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Influence of F-18 Bioactive Glass Incorporation into Whitening Gel on Properties of Bovine Dental Enamel

Influencia de la Incorporación de Vidrio Bioactivo F-18 al Gel Blanqueador sobre las Propiedades del Esmalte Dental Bovino

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ABSTRACT: Bioactive materials have shown positive results in reducing the deleterious effects of tooth whitening. However, their effects on whitening efficacy is still uncertain. Therefore, the aim of this study was to evaluate the effects of F-18 bioactive glass incorporation into a bleaching gel on color and microhardness of enamel after tooth whitening. Thirty bovine enamel-stained specimens were randomly divided into three groups (n=10) according to the whitening protocol: control group (unbleached), HP35 % (35 % hydrogen peroxide) and F-18 (F-18 bioactive glass incorporated into HP35 %). pH, color change and microhardness analyzes were performed at baseline, 24 hours and 7 days after tooth whitening. Color evaluation showed that experimental groups were different than control group for all coordinates ($p < 0.05$). However, no intragroup differences were observed ($p > 0.05$). No differences in enamel microhardness were found among the groups within the same evaluation time ($p > 0.118$). Regarding the intragroup comparisons, no differences were observed for control and F-18 groups ($p > 0.129$). It was concluded that F-18 bioactive glass incorporation did not affect the whitening efficacy and the enamel microhardness.

KEY WORDS: bioglass, enamel, hydrogen peroxide, tooth whitening, microhardness.

INTRODUCTION

Dissatisfaction with teeth color by patients is usual in dental practice. Among the treatment options, tooth whitening has gained popularity for being a simple and conservative procedure for changing tooth color. Hydrogen peroxide (HP) is the main whitening agent and acts degrading chromophore molecules through an oxidation-reduction process, changing the light absorption spectrum of the tooth, making it clearer (Alqahtani, 2014; Kwon & Wertz, 2015).

However, the high reactivity and low molecular weight of the free radicals released from the oxidation-reduction process result in reactions not only with the long carbon chains of the chromophores, but with the tooth structure itself, including its organic and inorganic matrix (Alqahtani, 2014; Kwon & Wertz, 2015). This non-selective action may lead to chemical and morphological changes on mineral content of the tooth (Soares *et al*., 2013; Borges *et al*., 2015; Vieira *et al*., 2020). Based on that, novel materials have been developed as less harmful and capable to promote remineralization of the dental surface (Borges *et al*., 2010; Crastechini *et al*., 2019; Ubaldini *et al*., 2020; Vieira *et al*., 2020).

Since the 70's, new materials have been developed to find remineralization and bioactive capacity. 45S5 bioactive glass is one of them (Hench, 2006; Pintado-Palomino & Tirapelli, 2015). The release of Na+ ions and corresponding dissolutions in Ca^{2+}, PO_4^{3-} and Si^{4+} ions from this glass results in calcium phosphate precipitation and formation of

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hydroxycarbonate apatite (Salonen *et al*., 2009; Deng *et al*., 2013; Jones, 2015), resulting in remineralization process. Another advantage of these materials is their antibacterial capacity, since they increased the pH of the microenvironment by releasing Ca^{2+}, $POPO_4^{3-}$ ions, which harms the proliferation of some bacteria (Begum *et al*., 2016).

In view of the importance of the benefits of a bioactive glass. Recently, the Vitreous Materials Laboratory of the Federal University of São Carlos (São Carlos, SP, Brazil) introduced a new highly-reactive glass, so-called F-18. Its formula consists of SiO_2–Na_2O–K_2O–MgO–CaO–P_2O_5. The differential of having K^+ and Mg^{2+} ions enabled important antimicrobial properties (Souza *et al*., 2017; Pitaluga *et al*., 2018). However, due to its high bioactivity, F-18 appears to promote the deposition of hydroxycarbonate apatite, making imperative the conduction of studies that investigate its remineralization capacity (Souza *et al*., 2017; Pitaluga *et al*., 2018).

It is interesting to evaluate the incorporation of F18 bioactive glass into the tooth whitening agent to reduce the harmful effects promoted by it. Previous studies have shown promising results from the evaluation of bioactive glass with bleaching (Deng *et al*., 2013; Pinta-do-Palomino *et al*., 2015; Khoroushi *et al*., 2016). However, a standardized protocol for this association has not been determined. Therefore, we evaluated the influence of F-18 bioactive glass incorporation into 35 % HP-based whitening agents on color and microhardness properties of bovine dental enamel before, 24 hours and 1 week after tooth whitening.

MATERIAL AND METHOD

Specimen's Preparation: Thirty bovine incisors without cracks and fissures were stored in 0.5 % thymol solution at 4°C until its use. The roots were sectioned and enamel/dentin bocks were obtained from the buccal crown surface (7x7x2 mm) using a metallographic cutter machine (Isomet 1000; Buehler Inc., Lake Bluff, IL, USA) (de Carvalho *et al*., 2020). The enamel surfaces of the dental blocks were polished for 30 seconds with #600, #1200, #1500 and #2500-grit silicon carbide sandpapers. After that, the specimens were submitted to ultrasonic bath in distilled water for 5 minutes (Cristofoli, Campo Mourão, PR, Brazil) 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 hours of drying at room temperature. The specimens underwent 4 complete cycles of the above protocol. After staining, specimens were stored for 1 week in distilled water to stabilize the color (Vaz *et al*., 2019).

Specimen's Allocation. The stained specimens were analysed using a Vita Easyshade V spectrophotometer (Wilcos, Petrópolis, RJ, Brazil). The initial value of L* of each specimen was used to stratify and standardize the random distribution of them into three groups (n=10) as homogeneously as possible (Vieira *et al*., 2020).

Experimental Groups. The groups are described below according to the treatment and incorporation or not of bioactive materials (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) performed with 7-day intervals. Each session had three 15-minutes applications. The manipulation of the whitening gel was performed according to the manufacturer in a 3:1 ratio between peroxide and thickener by 10 sec. A layer approximately of 1mm thick was applied over the enamel surface of each sample. After each bleaching session, the bleach was removed with air/water jets for 30 seconds and the teeth were stored in distilled water at 37 ºC.

· F-18: specimens were treated according to the 35 % HP group. However, the volume of the whitening and thickening gel drops (~ 50 μL each) was previously

Table I. Products used in the study.

Product	Abbreviation group	Manufacturer	Composition
Whiteness HP	35 % HP	FGM, Joinville, SC, Brazil	35 % hydrogen peroxide, thickeners, red pigment, glycol and water.
Bioactive glass F-18®	F-18	Vitreous Materials Laboratory - LAMAV, UFSCAr, São Carlos, SP, Brazil	Particles of bioactive bioglass (1–10μm) SiO_2–Na_2O–K_2O–MgO–CaO–P_2O_5

weighed in a precision balance (AR2140, OHAUS Corporation, Parsippany, NJ, USA). After that, F-18 particles were incorporated by means of a plastic spatula into the whitening gel corresponding to 10 % of the gel weight. The homogenization of both materials was carried out with circular movements of the spatula for 10 secs and then it was applied over the specimen surface. After each bleaching session, the whitening gel was removed with air/water jets for 30 seconds and the teeth were stored in distilled water at 37ºC.

Ph Measurement. The pH values of the whitening gel incorporated or not with F-18 bioactive glass were measured immediately after its manipulation. A digital pH meter (Ph-2600, Instrutherm, São Paulo, Brazil) previously calibrated with electrodes of surface (MI-401 Micro reference electrode, Microelectrodes.INC, Belford, New Jersey, USA) was used. All the measurements were performed in triplicate.

Color Evaluation. The specimens were subjected to an initial chromatic analysis using the CIE L* a* b* system, established by Comission Internacionale de l'Eclairaga, using a reflec- tance spectrophotometer (VITA Easyshade V, Wilcos, Petrópolis, RJ, Brazil). The a* and b* axes have right angles and represent the color dimension (a*: green-red ratio; b*: blue-yellow ratio).Third axis (L*) represents the lightness, perpendicular to a* and b* planes. The color change (DE) was evaluated by difference between the coordinates obtained before (baseline), 24 hours and 1 week after the final bleaching session, calculated based on the formula (Paul *et al*., 2002):

$$\Delta E = [(\Delta L^*)^{2+} (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

Microhardness Evaluation. Microhardness was determined using a Vickers diamond tester machine (MMT-3, Buehler Inc., Lake Bluff, IL, USA) settled with 100g during 10 seconds (Klaric *et al*., 2015; 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 data curation. The microhardness was evaluated before (baseline), 24 hours and 1 week after the final bleaching session.

Statistical Analysis. Shapiro-Wilk test (p>0.05) was used to verify the assumptions of normality. Two-way ANOVA for repeated measures and Bonferroni test was used to compare the groups and the evaluation times. 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 means of the whitening gel with and without F-18 bioactive glass were 7.3 and 6.7, respectively.

The color evaluation showed that experimental groups were different than control group for all coordinates (ΔL*, Δa*, Δb*, and ΔE) ($p < 0.05$). However, when the experimental groups were compared within the same evaluation time, no differences were observed ($p > 0.05$) for any coordinate. ΔE values indicated that all the groups did not show changes between the evaluation times ($p > 0.093$), which suggest a color stability over a week (Table II).

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

Groups	ΔL*		Δa*		Δb*		ΔE*	
	24 hours	7 days	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}	13.01 ± 1.86^{Aa}	12.38 ± 2.90^{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}	22.52 ± 4.14^{Ba}	23.66 ± 3.93^{Ba}
F18	8.92 ± 3.10^{Ba}	-6.81 ± 3.53^{Bb}	-6.37 ± 1.76^{Ba}	-6.55 ± 1.52^{Ba}	-18.66 ±2.10^{Ba}	-19.11 ± 1.74^{Ba}	21.79 ± 2.49^{Ba}	21.53 ± 3.81^{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*.

No differences in enamel microhardness were found between the groups within the same evaluation time (p > 0.118), except at 24 hours between 35 % HP and F-18 (p = 0.032). Regarding the intragroup comparisons, no differences were observed for control and F-18 groups (p > 0.129). However, for 35 % HP group, the enamel microhardness decreased after 7 days in comparison with baseline values (p=0.027) (Table III).

Table III. Mean ± standard deviation of Vickers microhardness (VHN) according to each evaluation time (n = 10).

Groups	Baseline	24 hours	7 days
Control	245.76 ± 13.43^{Aa}	245.58 ± 19.92^{ABa}	236.79 ± 10.74^{Aa}
35 % HP	244.62 ± 20.72^{Aa}	225.52 ± 22.45^{Aab}	232.19 ± 18.97^{Ab}
F-18	242.94 ± 20.92^{Aa}	253.35 ± 17.84^{Ba}	240.34 ± 15.26^{Aa}

Two-way repeated measures ANOVA followed by Bonferroni test (p < 0.05). Different uppercase letters mean statistically significant difference within the same evaluation time. Different lowercas Influence of F-18 Bioactive Glass Incorporation into Whitening

DISCUSSION

This study aimed to evaluate the influence of incorporating F-18 bioactive glass into the whitening gel on optical properties and surface microhardness of bovine teeth. The null hypothesis was partially accepted, since experimental groups showed the same whitening efficacy compared to control (p > 0.05) and in the evaluation of microhardness. F-18 showed statistical difference to the 35 % HP group, but not at 1 week. In the intragroup evaluation, the 35 % PH group presented a microhardness decrease after 1 week.

Different methodologies can affect the results when there is the same objective, such as the evaluation of color and microhardness. The difficulty of standardization is linked to several factors including protocol, type of gel, concentration of gels, pH of agents and even storage and tests performed (Borges *et al*., 2015; Lia Mondelli *et al*., 2015).

In order to avoid large discrepancies in the results, this study was carried out with a high concentration HP gel that is still considered the gold standard in the literature (Maran *et al*., 2020). The incorporation of F-18 bioactive glass into the gel is justified by do not require extra clinical step to the whitening protocol (Pintado-Palomino *et al*., 2015) and to validate whether its incorporation can actually reduce the adverse effects promoted by tooth whitening, as seen by Deng *et al*. (2013).

The specimens were standardized according to the initial measurements of the L* coordinate of the CIELAB system performed right after the staining protocol, in order to avoid the risk of variability among the specimens and to better predict the whitening efficacy of the treatments (Vieira *et al*., 2020). Another detail of the methodology is the use of distilled water as the storage solution. Although the use of artificial saliva is common in bleaching studies (Soares *et al*., 2013; Vieira *et al*., 2020), the authors preferred the use of distilled water to allow the remineralization capacity evaluation of the F-18 bioactive glass (Pintado-Palomino *et al*., 2015).

In the present study, we verify that the incorporation of F-18 bioactive glass microparticles into 35 % HP, at the mentioned concentration (10 %), promoted remineralization of the enamel surface enough to maintain the microhardness properties (Table II) without decreasing the 35 % HP whitening efficacy (Table III). We also noticed that the addition of F-18 increased the pH of the gel and it is known that the enamel demineralization may be a result of the acidity of the gel (Xu *et al*., 2011; Sa *et al*., 2012; Soares *et al*., 2016), while the microhardness test performed reflects the action of free radicals generated by HP on the mineral content and organic matrix of dental structure (Deng *et al*., 2013; Borges *et al*., 2014).

It is interesting to note that although 35 % HP had a slightly acidic pH (6.7) and above the critical enamel demineralization pH (5.5) (Frysh *et al*., 1995; Soares *et al*., 2016), the group showed a decrease in microhardness after 1 week, demonstrating that besides the pH, other factors such as composition and concentration of the gel can affect the mechanical properties.

Several approaches have already been tested to reduce the adverse effects of whitening gels on enamel surface (Borges *et al*., 2010; Khoroushi *et al*., 2016; Crastechini *et al*., 2019), including promising results with bioactive materials (Pinheiro *et al*., 2010; Deng *et al*., 2013; Pintado-Palomino *et al*., 2015). According to Kawamoto & Tsujimoto (2004), HP in higher concentrations generates greater amounts of free radicals and, consequently, greater whitening efficacy. Monitoring the evaluation of color and mechanical properties of the enamel over time is interesting, since the action of 35 % HP as well as the ions generated by the F-18 bioactive glass remain active for several days (Ubaldini *et al*., 2020).

F-18 bioactive glass is considered a highly reactive material capable to deposit hydroxycarbonate apatite and to increase the local pH through the release of its ions, conferring them antimicrobial properties (Souza *et al*., 2017). The benefits of F-18 use incorporated with bleaching have not yet been evaluated. However other bioactive glasses such as 45S5 bioglass and Biosilicate showed good remineralization capacity with this type of treatment (Hench, 2006; Ubaldini *et al*., 2020). It is interesting to mention that most bleaching agents used have a slightly acidic pH with the possibility of becoming even more acidic throughout the protocol (Xu *et al*., 2011; Sa *et al*., 2012; Soares *et al*., 2016; de Carvalho *et al*., 2020) and the increase in gel pH is less harmful to the tooth structure.

It is common to find studies that have already evaluated bioactive materials before bleaching, whether *in vitro* (Pinheiro *et al*., 2010; Khoroushi *et al*., 2015; Pintado-Palomino & Tirapelli, 2015; Ubaldini *et al*., 2020) or *in vivo* (Pintado-Palomino *et al*., 2015); however, as an additional step after the gel application. In our study, we preferred to test the incorporation of the material into the gel to favor the exchange of Ca^{2+}, Na^{+} and PO_4^{3-} ions with the tooth structure at the time of the bleaching process (Deng *et al*., 2013).

In this study, the difference between the experimental groups was verified 24 hours after the bleaching sessions, when there is a greater release of ions from the F-18 bioactive glass and free radicals from the bleaching gel. In the 1-week evaluation, the microhardness values were equivalent, which demonstrated a different result from Deng *et al*. (2013) when they also incorporated bioactive glass into the gel, but in an amount greater than used in this study. Thus, we believe that incorporate 10 % of the F-18 in relation to the weight of the bleaching drops is lower than what can actually be incorporated for better mineral deposition properties. Further investigations must be carried out to obtain the ideal amount that can interact with the bleaching chemistry.

The addition of F-18 bioactive glass should make it possible to reduce the adverse effects of bleaching, but without reducing the effectiveness of the treatment. Therefore, color analysis was also performed. The choice of evaluation times of 24 hours and 1 week after the bleaching sessions was similar to what was done in the microhardness tests, allowing a prolonged time for the chemical reactions of the treatment, but also allowing the rehydration and color stabilization of the specimens. Color analysis was performed using a spectrophotometer, a tool that is capable of measuring color coordinates that are imperceptible to the human eye (Pavarina *et al*., 2001; Turgut *et al*., 2018; Paravina *et al*., 2019).

Our results showed that the experimental groups exhibited whitening efficacy (ΔE) visible to human eye without significant differences in relation to control and with the same color stability in 1 week. The other coordinates ΔL, Δa and Δb were also presented in a similar way, with ΔL, the most perceptible coordinate to the human eye (Vieira *et al*., 2020; Fernandes *et al*., 2021), decreasing between the evaluation times due to the rehydration of the specimens (Table III). Satisfactory results with intensifying blue (Δb) and slightly greenish shades (Δa) were observed due to the breakdown of chromophores (Torres *et al*., 2019).

CONCLUSION

Within the limitations of the present study, it can be concluded that F-18 bioactive glass incorporation maintained the integrity of the enamel surface without affect the whitening efficacy. Therefore, the results demonstrate that the amount of bioactive glass incorporated and, consequently, the deposition of minerals in the specimens also had no influence on the action of HP, highlighting the hypothesis that the percentage of material was lower than what could actually be added. Further studies evaluating this interaction are encouraged both *in vitro* and *in vivo* to verify the optimum concentration, to confirm their real benefits, and to enable their clinical application.

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RESUMEN: Los materiales bioactivos han mostrado resultados positivos en la reducción de efectos nocivos del aclaramiento dental. Sin embargo, sus efectos sobre la eficacia del aclaramiento aún son inciertos. Por lo tanto, el objetivo de este estudio fue evaluar los efectos de la incorporación de vidrio bioactivo F-18 en un gel aclarador sobre el color y la microdureza del esmalte después del aclariamiento dental. Treinta especímenes bovinos teñidos con esmalte se dividieron aleatoriamente en tres grupos (n=10) de acuerdo con el protocolo de aclaramiento: grupo control (sin aclaramiento), HP35 % (peróxido de hidrógeno al 35 %) y F-18 (vidrio bioactivo F-18 incorporado en HP35 %). Se realizaron análisis de pH, cambio de color y microdureza al inicio del estudio, 24 horas y 7 días después del aclaramiento dental. La evaluación del color mostró que los grupos experimentales eran diferentes al grupo de control en todas las coordenadas ($p < 0,05$). Sin embargo, no se observaron diferencias intragrupo ($p > 0,05$). No se encontraron diferencias en la microdureza del esmalte entre los grupos dentro del mismo periodo de evaluación ($p > 0,118$). En cuanto a las comparaciones intragrupos, no se observaron diferencias para los grupos control y F-18 ($p > 0,129$). Se concluyó que la incorporación de vidrio bioactivo F-18 no afectó la eficacia de aclaramiento ni la microdureza del esmalte.

PALABRAS CLAVE: biovidrio, esmalte, peróxido de hidrógeno, aclaramiento dental, microdureza.

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