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Mineral Loss and Morphological Changes in Dental Enamel Induced by a 16% Carbamide Peroxide Bleaching Gel

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The aim of this study was to compare the effect of a 16% carbamide peroxide (CP) gel and a 10% CP gel on mineralized enamel content and morphology. Enamel blocks from bovine incisors were subjected to a 14-day treatment (8 h/day) with 10% or 16% CP gels. Knoop microhardness was evaluated before bleaching and at 1, 7 or 14 days after this treatment (50 g/15 s). Mineral content (energy-dispersive x-ray spectroscopy), surface roughness and topography (atomic force microscopy) were evaluated at the 14-day period. Data were analyzed statistically by two-way ANOVA and Tukey's test (α=0.05). Significant microhardness reduction was observed at the 7 th and 14 th days for 10% CP gel, and for all bleaching times for 16% CP gel ($p<0.05$). At the 14-day period, a significant decrease in Ca and P content, increase on surface roughness ($p<0.05$) as well as on picks and valleys distance were observed when both bleaching gels were used. These enamel alterations were more intense for 16% CP gel. It was concluded that both CP-based gels promoted loss of mineral structure from enamel, resulting in a rough and porous surface. However, 16% CP gel caused the most intense adverse effects on enamel.

Key Words: atomic force microscopy, carbamide peroxide, tooth enamel, hardness tests, tooth bleaching.

Introduction

Tooth bleaching is a popular esthetic procedure because of its simple technique, clinical efficacy and non-invasive nature, requiring no sound dental structure removal. However, a number of studies demonstrated that bleaching gels with high concentrations of hydrogen peroxide (HP), such as those used for in-office tooth bleaching, could promote pulp cell damage (1). This undesirable adverse effect has been related to the ability of HP to disrupt the mineral structure of enamel (2), which allows HP diffusion to the subjacent dentin and pulp chamber (3). Therefore, at-home tooth bleaching technique has been considered as an interesting alternative for bleaching teeth without causing toxic effects to pulp tissue. This esthetic procedure is based on patient wearing a custom-made tray filled with low concentrations of carbamide peroxide (CP) or HP gels (4).

Currently, several researchers are looking for tooth bleaching techniques that are at the same time effective and safe for dental tissues (1). Low-concentration HP (15-20%) gels for in-office therapy provide effective bleaching associated with less intense and prevalent tooth sensitivity (5,6). The CP-based gels have the advantage of promoting a slow and gradual release of HP, which prevents its quick diffusion through enamel and dentin at high concentrations (7). The 10% CP gel has been considered a safe bleaching technique for tooth structure, since several studies demonstrated low incidence of post-operative sensitivity (8) and low trans-enamel and trans-dentinal cytotoxicity (4). A recent *in vivo* study also demonstrated that night-guard vital bleaching with 10% CP gel provides patient satisfaction with minimal side effects up to 17 years post-treatment (9). However, some patients complain about the long treatment time required for this technique.

To fulfill patient requirement of faster tooth bleaching outcomes, CP-based gels with concentrations varying from 15 to 22% have been currently recommended. However, only a few studies evaluated the effects of these products on tooth structure (4,10-15). A recent investigation showed that 16% CP gel caused higher trans-enamel and trans-dentinal toxic effects to pulp cells than 10% CP gel, which may have been favored by the more intense enamel changes triggered by the 16% CP gel (4). However, as the effects of 16% CP gel on dental enamel as well as on pulp cells are still controversial, a rational approach is probably to carry out studies to clarify these topics. Therefore, the aim of this study was to evaluate the microhardness, mineral content, roughness and topography of dental enamel bleached with

16% CP gel compared with the widely used 10% CP gel.

Material and Methods

Preparation of Specimens

Thirty-six dental enamel blocks measuring 8x8 mm were obtained from buccal surface of sound bovine permanent incisors. The blocks were embedded in autopolymerizing acrylic resin (Vipi Flash; Vipi Ltda., Pirassununga, SP, Brazil) and the outer enamel surface was polished with wet 320-, 600-, 1200- and 1500-grit silicon carbide paper (T469-SF; Norton - Saint-Gobain Abrasivos Ltda., Jundiaí, SP, Brazil) in a polishing machine (Panambra DP-10; Panambra Industrial e Técnica S.A., São Paulo, SP, Brazil) followed by hand polishing with 6, 3 and 1 µm (Arotec; Arotec S.A., Cotia, SP, Brazil) diamond pastes intercalating ultrasonication with distilled water to eliminate paste residues. The specimens were rinsed with distilled water after the final polishing with 1 µm diamond paste.

Bleaching Procedure

Bleaching gels containing 10% or 16% CP (Whiteness HP; FGM, Joinville, SC, Brazil) were evaluated. These gels are identical in composition, except for the HP content, having a neutral pH. The specimens were randomly assigned to the following 3 groups (n=12): CG - control (no bleaching); G10 - 10% CP bleaching gel application; G16 – 16% CP bleaching gel application.

In G10 and G16, the surface of each enamel block was washed with 1 mL of sterile deionized water, dried with absorbent paper and then 30 mg of either of the bleaching gels was applied on enamel for 8 h at 37 °C. After this, the gel was aspirated, enamel surface was thoroughly rinsed with sterile deionized water and maintained in artificial saliva for 16 h at 37 °C. These procedures were repeated for 14 consecutive days. In CG, the enamel surface remained in contact with deionized water during the bleaching time (8 h) followed by storage in artificial saliva (16 h) (4).

Knoop Microhardness

Enamel microhardness was evaluated in 12 specimens *per* group using a microhardness meter with a square base diamond pyramid Knoop hardness indenter (Buehler, Lake Bluff, IL, USA). The analysis was performed before bleaching (baseline) and after 1, 7 and 14 days of bleaching protocol in each specimen. Each indentation was made with a 50 g static load for 15 s. Five indentations were made in each specimen at each evaluation period, within an area of 1.0 mm^2 on enamel surface, equidistant from each other 0.1 mm horizontally and 0.5 mm vertically. An average of the 5 indentations of each period was obtained. Data were submitted to two-way ANOVA ("group" and "period"), complemented by Tukey's test for multiple comparisons at a significance level of 5%.

Analysis of Mineral Content by Energy-Dispersive X-Ray Spectroscopy (EDX)

The mineral content of enamel was assessed in the same specimens used for microhardness analysis at the end of the experimental protocol. Six specimens of each group were examined by SEM-EDX at the 14 th day. An EDX probe coupled to a scanning electron microscope (Philips FEG XL 30; Oxford Instruments, Inc., Concord, MA, USA) at 25 kV accelerating voltage, 100 µA illuminating current and 100-second counting time was used. In each specimen, analyses of calcium (Ca) and phosphorus (P) content of enamel (%) were carried out in three equidistant points distributed diagonally along the test surface and the average value was statistically analyzed by two-way ANOVA and Tukey's test at a significance level of 5%.

Analysis of Surface Roughness and Topography by Atomic Force Microscopy (AFM)

In the other six specimens of each group subjected to the microhardness test, surface roughness and surface topography were evaluated with an atomic force microscope (Alpha 300AR; Witec, Ulm, Germany) at the 14 th day for each group. The topography of the specimens was examined within an area of 70 x 70 μm^2 in 3 different positions in each sample. All AFM measurements were conducted with the tip working in AC-mode (tapping mode) and the average values from the 3 measurements were submitted to statistical analysis (two-way ANOVA and Tukey's test; α=5%). The WSxMV 3.1 software (Nanotec Electrónica S.L., Tres Cantos, Madrid, Spain) transformed the obtained data in 3D images for subsequent analysis of the results. The results were three 3D images of the surface for each qualitatively examined specimen.

Table 1. Knoop microhardness (KHN) values for enamel according to carbamide peroxide concentration and duration of the bleaching protocol

Group	Duration of the bleaching protocol			
	Baseline	1 day	7 days	14 days
GC	284.9 (14.7)* A,a**	284.6 (15.1) A,a	286.8 (15.2) A,a	285.0 (16.7) A,a
G10	300.9 (11.4) A,a	286.3 (9.1) A,a	257.1 (22.9) B,b	262.4 (14.8) B,b
G16	285.5 (27.0) A,a	254.3 (14.6) B,b	239.6 (18.2) B,b	228.3 (16.3) C,b

* Values represent mean (standard deviation), n=12. ** Lowercase letters allow comparisons within rows (horizontal) while uppercase letters allow comparisons within columns (vertical). Different letters indicate statistically significant difference among groups (Tukey's test $p<0.05$).

Results

Knoop Microhardness

The Knoop microhardness values for enamel according to CP concentration and duration of the bleaching protocol are presented in Table 1.

In CG, enamel microhardness remained unaltered during the entire experimental period. Bleaching with 10% CP gel (G10) caused a significant decrease in enamel microhardness after 7 and 14 days of treatment compared with baseline ($p<0.05$). On the other hand, no significant difference was observed between these two periods ($p>0.05$). Enamel microhardness was significantly lower in G16 than in CG after all bleaching times ($p<0.05$). Comparing the three bleaching times within G16, no significant difference was found between the 1- and 7-day times ($p>0.05$); however, significantly lower microhardness values were observed after 14 days of bleaching treatment ($p<0.05$).

Analysis of Mineral Content by Energy-Dispersive X-Ray Spectroscopy (EDX)

No statistically significant difference was observed between G10 and G16 ($p>0.05$; Fig. 1A). However, both bleached groups presented a significantly lower Ca and P content on enamel compared with the non-bleached CG (Fig. 1) ($p<0.05$).

Analysis of Surface Roughness and Topography by Atomic Force Microscopy (AFM)

No significant difference was observed between G10 and G16 ($p>0.05$); but both bleached groups had a significantly higher surface roughness compared with the non-bleached CG (Fig. 2) ($p<0.05$). Regarding the topography, enamel surfaces in the bleached groups were more irregular than in CG, exhibiting more numerous and deeper peaks and valleys, suggesting a more evident microporous layer on this tissue (Figs. 3B and C), especially in the specimens bleached with the 16% CP gel (Fig. 3C).

Discussion

In spite of being considered a safe technique for dental structures, the in-home bleaching therapy using 10% CP bleaching gel requires a long time to reach a desirable esthetic outcome (2 to 4 weeks) (4). A recent study demonstrated that it is possible to speed up the whitening effect of this technique by increasing CP concentration (15). However, the negative effects of high concentration CP bleaching gels on this hard dental tissue structure were scarcely studied.

According to the results of the present study, higher CP concentration in the bleaching gel results in higher and faster decrease of dental enamel microhardness. This alteration has been correlated to the oxidative action of HP on the organic phase of this hard tissue (2). Disruption of

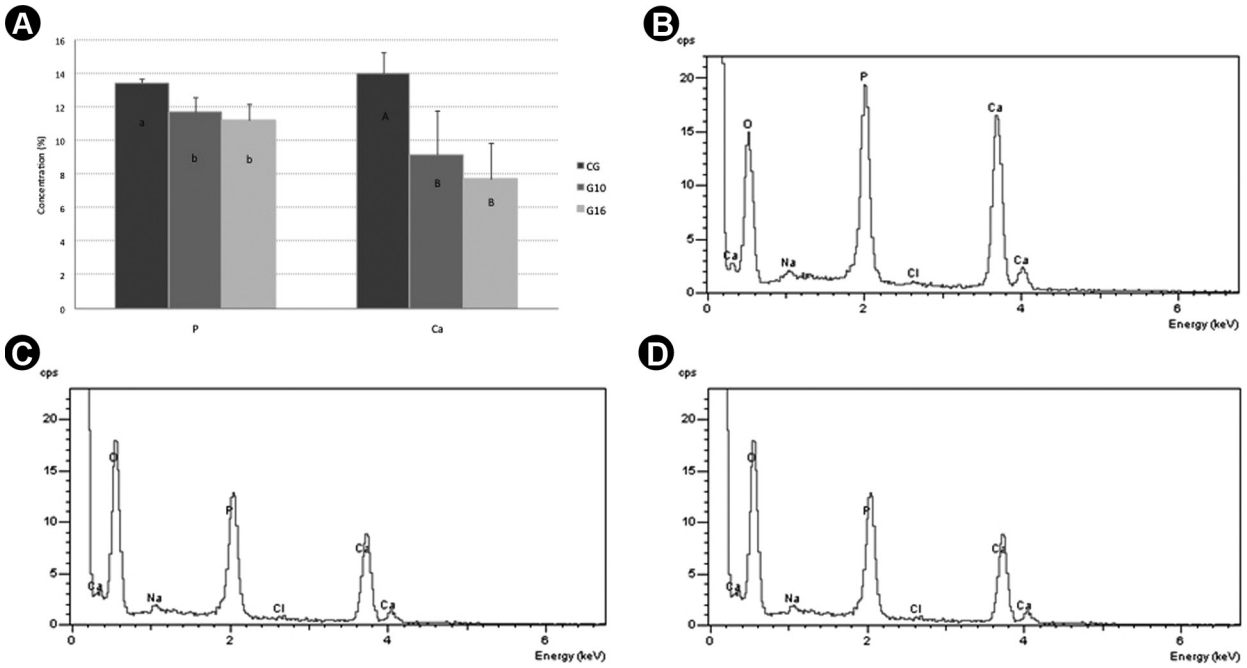

Figure 1. EDX Analysis. A: Means of Ca and P content (%) in GC, G10 and G16. Letters indicate comparisons for each element. Columns express means and error bars express standard deviations. Columns with same letters do not differ significantly (Tukey, $p>0.05$); B: Image representation of the mineral content in GC (keV x cps). C and D: Image representation of the mineral content in G10 and G16, respectively, showing the decrease in P and Ca peak in both groups.

the enamel organic matrix results in loss of the crystalline material sketched out of this matrix, leaving zones of erosion intercalated with areas of intact enamel, resulting in a rough surface (16,17). The presence of nitrogen and oxygen molecules on bleached enamel surface, probably due to the interaction of peroxide with the enamel proteins, suggests that HP is able of dissolving the enamel organic matrix (3). The results of the present investigation may confirm this hypothesis, since reduction of Ca and P content was observed after bleaching, associated with increase of enamel surface roughness. Therefore, as the compositions of both gels used in this study are identical except for the CP concentration, it may be suggested that the greater aggressiveness caused by 16% CP gel in the present study was due to the release of higher HP concentrations compared to the 10% CP gel.

Several studies demonstrated that the effects of bleaching agents on dental enamel might vary from insignificant up to deep alterations on its structure and morphology, depending on the bleaching protocol (18-20). As observed in the present investigation, some studies demonstrated that application of 16% CP gel also causes more intense morphological changes on enamel surface and reduction of enamel microhardness compared to low-concentration CP-gels (10-12). On the other hand, other studies did not observe significant differences on dental enamel structure between bleached or non-bleached surfaces (21-23), and also among different concentrations of the CP-gels (23). In these investigations, the enamel surface was bleached in an *in situ* environment. In this condition, there is an influx of saliva, which presents remineralizing ions such as fluoride, calcium and phosphates (18). However, at the moment of bleaching procedure, increase of enamel porosity allows HP penetration on deep enamel and consequently into the pulp chamber (2,3). A recent study demonstrated that even when applied onto remineralized enamel by fluoride, the 16% CP gel was still toxic to the odontoblast-like cells, with no significant difference from the non-fluoride treated enamel (24).

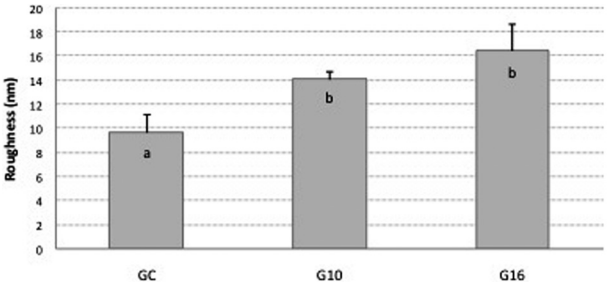

Figure 2. Means of enamel surface roughness (nm) by AFM for the control and bleached groups. Columns express means and error bars express standard deviations. Columns with same letters do not differ significantly (Tukey, p>0.05).

In this way, the high-concentration CP gels appear to promote greater alteration on dental structure, resulting in a more intense trans-enamel and trans-dentinal HP diffusion, as previously demonstrated (4). According to the authors, the slight increase in CP bleaching gel concentration (from 10% to 16% CP gel) may be accompanied by significantly greater deleterious effects to pulp cells. Based on the results of the present study, it is believed that this increased toxicity is a result of more alteration of the mineralized dental tissues, allowing a higher influx of HP into tooth structure. This hypothesis is confirmed by clinical studies, which demonstrated a significantly higher prevalence of tooth sensitivity for patients who used 20% CP gel (71.4%) compared with 10% CP gel (36.8%) (25). Other studies demonstrated that the 16% CP gel presents similar results of tooth sensitivity as the in-office bleaching protocol with 35% HP gel (13,14).

This way, since the higher CP concentration causes higher toxic effects in the pulp cells (3), more intense and prevalent tooth sensitivity (8,25), and higher modifications on the mineralized phase of teeth (10-12), with no benefits in the final esthetic outcome (14,15,25), the 10% CP gel appears to be an interesting alternative for tooth bleaching

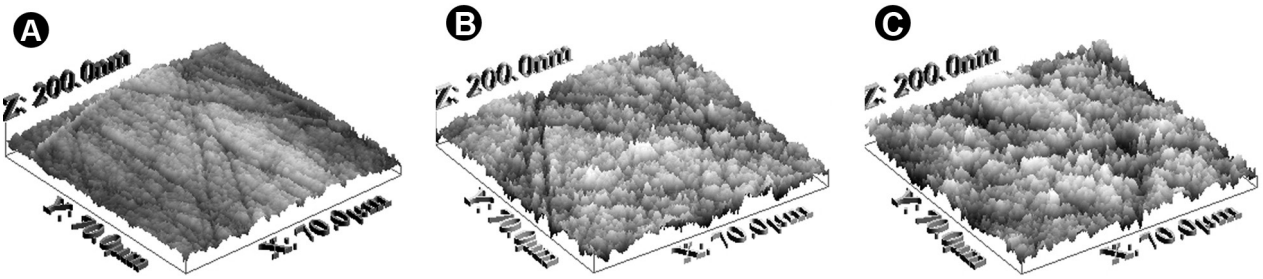

Figure 3. AFM 3D images (Witec, WSxMV 3.1 software). A: Non-bleached enamel (control) - smooth, polished surface. B: Enamel bleached with 10% CP - enamel surface is irregular, exhibiting grooves resulting from polishing of the specimens. C: Enamel bleached with 16% CP - enamel surface is even more irregular, exhibiting numerous pores.

associated with less intense adverse events. However, based on the fact that data from *in vitro* study cannot be directly extrapolated to clinical situations, it is strongly recommended that further *in vivo* studies should be carried out to evaluate the effects of 16% CP gel on enamel, dentin and pulp tissue of patients subjected to tooth bleaching.

According to the methodology used in this *in vitro* study, it may be concluded that both 10 and 16% CP bleaching gels reduced the mineral content and increased the surface roughness of dental enamel, producing a more irregular and porous surface. However, the 16% CP gel promoted the most intense alterations on enamel, even after a single 8-h application.

Resumo

O objetivo do presente estudo foi comparar o efeito de um gel com 16% de peróxido de carbamida (PC) sobre a estrutura mineral e morfologia do esmalte dental com os efeitos de um gel com 10% de PC. Blocos de esmalte provenientes de incisivos bovinos foram submetidos a 14 dias de tratamento (8 h/dia) com géis com 10 ou 16% de PC. A microdureza Knoop foi avaliada antes do clareamento e 1, 7 e 14 dias pós-tratamento (50 g/15 s). O conteúdo mineral (EDX), rugosidade de superfície e topografia (MFA) foram avaliados no período de 14 dias (ANOVA a dois critérios e teste de Tukey; α=0,05). Redução significante na microdureza foi observada nos períodos de 7 e 14 dias para o gel com 10% de PC, e em todos os períodos para o gel com 16% de PC ($p<0,05$). No período de 14 dias, uma diminuição significante no conteúdo de Ca e P, aumento na rugosidade de superfície ($p<0,05$), bem como na distância entre picos e vales foram observados para ambos os agentes clareadores usados. Estas alterações foram mais intensas para o gel com 16% de PC. Pôde-se concluir que ambos os géis à base de PC promoveram perda de estrutura mineral do esmalte, resultando em superfície mais rugosa e porosa. Porém, o gel com 16% de PC causou efeitos adversos mais intensos no esmalte dental.

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