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Is prolonged bleaching more harmful to dental enamel than daily dietary and hygienic oral habits?

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Abstract: The ultrastructural and mechanical properties of enamel surface were evaluated after prolonged bleaching treatments with 10% carbamide peroxide in the presence or absence of orange juice (erosive challenge) and toothbrushing (abrasive challenge). In total, 145 incisor bovine teeth were used in this study. Twenty-five samples were prepared for the ultrastructural evaluations, and 120 samples were prepared for microhardness and roughness tests. These 120 samples were divided into eight experimental groups (n = 15): G1- artificial saliva; G2- abrasion; G3- erosion; G4- dental bleaching; G5- erosion + abrasion; G6- bleaching + abrasion; G7- bleaching + erosion; and G8- bleaching + erosion + abrasion. All groups were tested at T0 (before treatment), T1 (14 days), T2 (21 days), and T3 (28 days). Two-way analysis of variance for repeated measures and the post hoc Sidak tests ($p \leq 0.05$) were used. The roughness evaluation demonstrated an increase in damage for all experimental groups with an increase in the time period. For microhardness, the groups exposed to artificial saliva (AS) and abrasive challenge did not show any differences at any time points, while the other groups showed a decrease in microhardness from T0 to T3. Ultrastructural evaluation showed different surface alterations in response to the treatments. Despite prolonged bleaching periods, the procedure caused lesser enamel surface alterations than exposure to orange juice alone or in combination with brushing.

Keywords: Dental Enamel; Tooth Bleaching; Tooth Erosion; Tooth Abrasion.

Introduction

Interest in esthetics has been increasing, partly due to media (or societal) pressure.[1] A harmonic smile is strongly connected to bright and well-aligned teeth.[2] From this point of view, tooth bleaching has gained great notoriety for being a fast, low-cost, and conservative (without dental wear) treatment option; it is occasionally used in more extensive aesthetic restoration/rehabilitation plans.[3] In this scenario, several bleaching products are launched in the dental market, indicated for professional or at-home use. The problem with at-home products is that users can overdo the recommended application time in an attempt to obtain increasingly white teeth.[3,4] Ideally, patients should not have free access to at-home products. Professionals must provide sufficient bleaching gels to fulfill the needs of the whole treatment.[5]

Hydrogen peroxide (H_2O_2) is the main active agent in bleaching products, even in those based on carbamide peroxide ($CH_6N_2O_3$), since it is its precursor.[2,5] Throughout the development of bleaching products, several modifications were included in their composition in an attempt to reduce possible side effects on the dental structure and on trans- and post-operatory sensitivity.[6,7] However, prolonged use has been shown to compromise enamel integrity.[4,5,8,9]

However, the integrity of the enamel can also be compromised by other injuries to the teeth, such as dental erosion, a phenomenon that occurs on the tooth surface when it is exposed to exogenous and endogenous acid substances, except those produced by bacteria.[10] The prevalence of dental erosion has increased in recent years and seems to be partially associated with our modern way of life, which includes the consumption of acidic fruit juice in the diet.[11,12] Associated with dental structure loss by erosion, abrasion (toothbrushing) is considered an important cofactor.[13] In this scenario, the damage to dental tissue can be even greater when brushing is performed in association with high abrasive dentifrices.[13,14]

Considering the problems mentioned above and the limited number of studies evaluating the consequences of prolonged tooth bleaching in association with various other habits, the objective of this study was to evaluate the effects of prolonged dental bleaching in combination with erosive (acid juice) and abrasive (simulated brushing) challenges on enamel surface characteristics. In this study, we hypothesized that prolonged tooth bleaching may be less damaging to the integrity of the enamel than other daily habits such as an acidic diet and/or toothbrushing.

Methodology

Ethical aspects, sample definition, and production of specimens

This study was performed in accordance with Law 11.794 of October 8, 2008 and the rules issued by the National Council for Control of Animal Experimentation and was approved by the Ethics Committee on Animal Use of the Federal University of Para (approval number #8031261217).

In total, 145 bovine incisors of *Bos taurus indicus* (mean age: 24 months) were used in this study. Only teeth with sound crowns and fully formed roots were included in the analysis. The teeth were analyzed after cleaning and disinfection under 40× magnification, and those that presented crown cracks or defects on the enamel surfaces that could impair their use later were discarded. Next, the selected teeth were stored at 4°C in distilled water.

The central part of the crown was lined off using a digital caliper (DIN 862; Mitutoyo, São Paulo, Brazil). Blocks of approximately 10 mm^2 were cut using an Isomet 1000 saw (Buehler Ltd., Lake Buff, USA). These dental blocks were embedded with acrylic resin in a 11 mm high round mold to expose the enamel surfaces for further flattening. After 24 h, the assembled blocks were sent to a semiautomatic polishing machine (Automet 250, Buehler Ltd., Lake Buff, USA) and ground wet at 200 rotations per minute and 20 N of pressure. The descending gridding sequence was #400 for 15 s, #600 for 30 s, #1500 for 1 min, #2500 for 3-min, and #4000 for 4 min. Between each granulation and after the final granulation, the specimens were cleaned in ultrasonic baths for 3 min in distilled water to avoid any abrasive interference, remove any remaining debris, and obtain fully polished enamel surfaces. Next, the specimens were randomly divided into the following groups: G1, AS; G2, abrasion; G3, erosion; G4, dental bleaching; G5, erosion + abrasion; G6, bleaching + abrasion; G7, bleaching + erosion; and G8, bleaching+ erosion + abrasion.

Tooth bleaching, erosive, and abrasive challenges

For the bleaching procedures, 0.1 ml of 10% CP bleaching gel (FGM, Joinville, SC, Brazil) and 0.05 mL of AS were mixed[15] and used for 4 h/day, as prescribed by the manufacturer, in the assigned experimental groups. A standard amount of gel was prepared and applied using a prefabricated acetate mold.[15] During 4 h of bleaching, the specimens were stored in plastic boxes, with a small amount of water at the bottom without encountering the surface of the specimens, in a biological oven at 37°C. After every bleaching treatment, the specimens were

washed with an air-water spray for 1 min and applied 5 cm away from the treated surfaces. Next, specimens exposed only to bleaching procedures were stored in AS (Table 1) at 37°C for 24 h. The other specimens were subjected to subsequent treatment accordingly.

Specimens submitted to erosive challenge (G3, G5, G7, and G8) were immersed for 10 min in 200 mL of orange juice (pH = 3; Del Valle Néctar, Rio de Janeiro, RJ, Brazil) poured into sterilized Becker glass under slight agitation[16] once a day for 28 days. Then, the specimens were washed in distilled water and blot dried. Specimens subjected only to erosive challenge were returned to AS at 37°C for 24 h, while the others were subjected to the next treatment.

With a pH meter, the pH of orange juice was constantly monitored before and after specimen immersion at the same temperature (25°C). At the end of each erosive challenge, the juice was discarded.

A tooth-brushing device was used for abrasion. Soft bristle toothbrushes (Colgate Essential Clean, São Bernardo do Campo, Brazil) were set at the end of the arms of the equipment, and under 200 g pressure, 45 brushing cycles (equivalent to 3 daily brushings)[17] were applied to the specimens positioned into small boxes immersed in a fresh dentifrice and water paste (1:2 weight volume).[18] Finally, the specimens were removed, washed for 30 s in distilled water and returned to the biological oven (37°C/24 h) immersed in AS, which was renewed daily.

Surface roughness, microhardness, and ultrastructure

A total of 120 specimens (n = 15) had their surfaces lined off in the middle to allow for MH (Knoop microhardness evaluation) and SR (Ra optics roughness evaluation) tests in different areas. An optical profilometer (Proscan 2100 3D, Scantron, Taunton, England) was used for the SR because it did not damage the specimen surface during readings; thus, subsequent profiles could be performed without surface injury risk. Readings were recorded at T0 (before testing), T1, 14 days; T2, 21 days; and T3, 28 days. The scanning parameters were axel X-2000 steps and 0.01-mm length; axel Y, only 1; time, 19 s. We calculated the mean values of the three readings from each surface.

Knoop MH measurements were performed using a microhardness testing equipment (HMV-2; Shimadzu, Kyoto, Japan). Three measurements, 100-µm apart, were completed under 50-g load for 20 s at T0, T1, T2, and T3, and the mean was calculated.

One specimen from each group at T1, T2, and T3 (except for T0, no treatment, when only one specimen for all groups was used) was prepared for scanning electron microscopy (SEM) using the Field Emission Gun tool (TESCAN, model Mira3, Tescan, LTD, Brno, Check Republic). Images were generated by a secondary electron beam using an output acceleration of 5 kV and applied at a distance of 15 mm, standardized at 11000× magnification. Morphological analysis was performed using the generated images.

Table 1. Materials, commercial names, and composition, according to the manufacturers.

Material (Manufacturer)	Composition
Colgate toothpaste Total 12 (Colgate-Palmolive, São Bernardo do Campo, Brazil)	Sodium fluoride (1450 F ppm), water, sorbitol, hydrated silica, Sodium laurylsulfate, PVM/MA copolimer, flavour, carragenine, Sodium hydroxide, triclosan, Titanium dioxide (CI 77891), dipenteno and RDA 70.
Colgate Essential Clean toothbrush (Colgate-Palmolive, São Bernardo do Campo, Brazil)	Plastic handle, round tip nylon bristles.
Whiteness Simple bleaching gel (FGM Produtos Odontológicos Ltda, Joinville, Brazil.)	10% carbamide peroxide, carbopol, potassium hydroxide, sodium fluoride, glycerol, deionized water and pH around 7.
Artificial Saliva (Prepared in the Biomaterial and Oral Biology laboratory- USP, Dental School)	Sodium bicarbonate 2190 mg, potassium phosphate 1270 mg, magnesium chloride 125 mg, calcium chloride 441 mg, potassium chloride 820 mg, sodium fluoride 4,5 mg, nipazol 100 mg, carboxymethylcellulose 8 mg, distilled water 3000 ml.
Del Valle orange juice (The Coca Cola Company, Rio de Janeiro, Brazil)	Water, sugar, concentrated orange juice, concentrated apple juice, vitamin C, acidity regulator citric acid, natural aroma, and pH=3

Statistical analysis

Statistical analysis was performed using SPSS (version 17.0, SPSS Inc., Chicago, USA). The Shapiro-Wilk test identified a normal distribution for the MH and SR results. Two-way analysis of variance for repeated measures and the post hoc Sidak test ($p \leq 0.05$) were applied to the results (mean ± standard deviation [SD]). SEM micrographs were qualitatively analyzed and represented enamel micromorphology shifts.

Results

Tooth bleaching and abrasive and erosive challenges caused changes in surface roughness, alone and in combination

An increase in the SR results was observed as a function of time when comparing T0 with T1 for all groups ($p < 0.05$). In G1 to G7, no statistical differences were observed between days 14 and 21 ($p > 0.05$). On the 28th day of bleaching, a statistical difference was found in G3, G5, G6, G7, and G8 in relation to T2. G8 (all combined treatments) was the only group with a statistically significant difference between all the times tested. Table 2 shows the mean ± SD of SR tests.

The erosive challenge alone affected microhardness over time more than any other treatments, alone or in combination

With respect to MH, there were no significant differences in G1 and G2 at any time point ($p < 0.05$). Significant differences were observed in the other groups, especially in G3, G7, and G8 ($p < 0,0001$) compared with T0 to T1, T2, and T3 (Table 3).

The combination of tooth bleaching and erosion challenge as well as all three treatments simultaneously caused severe damage to the interprismatic enamel

The electromicrographs displayed different views of the enamel surface of all groups at different times of bleaching. The dental enamel at T0 (no treatment) appeared as an enamel with a complete aprismatic layer, similar to that in groups G1 (AS) and

Table 2. Mean ± standard deviation for the SR analysis as a function of time for all groups.

Variable	T0- no treatment	T1- 14 days of treatment	T2- 21 days of treatment	T3- 28 days of treatment
G1- Artificial saliva	0.147 (±0.05)^{a}	0.172(±0.04)^{bc}	0.159(±0.04)^{abc}	0.168(±0.04)^{c}
G2- Abrasion	0.125(±0.04)^{a}	0.153(±0.03)^{bc}	0.193(±0.05)^{cd}	0.222(±0.06)^{d}
G3- Erosion	0.129(±0.04)^{a}	0.462(±0.06)^{bc}	0.505(±0.07)^{c}	0.636(±0.15)^{d}
G4- Dental bleaching	0.141(±0.04)^{a}	0.201(±0.03)^{bc}	0.208(±0.03)^{cd}	0.237(±0.02)^{d}
G5- Erosion+abrasion	0.141(±0.04)^{a}	0.443(±0.06)^{bc}	0.450(±0.05)^{c}	0.503(±0.06)^{d}
G6- Bleaching+abrasion	0.117(±0.02)^{a}	0.319(±0.05)^{bc}	0.329(±0.07)^{c}	0.422(±0.03)^{d}
G7- Bleaching+erosion	0.149(±0.05)^{a}	0.456(±0.05)^{bc}	0.479(±0.03)^{c}	0.575(±0.09)^{d}
G8- Bleaching+erosive+abrasion	0.132(±0.03)^{a}	0.468(±0.03)^{b}	0.516(±0.05)^{c}	0.663(±0.08)^{d}

*Different lowercase letters indicate significant intragroup differences at different times

Table 3. Mean ± SD values for MH tests, analysed by two-way ANOVA for repeated measures as a function of time.

Variable	T0- no treatment	T1- 14 days of treatment	T2- 21 days of treatment	T3- 28 days of treatment
G1- Artificial saliva	352.4(± 52.81)^{a}	326.8(± 41.86)^{a}	321.9(± 33.53)^{a}	314.2(± 33.19)^{a}
G2- Abrasion	323.9(± 42.73)^{a}	341.6(± 35.69)^{a}	332(± 52.36)^{a}	322.7(± 38.47)^{a}
G3- Erosion	289.8(± 63.30)^{a}	59.8(± 23.57)^{bcd}	46.4(± 14.25)^{c}	65.1(± 16.90)^{d}
G4- Dental bleaching	274 (± 71.97)^{a}	245.7 (± 79.04)^{ac}	277.1(± 43.18)^{a}	220.7(± 54.28)^{bc}
G5- Erosion+abrasion	292.5(± 63.22)^{a}	176.2(± 58.31)^{bcd}	199.2(± 40.59)^{cd}	194.2(± 48.24)^{d}
G6- Bleaching+abrasion	323.5(± 57.22)^{a}	266.4(± 59.46)^{ab}	288.5(± 68.79)^{ab}	272.1(± 53.87)^{b}
G7- Bleaching+erosion	299.9(± 48.27)^{a}	148.3 (± 47.60)^{bc}	146.8 (± 46.13)^{c}	90.3(± 32.22)^{d}
G8-bleaching+erosive+abrasion	328.3(± 61.78)^{a}	183.8 (± 53.04)^{bcd}	175.5(± 42.17)^{cd}	179.3(± 62.42)^{d}

*Different lowercase letters indicate significant intragroup differences at different times.

G2 (brushing) at all time points and in G4 (bleaching) at T1 and T2 (21 days considered prolonged time) and in group G6 (bleaching and brushing for 14 days) at T1. The erosive challenge (G3) at all time points (T1, T2, and T3) exposed a pattern of discontinuity of the aprismatic enamel with a small exposure of the prismatic enamel, similar to the images of the bleaching in G4 at T3 (28 days) and in G5 (erosion + brushing) at T2 and T3. The most severe and evident changes were observed in G7 (bleaching + erosion) and G8 (all treatments), exposing the prisms in a generalized manner (Figure).

Discussion

This study is the first to demonstrate that prolonged at-home dental bleaching, combined with erosive and abrasive challenges, can cause severe damage to the dental enamel. Dental bleaching affected enamel SR, even when the manufacturer's instructions were followed (14 days). The values observed for the MH test were affected by the duration of the treatment, with the lowest value at day 28. In addition, the two characterized properties (SR and MH) were more severely influenced when bleaching was associated with abrasive and erosive challenges, mainly by the acid in orange juice (erosive).

Morphological alterations of the dental enamel are attributed to the loss of mineral components due to the low pH of some bleaching agents,[19] although research[20,21] has shown that these alterations occur with the use of bleaching gels with neutral pH, as the agent used in the present study. One possible explanation for these alterations is related to the low molecular weight of peroxides, which attack not only the chromatogenic molecules present in the pigments but also the substrate organic matrix, which is responsible for the degradation process.[21] These abovementioned features might explain the decrease in MH values (although not significant) when the manufacturer's recommendations were followed (14 days) and more pronounced decrease at 28 days even when using a neutral pH-bleaching agent.

The gel used in this study contains potassium hydroxide, which is a strong alkaline component that may be able to neutralize acids in aqueous environments.[22] Another component present in the formulation of the bleaching gel is sodium fluoride, which is broadly added to toothpastes as a caries preventive element. According to the manufacturer, these components play an important role in desensitizing mechanisms, preventing demineralization development and loss of hardness. Another feature that may have protected the substrate

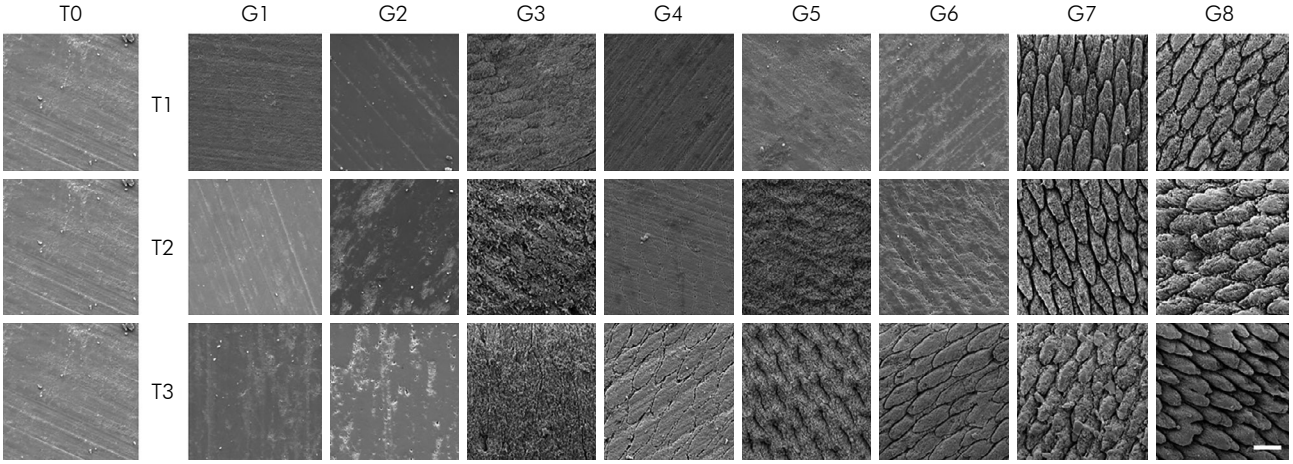

Figure. SEM micrographs depicting the different treatments as a function of time. Columns indicate the following groups: G1, AS; G2, toothbrushing; G3, orange juice; G4, 10% carbamide peroxide; G5, orange juice + toothbrushing; G5, 10% carbamide peroxide + toothbrushing; G6, 10% carbamide peroxide + toothbrushing; G7, 10% carbamide peroxide + orange juice; and G8, 10% carbamide peroxide + orange juice + toothbrushing. Lines indicate the following times: T0 (no treatment), T1 (14 days, positive control), T2 (21 days), and T3 (28 days). Scale bar: 5 μm.

against the demineralizing action of the bleaching gel was the presence of calcium and other mineralizing elements in the AS formulation.

One of the assumptions of this study was that prolonged at-home bleaching would be less harmful to the enamel structure compared to daily challenges, such as toothbrushing and/or diet including acid substances. Orange juice was chosen because of its acidic nature, an erosive potential widely tested and documented in the literature and because of its worldwide consumption.[23,24,25] In addition, a recent study[26] showed that orange juice was more suitable for erosive laboratory studies than isolated citric acid.

In vitro studies have shown that short-term erosive challenges (1–3 min) cause weakened and softened partially demineralized areas on the enamel, which may reach some nanometers in depth and are removed during toothbrushing.[17,27] Regarding these features, Scaramucci et al.[28] have demonstrated that this softened layer may lead to larger marked areas indented during MH tests and, thus, lower reading values. These features were also observed in experimental group G3, where the treatment included only an erosive challenge without abrasive action.

It is plausible to consider that abrasive action can intensify the erosive process.[29] In this study, it is feasible to admit that the abrasive brushing action could have the capacity to remove the outer acid attacking the enamel layer (G5, erosive + abrasive challenge and G8, erosive + abrasive challenge + bleaching), contributing, at least partially, to the better MH values than those reported for G3 (acid challenge only). However, when SR was measured, although the eroded portions were eliminated by the brushing action, the exposition of interprismatic areas could have negatively affected the SR results.

An *in vitro* study[30] demonstrated that when different abrasive toothpastes with and without fluoride in their composition were used, the abrasion caused by non-fluoride toothpastes was higher. Therefore, it is reasonable to believe that in the G5 (erosive + abrasive challenge), G6 (abrasive challenge + bleaching), and G7 (erosive challenge + bleaching), where the specimens were exposed to abrasive action, the fluoride protective mechanism as well as the presence of some mineral compounds in the storage medium (AS) may be responsible for the improved MH results compared to those of G3 (orange juice only) since brushing treatment was accomplished immediately after the erosive challenge. To date, there are insufficient data to support the precise harmless level of abrasive dentifrices. Therefore, the use of highly abrasive toothpastes (RDA 250) for brushing our teeth must be avoided.[29]

The most damaging results were observed when the brushing action was performed immediately after the erosive cycles, as mentioned in another study.[31] The literature shows that in SR beyond 0.2 micron, the biofilm accumulation is higher.[32] Special attention must be given to G8 results, which showed higher values of SR. In this particular group, the three treatments were supplementary (bleaching/abrasion/erosion). Since toothbrushing cannot be avoided as a daily hygienic procedure, patients must be instructed regarding the damaging action of the association of acidic juices and bleaching.

The pH of the AS used in this study was adjusted to neutral pH (pH = 7). The capacity for remineralization was related to the saturation of calcium and phosphorus.[33] Thus, it is believed that a possible non-significant decrease in the MH values in the studied groups was due to the remineralizing ability of the storage medium. Several studies have demonstrated the remineralizing capacity of AS in the initial stages of the erosion process.[33,34,35] Another study on enamel bleaching for prolonged periods,[5] using X-ray diffraction, found changes in the amounts of certain chemical elements (O, P, and Ca) without changes in the structure of the hydroxyapatite crystals. These data may be related to transient demineralization during bleaching treatments, as suggested by the microhardness results in G4. Therefore, tooth bleaching itself, even when performed for longer periods than those recommended by the manufacturers, does not seem to cause significant damage to the enamel. However, clinically, erosion and abrasion attacks would take place simultaneously; therefore, it would be expected to cause more intense damage to tooth structure, especially if prolonged bleaching is performed (as observed in G8, Tables 2 and 3).

Determination of surface loss using an optical profiler is the gold standard for estimating the effects of erosive processes.[17,30] This test requires the placement of specific tapes that are removed at the time of analysis. Although we considered using this methodology, the need to repeat the analysis at the same locations and the difficulty of replacing the tape exactly at the same spot made us choose the surface roughness analysis instead. In this sense, our choice could be considered a limitation of the present study. Another limitation was related to the non-use of human saliva as the specimen storage media, which did not allow the formation of an acquired pellicle on the enamel surface. Therefore, considering the protective aspect of this pellicle, less pronounced damage to the enamel would be expected. In this sense, more studies (for example, in situ studies) are needed.

Within the limitations of this *in vitro* study, we can conclude that bleaching treatment, even when performed for long periods, causes less damage to enamel surfaces than exposure to acidic substances, such as orange juice, both in isolation and in combination with brushing.

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