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Can the efficacy of in-office tooth bleaching change in the presence of orthodontic brackets? An *in vitro* study

Clareamento dental in office durante o uso de bráquete ortodôntico pode alterar sua eficácia? Estudo *invitro*

El blanqueamiento dental en la oficina durante el uso de pulsera ortodóntica ¿puede cambiar su efectividad? Estudio *invitro*

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Abstract
*Objective***:** To assess the color change of bovine enamel after tooth bleaching while using orthodontic brackets. *Methodology***:** This *in vitro* study selected 48 bovine incisors without visible fractures or cracks (cut in blocks of 8x12x3 mm). They were distributed in four groups: no staining and with brackets (NSWB), no staining and no brackets (NSNB), with staining and brackets (WSWB), and with staining and no brackets (WSNB) (n=12). The color was initially assessed with a spectrophotometer for NS (no staining). The teeth in WS (with staining) groups were immersed in an instant coffee solution for 15 days and the initial color was also assessed. After prophylaxis and adhesive treatment, the brackets were bonded to the teeth. The teeth were bleached with 35% hydrogen peroxide and the color was analyzed 30 days after bleaching. A two-way Analysis of Variance was applied as well as a subsequent multiple comparison analysis ($p>0.05$). *Results***:** According to the data obtained, the factor of the presence of brackets ($p=0.569$) had no significant influence. This differed from the factor of tooth staining ($p<0.001$). Moreover, there was no interaction between them ($p=0.863$). *Conclusion***:** The presence of brackets in non-stained teeth did not affect bleaching effectiveness. The ΔE of stained teeth was always lower than non-stained teeth, especially for the NB (no brackets) group.
Keywords: Bleaching agents; Tooth bleaching; Orthodontics.

Resumo
*Objetivo***:** Avaliar alteração de cor do esmalte bovino após o clareamento dentário com o uso de bráquetes ortodônticos. *Metodologia***:** Neste estudo *in vitro, 48* incisivos bovinos, sem fraturas ou trincas visíveis (cortados em blocos de 8X12X3mm) foram selecionados. Estes foram distribuídos em quatro grupos: grupo sem manchamento com bráquete (SMCB), grupo sem manchamento sem bráquete (SMSB), grupo com manchamento com bráquete (CMCB) e grupo com manchamento sem bráquete (CMSB) (n=12). A cor foi avaliada inicialmente com espectrofotômetro para SM (sem manchamento), os dentes dos grupos CM (com manchamento) foram imersos em solução de café solúvel por quinze dias e cor inicial também avaliada. Após a profilaxia e tratamento adesivo, os dentes tiveram os bráquetes colados. Os dentes foram submetidos ao clareamento com peróxido de hidrogênio a 35% e a cor foi analisada após 30 dias do clareamento. O teste aplicado foi Análise de Variância a 2 critérios e uma subsequente análise de comparações múltiplas ($p>0,05$). *Resultados***:** De acordo com os dados obtidos, O fator presença de bráquetes ($p=0,569$) não exerceu influência significante. Diferentemente do fator manchamento dentário ($p<0,001$). Ademais, não houve interação entre eles ($p=0,863$). *Conclusão***:** A presença de bráquete em dente não manchado não afetou a efetividade do clareamento. O ΔE, quando houve manchamento dentário, foi sempre inferior que aquele quando não houve manchamento, principalmente para o grupo SB (Sem Bráquete).

Palavras-chave: Clareadores; Clareamento dental; Ortodontia.

Resumen

*Objetivo***:** Evaluar el cambio de color del esmalte bovino tras el blanqueamiento dental mediante brackets de ortodoncia. *Metodología*: En este estudio in vitro se seleccionaron 48 incisivos bovinos, sin fracturas ni fisuras visibles (cortados en bloques de 8X12X3mm). Estos se dividieron en cuatro grupos: grupo sin tinción de brackets (SMCB), grupo sin tinción de brackets (SMSB), grupo con tinción de brackets (CMCB) y grupo con tinción de brackets (CMSB) (n = 12). El color se evaluó inicialmente con un espectrofotómetro para SM (sin tinción), los dientes de los grupos CM (con tinción) se sumergieron en solución de café soluble durante quince días y también se evaluó el color inicial. Después de la profilaxis y el tratamiento adhesivo, los brackets se adhirieron a los dientes. Los dientes se blanquearon con peróxido de hidrógeno al 35% y se analizó el color 30 días después del blanqueamiento. La prueba aplicada fue Análisis de Varianza con 2 criterios y posterior análisis de comparaciones múltiples (p> 0.05). *Resultados***:** Según los datos obtenidos, el factor de presencia de brackets (p = 0,569) no tuvo una influencia significativa. A diferencia del factor de tinción dental (p <0,001). Además, no hubo interacción entre ellos (p = 0,863). *Conclusión***:** La presencia de brackets en dientes no contaminados no afectó la efectividad del blanqueamiento. El ΔE, cuando hubo tinción dentaria, fue siempre menor que cuando no hubo tinción, principalmente para el grupo SB (Sin Bracket).

Palabras clave: Blanqueamiento; Blanqueamiento dental; Ortodoncia.

1. Introduction

Considering extrinsic stains, the constituents of the human diet change tooth color in different ways, often because of the association of salivary proteins with components of these foods, such as polyphenols (Proctor *et al*., 2005). Some beverages rich in polyphenols, such as tea, wine, and coffee, produce color changes in teeth over time, which is affected by the low pH and the color of such foods. Tooth bleaching is one of the treatment options to solve or minimize tooth staining (Brook *et al*., 2007; Hattab *et al*., 1999; Nathoo, 1997; Sulieman, 2005).

Theoretically, the diffusion of hydrogen peroxide in the bleaching gel occurs due to the oxidation caused by the products resulting from its decomposition. Thus, low-weight molecules diffuse through the dental tissues in alkaline conditions, breaking chromophores into molecules of lower complexity that reflect less light (Araújo *et al*., 2010; Consolaro *et al*., 2013; Eimar *et al*., 2012; Goldberg *et al*., 2010; Joiner, 2006; Markowitz, 2010; Thiesen *et al*., 2013), bleaching the teeth (Gomes *et al*., 2017; Jadad *et al*., 2011; Montenegro-Arana *et al*., 2016).

It is mentioned that using brackets may prevent/decrease the diffusion of hydrogen peroxide through the adjacent enamel (Castro *et al*., 2017; Consolaro *et al*., 2013; Lunardi *et al*., 2014). However, authors have shown that teeth can be bleached satisfactorily even in the presence of orthodontic brackets, in comparison to bleaching without brackets, without presenting an aesthetic loss (Gomes *et al*., 2017; Jadad *et al*., 2011; Montenegro-Arana *et al*., 2016). Jadad *et al*. (2011) compared the tooth bleaching treatment with and without brackets using 8% hydrogen peroxide and showed that the product was effective with a similar degree of bleaching in both cases, which suggests the occurrence of diffusion under the brackets (Jadad *et al*., 2011).

Scientific evidence has shown that teeth can be bleached while using orthodontic appliances (Castro et al., 2017; Feitosa et al., 2020; Gomes et al., 2017; Jadad et al., 2011; Lunardi et al., 2014; Montenegro-Arana et al., 2016). Such procedure would allow performing esthetic restorations immediately after removing the brackets, accelerating the esthetic conclusion of the case (Castro *et al*., 2017; Gomes *et al*., 2017; Lunardi *et al*., 2014; Márquez *et al*., 2012; Shibasaki *et al*., 2019). It would also promote better acceptance of the orthodontic treatment among patients who are reluctant to use brackets (Jadad *et al*., 2011) and optimize hygiene and the search for more esthetic procedures due to the lighter tooth surface (Bulut *et al*., 2006; Christensen, 1997).

Considering that the mode of diffusion of bleaching agents in the scientific literature is not fully explained, as there are only theories about the true mechanism of gel propagation through the teeth (Benetti *et al*., 2004; Eimar *et al*., 2012; Kwon

& Wertz, 2015), and that the possibility of finishing an orthodontic treatment with teeth already aligned and bleached may encourage patients to accept the treatment sequence (Gomes *et al*., 2017), new studies have been required to show bleaching efficacy while using orthodontic brackets.

2. Methodology

This work corresponded to an *in vitro* study that assessed color change (ΔE) in bovine enamel caused by artificial staining (I- coffee immersion and II- deionized water immersion) and the subsequent tooth bleaching (TB) in the treatment with orthodontic bracket bonding, performed in a laboratory with 48 bovine incisors selected for the study. The ΔE values were measured as reference/baseline.

In this experimental research, the variable of main response involved the analysis of color changes that occurred under orthodontic brackets after tooth bleaching in enamel when it was still cemented.

Forty-eight bovine incisors were selected. Teeth of approximate size were included and teeth with visible crown fractures or cracks in the direct visual assessment were excluded. All teeth were stored in saline solution at room temperature.

Based on previous studies and using the IBM SPSS Statistics for Windows software (Version 27.0. Armonk, NY: IBM Corp), the mean standard deviation of 2.65 was used to calculate the sample. By using a 5% significance level and 80% test power to detect a minimal difference of 4.40 of color change among the groups, at least 10 specimens per group were required (Bittencourt, 2014; Castro *et al*., 2017; Lunardi *et al*., 2014). It was decided to use 12 specimens per group, preventing potential complications that may exclude some specimens, not reaching the minimum number.

The bovine teeth were cut in dental crown blocks of 8 mm x 12 mm of length with approximately 3 mm of thickness, using a double diamond disc (KG Sorensen, Rio de Janeiro, RJ, Brazil) at low rotation and under constant water cooling. The thicknesses were verified with a digital caliper (Digimess, São Paulo, SP, Brazil). The enamel surface was flattened with carborundum discs (#320, 600, and 1200 Al_2O_3 discs; Buehler, Lake Bluff, IL, USA), which resulted in the removal of approximately 100 µm of depth. Polishing was performed with a silicon carbide polishing tip (American Burrs, Palhoça, SC, Brazil), a felt disc, and diamond paste, at low rotation for 20 seconds.

The specimens were distributed equally in four groups with a simple allocation through a random draw of numbers 1 through 48, using envelopes according to the treatment (NS- no staining, WS- with staining, WB- with bracket, NB- no bracket).

The teeth in the artificial staining group were immersed in a coffee solution prepared with 25 g of instant coffee (Nescafé Tradição, Nestlé Brasil Ltda., Araras, SP, Brazil) and 100 mL of distilled water at room temperature (Torres *et al*., 2013) for 15 days. The solution was changed every 24 hours and the specimens were washed at each exchange.

Fifteen days after tooth staining in the WS group, prophylaxis was performed with pumice and Robinson brush in the dental blocks, as well as cleaning with water spray.

For bracket bonding, a 37% phosphoric acid (Condac - FGM) etching was performed in the enamel for 30 seconds at the site of accessory bonding, abundantly washed for 60 seconds, and dried by air blast. The Transbond™ XT adhesive (3M Unitek, Landsberg, Germany) was applied and light-cured for 20 seconds with the Radii-cal device (SDI, Victoria, Australia). Next, stainless steel orthodontic metal brackets, Roth prescription (Morelli, Sorocaba, SP, Brazil) were bonded. They were assigned to upper right central incisors, slot 0.22, with Transbond™ XT resin (3M Unitek, Landsberg, Germany). The resin excess was removed with an orthodontic spatula and the set was polymerized for 20 seconds. The specimens were stored in 0.5 mL of deionized water, in a lidded plastic box, at room temperature, for 24 hours.

A single evaluator read the specimens with an X-Rite Color 962 spectrophotometer (Danaher Corporation, Michigan, USA) of color reflectance. The measurement was performed and repeated three times and a mean of the values was calculated.

The measures were taken at three different times: 1- Before the procedures; 2- After staining; and 3- Thirty days after bleaching. The spectrophotometer was calibrated according to the manufacturer's specifications, using SAV (small area variation) of 3 mm.

Each specimen was marked with a black nail polisher (Colorama, Rio de Janeiro, Brazil) on the posterior surface to standardize the specimen number during the color assessment.

For the readings after bleaching, the brackets were removed with bracket remover orthodontic pliers #346R (ICE, São Paulo, SP, Brazil). The remaining resin was removed with a multilaminated low-rotation zirconia drill (Morelli, Sorocaba, SP, Brazil) and polishing was performed with a silicon carbide polishing tip (American Burrs, Palhoça, SC, Brazil). Next, the teeth were stored in deionized water to rehydrate the enamel for 24 hours as aforementioned.

In the bracket location (research interest area), a matrix with a 3-mm hole was placed to reduce the size of the reading point of the spectrophotometer.

For color assessment, the model proposed by the *Commission Internationale de l'Eclairage* (CIE) L* a* b* was used. Measuring the color differences [ΔE] obtained after bleaching the dental blocks required using formulas recommended by the CIE method: $\Delta E = \sqrt{[\Delta L]^2 + [\Delta a]^2 + [\Delta b]^2}$, where ΔL = L1[final] - L0 [initial], Δa = a1 [final] - a0 [initial], Δb = b1 [final] - b0 [initial]. This system presents L* as the luminosity variable and a* and b* as the chromaticity coordinates, with ranges of a* between green (-) and red (+) and b* between blue (-) and yellow (+).

One day after staining and 24 hours after bracket bonding on the buccal surface, the specimens were subjected to the first high-concentration tooth bleaching session with 35% hydrogen peroxide Total Blanc Office (DFL, Rio de Janeiro, RJ, Brazil), following the manufacturer's instructions: applying three times for 15 minutes/session, three sessions (once/week). After bleaching, the specimens were washed to fully remove the bleaching agent and stored in 0.5 ml of deionized water, in a lidded plastic box, at room temperature, for 30 days. The water was changed daily.

Color analyses were performed 30 days after bleaching as described previously.

Regarding the response variable (continuous quantitative), two-way Analysis of Variance and subsequent multiple comparison analysis was used at a 5% significance level. The SigmaPlot 13 statistical software (Systat Software, Inc., San Jose, CA, USA) was used.

3. Results

The analysis of the data obtained shows that, differing from the presence of brackets (p=0.569), the factor of tooth staining significantly affected the results (p<0.001). Moreover, there was no interaction between them (p=0.863).

Table 1 describes the mean ΔE values (CIELab, 30 days after treatment minus initial condition) of the enamel with brackets (WB) or no brackets (NB), and with staining (WS) or no staining (NS).

Table 1 - Mean±standard deviation of ΔE values (CIELab, 30 days after treatment minus initial condition) of the enamel with brackets (WB) or no brackets (NB), and with staining (WS) or no staining (NS).*

	NB	**WB**
NS	11.00 ± 2.91^{Aa}	11.29 ± 3.36^{Aa}
WS	8.18 ± 0.97^{Ba}	8.70 ± 0.93^{ABa}

*Different upper-case letters indicate a statistically significant difference (p<0.05) in each column for the factor of tooth staining (NS, WS). Different lower-case letters indicate a statistically significant difference (p<0.05) for the factor of the presence of brackets (NB, WB). Source: Authors.

The results obtained (Table 1) confirm the color change in bovine teeth with and without brackets subjected to in-office bleaching after 30 days. The null hypothesis that the use of orthodontic brackets and staining did not affect the color change and stability of bovine teeth after 30 days should therefore be rejected.

4. Discussion

Considering teeth with and without brackets and not stained, tooth bleaching was effective. Thus, there is evidence that hydrogen peroxide penetrated the enamel and permeated the dentin, bleaching the structures. The hydrogen peroxide molecule presents a very low molecular weight (34 mg Mol) (Kugel *et al*., 2007) and perhaps this is why it is one of the chemical molecules with the highest penetrating power in the tooth structure. When applied close to orthodontic brackets, it penetrates the subsurface, thus bleaching the tooth structure (Consolaro *et al*., 2013). Theoretically, its penetration produces free radicals that are conducted multi/polydirectionally, thus working under resin restorations and orthodontic brackets (Jadad *et al*., 2011). Conversely, the scientific literature reports that areas covered by gingiva or in contact with another tooth will not receive the bleaching agent as equally as uncovered areas, producing a high risk of irregularities in color and staining (Consolaro *et al*., 2013). It is also mentioned that, after removing the orthodontic bracket, the enamel presents irregular surface and color (Consolaro *et al*., 2013; Lunardi *et al*., 2014), and it is not known whether hydrogen peroxide works uniformly through the adamantine fluid (Ayres *et al*., 2016; Consolaro *et al*., 2013).

Nonetheless, the present study used a high-concentration gel (Araújo et al., 2010; Ayres et al., 2016; Feitosa et al., 2020; Gomes et al., 2017; Horning et al., 2013; Lima et al., 2020; Lunardi et al., 2014), which increases the penetration power of the product in the tooth enamel (Abouassi *et al*., 2011; Benetti *et al*., 2004; Bistey *et al*., 2007) despite reports of low-concentration formulations (Jadad *et al*., 2011; Montenegro-Arana *et al*., 2016) that were also effective.

Even with the use of a 35% hydrogen peroxide bleaching gel, when comparing the group with and without staining, the group with staining presented lower ΔE values than the group without staining, particularly the WSNB group. A probable justification for the non-stained group to have presented a greater color difference can be the insufficient gel application. Another hypothesis is that coffee staining would have made it difficult for the teeth to obtain bleaching as effectively as those immersed only in water. Considering that all diets are somehow pigmented but not homogeneously, the degree of staining would be a factor worth considering when deciding on bleaching with brackets in more pigmented teeth. Moreover, the variation of the amount of enamel and dentin between the specimens may have contributed, considering that factors such as the thickness of dentin, enamel, and cement affect the penetration of the hydrogen peroxide molecule. The higher the amount of tooth structure, the lower the diffusion of the bleaching agent (Palo *et al*., 2012).

Coffee staining was performed as a way to compare the groups with more and less visible extrinsic stains, aiming to simulate the accumulation of food pigments adhered for the stained group. Hence, the specimens were pigmented with coffee, similar to Araújo (2010) and Torres *et al* (2013), for 15 days. Another product that is extensively used for the same purpose is black tea (Castro *et al*., 2017; Joiner *et al*., 2003; Lunardi *et al*., 2014).

To assess this color change, a spectrophotometer was used (Araújo et al., 2010; Castro et al., 2017; Eimar et al., 2012; Feitosa et al., 2020; Jadad et al., 2011; Lunardi et al., 2014; Montenegro-Arana et al., 2016) because it provides quantitative color information, which allows a more reliable comparison. Moreover, the CIELab protocol was used to assess the color, aiming to standardize the values found (Caneppele et al., 2013; Castro et al., 2017; Feitosa et al., 2020; Lunardi et al., 2014). Using the VITA scale with the equivalence of 16 colors in the guide with a numerical scale (Jadad *et al*., 2011; Montenegro-Arana *et al*., 2016) could produce a subjective assessment.

Also for the use of the spectrophotometer, reducing the reading area (SAV) to 3 mm allowed a satisfactory visualization of color difference in the area under the brackets, which is an important decrease to show color heterogeneity, considering that a larger opening does not allow viewing this variation between both areas (Lunardi *et al*., 2014).

Enamel and dentin dehydration interferes with tooth color due to the change in the air/water refraction index. During bleaching, besides the relative isolation, the bleaching gel may affect tooth hydration, providing less water (Matis *et al*., 1998; Spalding *et al*., 2003). In the present study, to prevent dehydration and color change, the teeth were stored in distilled water (Castro *et al*., 2017), but another common type of storage could be artificial saliva (Lunardi *et al*., 2014; Pinheiro *et al*., 2011).

Some authors discuss, separately, the difference of L, a, and b values (Bengel, 2003; Caneppele *et al*., 2013; Dietschi *et al*., 2006). For Dietschi, Rossier, and Krejci (2006), a and b values, relative to saturation, would be less important, and the L value would be the most significant. The present study considers essential the total ΔE value, which are rapidly perceptible values visible to the naked eye.

Considering the divergences of results and the small number of studies correlating tooth bleaching to the use of orthodontic brackets, further research is required to compare the effectiveness of high-concentration bleaching products during the orthodontic treatment and without using orthodontic appliances.

5. Conclusion

Based on the methodology used and the results obtained, it is concluded that tooth bleaching is effective even in the presence of orthodontic brackets. However, the degree of tooth staining should be assessed previously, considering it may be required to increase the number of hydrogen peroxide applications to obtain a satisfactory result.

Thus, performing randomized clinical trials is suggested to provide data that are increasingly safer for the dental clinic.

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