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An In Situ Study of the Influence of Staining Beverages on Color Alteration of Bleached Teeth

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Clinical Relevance

Although overall tooth color change was not affected at the end of at-home bleach treatment, consumption of staining beverages should be avoided since important color dimensions are altered.

SUMMARY

The aim of this study was to evaluate overall color change in bovine tooth fragments submitted to dental bleaching treatment performed simultaneously with the ingestion of beverages containing dyes. For this purpose, tooth fragments assembled into intraoral devices were submitted to at-home dental bleaching using 10% carbamide peroxide (CP) for 14 days and to immersion in staining beverages for 10 minutes daily. The specimens were divided into the following study groups according to bleaching treatment and staining substance (n=12): G I (negative control): no bleaching + distilled water; G II (positive control): bleaching + distilled water; G III: bleaching + coffee; and G IV: bleaching + grape juice. Twelve volunteers used the device continually, except during meals, oral hygiene, dental bleaching, and pigment challenge. Color readings were performed using a spectrophotometer both before the bleaching treatment and after each treatment week. The results were submitted to the normality test.

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The data obtained were submitted to analysis of variance and the Tukey or Kruskal-Wallis and Dunn tests (α=0.05). All bleached groups showed similar ΔE results at the end of treatment. Staining beverages generated negative ΔL mean values, and the lowest result was obtained in the treatment with coffee after 14 days. The Δa values in the groups that received treatment with staining beverages were higher when compared to the control groups. Dental bleaching associated with the consumption of staining substances may not affect overall tooth color change by the end of the treatment, although the consumption of staining substances did influence the different color dimensions.

INTRODUCTION

Several factors can alter the esthetics of a smile, including changes in the shape, texture, position, and color of teeth. The color and the appearance of teeth involve complex phenomena that are influenced by the type of ambient light, light scattering, translucency, opacity, and brightness of the substrate.[1,2]

Many esthetic treatments have been proposed seeking to improve dental esthetics; however, dental bleaching has gained great popularity by presenting itself as a conservative and effective technique.[1-3] The at-home bleaching technique is the most frequently used treatment for vital teeth due to its efficacy and biosafety and is considered the gold standard treatment among the different bleaching therapies.[4] It is known, however, that dental bleaching products change, although usually temporarily, the microhardness, surface roughness, and enamel surface morphology, increasing tooth porosity.[5-8] Thus, many professionals and manufacturers recommend that patients avoid eating pigment-rich foods during bleaching in order to avoid compromising the esthetic results.[9-11]

Despite the occurrence of these alterations in the enamel, studies have not been conclusive about the increased risk of pigmentation during bleaching,[12] especially considering that most of these investigations have not taken into account the challenging conditions of the oral environment.

In situ studies represent an intermediate step between laboratory experiments and clinical trials. *In situ* studies can more accurately examine the biological influences and protective effects of saliva under experimental conditions.[6,13] Therefore, intraoral models provide a clinical reality approach while preserving the sensitivity of laboratory models since the analysis can be carried out outside the oral cavity, using sensitive and accurate methods.[10]

Furthermore, few studies regarding the evaluation of the effects of bleaching have considered the three color dimensions separately, the value (ΔL), the amount of red and green (Δa), and the amount of yellow and blue (Δb).[3,11,14] These color parameters are related to human eye color perception and participate in the calculation of the overall change in tooth color (ΔE), representing an important factor in obtaining the final result.[1,2]

Since limited information regarding overall change in tooth color using the *in situ* study design is available and these effects have not been thoroughly investigated, the aim of this *in situ* study was to evaluate the different aspects of overall color change in bovine teeth exposed to staining beverages after undergoing dental bleaching using 10% carbamide peroxide. The tested null hypotheses were 1) that staining beverages do not interfere with the ΔE at the end of the bleaching treatment and 2) that staining beverages do not interfere in the three color dimensions, the value (ΔL), the amount of red and green (Δa), and the amount of yellow and blue (Δb) of the bleached specimens at the end of treatment.

METHODS AND MATERIALS

Volunteer Selection

This crossover, randomized, and double-blind *in situ* study was carried out in accordance with the ethical standards of the committee responsible for human experimentation and with the Helsinki Declaration and was approved by the institutional Research and Ethics Committee (03731512.0.0000.5420). Twelve volunteers were selected after anamnesis and clinical exams. The exclusion criteria were use of removable prosthesis, smokers, expectant mothers, mothers who were breast-feeding, use of drugs that affect salivary flow (antidepressants, narcotics, and diuretics), presence of decayed teeth, periodontal disease, and digestive disorders. Following selection, the volunteers received oral and written information regarding the study and, after agreeing to participate, signed an informed consent statement. Subsequently, they received oral hygiene instructions, a list of guidelines, and a case for storing their devices during meals.

The subjects were also instructed to wear a removable palatine intraoral device for 14 consecu-

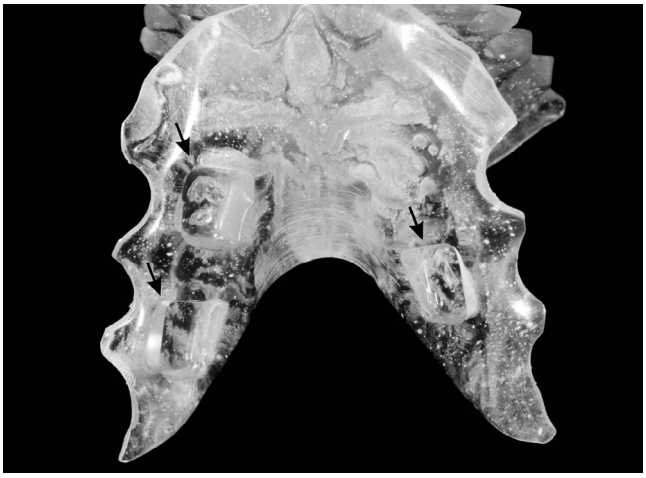

Figure 1. *Intraoral device containing three niches to assemble the experimental samples.*

tive days. Three bovine tooth samples were embedded into each experimental device, one for each study group (n=12). The bleaching treatments and the pigment challenges were performed daily and outside of the oral cavity.

Specimen Preparation

Thirty-six permanent bovine teeth from 24- and 30-month-old steers were selected. The experimental units (enamel/dentin discs) were obtained from bovine incisors. The 4.7-mm-diameter discs were obtained from the middle third of the buccal surface of the teeth. Those that presented with cracks, enamel stains, morphological crown alterations, and/or excessive wear on the incisal edge were excluded. The selected teeth were mechanically cleaned using a scalpel blade, followed by prophylaxis with pumice and water. Afterward, the dentin surface was wet-ground using fine (#400) and extra fine (#600) aluminum oxide sandpaper (T469-SF-Noton, Saint-Gobam Abrasivos Ltd, São Paulo, Brazil), until 1.0 mm (±0.2 mm) of enamel and 1.0 mm of dentin remained. Thereafter, the dentin tissue was impermeabilized with the application of two coats of clear nail polish (Risqué, NIASI, São Paulo, Brazil) so that only the enamel maintained contact with the staining substances.

Intraoral Device

After volunteer selection, impressions were made and working models fabricated. The intraoral palatal devices were made using acrylic resin (Jet, Artigos Odontológicos Clássico Ltd, São Paulo, Brazil) containing three niches of $5 \times 5 \times 4$ mm to hold the experimental samples (Figure 1). The specimens were sterilized in ethylene oxide and randomly placed in the intraoral devices using sticky wax (Kota Ind. e Com. Ltd, São Paulo, Brazil) and positioned 2 mm below the surface of the resin. They were carefully handled to prevent lateral cracks between the block and the wax. A polyethylene screen was fixed in the acrylic covering the specimens to keep the bleaching agent on the enamel samples during treatment.

Experimental Groups

The study groups were divided according to the bleaching treatment and the staining procedure. Before carrying out the experimental procedures, the pH of each product was measured with a previously calibrated digital pH meter (Crison Instruments SA, Barcelona, Spain).

In G I (negative control), no bleaching agent was used, and the samples were immersed in 1 mL of distilled water. In G II (positive control), the volunteers were instructed to deposit 0.04 mL of the 10% carbamide peroxide bleaching product Whiteness Perfect (FGM Produtos Odontológicos Ltd, Santa Catarina, Brazil) on specimens placed in the intraoral device, with the bleaching product remaining in contact with the enamel for 4 hours for each of the 14 days. Bleaching treatment was performed inside the oral cavity, and, following the product application, the specimens were washed in running water. After 1 hour, the samples were immersed in 1 mL of distilled water. In G III (coffee) and IV (grape juice), bleaching treatment using the 10% carbamide peroxide was performed as described for G II. After 1 hour, the volunteers took the device to the researcher for samples to be exposed to the staining beverages. Specimens exposed to coffee (G III) were immersed in 1 mL of coffee infusion at room temperature. The infusion was obtained using 8 g of Nescafe (Nestle SA, Vevay, Switzerland; pH 5.21) dissolved in 50 mL of purified water. Specimens exposed to grape juice (G IV) were immersed in 1 mL of industrialized grape juice at room temperature (Del Valle Mais, Coca-Cola Company, Rio de Janeiro, Brazil; pH 2.59).

For pigment treatments, the samples were carefully removed from the intraoral device and exposed to the appropriate staining beverages for 10 minutes daily. After exposure to these substances, the samples received prophylaxis, were rewashed and repositioned in the intraoral device (Figure 1).

Table 1: *Mean Values (SD) of ΔE in the Different Experimental Conditions and Evaluation Times*[a]

Evaluation Times	G I (Negative Control)	G II (Positive Control)	G III (Coffee)	G IV (Grape Juice)
T0	0.00 (0.0) Ba	0.00 (0.0) Ba	0.00 (0.0) Ba	0.00 (0.0) Ba
T1	1.26 (0.7) Ac	4.26 (1.6) Aa	3.41 (1.8) Aab	2.23 (1.3) Abc
T2	1.96 (0.9) Ab	5.53 (2.5) Aa	4.93 (2.4) Aa	4.22 (2.2) Aab

[a] *Means followed by different letters (uppercase in vertical, lowercase in horizontal) represent significant difference according to statistical analysis (p<0.05). One-way analysis of variance and Tukey test were used when comparing evaluation times in positive control and when comparing groups within T2. Kruskal-Wallis and Dunn tests were used for other comparisons.*

Spectrophotometry Analysis

Before each measurement, the specimens were submitted to prophylaxis with a Robinson-type brush to remove excess dye and other impurities that could interfere with the color measurement. Three color measurements were performed at each analysis, using an ultraviolet-visible reflection spectrophotometer (model UV-2450, Shimadzu Corporation, Kyoto, Japan). Arithmetic means were used to perform the statistical analysis.

The spectrophotometer used the CIE L*a*b* color model, established by the Commission Internacionale de l'Eclairage (CIE; International Commission on Illumination), which allows for the specification of color perceptions in three-dimensional models. Readings were taken on the buccal surface of the specimens. The axial "L" indicates the color value and extends from 0 (black) to 100 (perfect white). The coordinate "a" represents the amount of red (positive values) and green (negative values), while the coordinate "b" is the amount of yellow (positive value) and blue (negative values). The system CIE L*a*b* calculates the ΔE between two points using the formula $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$. Readings were performed before the start of the bleaching treatment and after the pigment application on the seventh day (T1) and the 14th day (T2) of 10% CP application. All baseline parameters had means and standard deviations as follows: L = 67.90 (1.99); a = −1.95 (0.16), and b = 3.56 (0.42).

Statistical Analysis

The results were submitted to the normality test (Shapiro-Wilk). Analysis of variance and Tukey tests were used for ΔE positive control, ΔL negative control, and grape juice when comparing evaluation times within the same group. Similar analyses were performed for Δb in T1 and for ΔE, ΔL, and Δb in T2 when comparing groups within the same evaluation time. Other comparisons did not pass the normality test; therefore, the Kruskal-Wallis and Dunn tests were used. All analyses were performed at a 5% significance level using SigmaPlot 12.0 statistics software.

RESULTS

The statistical analysis showed that the study groups presented considerable differences in ΔE after the first week (T1). All groups presented a continuing increase in the mean value of ΔE but without differences between T1 and T2. The comparison performed between groups showed that, at the seventh day of treatment (T1), the group exposed to grape juice and the negative control group (G IV and G I) had lower ΔE when compared to the positive control group exposed to distilled water and the group exposed to coffee (G II and G III). However, all bleached groups (G II, G III, and G IV) showed similar results at the end of treatment (T2). In general, the positive control had the highest mean ΔE values, unlike the negative control, which presented the lowest mean values until the end of the treatment (Table 1).

When analyzing the ΔL values, Table 2 shows that the bleached groups had similar results to the group that did not receive the bleaching treatment (G I) after the seventh day of treatment (T1). On the other hand, the positive control group (G II) showed the

Table 2: *Mean Values (SD) of ΔL in the Different Experimental Conditions and Evaluation Times*[a]

Evaluation Times	G I (Negative Control)	G II (Positive Control)	G III (Coffee)	G IV (Grape Juice)
T0	0.00 (0.0) ABa	0.00 (0.0) Ba	0.00 (0.0) Aa	0.00 (0.0) Aa
T1	0.51 (0.7) Aab	4.73 (4.1) Aa	−1.90 (2.8) Bb	0.19 (2.1) ABb
T2	−0.80 (1.2) Bb	4.59 (3.6) Aa	−3.13 (3.5) Bb	−2.51 (3.1) Bb

[a] *Means followed by different letters (uppercase in vertical, lowercase in horizontal) represent significant difference according to statistical analysis (p<0.05). One-way analysis of variance and Tukey test were used when comparing evaluation times in negative control and grape juice, and when comparing groups within T2. Kruskal-Wallis and Dunn tests were used for other comparisons.*

Table 3: *Mean Values (SD) of Δa in the Different Experimental Conditions and Evaluation Times*[a]

Evaluation Times	G I (Negative Control)	G II (Positive Control)	G III (Coffee)	G IV (Grape Juice)
T0	0.00 (0.0) Ba	0.00 (0.0) Ba	0.00 (0.0) Ba	0.00 (0.0) Ba
T1	0.35 (0.2) Ac	0.13 (0.5) ABc	1.35 (0.3) Aab	0.96 (0.2) Ab
T2	0.58 (0.2) Abc	0.42 (0.3) Ac	1.93 (0.3) Aa	1.26 (0.5) Aab

[a] *Means followed by different letters (uppercase in vertical, lowercase in horizontal) represent significant difference according to statistical analysis ($p<0.05$). Kruskal-Wallis and Dunn tests were used for all comparisons.*

highest luminescence values (ΔL) at T2. It was also noted that the pigment treatments generated negative ΔL mean values and that the lowest value obtained in the treatment was with coffee at T2.

Table 3 shows that the negative and positive control groups had statistically similar Δa values throughout the study. All groups presented a continuing increase in mean value of Δa but without differences between T1 and T2. It was also verified that the Δa values in the groups receiving treatment with staining beverages (G III and G IV) were higher when compared to the control groups (G I and G II).

Table 4 indicates that there were no statistically significant differences in Δb between the evaluation times for all groups.

DISCUSSION

In the present study, bovine teeth were used, as they are easy to obtain and standardize. Bovine teeth have been commonly used in laboratory research since they have low variations of experimental responses due to their uniform composition and are similar to human teeth with respect to morphology and histology.[15]

The recommendation to avoid foods rich in dyes when undergoing bleaching procedures is routine among dental professionals and manufacturers, even though the evidence of the effect of such foods is based on *in vitro* studies.[9,10] In the present study, the *in situ* experimental design was used to account for both the effect of bleaching products and of staining beverages and the action of saliva and thermal/chemical variations in the oral cavity. As such, it was possible to reproduce similar conditions to those found in patients that perform the bleaching treatment, and the current results most likely approximate the potential results that would be observed in a clinical setting.[13]

The overall tooth color change results showed that the exposure of dental fragments to grape juice during at-home dental bleaching resulted in a ΔE that was different from the group that received only the bleaching treatment after seven days. However, all of the bleached study groups presented similar color alterations at the final stage of treatment, accepting the first hypothesis of the study. Similar results were recently reported by Matis and others,[11] who noted that, based on data obtained in previous clinical trials, dark beverages do not negatively influence the overall tooth color change final values. Despite this result, it is been suggested that bleached teeth may be susceptible to staining after whitening treatment.[10] It is also worth mentioning that some studies have indicated that the acidic pH of pigment solutions promotes a loss of mineral in tooth structure,[16,17] favoring the penetration of these solutions into the demineralized structure, thus influencing the overall tooth color change during the bleaching treatment.

Although clinical trials show that at-home dental bleaching requires 15 to 21 days to achieve the best overall tooth color change, ΔE values obtained in G II from T2 were the same as those observed in studies that performed longer treatments.[18-20] It is possible that the present results were obtained faster (in seven days) because of the dimensions of the experimental samples, which were tapered (preserving 1.5 mm of dentin) so that they could be placed into intraoral devices, causing minimal discomfort for the volunteers. It is important to note

Table 4: *Mean Values (SD) of Δb in the Different Experimental Conditions and Evaluation Times*[a]

Evaluation Times	G I (Negative Control)	G II (Positive Control)	G III (Coffee)	G IV (Grape Juice)
T0	0.00 (0.0) Aa	0.00 (0.0) Aa	0.00 (0.0) Aa	0.00 (0.0) Aa
T1	0.39 (1.1) Aa	−0.68 (1.5) Aa	0.22 (1.3) Aa	−0.63 (1.1) Aa
T2	−0.03 (1.6) Aa	−1.18 (1.4) Aa	0.49 (2.2) Aa	−0.63 (2.4) Aa

[a] *Means followed by different letters (uppercase in vertical, lowercase in horizontal) represent significant difference according to statistical analysis ($p<0.05$). One-way analysis of variance and Tukey test were used when comparing groups within T1 and T2. Kruskal-Wallis and Dunn tests were used for other comparisons.*

that in clinical studies, the dentin, which supposedly houses most of the chromophore molecules, presents considerably greater thickness, making overall tooth color changes easier to observe even after 15 days of treatment.[18,19,21,22]

With the aim for an accurate analysis of the overall tooth color changes promoted by the bleaching treatment and the staining substances, three color dimensions were also analyzed—ΔL, Δa, and Δb—since dental bleaching outcome is characterized mainly by alterations in these values.[23,24] G II was found to have a higher value (ΔL) when compared to G III and G IV at all evaluation times. These results corroborate the findings of Attin and others,[3] who demonstrated that bleached specimens simultaneously pigmented with black tea had a darker appearance than the specimens that were only bleached. These data suggest that the use of staining beverages during dental bleaching may darken or delay the desired esthetic result.

A previous report affirms that beverages/foods that are not considered part of a white diet do not negatively affect the bleaching process.[11] However, the present research used the exact bleaching agent methodology and staining substances from the above-mentioned study, simulating a more intense consumption of these beverages.

The Δa analysis showed that specimens treated with coffee and grape juice presented a more reddish color than those from G I and G II due to the dye used in these substances, indicating that the bleaching treatment was not fully effective in breaking down the pigment molecules from these beverages.

On the other hand, negative values were obtained for G I, G II, and G IV for Δb, indicating that they became less yellow, indicating that the bleaching agent was able to bind with the pigments and break their complex chains, making the samples more blue.[19,25]

In general, the CIE L*a*b* analyses indicated that beverages with a high potential for pigmentation did in fact influence the color of the experimental samples submitted to bleaching, allowing us to reject the second null hypothesis.

All of these observations related to the assessment of color indicate that the isolated study of ΔE is not sufficient when analyzing the effectiveness of bleaching therapies in cases where substances with antagonistic effects (bleaching substances and pigments) are used; the study of ΔE should be complemented by analyses of Δa, Δb, and ΔL. The analyses of these data show that the staining substances may affect the outcome of the bleaching treatment.

The present study may contribute to the understanding of the mechanisms of pigmentation during bleaching procedures, although further studies considering some important factors that influence the dental esthetics, such as fluorescence, glow, and other optical properties, are needed to effectively analyze these effects.

CONCLUSION

Under the experimental conditions of this clinical study, it can be concluded that dental bleaching associated with the consumption of staining substances may not affect overall tooth color change by the end of the treatment, although the consumption of staining substances did influence the different color dimensions.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Institutional Research and Ethics Committee. The approval code for this study is 03731512.0.0000.5420.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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