ORIGINAL RESEARCH

Staining Power of Natural and Artificial Dyes after At-home Dental Bleaching

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Abstract

Introduction: Bleached enamel surfaces may undergo changes and retain more dye, which is a reason to recommend the reduction/suspension of foods with dyes during dental bleaching.

Aim: Evaluate the effects of the action of natural and artificial dyes on the bleached enamel of extracted human teeth.

Materials and methods: Fifty human premolars were used, which were distributed in 5 groups (n = 10) according to the following staining solutions: GW (distilled water); GB (beet); Gca (caramel); GC (carmine); and GR (red 40). After the removal of the root and pulp section, the teeth were embedded in acrylic resin blocks, stored in artificial saliva, and kept at 37ºC. At-home bleaching was performed using 16% carbamide peroxide (CP) for 3 hours daily for 3 weeks. After each daily session of bleaching, the specimens were exposed to the dye solution twice a day for 5 min; one of these exposures was performed immediately after bleaching. The color was recorded using a spectrophotometer according to the CIE Lab system (ΔE) for the following periods: baseline, during bleaching (after 1st, 2nd, and 3rd week) and post-bleaching (after 1 week and 1 month). The color was evaluated by two-way analysis of variance and Tukey's test (α=0.05).

Results: There was effective dental bleaching for all groups: GW (18.5 ± 6.1), GB (19.9 ± 4.4), Gca (18.9 ± 6.1), GC (20.2 ± 4.6), and GR (19.3 ± 4.2), $p < 0.01$. No color rebound was observed after 1 week and 1 month ($p > 0.05$).

Conclusion: The exposure to beet, carmine, caramel, and red 40 dyes did not interfere with the effectiveness of dental bleaching using 16% CP.

Clinical significance: Dyes consumption during bleaching did not affect the effectiveness of dental bleaching.

Keywords: Coloring agents, Hydrogen peroxide, Laboratory research, Tooth bleaching.

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Introduction

The search for a pleasant smile has motivated many patients with discolored teeth to perform dental bleaching. This is because the color of teeth is considered to be one of the most important factors in relation to esthetics. Many people believe that lighter-colored teeth reflect health and youthfulness, and lighter teeth can also have a positive effect on patients' self-esteem.[1]

Color changes to teeth can be caused by many factors of intrinsic and extrinsic origin.[2,3] Changes that are of intrinsic origin can occur due to dental trauma, pulp necrosis, drug use, fluorosis, and the natural aging process. The most important extrinsic factors are smoking, the use of medication, mouthwashes containing chlorhexidine, the accumulation of plaque, and the intake of beverages and foods containing dyes such as coffee, tomato sauce, carrots, beets, jellies, soft drinks, red wine, and black tea.[4,5]

Dental bleaching is an excellent option for the treatment of discolored teeth due to the fact that it is effective and that it preserves the tooth structure.[6] Nevertheless, adverse effects can occur because the use of bleaching agents causes changes in the enamel surface, leaving it rougher, porous, and permeable.[7-9] Therefore, it is common to suspend the intake of foods containing dyes during dental bleaching because changes in the bleached tooth surface could result in better retention of dyes, thereby reducing the bleaching effect.[10,11] However, the length of time for dental bleaching may take 2–4 weeks,[6] depending on the gel concentration and technique[12], which makes it difficult to restrict food/drinks containing dyes over a long period because food dyes are present in most foods consumed by many people daily.[13,14]

Some authors claim that these dyes do not interfere with the effectiveness of dental bleaching,[5,15-17] while other studies have found that bleached tooth enamel is more susceptible to staining by absorbing greater amounts of pigments,[18,19] as well as showing less stable results over time.[20] It is known that dental staining is not only related to the pH of substances but also to different kinds of dyes present in foods, as well as exposure time.[19,21-23]

The food industry adds different types of natural or artificial dyes to foods to make them more attractive and to increase consumption, although they have no nutritive value. Natural plant, animal, and mineral substances have been gradually replaced by synthetic dyes because they have more powerful tinting capacity, are more uniform, and are more stable.[13,14] However, some artificial dyes can be harmful to health, causing adverse effects like allergies, itching, and headaches, so the use of natural dyes is now encouraged.[13,14] The natural dyes carmine, beet, and caramel are among those most widely used because of their fixing and

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tinting strength. Besides its tinting strength, the artificial dye red 40 is widely used because of its low absorption by the body and because it is allowed for commercial use.[24]

In reviewing the literature it was observed that several studies have tested various food and beverage containing dyes.[15-19,21,23] However, no studies were found that evaluated the effect of exposure to different natural and artificial dyes on bleached enamel. Consequently, the aim of this in vitro study was to evaluate the effectiveness of the dental bleaching of teeth exposed to natural (carmine, beet, and caramel) and artificial (red 40) dyes during bleaching treatment. In the present study, we tested the hypothesis that the use of natural and artificial dyes during at-home dental bleaching does not interfere with the effectiveness of tooth whitening.

Materials and Methods

After approval by the ethics committee of the local university (124/2011), 50 extracted human premolars were selected from the human teeth bank of the same university, which met the following inclusion criteria: healthy teeth, without stains, cracks and fractures in the enamel, and an initial color of A2, which was assessed using a Vita Easyshade spectrophotometer (Vita Zahnfabrik, BadSäckingen, Germany). All the specimens were manipulated using protective equipment (goggles, gloves, mask, cap, and apron).

The teeth were submitted to manual scraping with periodontal curettes (Gracey 11/12) and scraping with an ultrasonic tip, as well as dental prophylaxis to remove organic debris. The roots were cut 5 mm below the enamel-cement boundary with the aid of a carborundum disk. The remaining pulp was extirpated with endodontic instruments and the inlet opening of the pulp cavity was sealed with utility wax.[5]

The specimens were then randomly allocated into the following 5 groups (n = 10) according to the dye solution: distilled water, beet, caramel, carmine, and red 40. The teeth were embedded in 5 resin blocks (Jet Incolor Clássico, São Paulo, São Paulo, Brazil) containing 10 premolars in each block. Individual 1 mm thick ethylene vinyl acetate molds were made (Whiteness Placas para Moldeiras, FGM, Joinville, Santa Catarina, Brazil) for each block. At-home dental bleaching was performed with 16% carbamide peroxide (CP) (Whiteness Perfect, FGM, Joinville, Santa Catarina, Brazil) for a period of 3 hours daily for 3 weeks[15], as recommended by the manufacturer. One drop of the product was placed on the mold in the region corresponding to the buccal surface of the specimens. After this period, the specimens and the molds were washed in water to remove the product.[5]

The dye solutions for the immersion of the specimens were prepared daily as follows: for the beet, carmine, and caramel dyes, 1 g dye was added to 100 g of distilled water. For the red 40 dye, 0.35 g of dye was added to 100 g of distilled water. This ratio was based on the final dosage for food coloring products (Sensient Technologies Brasil, Jundiaí, São Paulo, Brazil), the maximum dose allowed by law. To make a solution, the manufacturers responsible for the dyes (Sensient Technologies Brasil, Jundiaí, São Paulo, Brazil) suggested diluting the powder in water by weight.

After bleaching, the teeth were exposed to the respective dye solutions, twice a day for 5 min, and one of these occurrences took place immediately after bleaching. After the 5 minutes, each group was removed from the dye solution, rinsed with tap water for 5 s, immersed in containers containing artificial saliva, and kept at 37°C[5,16,18] throughout the experiment. The artificial saliva was changed every 3 days.

The color evaluation was performed using a Vita Easyshade spectrophotometer (Vita Zahnfabrik, BadSäckingen, Germany) according to the CIELab[5,12,15,16,22] system during the following three periods: baseline; during the bleaching treatment (after the 1st, 2nd and 3rd weeks); and post-treatment (after 1 week and 1 month). The baseline period refers to initial color before the staining and bleaching process for periods of 1, 2, and 3 weeks. Readings were performed after the sequences of staining and bleaching. During the periods of 1 week and 1 mo post-whitening, the teeth were stored in artificial saliva and were not exposed to dyes and bleaching. In order to narrow the window of the spectrophotometer reading, a matrix with condensation silicone was performed (Coltoflax e Perfil Cub, Vigodent, Rio de Janeiro, RJ, Brazil), perforated with a circular scalpel and with a diameter that was compatible with the active end of the spectrophotometer.[12,15]

The color analysis was performed using the same room, the same lighting, at the same time, and with the silicone guide in a position to prevent possible interference in the evaluation of color.

The color comparison was calculated using the formula $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^2$, where L* represents the value from 0 (black) to 100 (white), where a* is the measurement along the red-green axis and b* is the measurement along the yellow-blue axis, as in previous studies.[5,12,15]

The comparison of the effectiveness of bleaching was performed with two-way analysis of variance (ANOVA) of the repeated measures and Tukey's test for the contrast of means ($\alpha = 0.05$).

Results

The means and standard deviation of color variation (ΔE) at the different periods of evaluation are depicted in Table 1. The interaction of the factors group *vs.* time ($p = 0.479$) and the main factor group ($p = 0.076$) were not statistically significant. Only the time factor ($p < 0.001$) was statistically significant. The use of the dyes did not affect the effectiveness of dental bleaching in any of the study periods. A higher degree of whitening was observed after the 2nd week of bleaching, which was statistically similar to the color obtained in the 3rd week of whitening. No color rebound was observed 1 week and 1 month after the end of the bleaching period ($p > 0.05$).

Discussion

The tested dyes (carmine, beet, caramel, and red 40) were chosen because they are widely used in the food industry and are present in products that are routinely consumed, in addition to their tinting strength.[13,14] Three natural dyes and only one artificial dye were tested due to the trend of replacing synthetic dyes by natural dyes in the food industry.[13,14] The exposure time of the whitened teeth to the coloring process totaled 10 minutes a day, which simulated the time that the bleached enamel would be in contact with the dye in the oral environment. This also represented the excessive consumption of dyes by the general population.[5,15]

The selected technique was at-home dental bleaching because it is an established technique used in dentistry to treat discolored teeth.[6,10,25] The gel used was 16% CP, which has proven efficiency,[15,25,26] and which contains remineralizing agents including potassium nitrate and sodium fluoride, which help to minimize changes in dental tissue.[26,27]

The results are in agreement with previous literature studies,[5,15,17] which observed that dyes do not interfere with the

Table 1: Values ΔE (means and standard deviations) for the groups evaluated

	Periods				
Groups	*Baseline vs 1 week*	*Baseline vs 2 week*	*Baseline vs 3 week*	*Baseline vs 1week post-bleaching*	*Baseline vs 1 month post-bleaching*
Distilled water	16.6 ± 5.4 Ab	18.2 ± 5.4 Aa	18.5 ± 6.1Aa	18.5 ± 5.2 Aa	19.1 ± 6.1Aa
Beet	18.2 ± 3.8 Ab	18.5 ± 4.9 Aa	19.9 ± 4.4 Aa	20.7 ± 5.2 Aa	23.5 ± 6.6 Aa
Caramel	16.2 ± 2.9 Ab	18.1 ± 2.1Aa	18.9 ± 6.1 Aa	17.5 ± 4.5 Aa	20.0 ± 3.4 Aa
Carmine	19.1 ± 4.2 Ab	19.8 ± 6.2 Aa	20.2 ± 4.6 Aa	20.5 ± 5.4 Aa	22.7 ± 6.2 Aa
Red 40	15.0 ± 3.9 Ab	18.3 ± 3.7 Aa	19.3 ± 4.2 Aa	18.9 ± 4.0 Aa	21.7 ± 3.4 Aa
Main factor time	17.5 ± 4.7 b	18.6 ± 4.6 a	19.4 ± 5.0 a	19.3 ± 4.9 a	21.4 ± 5.1 a

*Similar capital letters indicate statistical similarity between each column. Similar lowercase letters indicate statistical similarity in each row

effectiveness of dental bleaching. Some methodological aspects of this study may have supported our findings. We used a whitening gel containing remineralizing agents and neutral pH. Moreover, the specimens were stored in artificial saliva, which has an excellent remineralizing potential.[16,17]

Another feature in the present study that was similar to other studies, in which no influence of dye on the effectiveness of dental bleaching was found, is that the specimens were exposed to the dyes concomitantly with the bleaching procedure. In the study design, the peroxide acts to remove the dye adsorbed on the surface (extrinsic pigment) and also promotes the breakage of the chromophore molecules located in the dentin.

The findings of this study are in agreement with a recently published clinical study by Rezende et al.,[15] which found that exposure to coffee during bleaching did not affect the effectiveness of at-home dental bleaching using carbamide peroxide. However, the results of the present study differ from those of other researchers who have observed a greater potential for the staining of bleached dental enamel when the enamel is exposed to dye solutions.[18,22] In the aforementioned studies,[17,21] the specimens were bleached and immersed in dye. As well as failing to simulate clinical conditions, these specimens did not undergo further exposure to a bleaching agent or dental prophylaxis.

Another factor that may explain the lack of consensus in the literature relates to the type of bleaching agent, its concentration, and the type of dye solution that is used. Liporoni et al.[19] reported that specimens bleached with light-activated 35% hydrogen peroxide and exposed to wine showed a higher level of darkening than those exposed to coffee. This may have been due to differences in the concentration of the bleaching gel that was used because a higher concentration of gel could have contributed to an increase in surface changes and staining.[11] Another difference between the two solutions used in this study is the pH of beverages. The fact that red wine is more acidic than coffee may also have contributed to an increase in the permeability of the enamel.[5,23]

Dental enamel serves as a semi-permeable barrier, i.e., only ions and small molecules can permeate through the enamel. In the present study, the tested dyes, regardless of their individual characteristics, consisted of large molecules, with molecular weights ranging from 551.41 g/mol (beet dye) to 492.39 (carmine dye), which precluded the penetration of the enamel.[28]

Finally, the limitations of this study should be reported. We used a whitening gel containing remineralizing agents and neutral pH, maybe the use products that demonstrably more alter the substrate, such as a gel that does not contain in its formulation remineralizing agents, such as sodium fluoride, could corroborate more change of the substrate and hence greater pigmentation.

Other laboratory studies should be conducted to test different products and methodologies in order to determine whether other dyes influence the effectiveness of dental bleaching in removing these pigments.

Conclusion

We concluded that the different types of dyes tested, whether natural or artificial, did not interfere with the effectiveness of bleaching using 16% carbamide peroxide.

Clinical relevance

Dyes consumption during bleaching did not affect the effectiveness of dental bleaching.

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