Effect of 10% Carbamide Peroxide Bleaching on Sound and Artificial Enamel Carious Lesions

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This study evaluated the effect of 10% carbamide peroxide (CP) bleaching on Knoop surface microhardness (KHN) and morphology of sound enamel and enamel with early artificial caries lesions (CL) after pH-cycling model (pHcm). Human dental enamel blocks were randomly divided into 6 groups (n=10): 1 - sound enamel bleached (S) with CP (Rembrandt/Den-Mat); 2 - S and submitted to pHcm; 3 - CL bleached with CP; 4 - CL stored in artificial saliva and submitted to pHcm; 5 - CL treated with placebo gel and submitted to pHcm; 6 - CL bleached with CP and submitted to pHcm. Enamel blocks with known initial KHN values were demineralized (groups 3 to 6) and submitted to 12 day pHcm (groups 2, 4, 5 and 6). After demineralization and treatments, KHN was determined and the specimens were examined using scanning electron microscopy (SEM). Data were analyzed statistically by ANOVA and Tukey's test at 5% significance level. The results showed that among CL groups (3 to 6) only the group 3 presented remineralization after treatments. S groups (1 and 2) showed higher KHN and presented less formation of porosities on enamel surface than CL groups after treatments. In conclusion, bleaching procedures on enamel with CL did not exacerbate the demineralization, but should be indicated with caution.

Key Words: carbamide peroxide, caries lesions, dental enamel, microhardness, scanning electron microscopy.

INTRODUCTION

Carbamide peroxide is the active ingredient in most home-bleaching agents. Since the introduction of nightguard vital whitening, concern has been expressed regarding the potential effects of carbamide peroxide solution on dental hard tissues (1). As the bleaching technique of vital teeth comprises the direct contact of the whitening gel on the outer enamel surface, the oxidation reaction for an extended period of time can be related to demineralization effects (2,3).

Various effects of carbamide peroxide bleaching on teeth have been studied. Scanning electron microscopy (SEM) investigations have shown that 10% carbamide peroxide changes enamel, such as surface dissolution and topographical alterations, exposing a porous surface (4-6). The morphological changes and reduction of enamel microhardness are not limited to the enamel surface. Alterations have also been detected in the subsurface enamel layer (7-9).

Moreover, some studies have shown alterations of the histological aspects and composition of sound dental enamel after carbamide peroxide gel application (9,10). The bleaching treatment with 10% carbamide peroxide slightly increased caries susceptibility of enamel (11,12). However, it is not known the effect of carbamide peroxide gels on enamel with early caries lesions. The objective of this study was to evaluate the effect of 10% carbamide peroxide bleaching on surface microhardness and morphology of sound enamel or

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enamel with early artificial caries lesions during pH-cycling model. The hypothesis tested was that the bleaching treatment can alter enamel microhardness and morphology, increasing the mineral loss of enamel with early artificial caries lesions.

MATERIAL AND METHODS

Experimental Design

Erupted human third molars, stored in saturated thymol solution at 5^oC for at least one month were used in this study. The teeth were obtained after informed consent by the patients and under protocol (#141/2002) approved by the local Research Ethics Committee of Piracicaba Dental School, State University of Campinas, Brazil.

Dental enamel blocks (4x4x3 mm) were obtained from teeth and enamel surfaces were wet-polished with 600- and 1200-grit aluminum oxide abrasive papers and diamond paste (1 μm). Sixty enamel blocks with known mean microhardness (404.6 ± 4.5 Knoop Hardness Number - KHN) were randomly divided into two groups: sound group and group that was exposed to a demineralization solution. After induction of artificial early carious lesions, 40 blocks with known surface microhardness were selected for the study and divided into 4 treatments. The experimental groups (n=10) are described bellow and summarized in Table 1: 1) S+10%CP+AS - sound enamel bleached with 10% carbamide peroxide gel (Rembrandt, Den-Mat Corp., Santa Maria, CA, USA) (pH = 6.23) and stored in

artificial saliva at 37°C after each daily bleaching treatment; 2) S+10%CP+pHcm - sound enamel bleached with 10% carbamide peroxide gel and subjected to pH-cycling model; 3) CL+10%CP+AS - enamel with early artificial caries lesions bleached with 10% carbamide peroxide gel and stored in artificial saliva at 37°C after each daily bleaching treatment; 4) CL+AS+pHcm - enamel with early artificial caries lesions stored in artificial saliva at 37°C and subjected to pH-cycling model; 5) CL+pl+pHcm - enamel with early artificial caries lesions treated with placebo gel (5% glycerin, 1.2% carbopol 940, pH 7.0 /Proderma Pharmacy, Piracicaba, SP, Brazil) and submitted to pH-cycling model; 6) CL+10%CP+pHcm - enamel with early artificial caries lesions bleached with 10% carbamide peroxide gel and subjected to pH-cycling model.

After these treatments, Knoop surface microhardness was determined for each group.

Induction of Artificial Caries Lesions

Forty surface enamel blocks with known surface microhardness were coated with acid-resistance varnish, excepted for an area of 8 mm^2. The blocks (groups 3 to 6) were submitted to demineralized solution containing 50 mM acetate buffer; 1.23 mM Ca; 0.73 mM P, 0.1 ppm F, pH 5.0 for 32 h at 37^oC using 2 mL of solution to each 1 mm^2 of exposed area (13,14). Enamel blocks with surface microhardness mean of 28.9 ± 0.4 KHN units were selected.

Treatments and pH-Cycling Regimen

The blocks (groups 2,4,5,6) were subjected to a 12-day pH-cycling (13,14) consisted of five phases: 1) 1-min soak in fluoridated dentifrice (1100 ppm F as NaF)/water slurries twice a day to simulate daily toothbrushing; 2) between the treatments with dentifrice, samples were individually immersed in artificial saliva at 37^oC; 3) to simulate the daily acid challenge, enamel blocks were individually immersed in demineralized solution for 2 h at 37^oC, with the same composition of the solution used in the initial carious lesion procedure; 4) the samples were immersed in human saliva during 2 h at 37^oC before the bleaching treatment to promote acquired pellicle formation; 5) to simulate daily treatment, the samples were exposed to artificial saliva (group 4), placebo gel (group 5), whitening gel

Table 1. Experimental groups evaluated in this study.

Groups	S	CL	AS	10%CP	pH-cycling
1	Yes	No	Yes	Yes	No
2	Yes	No	No	Yes	Yes
3	No	Yes	Yes	Yes	No
4	No	Yes	Yes	No	Yes
5	No	Yes	No	No (PL)	Yes
6	No	Yes	No	Yes	Yes

S = sound enamel; CL = Enamel with early artificial caries lesions; AS = immersion in artificial saliva; 10%CP = treatment with 10% carbamide peroxide; PL = treatment with placebo.

(groups 2 and 6) for 8 h (nocturnal period) at 37°C. In each specimen, 0.1 mL of the artificial saliva, placebo gel or whitening gel and 0.05 mL of artificial saliva, placebo gel or whitening gel) and 0.05 mL of artificial saliva were applied on enamel surface and covered with an individual tray. During bleaching, the specimens were placed in 100% relative humidity at 37°C. After bleaching, the specimens were rinsed with an air/water spray for 10 s and stored in humidity environmental until analysis.

Microhardness Analysis

Surface microhardness was determined in the enamel blocks before (baseline), after demineralization and after experimental treatments. A microhardness tester (FM-1e; Future Tech, Tokyo, Japan) with a Knoop indentor was used under a 50-g load for 5 s. Five indentations spaced 100 μm from each other, from the baseline and from those made after demineralization were performed. Data were analyzed statistically by two-way ANOVA and Tukey's test at a 5% level of significance.

Scanning Electron Microscopy

Two specimens of each group were prepared for analysis with a scanning electron microscope (JSM - 5600; JEOL USA, Inc., Peabody, MA, USA). Specimens were sputter coated with gold in a vacuum evaporator (Desk II; Denton Vacuum Inc., NJ, USA, USA) and SEM micrographs of representative areas of the surfaces were taken at ×5,000 magnification.

RESULTS

Table 1 presents the mean KHN values and standard deviations for experimental groups. Two-way ANOVA revealed statistically significant difference for both factors (treatments and interval measurements) ($p < 0.01$), and significant interaction between them ($p < 0.01$). The groups presented similar baseline mean KHN values ($p > 0.05$). The enamel blocks (groups 3 to 6) that were exposed to the demineralizing solution did not differ statistically among each other ($p > 0.05$). Also, these groups did not show statistically difference after treatments ($p > 0.05$). However, group 3 showed mineral gain after treatments ($p < 0.05$). Sound enamel surfaces submitted to bleaching procedures and not submitted to pH-cycling model (group 1) showed the highest KHN values post-treatment, although, the bleaching treatment reduced the mean KHN value of sound enamel when compared to the baseline value ($p < 0.05$).

Changes in enamel morphology after treatments were observed for all groups, with different levels of surface changes (Figs. 1 and 2). When the enamel was sound, minor alterations was induced by treatments (Figs. 1a and 1b). The carious enamel submitted to bleaching treatment and pH-cycling model presented dissolution of some enamel superficial areas (Fig. 2d).

DISCUSSION

Recent studies have shown that treatment of enamel with either fluoridated dentifrice or acidic carbamide peroxide bleaching gels result in increased susceptibility to subsequent acid erosion and demineralization (15-17). However, it is not known if the 10% carbamide peroxide can promote similar effects on enamel with early caries lesion. The present study evaluated the effect of carbamide peroxide on artificial caries lesion during a pH-cycling model.

According to surface microhardness analysis (Table 2), the groups did not differ significantly in the baseline KHN values. Also, the groups that

Table 2. Mean Knoop Hardness Number (SD) for experimental groups (n=10).

Groups	Baseline	After demineralization	After treatments
1) S+10%CP+AS	402.06 (19.91) Aa	---------	362.64 (48.68) Ab
2) S+10%CP+pHcm	401.50 (21.15) Aa	---------	171.92 (71.97) Bb
3) CL+10%CP+AS	410.47 (25.36) Aa	28.89 (12.02) Bc	44.58 (24.85) Cb
4) CL+AS+pHcm	399.11 (28.30) Aa	28.35 (9.75) Bb	38.43 (19.83) Cb
5) CL+PL+pHcm	405.63 (24.18) Aa	29.30 (12.42) Bb	30.43 (9.24) Cb
6) CL+10%CP+pHcm	403.81 (21.07) Aa	28.77 (10.42) Bb	29.34 (7.15) Cb

Different uppercase letters in columns and lowercase letters in rows indicate statistically significant difference (Tukey's test; $p < 0.05$). S = sound enamel; CL = Enamel with early artificial caries lesions; AS = immersion in artificial saliva; 10%CP = treatmen with 10% carbamide peroxide; PL = treatment with placebo.
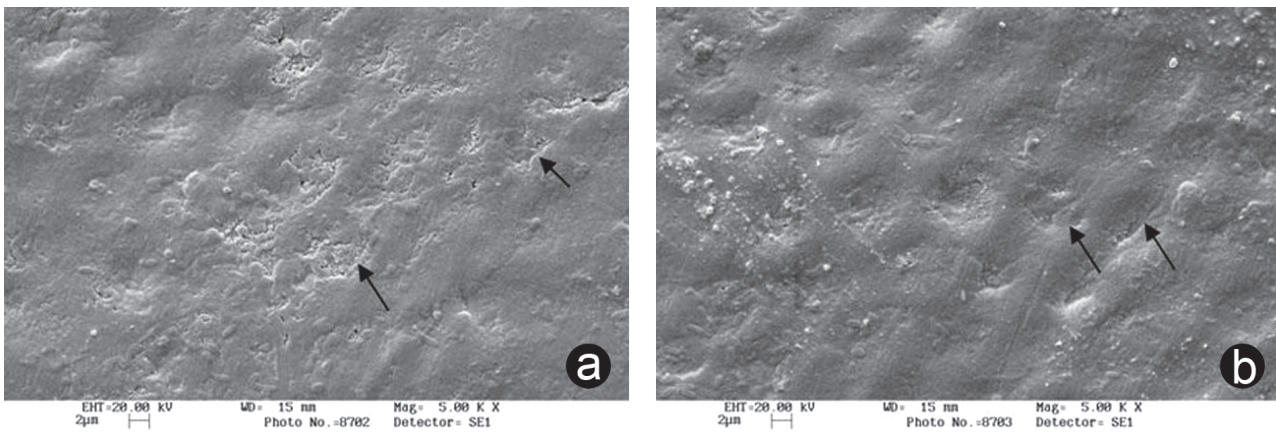
Figure 1. SEM micrographs illustrating the changes in enamel morphology after treatments. A= Sound enamel exposed to 10% carbamide peroxide; B = Sound enamel exposed to 10% carbamide peroxide and subjected to a pH-cycling model. Affected interprismatic structures (arrows) and porosities created by treatments can be noted on enamel surface.
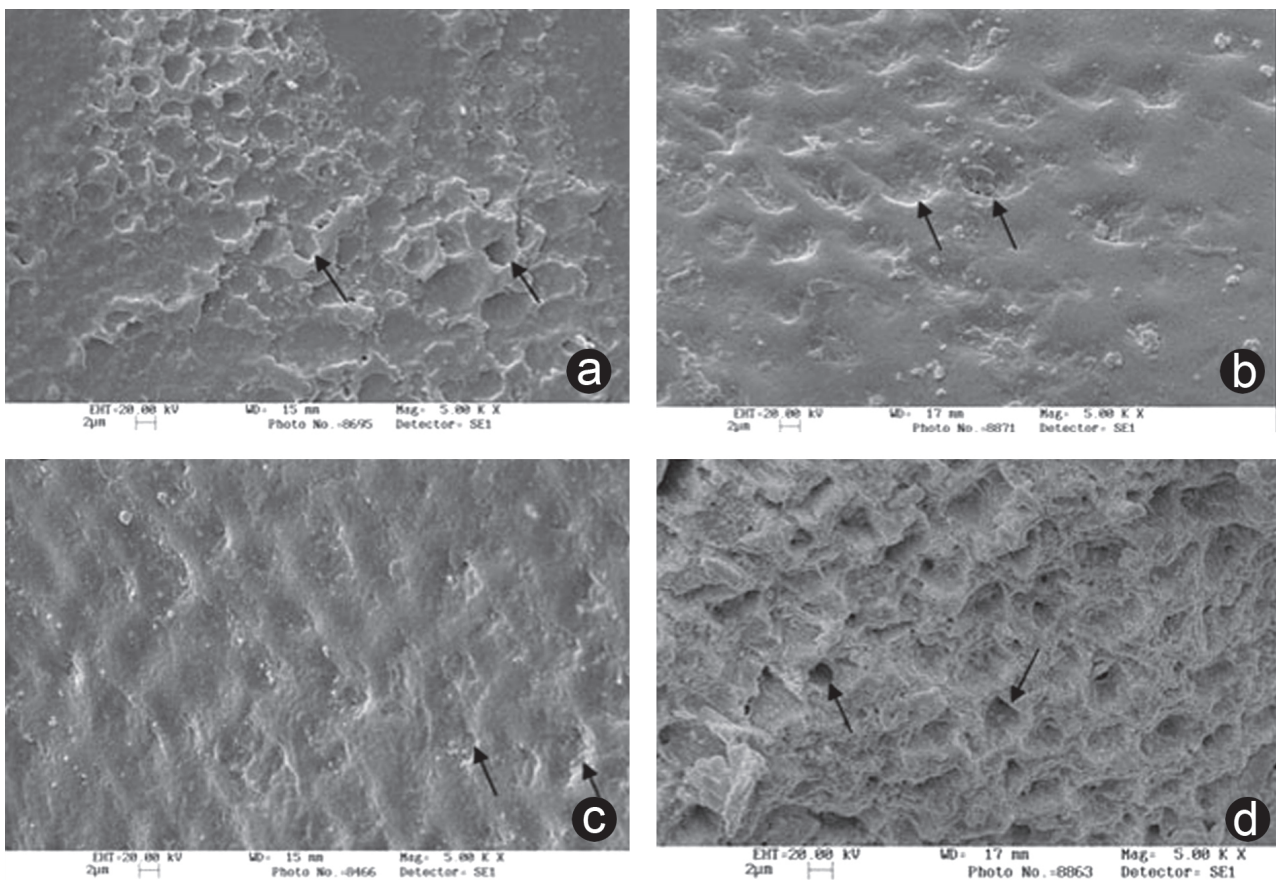

Figure 2. SEM micrographs of carious enamel submitted to bleaching treatment and pH-cycling. A= Enamel with caries lesions exposed to 10% carbamide peroxide; B= Enamel with artificial caries lesions immersed in saliva and subjected to a pH-cycling model; C= Enamel with artificial caries lesions exposed to placebo gel and subjected to pH-cycling; D= Enamel with caries lesions exposed to 10% carbamide peroxide and submitted pH-cycling. Affected interprismatic structures (arrows) can be noted. Erosion and total intraprismatic structure dissolution were observed only in group 6 (Fig. d).

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were exposed to a demineralization solution presented similar KNH values. The study showed homogeneity among the treatments before and after demineralization challenge and so the effect of the treatments could be tested in these groups. After treatments, the results showed that the sound groups (1 and 2) differed from each other and from the caries lesion groups (3, 4, 5 and 6). Groups 3, 4, 5 and 6 did not differ among each other.

Regarding the posttreatment analysis, group 1 (S+10%CP+AS) showed mineral loss when compared to the baseline KHN values. These data confirmed the effect of carbamide peroxide in promoting enamel mineral loss (1-4,16,18) and surface morphological changes (4-9,16) (Fig. 1a). Group 2 (S+10%CP+pHcm) showed higher mineral loss than group 1most likely because it also underwent a 12-day pH-cycling model simulating daily cariogenic challenge. Although, the enamel was treated twice with fluoridated dentifrice, it was not able to inhibit the demineralization because it was exposed to a combination of effects, such as 10% carbamide peroxide, which already increased the mineral loss (group 1), and pH-cycling. This non-effect of fluoride in inhibiting mineral loss can be attributed to the fact that carbamide peroxide could promote enamel dissolution or erosion (e.g.: intraprismatic structure removal) (4,9,16), impairing accurate surface microhardness measurement; or perhaps the fluoridated dentifrice actually inhibited higher mineral loss, since it is established the effect of fluoride dentifrice on remineralizing artificial caries lesions (13,14,19).

With respect to the groups with artificial caries lesion, only group 3 (CL+10%CP+AS) showed statistically significant remineralization when compared to the post-demineralization KHN values. This group also showed the formation of porosities (Fig. 2a) due to exposure to carbamide peroxide and artificial saliva, while the other groups were subjected to pH-cycling. Although the enamel blocks received daily treatment of 10% carbamide peroxide, these data suggested that artificial saliva was able to enhance surface microhardness, confirming the role of saliva in remineralizing caries lesion (13,19,20).

Groups 4 (CL+AS+pHcm), 5 (CL+pl+pHcm) and 6 (CL+10%CP+pHcm) did not show statistically significant remineralization after pH-cycling (Table 1). According to these results it can be suggested that the 10% carbamide peroxide can be indicated in situations of caries activity, since saliva and fluoridated dentifrice

seems to inhibit higher mineral loss. Although 10% carbamide peroxide bleaching (group 6) did not show statistically higher mineral loss when compared to the other groups (group 4-treated with artificial saliva and group 5- treated with placebo gel), the SEM micrographs revealed intraprismatic dissolution and lack of smoothness in some areas of enamel surface (Fig. 2d).

Some hypotheses could explain the non-statistically significant results among groups 4, 5 and 6. the small number of samples (n=10) and the variability of samples, leading to high standard deviations; 2. The demineralizing solution used in this study promotes high mineral loss without erosion and, perhaps, it was too aggressive showing 28.9 ± 0.4 KHN values. This high mineral loss could impair surface mineral recovery because the prisms could be totally disorganized, as reported by several authors (4-6) and observed in Figure 2d; 3. The method used to evaluate mineral changes, Knoop microhardness test is an excellent method to determine the remineralization of caries lesions with fluoridated dentifrice, and maybe, the treatment with bleaching gel could alter the surface structure, masking the remineralization effect of saliva and fluoridated dentifrice; 4. The results of this study do not agree with those of Costa and Mazur (18), who observed that remineralization of bleached enamel is improved by application of fluoride. However, those authors used high fluoride concentration while the present study used fluoride solution with the same dilution that occurs in mouth during toothbrushing.

In conclusion, the findings of the present study showed that carbamide peroxide bleaching promoted mineral loss of sound enamel. Although, the bleaching procedure did not exacerbate mineral loss of the carious enamel, it should be indicated with caution on enamel with caries activity.

RESUMO

Este estudo analisou o efeito do peróxido de carbamida a 10% (PC) na microdureza Knoop de superfície (KHN) e morfologia do esmalte hígido e com lesões iniciais de cárie artificial (EC), após modelo de ciclagem de pH (cpH). Blocos de esmalte dental humano foram divididos aleatoriamente em 6 grupos (n=10): 1- esmalte hígido clareado (EHC) com PC (Rembrandt/Den-Mat); 2- EHC e submetido a cpH; 3- EC clareado com PC; 4- EC armazenado em saliva artificial e submetido a cpH; 5- EC tratado com gel placebo e submetido a cpH; 6- EC clareado com PC e submetido a cpH. Blocos de esmalte com a KHN conhecida eram desmineralizados (grupos 3 a 6) e submetidos a cpH (grupos 2, 4,

5 e 6). KHN foi determinada após a desmineralização e os tratamentos. Os espécimes foram examinados através de Microscopia Eletrônica de Varredura (MEV). Os dados foram analisados através de ANOVA e teste de Tukey (p<0,05). Os resultados indicaram que entre os grupos com EC (3 a 6) somente o grupo 3 apresentou remineralização após os tratamentos. Os grupos EHC (1 e 2) mostraram maior KHN e menor formação porosidades quando comparados aos grupos EC após os tratamentos. Os procedimentos clareadores no esmalte com lesão de cárie não exacerbaram a desmineralização, entretanto precisam ser indicados com cautela.

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