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

Influencia del Agente Blanqueador que Contiene Vidrio Bioactivo sobre el Color y la Microdureza del Esmalte Bovino

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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^*, $\Delta E00$ and WID) showed a difference between the control group and the experimental ones ($p<0.05$). $\Delta E00$ 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 (H_2O^-) and superoxide (O_2) 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 (Crastechini *et al*., 2019; Torres *et al*., 2019; Vieira *et al*., 2020; Ubaldini *et al*., 2020).

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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 Ca^{2+}, PO_4^{3-} and Si^{4+} 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 Ca^{2+}, PO_4^{3-}, 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 37ºC (± 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 ºC) 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.

Product	Acronym	Manufacturer	Composition
Whiteness HP	35 % HP	FGM, Joinville, SC, Brazil	35 % hydrogen peroxide, thickeners, red pigment, glycol and water.
Bioglass 45S5 ®	BG45S5	Vitreous Materials Laboratory - LAMAV, UFSCAr, São Carlos, SP, Brazil	Particles of bioactive bioglass (1–10μm) P_2O_5–Na_2O–CaO–SiO_2
Biosilicate®	BIO	Vitreous Materials Laboratory - LAMAV, UFSCAr, São Carlos, SP, Brazil	Particles of bioactive ceramic crystalline (1–10μm) P_2O_5–Na_2O–CaO–SiO_2

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 ºC).

· BG45S5: 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 BG4S5 particles using a plastic spatula present in the bleaching agent handling kit. The peroxide and thickener were homogenously 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 ºC).

· 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 BG45S5. 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 ºC).

Ph Measurement: The pH values of the bleaching gel with and without BG were obtained after its manipulation using a digital pHmeter (Ph-2600, Instrutherm, São Paulo, SP, Brazil) previously calibrated with surface 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 (ΔE_{00}), CIELAB-based Whiteness Index for Dentistry (W_{ID}) 19, ΔL* (difference in lightness), Δa* (difference in red * green) and Δb* (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 BG45S5 (7.7), gel with BIO (7.15), and gel without any BG (6.7).

All the coordinates (ΔL*, Δa*, Δb*) 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).

ΔE 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).

WID 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 ($p < 0.05$).

Groups	ΔL*		Δa*		Δb*	
	24 hours	7 days	24 hours	7 days	24 hours	7 days
CONTROL	-7.68±2.71^{Aa}	-8.17±2,97^{Aa}	-2.84±1.77^{Aa}	-2.02±1.69^{Aa}	-9,44±2.75^{Aa}	-8.51 ±2,83^{Aa}
35 % HP	11.99 ± 5.29^{Ba}	10.19 ± 4.83^{Bb}	-7.76 ± 1.57^{Ba}	-7.9 ± 1.72^{Ba}	-16.73 ± 3.55^{Ba}	-19.31 ± 3.47^{Bb}
BG45S5	7.24 ± 2.75^{Ba}	5.53 ± 2.34^{Ba}	-5.62 ± 1.10^{Ba}	-5.94 ± 1.15^{BCb}	-17.61 ± 2.88^{Ba}	-19.11 ± 1.74^{Ba}
BIO	7.18 ± 3.00^{Ba}	6.14 ± 3.20^{Bb}	-6.53 ± 1.59^{B}	-6.29 ± 1.30^{Cc}^{a}	-19.22 ±4.16^{Ba}	-19.14 ± 3.59^{Ba}

· Different uppercase letters mean statistically significant difference within the same evaluation time for each Δ*.
· Different lowercase letters mean statistically significant difference within the same group for each Δ*.

Table III. Mean ± standard deviation of ΔE* (CIEDE 2000) and WID according to each evaluation time (n = 10). Two-way repeated measure ANOVA followed by Bonferroni test ($p < 0.05$).

Groups	ΔE*		WI_D	
	24 hours	7 days	24 hours	7 days
Control	7.23 ± 1.45^{Aa}	7.25 ± 1.92^{Aa}	-10.36 ± 12.79^{Aa}	-13.54 ± 12.27^{Aa}
35 % HP	12.09 ± 2.59^{Ba}	12.26 ± 2.33^{Ba}	21.00 ± 5.36^{Ba}	23.25 ± 4.97^{Ca}
BG45S5	9.82 ± 1.91^{ABa}	9.67 ± 1.69^{AB}^{a}	17.19 ± 4.12^{Ba}	17.66 ± 4.63^{Ba}
BIO	10.67 ± 2.50^{Ba}	10.24 ± 2.16^{ABa}	18.46 ± 7.03^{Ba}	17.28 ± 5.86^{Ba}

· Different upper case letters mean statistically significant difference within the same evaluation time for each Δ*.
· Different lower case letters mean statistically significant difference within the same group for each Δ*.

Table IV. Mean ± standard deviation of Vickers microhardness (VHN) according to each evaluation time (n = 10). Two-way repeated measure ANOVA followed by Bonferroni test ($p < 0.05$).

Groups	Baseline	24 hours	7 days
Control	245.76 ± 13.43^{Aa}	245.58 ± 19.92^{Aa}	236.80 ± 10.75^{Aa}
35 % HP	244.62 ± 20.72^{Aa}	225.51 ± 22.45^{Aab}	232.18 ± 18.96^{Ab}
BG45S5	231.62 ± 17.09^{Aa}	250.57 ± 10.58^{Ab}	240.05 ± 13.11^{Aab}
BIO	227.94 ± 33.13^{Aa}	231.39 ± 23.08^{Aa}	228.57 ± 12.69^{Aa}

· Different upper case letters mean statistically significant difference within the same evaluation time.
· Different lower case letters mean statistically significant difference within the same group.

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 eyes (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.

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RESUMEN: Para intentar reducir los efectos nocivos del aclaramiento dental, se han usado materiales bioactivos. Cuarenta bloques de esmalte/dentina se tiñeron con té oscuro y se asignaron al azar en cuatro grupos (n=10): grupo de control (sin blanquear), HP35% (peróxido de hidrógeno al 35 %), BG45S5 (Bioglass 45S5® incorporado en HP35%) y BIO (Biosilicato® incorporado a HP35%). El análisis colorimétrico y la evaluación de la microdureza se realizaron al inicio del estudio, 24 horas y 7 días después de la última sesión de blanqueamiento. Se utilizó ANOVA de dos vías para medidas repetidas y la prueba de Bonferroni a un nivel de significancia del 5 %. Todas las coordenadas (ΔL*, Δa*, Δb*, ΔE00 y WID) mostraron diferencia entre el grupo control y el experimental ($p<0.05$). Los valores de ΔE00 indicaron que todos los grupos no presentaron cambios entre los tiempos de evaluación ($p>0.05$), lo que sugiere una estabilidad del color durante una semana. En cambio, a los 7 días, el WID mostró que el control y el PH35 % eran diferentes a los demás grupos ($p < 0,023$), no obstante, diferencias entre los grupos BG45S5 y BIO ($p = 1,000$) no fueron observadas. No se encontraron diferencias en la microdureza del esmalte entre los grupos dentro del mismo tiempo de evaluación ($p>0.05$). La microdureza no cambió con el tiempo ($p>0.05$), excepto para 35 % HP. En conclusión Bioglass 45S5® y Biosilicate® previnieron el daño del esmalte sin afectar negativamente la eficacia del blanqueamiento.

PALABRAS CLAVE: vidrio bioactivo, esmalte, peróxido de hidrógeno, blanqueamiento dental, microdureza, color del diente.

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