tooth structure, making the color appearance lighter (Alqahtani, 2014; Kwon & Wertz, 2015).

HP has a great ability to diffuse through the tooth structure and to degrade into free radicals such as hydroxyl (OH·), peridroxyl (H2O2) and superoxide (O2-) ions. These radicals have low molecular weight and are highly reactive, interacting not only with the chromophoric molecules but also with the organic and inorganic matrix of the tooth (Alqahtani, 2014; Kwon & Wertz, 2015).

Although bleaching agents are considered safe, there is concern about possible physical and morphological changes in the enamel structure, such as increased roughness, decreased microhardness and altered mineral content (Soares et al., 2013; Vieira et al., 2020). In order to minimize or even avoid changes in the bleached enamel structure, different remineralizing compounds have been evaluated to prevent demineralization promoted by whitening treatments (Crasitechini et al., 2019; Torres et al., 2019; Vieira et al., 2020; Ubaldini et al., 2020).
Bioactive glasses (BG) have been applied in dentistry with the objective of promoting dentin remineralization (Hench, 2013; Pintado-Palomino & Tirapelli, 2015). The contact of BG with body fluids promotes the release of Na⁺ ions and corresponding dissolution in Ca²⁺, PO₄³⁻ and Si⁴⁺ ions occur on the glass surface with subsequent precipitation of calcium phosphate not only at the glass/tissue interface but also in distant living tissues. As a result, formation of hydroxycarbonate apatite (HCA) (Deng et al., 2013; Jones, 2015) can be verified, improving the sealing of dentinal tubules. In addition, BG have antibacterial potential due to increase in pH during the release of ions Ca²⁺, PO₄³⁻, that prevent the proliferation of some bacteria (Begum et al., 2016).

Based on their benefits, the incorporation of BG into tooth whitening agents could apparently promote mineral deposition on the tooth surface and reduce the adverse effects of tooth whitening. Some studies regarding the BG incorporation have demonstrated good interaction between BG and whitening gel, preserving the integrity of the dental enamel (Deng et al., 2013; Khoroushi et al., 2015; Pintado-Palomino & Tirapelli, 2015; Khoroushi et al., 2016). Nonetheless, a standardized protocol has not yet been determined for this association.

In view of the potential and benefits of bioactive materials in terms of mineral deposition, the aim of this paper was to evaluate the impact of bleaching agents containing BG on color and microhardness of bovine dental enamel. The null hypothesis is that the incorporation of the materials will not influence the different properties mentioned.

MATERIAL AND METHOD

Specimen preparation: Forty bovine incisors with no visible cracks or enamel defects were selected and stored in 0.5 % thymol solution, at 4 °C until the moment of use. Enamel/dentin blocks (7x7x2 mm) from the crown were obtained using a metallographic cutter machine (Isomet 1000; Buehler Inc., Lake Bluff, IL, Umm SA) (de Carvalho et al., 2020). The enamel surfaces of the blocks were polished for 30 seconds using silicon carbide sandpapers with decreased granulation (#600, #1200, #1500 and #2500). After polishing, the specimens were cleaned in an ultrasonic tank (Cristófoli, Campo Mourão, PR, Brazil) with distilled water for 5 min, and then stored in distilled water at 37°C (±1°C) until the staining procedures.

The specimens were stained with a concentrated dark tea solution prepared with 500 mL of water and 16 g of tea (10 sachets). The staining protocol consisted of 18 hours of immersion in dark tea with 6 h of drying at room temperature. The specimens were subjected to 4 complete cycles of the protocol described above. After staining, specimens were stored for 7 days in distilled water for color stabilization (Costa et al., 2021).

Specimen’s allocation: The stained specimens had their surfaces analyzed by digital spectrophotometer (Vita Easyshade V, Wilcos, Petrópolis, RJ, Brazil) and the initial L* value of each specimen was used to standardize the random distribution into 4 groups (n = 10) to select specimens with homogenous colors (Vieira et al., 2020).

Experimental groups: The experimental groups are described below according to the treatment and incorporation or not of BG (Table I):

- Control: unbleached enamel, no treatment; the specimens were kept in distilled water (at 37 °C) during the entire period of study.
- 35 % HP: Three Whiteness HP sessions (FGM, Joinville, SC, Brazil) were performed with 7-day intervals. Each session consisted of a 15-min

Table I. Products used in the study.

<table>
<thead>
<tr>
<th>Product</th>
<th>Acronym</th>
<th>Manufacturer</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whiteness HP</td>
<td>35 % HP</td>
<td>FGM, Joinville, SC, Brazil</td>
<td>35 % hydrogen peroxide, thickeners, red pigment, glycol and water.</td>
</tr>
<tr>
<td>Bioglass 45S5</td>
<td>BG45S5</td>
<td>Vitreous Materials Laboratory - LAMAV, UFSCAr, São Carlos, SP, Brazil</td>
<td>Particles of bioactive bioglass (1–10µm) P₂O₅–Na₂O–CaO–SiO₂</td>
</tr>
<tr>
<td>Biosilicate®</td>
<td>BIO</td>
<td>Vitreous Materials Laboratory - LAMAV, UFSCAr, São Carlos, SP, Brazil</td>
<td>Particles of bioactive ceramic crystalline (1–10µm) P₂O₅–Na₂O–CaO–SiO₂</td>
</tr>
</tbody>
</table>
application. The manipulation of the bleaching gel was carried out according to the manufacturer in the ratio of 3:1 between peroxide and thickener for 10 s. A layer of approximately 1mm thick was applied over the enamel surface of each sample. After each whitening session, the gel was removed with air/water jets for 30 seconds and the teeth were kept stored in distilled water (37 °C).

- BG455S: The specimens were bleached according to the 35 % HP group. However, the volume of the bleaching gel and thickener drops (~ 50 µL of each material) were previously weighed in a precision balance (AR2140, OHAUS Corporation, Parsippany, NJ, USA). Based on the weight of the bleaching agent drops, the corresponding 10 % of the value previously weighed on a precision balance was incorporated with BG455 particles using a plastic spatula present in the bleaching agent handling kit. The peroxide and thickener were homogeneously mixed by circular movements for 10 s using a spatula. After homogenization, the whitening gel containing BG was applied on the enamel surface of the specimens. After each whitening session, the gel was removed with air/water jets for 30 seconds and the specimens were kept stored in distilled water (37 °C).

- BIO: The specimens were bleached according to the 35 % HP group. However, the volume of the bleaching gel and thickener drops were previously weighed in a precision balance (AR2140, OHAUS Corporation, Parsippany, NJ, USA). Based on the weight of the bleaching agent, the corresponding 10 % of the value was incorporated with BIO particles, similar to BG455. After homogenization, the whitening gel containing BG was applied on the enamel surface of the specimens. After each whitening session, the gel was removed with air / water jets for 30 s and the specimens were kept stored in distilled water (37 °C).

**Ph Measurement:** The pH values of the bleaching gel with and without BG were obtained after its manipulation using a digital pH meter (Ph-2600, Instrutherm, São Paulo, SP, Brazil) previously calibrated with standard electrodes (MI-401 Microreference electrode, MICROELECTRODES INC, Belford, New Jersey, USA). All the measurements were performed in triplicate.

**Color evaluation:** The specimens were subjected to an initial chromatic analysis using the CIEDE2000 colorimetry system ($\Delta E_{\text{CIE}}$), CIELAB-based Whiteness Index for Dentistry ($W_{\text{ID}}$) 19, $\Delta L^*$ (difference in lightness), $\Delta a^*$ (difference in red * green) and $\Delta b^*$ (difference in yellow * blue). The coordinates were obtained by a reflectance spectrophotometer (VITA Easyshade IV, Wilcos, Petrópolis, RJ, Brazil) and the color change (DE) was evaluated before, 24 hours and 1 week after bleaching (Costa et al., 2021).

**Microhardness evaluation:** Microhardness was determined using a Vickers hardness tester machine (MMT-3, Buehler Inc., Lake Bluff, IL, USA) at a load of 100 g for 10 s (Costa et al., 2021). Three measurements with 100 µm of distance between them were performed in each specimen. The arithmetic mean of the three indentations was calculated for each sample for the statistical analysis. The microhardness was evaluated before (baseline), 24 h and 1 week after the final bleaching session.

**Statistical analysis:** The assumptions of normality were verified by Shapiro-Wilk test (p>0.05). To compare the groups and the evaluations times, two-way ANOVA for repeated measures followed by Bonferroni test was applied. All the tests were employed at a significance level of 5 % using the SPSS statistical software package version 20 (IBM Corporation, Armonk, NY, USA).

**RESULTS**

The pH mean of the bleaching agents were: gel with BG455S (7.7), gel with BIO (7.15), and gel without any BG (6.7).

All the coordinates ($\Delta L^*$, $\Delta a^*$, $\Delta b^*$) showed a difference between the control group and the experimental ones (p < 0.05). However, when the experimental groups were compared within the same evaluation time, no differences were observed (p > 0.05) for any coordinate (Table II).

$\Delta E$ values indicated that all the groups did not show changes between the evaluation times (p > 0.354), which suggest a color stability over a week. However, the intergroup comparison within the same evaluation time showed that control was different than PH 35 % (P = 0.004) and BIO (p = 0.05) at 24 hours. After 7 days, no differences were observed among the groups, except between control and PH35 % (p = 0.005) (Table III).

$W_{\text{ID}}$ also showed no intragroup difference (p >
0.081). However, when the groups were compared within 24 hours evaluation time, control group was different than the experimental ones (p < 0.002), with no difference among those (p = 0.101). After 7 days, control and PH35 % were different than the other groups (p < 0.023), although no differences were observed between BG45S5 and BIO groups (p = 1.000) (Table III).

No differences in enamel microhardness were found between the groups within the same evaluation time (p > 0.118). Furthermore, the microhardness did not change over time for control and BIO groups, since no differences were observed after 24 hours and 7 days (p > 0.297). For BG45S5 group, the microhardness increased after 24 hours (p = 0.025), but after 7 days the values decreased, with no difference compared to baseline (p = 0.972). The 35 % HP group exhibited a microhardness decrease after 7 days in comparison with baseline values (p = 0.027) (Table IV).

Table II. Mean ± standard deviation of ΔL*, Δa* and Δb* according to each evaluation time (n = 10). Two-way repeated measure ANOVA followed by Bonferroni test (p < 0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>ΔL*</th>
<th>Δa*</th>
<th>Δb*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hours</td>
<td>-7.68 ± 2.71 Aa</td>
<td>-8.17 ± 2.97 Aa</td>
<td>-2.02 ± 1.69 Aa</td>
</tr>
<tr>
<td>7 days</td>
<td>-9.44 ± 2.75 Aa</td>
<td>-8.51 ± 2.83 Aa</td>
<td>-1.77 ± 2.02 Aa</td>
</tr>
<tr>
<td>35 % HP</td>
<td>11.99 ± 5.29 Ba</td>
<td>10.19 ± 4.83 Bb</td>
<td>7.76 ± 1.57 Bd</td>
</tr>
<tr>
<td>24 hours</td>
<td>-7.9 ± 1.72 Ba</td>
<td>-6.73 ± 3.55 Ba</td>
<td>5.94 ± 1.15 BcB</td>
</tr>
<tr>
<td>7 days</td>
<td>-17.61 ± 2.88 Ba</td>
<td>-19.11 ± 1.74 Ba</td>
<td>-17.19 ± 2.98 Ba</td>
</tr>
<tr>
<td>BG45S5</td>
<td>7.24 ± 2.75 Ba</td>
<td>5.62 ± 1.10 Bb</td>
<td>6.29 ± 1.59 Ba</td>
</tr>
<tr>
<td>24 hours</td>
<td>5.94 ± 1.15 BcB</td>
<td>17.66 ± 4.63 Bc</td>
<td>-17.19 ± 3.47 Ba</td>
</tr>
<tr>
<td>7 days</td>
<td>17.19 ± 4.12 Bc</td>
<td>17.28 ± 5.86 Ba</td>
<td>23.25 ± 4.97 Ca</td>
</tr>
<tr>
<td>BIO</td>
<td>7.18 ± 3.00 Ba</td>
<td>6.14 ± 3.20 Bb</td>
<td>6.29 ± 1.59 Ba</td>
</tr>
<tr>
<td>24 hours</td>
<td>5.94 ± 1.15 BcB</td>
<td>17.66 ± 4.63 Bc</td>
<td>-17.19 ± 3.47 Ba</td>
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</tr>
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</table>

DISCUSSION

This study evaluated the influence of BG incorporation (45S5® and Biosilicate®) into hydrogen peroxide bleaching gel on the optical properties and microhardness of bovine enamel. The null hypothesis was partially accepted, since all the bleaching protocols showed the same effectiveness in relation to the control (p > 0.05). However, regarding the microhardness evaluation, HP and BG45S5 showed differences among the evaluation times, but no differences in intergroup values were detected.
Although tooth whitening is a very common dental procedure, its side effects are still controversial (Vieira et al., 2020). Contradictory results regarding the ability of bleaching agents to cause morphological changes on enamel are reported. This variation may be associated with: methodology used, whitening protocol, concentration and pH of the bleaching agents, storage method, and the analyzes performed (Mondelli et al., 2015; Borges et al., 2015).

In this study, we used a high concentration bleaching agent (35 %) since is still considered the gold standard in in-office tooth whitening (Maran et al., 2020), while 45S5 bioglass is a material with high bioactivity and remineralization capacity (Hench, 2013; Ubaldini et al., 2020), and the Biosilicate® emerged as a glass-ceramic with enhanced mechanical properties (Crovace et al., 2016; Ubaldini et al., 2020). Previous studies have evaluated both in vitro (Pintado-Palomino & Tirapelli, 2015; Khoroushi et al., 2015, 2016; Ubaldini et al., 2020) and in vivo 15 the effectiveness of BGs as an additional step in the whitening procedure, increasing the clinical time. The idea of incorporating the materials into the whitening gel seeks to enable a combined action of the two materials and avoids additional clinical time. Furthermore, Deng et al. (2013) reported that the application of BG45S5 during HP application acts as a protective barrier to prevent/restore the enamel. Based on that results, we evaluated the BG incorporation into the HP-based bleaching agent. The penetration and action of 35 % HP in the dental structure is active for several days, as well as the release of ions from BG in contact with the dental surface (Ubaldini et al., 2020). For this reason, we evaluated the specimens 7 days after the final whitening session to verify the bioactivity over time.

The standardization of the initial readings was determined by the value of the L* coordinate using the CIELAB system, since the reduction of the initial variability allows more accurate statistical comparisons and better evaluate the whitening efficacy (Vieira et al., 2020). Another observation regarding the present study is the storage of specimens in distilled water. Although some studies use artificial saliva for this purpose (Soares et al., 2013; Vieira et al., 2020), we choose to store the specimens in distilled water to verify only the influence of BGs on enamel remineralization (Pintado-Palomino et al., 2015).

The incorporation of 10 % BGs into 35 % HP promoted remineralization of the enamel surface enough to maintain the microhardness without compromise the whitening efficacy. The pH values of the bleaching agents containing BG increased in comparison of the control group (without BG). It is known that enamel demineralization can be influenced by the acidity of the gel (Xu et al., 2011; Soares et al., 2016), considering that the analyzes performed reflects the loss of mineral content and organic matrix promoted by the degradation of HP in free radicals (Deng et al., 2013; Borges et al., 2014). Although 35 % HP showed a slightly acidic pH (6.7) and above the critical enamel demineralization pH value (5.5) (Xu et al., 2011), it was verified that the HP35 group promoted a decrease in microhardness after 1 week of the final whitening session (p < 0.05), suggesting that other factors such as composition and concentration of the gel can affect the mechanical properties of enamel. According to our results, it is possible to assume that the mineral deposition and the buffering of the whitening gel acidity promoted by the bioactive glasses maintained the integrity of the enamel. That results raise questions regarding the use of greater amounts of material incorporation may or may not increase the microhardness of the enamel.

In this study, although the microhardness values of the groups incorporated with BG were higher than the positive control group 35 % HP, no statistically significant difference was observed between the groups. This result differs from a previous study that reported an increase in microhardness after the use of BG during the use of HP (Deng et al., 2013). We attribute these divergent results both to the methodology and to the amount of BG weight that was added into the bleaching agent, since our BG weight was much lower (~0.1 g) than the used in a previous study by Deng et al. (2013) (2.16 g). In this way, further studies are needed to evaluate the ideal percentage of BG incorporation without negatively affect the whitening efficacy.

Herein we sought solutions to minimize the adverse effects of tooth whitening, without losing its whitening efficacy; therefore, the colorimetric analysis is crucial. The evaluated times were standardized for initial rehydration with distilled water (24 hours) and color stabilization after 1 week. Objective analysis with a digital spectrophotometer is interesting to measure color conditions that the human eye is not able to perceive (Turgut et al., 2018; Paravina et al., 2019). In addition, the parameters adopted in this article (CIEDE 2000 and WID) are interesting to demonstrate the color change and whether this change leads to an increase in whiteness. In this study, all groups showed
whitening efficacy perceptible to the human eye (ΔE00 ≥ 2.73) (Samra et al., 2008; Paravina et al., 2015), with no significant differences between them and with the same color stability after 7 days (Table III).

The WID clearly shows the effectiveness of the whitening of the experimental groups in relation to the control group, which presented values above 10 (Table III). According to Pérez et al. (Pérez et al., 2016), values above 0.61 would be enough to have some perception by naked eyes. Therefore, we can verify that the 3 bleaching sessions were enough to promote perceptible changes regardless of the presence of material incorporation or not. However, after 7 days, the experimental groups with bioactive glasses differed significantly from the 35 % HP group, which raises the hypothesis that the release of Ca2+, Na+ and PO43- ions and the consequent mineral deposition in 1 week could harm the action of free radicals on the tooth structure.

ΔL represents the luminosity and is often noticed by the human eye. In our study, ΔL values were similar between the experimental groups within the same evaluation time (p > 0.05). In addition, Δa and Δb presented negative values, which is in accordance with previous studies (Deng et al., 2013; Vieira et al., 2020; de Carvalho et al., 2020). Based on our results, the bleaching procedures promoted an increase in green-blue tones in relation to red-yellow ones, suggesting satisfactory results in tooth whitening (Table II) (Torres et al., 2019).

This study encourages further studies in the field. Long-term evaluations and the assessment of different percentages of material addition are essential for a deeper understanding of the interaction between BG and whitening agent. In vivo studies are equally important, since the results can be enhanced by the presence of the remineralization capacity of the saliva (Crastechini et al., 2019).

CONCLUSION

The incorporation of 45S5 Bioglass and Biosilicate® into 35 % HP gel maintained the integrity of the enamel surface without compromise the whitening efficacy, with only difference in the WID after 7 days of the sessions. Further studies incorporating greater amounts of BG and with direct measures for assessing mineral content are encouraged to enable its clinical application in the future.

REFERENCES


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