The effect of 10% carbamide peroxide, carbopol and/or glycerin on enamel and dentin microhardness

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The Effect of 10% Carbamide Peroxide, Carbopol and/or Glycerin on Enamel and Dentin Microhardness

RT Basting • AL Rodrigues, Jr • MC Serra

Clinical Relevance

Changes in enamel and dentin microhardness may be related not only to carbamide peroxide, but also to the presence of other components in bleaching agents, such as carbopol and glycerin. Carbopol and its associations may cause alterations in microhardness compared to Opalescence. None of the treatment agents or associations evaluated was inert for dental microhardness, although glycerin seemed to affect enamel and dentin to a lesser degree.

SUMMARY

This study evaluated the effects of 10% carbamide peroxide, carbopol and glycerin and their associations on microhardness over time on enamel and dentin. Eight treatment agents were evaluated: a commercial bleaching agent containing 10% carbamide peroxide (Opalescence 10% Ultradent), 10% carbamide peroxide, carbopol, glycerin, 10% carbamide peroxide + carbopol, 10% carbamide peroxide + glycerin, carbopol + glycerin and 10% carbamide peroxide + carbopol + glycerin. Three hundred and twenty human dental fragments, 80 sound enamel fragments (SE), 80 demineralized enamel fragments (DE), 80 sound dentin fragments (SD) and 80 demineralized dentin (DD) fragments, were exposed to the treatment agents (n=10). These agents were applied onto the surface of the fragments eight hours a day for 42 days. After eight hours, they were washed from the dental fragment surfaces after five back-and-forth movements with a soft bristle toothbrush under distilled and deionized running water. During the remaining time (16 hours per day), the fragments were kept in individual vials in artificial saliva. After the 42-day treatment period, the specimens were kept individually in artificial saliva for 14 days. Knoop microhardness measurements were performed at baseline, after eight hours, and 7, 14, 21, 28, 35 and 42 days, and 7 and 14 days post-treatment (corresponding to 49 and 56 days after the initial treatment agent applications). The non-parametric Kruskal-Wallis analysis showed significant differences among the agents at each time interval, except at baseline for sound and demineralized enamel and dentin. For SE, SD and DD, there was a decrease in microhardness values during treatment with all agents. There was a tendency towards lower microhardness values after treatment with carbopol and its associations for

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sound tissues. DD showed low microhardness values during and after treatment with CP and its associations. For DE, there was an increase in microhardness values during treatment with all agents and in the post-treatment phase. The baseline microhardness values were not recovered during the 14-day post-treatment phase. Opalescence 10%, carbamide peroxide, carbopol, glycerin and their associations may change the microhardness of sound and demineralized dental tissues, even in the presence of artificial saliva.

INTRODUCTION

Bleaching procedures with 10% carbamide peroxide agents have been used as a simple and effective technique for the removal of intrinsic and extrinsic stains (Haywood, 1994; Haywood, 2000). The clinical protocol employs a bleaching agent in a tray for two to eight hours during the day or night for two to six weeks of treatment (Haywood & Heymann, 1989; Haywood, 2000; Ritter & others, 2002).

Ten percent carbamide peroxide seems to be effective and safe (Curtis & others, 1996; Ritter & others, 2002) and has the American Dental Association acceptance seal for some brands (Haywood, 1993; Haywood & Robinson, 1997). The addition of carbopol and glycerin as thickening agents improves adherence of the bleaching agent to the surface of dental structure, allowing for a prolonged time for the release of carbamide peroxide (Haywood, 1994; McCracken & Haywood, 1996).

Because the bleaching of vital teeth involves direct contact of the whitening agent with the outer surface of enamel and dentin in areas of defects, abfraction or abrasion lesions, exposed root surfaces and marginal areas between teeth and restorations, many studies have evaluated the potential effects of these agents on superficial micromorphology, changes in mineral content and microhardness. Scanning electron microscopic evaluations have reported porosities and erosion on enamel (Ben-Amar & others, 1995; Bitter, 1998; Bitter & Sanders, 1993; Ernst, Marroquin & Willershausen-Zonnchen, 1996; Flaitz & Hicks, 1996; Josey & others, 1996; Shannon & others, 1993; Smidt, Weller & Roman, 1998; Zalkind & others, 1996) and dentin (Zalkind & others, 1996). In vitro studies have also reported some alterations in mineral content and both enamel and dentin microhardness after exposure to 10% carbamide peroxide (Attin & others, 1997; Basting, Rodrigues & Serra, 2003; Freitas & others, 2002; Oliveira & others, 2003; McCracken & Haywood, 1995, 1996; Pécora & others, 1994; Rodrigues & others, 2001; Rotstein & others, 1996; Smidt & others, 1998; Seghi & Denry, 1992).

Changes in enamel and dentin microhardness may be related not only to the acidic pH of the bleaching agents, which is responsible for a prolonged storage time of the product, but also to the presence of other components in commercial bleaching agent products. McCracken and Haywood (1995) verified a significant decrease in microhardness in the outer 25.0 µm of enamel surface after treatment with a product containing carbopol. Basting and others (2003) also reported a significant decrease in enamel surface microhardness when using a placebo agent with carbopol and glycerin with a neutral pH, even in the presence of artificial saliva. Freitas and others (2002) showed the same behavior for this product in dentin. However, no in vitro studies evaluated the effects of bleaching agents on demineralized dental tissues. Bleaching agents have possibly been applied to active carious lesions in enamel and dentin.

In an *in situ* study, Basting, Rodrigues and Serra (2001) observed significant differences in enamel microhardness after treatment with 10% carbamide peroxide bleaching agent and a placebo containing carbopol and glycerin. The sound and demineralized enamel submitted to the 10% carbamide peroxide bleaching agent showed significantly lower microhardness values than that submitted to a placebo agent. However, no differences were found between the sound and demineralized dentin treated with bleaching or placebo agents, but slightly higher microhardness values for dentin exposed to a bleaching product.

However, the isolated effects of carbopol, glycerin and 10% carbamide peroxide, and even the combined effects of those components on the microhardness of sound and demineralized enamel and dentin tissues, are also unknown.

This study evaluated *in vitro* the effects of 10% carbamide peroxide, carbopol, glycerin and their associations on the microhardness of sound and demineralized enamel and dentin tissues and compared their values with those of a 10% carbamide peroxide commercial bleaching product at different time intervals.

METHODS AND MATERIALS

Experimental Design

The factors under study were:

Treatment agents (eight levels): Opalescence 10% Ultradent, 10% carbamide peroxide; carbopol, glycerin, 10% carbamide peroxide + carbopol, 10% carbamide peroxide + glycerin, carbopol + glycerin, and 10% carbamide peroxide + carbopol + glycerin.

Time (nine levels): baseline, 8 hours, and 7, 14, 21, 28, 35 and 42 days of treatment, and 7 and 14 days post-treatment period (corresponding to 49 and 56 days after the beginning of the bleaching treatment).

The experimental units consisted of 320 dental slabs: 80 sound enamel slabs; 80 demineralized enamel slabs; 80 sound dentin slabs and 80 demineralized dentin slabs. Ten dental fragments of each dental tissue (n=10)

were randomly and evenly assigned to the eight different treatment agents. The effects of the different treatment agents on enamel were not compared to dentin, neither were the effects of sound tissues compared to the demineralized ones.

Three repeated measurements of Knoop microhardness were taken from the surface of each specimen at each time interval.

Dental Fragments Preparation

This study had the approval of the FORP/USP Ethical Committee Guidelines in accordance with the National Health Council (Conselho Nacional de Saúde, 2003). Seventy-seven non-erupted third molars were used. Immediately after extraction for reasons other than the experiment, the teeth were kept in 0.1% thymol. They were sectioned with double-faced diamond discs (KG Sorensen, Barueri, SP, Brazil) at a low motor speed (Kavo do Brasil, Joinville, SC, Brazil), dividing the root from the coronary portion to obtain 320 dental slabs with 3 mm x 3 mm x 2 mm (160 enamel slabs and 160 dentin slabs). In the root, the apical third was discarded and only the cervical region was used. Care was taken not to leave the dental fragments dehydrated for long periods. Those slabs that presented stains or cracks after observation under stereomicroscope loupe (Meiji Techno EMZ Series, Saitama, Japan) at 30x were discarded.

The dental fragments were embedded individually in a self-curing polyester resin in a polyvinylchloride ring mold 2.0-cm in diameter, with the external surface of the enamel or dentin exposed. The molds were removed and the external surfaces of the dental fragments were leveled by a water-cooled mechanical grinder (Maxgrind/Solotest, São Paulo, Brazil). These procedures were conducted to form parallel planar surfaces for the Knoop microhardness tests. For the enamel surfaces, aluminum oxide discs of 400, 600 and 1000 grit were used sequentially (Carborundum/3M do Brasil Ltda, Sumaré, Brazil) with water cooling. Polishing was performed using polishing cloths (Top, Gold and Ram, Arotec Ind e Com Ltda, Cotia, Brazil) and diamond pastes of 6, 3, 1 and 1/4 µm (Arotec Ind e Com Ltda) with mineral oil cooling (Red lubricant, Arotec Ind e Com Ltda). For the dentin fragments, only aluminum oxide discs were used in a sequential granulation of 600, 1000 and 1200 grit (Carborundum/3M do Brasil Ltda, Sumaré, Brazil) with water cooling. Between each sequential disc, the dental fragments were immersed in an ultrasonic distillated water bath for 10 minutes.

Dental Slabs Preparation

To obtain 80 demineralized enamel slabs and 80 demineralized dentin slabs, caries-like lesions were generated by a dynamic model of demineralization and remineralization cycles similar to the model proposed by Featherstone and others (1986) and modified by Delbem and Cury (2002).

The enamel and dentin fragments were submitted to cycles of de-remineralization. The group that made up the sound group of each dental tissue was not submitted to the de-remineralization cycles; instead, the specimens were stored in a humid environment.

Specification of the Treatment Agents

The treatment agents are presented at Table 1 according to composition and manufacturer.

A 10% carbamide peroxide commercial bleaching agent (Opalescence 10% Ultradent, South Jordan, UT, USA) was used as a control as it is accepted as safe and effective by the American Dental Association (ADA). It contains 10% carbamide peroxide and amounts of carbopol and glycerin not specified by the manufacturer. The flavor tested was "regular."

The treatment agents evaluated include 10% carbamide peroxide (CP), carbopol (C) and glycerin (G). Their associations were also tested: 10% carbamide peroxide + carbopol (CP + C), 10% carbamide peroxide + glycerin (CP + G), carbopol + glycerin (C + G) and 10% carbamide peroxide + carbopol + glycerin (CP + C + G). These products were freshly obtained and/or prepared in a dispensing pharmacy. The consistency of the asso-

Table 1: *Composition and Manufacturer of Each Treatment Agent*

Treatment Agents	Composition	Manufacturer
Opalescence 10% (OPA) carbopol; glycerin; flavoring*	10% carbamide peroxide;	Ultradent Products Inc, South Jordan, UT, USA
10% carbamide peroxide (CP)	10% carbamide peroxide	Proderma – Pharmacy, Piracicaba, Brazil
Carbopol (C)	Carbopol	Proderma – Pharmacy, Piracicaba, Brazil
Glycerin (G)	Glycerin	Proderma – Pharmacy, Piracicaba, Brazil
10% carbamide peroxide + carbopol (CP + C)	10% carbamide peroxide + carbopol	Mixed formula, Proderma – Pharmacy, Piracicaba, Brazil
10% carbamide peroxide + (CP + G)	10% carbamide peroxide + glycerin	Mixed formula, Proderma – Pharmacy, Piracicaba, Brazil
Carbopol + glycerin (C + G)	Carbopol + glycerin	Mixed formula, Proderma – Pharmacy, Piracicaba, Brazil
10% carbamide peroxide + carbopol + glycerin (CP + C + G)	10% carbamide peroxide + carbopol + glycerin	Mixed formula, Proderma – Pharmacy, Piracicaba, Brazil

**The manufacturer does not indicate the percentage of each component.*

ciation of carbamide peroxide + carbopol + glycerin and carbopol + glycerin was similar to the commercial product.

Application of Treatment Agents

Prior to treatment, an individual tray for each specimen was manufactured from a 1.0-mm thick flexible ethyl vinyl acetate polymer (Bio-Art Equipamentos Odontológicos Ltda, São Carlos, Brazil) placed in a vacuum-forming machine (P7, Bio-Art Equipamentos Odontológicos Ltda).

Both the sound and demineralized enamel and dentin fragments were exposed to the treatment agents eight hours a day for 42 days. A syringe was used to apply 0.02 mL of each agent to each specimen. The specimens were individually covered with a tray and soaked in individual closed vials with 13.5 mL of artificial saliva (pH 7.0) at 37°C ± 2°C.

After eight hours, the treated specimens were taken out of the storage media and the trays removed. The treatment agents were removed from the dental fragment surfaces by making five back-and-forth movements with a soft bristle toothbrush (Oral B 35/Gillette do Brasil Ltda, Manaus, Brazil) to remove the viscous film that formed on the fragment surfaces, which was then washed under distilled and deionized running water for five seconds.

During the remaining daily time (16 hours per day), the fragments were kept in individual vials with 13.5 mL of artificial saliva (pH 7.0) at 37.0°C ± 2.0°C. The artificial saliva was changed every two days and consisted of a remineralization solution proposed by Featherstone and others (1986) and modified by Delbem and Cury (2002). It contained calcium hydroxide, phosphoric acid, potassium chloride, buffering agent and deionized and distilled water.

This cycle was repeated for 42 days, corresponding to the maximum clinical period for a bleaching treatment of six weeks as recommended by Haywood and Heymann (1989).

Post-treatment Phase

After the 42-day treatment period, the specimens were kept in their individual vials with 13.5 mL of artificial saliva (pH 7.0) at 37.0°C ± 2.0°C during 14 days. The artificial saliva was also changed every two days. Thus, the possible remineralizing effects of the artificial saliva on the microhardness of sound and demineralized dental fragments could be evaluated.

Microhardness Testing

Microhardness measurements were taken before initial exposure to the treatment agents (baseline) after 8 hours and 7, 14, 21, 28, 35 and 42 days, and 7 and 14 days post-treatment (corresponding to 49 and 56 days after initial application of the treatment agents). A Knoop indenter was used; the long axis of the diamond was kept parallel to the dentinal surface in a microhardness testing machine (Future Tech, FM-1e, Tokyo, Japan). Three microhardness measurements were taken on each specimen at different time intervals. A load of 25.0 grams was used for the enamel fragments and a load of 10 grams was used for the dentin for five seconds.

Statistical Analysis

Knoop microhardness responses were statistically evaluated by Kruskal-Wallis test, followed by pair-wise multiple comparison (Conover, 1971), according to dental slab tissue/type (enamel or dentin, sound or demineralized). Means were obtained after triplicates were averaged. The response value at each time was then subtracted from its respective baseline mean, yielding the ultimate response value.

RESULTS

Statistical analysis showed significant differences among the agents at each time interval, except at baseline, for sound and demineralized enamel and dentin.

Table 2 and Figure 1 show the means and results of the pair-wise multiple comparisons for the sound

Table 2: *Means and the Results of Pair-wise Comparisons of the Knoop Microhardness Difference Values for Sound Enamel Slabs*

Time (hours)	Treatment Agents							
	OPA	CP	C	G	CP + C	CP + G	C + G	C + G + CP
8	-71.4 d	-32.6 g	-200.8 a	-34.1 f	-205.3 a	-73.8 e	-113.1 b	-84.6 c
168	-103.2 b	-50.4 d	-297.8 a	-83.9 c	-303.3 a	-140.3 b	-306.5 a	-87.9 c
336	-83.8 c	-26.1 c	-314.0 a	-71.2 d	-310.0 a	-83.0 c	-302.6 a	-89.2 b
504	-106.9 c	-29.8 d	-317.7 a	-44.8 d	-311.7 b	-108.9 c	-325.3 ab	-95.1 c
672	-140.4 b	-14.3 e	-316.7 a	-54.6 d	-310.7 a	-106.6 c	-324.7 a	-97.4 c
840	-107.8 c	-4.9 f	-318.4 a	-56.5 e	-309.0 ab	-70.7 d	-305.0 b	-107.7 c
1008	-120.3 b	-7.0 e	-317.9 a	-27.5 d	-305.4 a	-99.7 c	-313.4 a	-109.6 b
1176	-90.2 b	18.9 d	-301.7 a	-27.1 c	-308.8 a	-60.2 c	-301.0 a	-94.8 b
1344	-76.7 c	1.5 ed	-298.4 a	-52.7 e	-298.7 a	-44.3 d	-303.4 a	-82.0 b

**Equal letters horizontally indicate mean values that are not significantly different.*

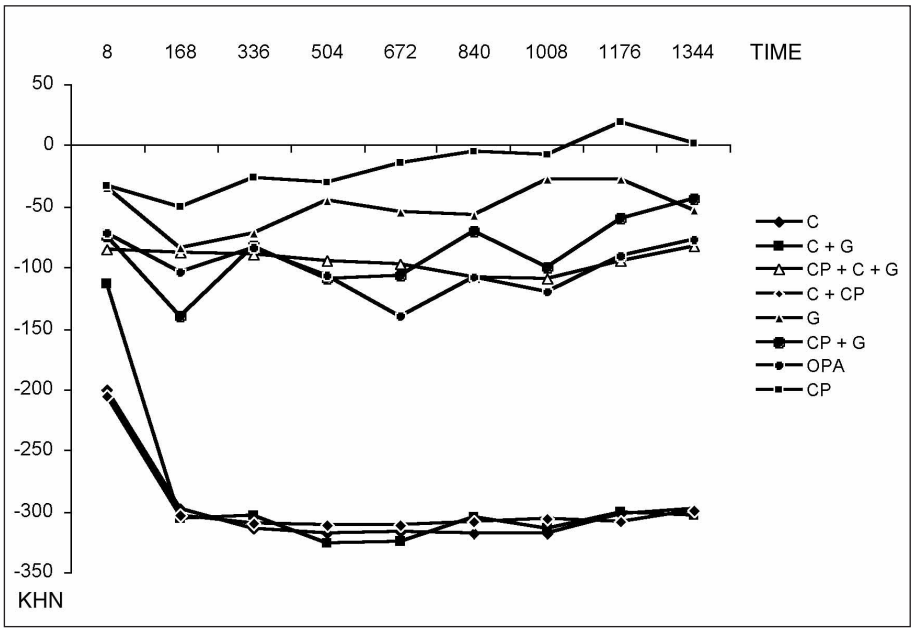

Figure 1: *Linear diagram of the means of Knoop Microhardness Number (KHN) differences for each treatment agent over time for sound enamel slabs.*

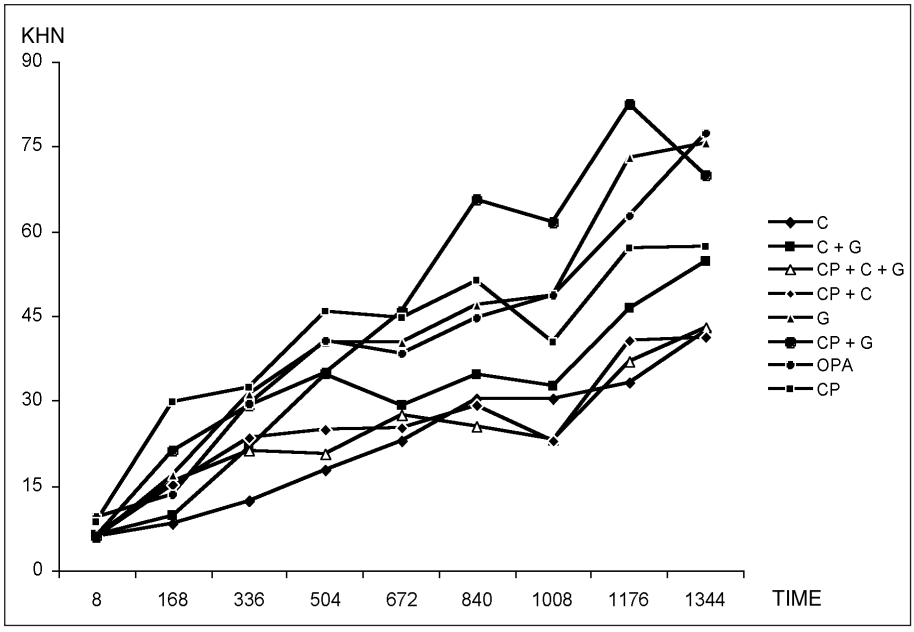

Figure 2: *Linear diagram of the means of Knoop Microhardness Number (KHN) differences for each treatment agent over time for demineralized enamel slabs.*

enamel fragments. There was a decrease in enamel microhardness values over time for all agents evaluated. Lower microhardness values were obtained after treatment with C, C + G and CP + C, even eight hours after application and during the post-treatment period. An increase in microhardness values above baseline was observed when using CP during the post-treatment phase.

For the demineralized enamel fragments, there was an increase in microhardness values during treatment with all the agents and in the post-treatment phase (Table 3 and Figure 2).

There was a decrease in microhardness values for sound dentin fragments after treatment with all agents; however, lower values were obtained with the use of OPA, C, CP + C, CP + G and CP + C + G (Table 4 and Figure 3).

The demineralized dentin fragments showed a decrease in microhardness values during the treatment period for all agents, mainly after the application of CP, CP + G, C + G and CP + C + G. However, these fragments showed an increase in microhardness values during the post-treatment phase, which was not observed for the other dental tissues (Table 5 and Figure 4).

DISCUSSION

Although research has been conducted to evaluate the effects of bleaching agents on enamel and dentin (Attin & others, 1997; Ben-Amar & others, 1995; Bitter, 1998; Bitter & Sanders, 1993; Ernst & others, 1996; Flaitz & Hicks, 1996; Josey & others, 1996; McCracken & Haywood, 1995, 1996; Nathoo, Chmielewski & Kirkup, 1994; Pécora & others, 1994; Rodrigues & others, 2001; Rotstein & others, 1996; Seghi & Denry, 1992; Shannon & others, 1993; Smidt & others, 1998; Zalkind & others, 1996), it does not consider the isolated effects of each component of these products, which may adversely affect dental hard tissues. Different brands of 10% carbamide peroxide bleaching agents present different effects on enamel and dentin, and this variation may be related to the composition of each product (Basting & others, 2003; McCracken & Haywood, 1996).

The chemistry of carbamide peroxide bleaching agents is based on its ability to generate free radicals, which are highly reactive. The free radicals of hydrogen peroxide are non-specific, extremely unstable and can potentially react not only with the pigmented carbon rings, but also with other organic molecules to achieve stability (Goldstein & Garber, 1995). Thus, changes in the chemical or morphological structure of a tooth must

be of concern when using bleaching techniques as a treatment for whitening teeth. Although some studies have reported no significant changes in dental microhardness when using short-term regimens of carbamide peroxide (Nathoo & others, 1994; Potocnik, Kosec, Gaspersic, 2000; Seghi & Denry, 1992; Shannon & others, 1993), others observed a decrease in enamel and dentin microhardness when using these bleaching agents for two weeks or more, even with the use of artificial saliva or fluoride solutions (Attin & others, 1997; Basting & others, 2003; Freitas & others, 2002; McCracken & Haywood, 1995; Oliveira & others, 2003; Rodrigues & others, 2001; Smidt & others, 1998).

In this study, a decrease in microhardness for sound enamel and dentin was found even after eight hours of treatment with all agents. Although the remineralizing effect of saliva was expected during the 16-hours of immersion in artificial saliva, a slow, continuing decrease and maintenance of low values of enamel and dentin microhardness was observed throughout the experimental phase.

Some of the thickening agents in saliva substitutes generally use carbopol, carboxymethylcellulose or other polymers (Christersson, Lindh & Arnebrandt, 2000; Van der Reijden & others, 1997). In this study, the artificial saliva used was supersaturated in minerals and no salivary proteins were considered (Featherstone & others, 1986), but its remineralization effect was observed during the post-treatment period.

Polymers used as thickening agents for saliva substitutes largely inhibited further demineralization, except carbopol, which causes demineralization, especially in a remineralization solution. Carbopol completely inhibited hydroxyapatite crystal growth because of its high calcium-binding capacity (Van der Reijden & others, 1997). Carbopol was not added as an ingredient to the artificial saliva used in this study, but it was evaluated alone or in combination with glycerin and carbamide peroxide. A decrease in microhardness values for sound enamel and dentin during treatment with almost all agents containing carbopol was observed, showing a continuing demineralization of enamel and dentin at neutral pH. In a microhardness evaluation comparing the effects of two 10% carbamide peroxide bleaching agents with and without carbopol on enamel, McCracken and Haywood (1995) showed a significant decrease in microhardness in the outer 25 µm of the enamel surface after treatment with the product containing carbopol. This difference was related not only to the pH

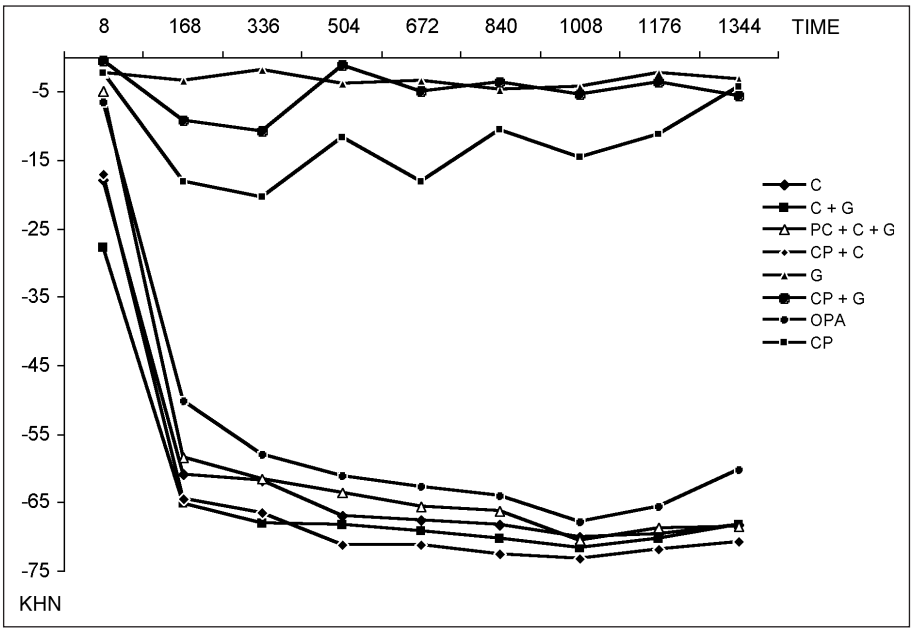

Figure 3: *Linear diagram of the means of Knoop Microhardness Number (KHN) differences for each treatment agent over time for sound dentin slabs.*

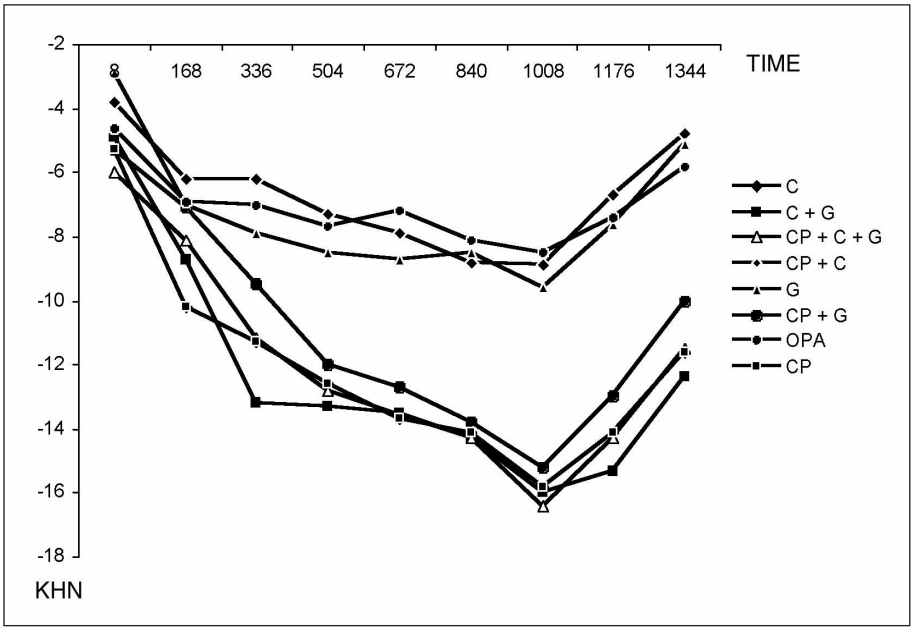

Figure 4: *Linear diagram of the means of Knoop Microhardness Number (KHN) differences for each treatment agent over time for demineralized dentin slabs.*

Table 3: *Means and the Results of Pair-wise Comparisons of the Knoop Microhardness Difference Values for Demineralized Enamel Slabs*

Time (hours)	Treatment Agents							
	OPA	CP	C	G	CP + C	CP + G	C + G	C + G + CP
8	9.4 d	8.5 d	6.1 ab	5.8 a	6.0 ab	5.9 c	6.3 abc	6.4 bc
168	13.5 c	29.7 h	8.2 a	17.0 f	15.2 d	21.3 g	9.7 b	15.7 e
336	29.5 d	32.3 d	12.4 a	31.2 d	23.5 c	29.2 d	21.8 c	21.2 b
504	40.7 f	46.0 f	17.9 a	40.4 e	24.8 b	35.0 d	34.8 c	20.7 a
672	38.4 c	44.7 d	23.0 a	40.3 c	25.2 a	45.9 e	29.1 b	27.6 a
840	44.8 d	51.4 d	30.4 b	47.0 d	29.3 a	65.7 e	34.8 c	25.4 a
1008	48.6 e	40.5 d	30.3 b	48.7 e	22.9 a	61.5 f	32.8 c	23.3 a
1176	62.8 d	57.1 c	33.2 a	73.2 d	40.8 a	82.6 e	46.4 b	36.9 a
1344	77.5 f	57.3 d	42.5 b	75.8 e	41.2 a	69.9 e	54.7 c	43.1 b

**Equal letters horizontally indicate mean values that are not significantly different.*

Table 4: *Means and the Results of Pair-wise Comparisons of the Knoop Microhardness Difference Values for Sound Dentin Fragments*

Time (hours)	Treatment Agents							
	OPA	CP	C	G	CP + C	CP + G	C + G	C + G + CP
8	-6.6 c	-2.2 e	-18.0 b	-2.3 e	-17.0 ab	-0.5 e	-27.8 a	-4.9 d
168	-50.1 e	-18.1 f	-60.8 c	-3.3 h	-64.4 b	-9.2 g	-65.1 a	-58.4 d
336	-58.0 e	-20.4 f	-61.9 c	-1.9 h	-66.4 b	-10.7 g	-68.0 a	-61.5 d
504	-61.2 e	-11.7 f	-67.0 c	-3.8 g	-71.3 a	-1.1 g	-68.2 b	-63.6 d
672	-62.7 d	-18.1 e	-67.5 c	-3.3 f	-71.3 a	-5.0 f	-69.1 b	-65.6 c
840	-64.0 e	-10.6 f	-68.3 c	-4.8 g	-72.6 a	-3.6 g	-70.3 b	-66.2 d
1008	-67.9 c	-14.6 d	-70.0 b	-4.2 e	-73.2 a	-5.3 e	-71.6 a	-70.5 a
1176	-65.6 d	-11.3 e	-69.7 c	-2.3 f	-71.9 a	-3.6 f	-70.2 ab	-68.8 b
1344	-60.3 c	-4.3 de	-68.3 b	-3.1 e	-70.7 a	-5.7 d	-68.3 ab	-68.4 ab

**Equal letters horizontally indicate mean values that are not significantly different.*

Table 5: *Means and the Results of Pair-wise Comparisons of the Knoop Microhardness Difference Values for Demineralized Dentin Fragments*

Time (hours)	Treatment Agents							
	OPA	CP	C	G	CP + C	CP + G	C + G	C + G + CP
8	-4.6 b	-5.3 c	-5.3 e	-2.9 d	-3.8 a	-5.3 cd	-4.9 b	-6.0 b
168	-6.9 de	-10.2 e	-10.2 g	-7.0 f	-6.2 c	-7.1 d	-8.7 a	-8.1 b
336	-7.0 d	-11.3 f	-11.3 h	-7.9 g	-6.2 c	-9.5 e	-13.2 b	-11.2 a
504	-7.7 c	-12.6 d	-12.6 f	-8.5 e	-7.3 b	-12.0 d	-13.3 b	-12.8 a
672	-7.2 b	-13.7 d	-13.7 e	-8.7 d	-7.9 a	-12.7 c	-13.5 a	-13.6 a
840	-8.1 c	-14.1 d	-14.1 e	-8.5 d	-8.8 a	-13.8 d	-14.3 b	-14.3 ab
1008	-8.5 c	-15.8 e	-15.8 f	-9.6 e	-8.9 a	-15.2 d	-16.0 b	-16.4 a
1176	-7.4 c	-14.1 de	-14.1 f	-7.6 e	-6.7 b	-13.0 d	-15.3 b	-14.3 a
1344	-5.8 b	-11.6 c	-11.6 f	-5.1 c	-4.8 a	-10.0 d	-12.4 a	-11.5 a

**Equal letters horizontally indicate mean values that are not significantly different.*

level of the products, but also to the presence of carbopol. Probably, the neutralizing effect of saliva in the mouth and the combination of carbopol with other components of bleaching agents may reduce its negative effect on dental microhardness, although other formulations may be developed for reducing the hazardous effects of this product on dental mineral content.

Although carbamide peroxide was thought to significantly change microhardness values for sound dental tissues due to the release of hydrogen peroxide and urea, this agent and its association with glycerin showed slight decreases compared to other agents evaluated, probably due to the rise in the hydrogen ion concentration (pH) of the solution (Haywood & Heymann,

1989). Urea is capable of penetrating into enamel and affecting not only the surface, but also the interprismatic regions of enamel. The increase in enamel permeability may cause structural changes (Arends & others, 1984; Goldberg & others, 1983) due to the dissociation of H-bonds between the CO and NH groups (Goldberg & others, 1983). It denatures proteins and causes conformational changes, although the increased porosity of the outer enamel surface shown by Hegedüs and others (1999) may be caused mainly by nascent oxygen when released in the inner structure. When using 10% carbamide peroxide on sound dental tissues for seven days, Zalkind and others (1996) showed moderate morphological changes in the dentin surface, but none in enamel. Rotstein and others (1996) also showed an increase in the calcium levels of enamel following treatment with 10% carbamide peroxide, although there was a decrease in the calcium/phosphorus ratio and potassium levels of dentin. Changes in the levels of these minerals may indicate damage to the organic component of the matrix, especially in dentin, due to the higher organic concentration.

Glycerin also presented slight decreases in microhardness for sound enamel and dentin, similar to the effect of carbamide peroxide. It could act as an adsorbed layer barrier to artificial saliva and to a remineralizing effect.

For demineralized enamel fragments, treatment with all agents and daily immersion in artificial saliva contributed to a remineralization process shown as an increase in microhardness values. However, microhardness decreases were observed for demineralized dentin. Haywood and Robinson (1997) have advocated the use of carbamide peroxide bleaching agents for initial caries lesions, mainly root caries, as the caries progression is retarded or stopped during bleaching. For demineralized dentin, the effect is a high decrease in microhardness values that could increase the depth of lesion formation, and bleaching should not be indicated as a common procedure. Although the post-treatment period seems to allow for an increase in microhardness values, probably due to a mineral deposition on dentin through a remineralization process, immersion of the fragments in artificial saliva did not provide recovery of the baseline values.

The demineralizing effects of agents, other than carbamide peroxide contained in bleaching agents, may play a role. As a general trend, 10% carbamide peroxide, carbopol, glycerin and their association seem to decrease sound enamel and dentin microhardness and demineralized dentin. Carbopol and its associations cause severe alterations in microhardness compared to Opalescence, which is a commercial brand available on the market. None of the agents evaluated were inert for dental microhardness, although glycerin seemed to affect enamel and dentin to a lesser degree. Thus, these results may be an advice warning to manufacturers to re-formulate the composition of some bleaching products or provide a better agent that does not cause enamel or dentin demineralization. The damage to sound and demineralized enamel and dentin in this experiment does not, however, necessarily imply demineralization *in vivo*, but should be kept in mind in further research.

CONCLUSIONS

Ten percent carbamide peroxide, carbopol, glycerin and their association decreased sound enamel and dentin microhardness and demineralized dentin. Carbopol and its associations caused alterations in microhardness, although glycerin seemed to affect enamel and dentin to a lesser degree.

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References

Arends J, Jongebloed WL, Goldberg M & Schuthof J (1984) Interaction of urea and human enamel *Caries Research* **18** 17-24.

Attin T, Kielbassa AM, Schwanenberg M & Hellwig E (1997) Effect of fluoride treatment on remineralization of bleached enamel *Journal of Oral Rehabilitation* **24** 282-286.

Basting RT, Rodrigues AL Jr & Serra MC (2001). The effect of 10% carbamide peroxide bleaching material on microhardness of sound and demineralized enamel and dentin *in situ* *Operative Dentistry* **26** 531-539.

Basting RT, Rodrigues AL Jr & Serra MC (2003) The effects of seven carbamide peroxide bleaching agents on enamel microhardness over time *Journal of the American Dental Association* **134** 1335-1342.

Ben-Amar A, Liberman R, Gorfil C & Bernstein Y (1995) Effect of mouthguard bleaching on enamel surface *American Journal of Dentistry* **8** 29-32.

Bitter NC (1998) A scanning electron microscope study of the long-term effect of bleaching agents on the enamel surface *in vivo General Dentistry* **46** 84-88.

Bitter NC & Sanders JL (1993) The effect of four bleaching agents on enamel surface: A scanning electron microscopic study *Quintessence International* **24** 817-824.

Christersson CE, Lindh L & Arnebrandt T (2000) Film-forming properties and viscosities of saliva substitutes and human whole saliva *European Journal of Oral Science* **108** 418-425.

Conover WJ (1971) *Practical Non-parametric Statistics* New York John Wiley & Sons.

Conselho Nacional de Saúde (2003) [Diretrizes e normas regulamentadoras de pesquisas envolvendo seres humanos] *Pesquisa Odontológica Brasileira* **17** 33-41.

Curtis JW, Dickinson GL, Downey MC, Russell CM, Haywood VB, Myers ML & Johnson MH (1996) Assessing the effects of 10 percent carbamide peroxide on oral soft tissues *Journal of the American Dental Association* **127** 1218-1223.

Delbem AC & Cury JA (2002) Effect of application time of APF and NaF gels on microhardness and fluoride uptake of *in vitro* enamel caries *American Journal of Dentistry* **15** 169-172.

Ernst CP, Marroquin BB & Willershausen-Zonnchen B (1996) Effects of hydrogen peroxide-containing bleaching agents on the morphology of human enamel *Quintessence International* **27** 53-56.

Featherstone JDB, O'Really MM, Shariati M & Brugler S (1986) Enhancement of remineralization *in vitro* and *in vivo* in Leach SA *Factors Relating to Demineralization and Remineralization of the Teeth* Oxford IRL 23-34.

Flaitz CM & Hicks MJ (1996) Effects of carbamide peroxide whitening agents on enamel surfaces and caries-like lesion formation: An SEM and polarized light microscopic *in vitro* study *Journal of Dentistry for Children* **63** 249-256.

Freitas PM, Basting RT, Rodrigues Jr AL & Serra MC (2002) Effects of two 10% carbamide peroxide bleaching agents on dentin microhardness at different time intervals *Quintessence International* **33** 370-375.

Goldberg M, Arends J, Jongebloed WL, Schuthof J & Septier D (1983) Action of urea solutions on human enamel surfaces *Caries Research* **17** 106-112.

Goldstein GR & Garber DA (1995) *Complete Dental Bleaching* Chicago Quintessence Books 165.

Haywood VB & Heymann HO (1989) Nightguard vital bleaching *Quintessence International* **20** 173-176.

Haywood VB (1992) History, safety, and effectiveness of current bleaching techniques and applications of the nightguard vital bleaching technique *Quintessence International* **23** 471-488.

Haywood VB (1993) The Food and Drug Administration and its influence on home bleaching *Current Opinion in Cosmetic Dentistry* 12-18.

Haywood VB (1994) Consideration and variations of dentist-prescribed, home applied vital tooth-bleaching techniques *Compendium* **17** S616-621.

Haywood VB (2000) Current status of nightguard vital bleaching *Compendium for Continuing Education Dentistry* **28** S10-17.

Haywood VB & Robinson FG (1997) Vital tooth bleaching with Nightguard vital bleaching *Current Opinion in Cosmetic Dentistry* **4** 45-52.

Hegedüs C, Bistey T, Flora-Nagy E, Keszthelyi G & Jenei A (1999) An atomic force microscopy study on the effect of bleaching agents on enamel surface *Journal of Dentistry* **27** 509-515.

Josey AL, Meyers IA, Romaniuk K & Symons AL (1996) The effect of a vital bleaching technique on enamel surface morphology and the bonding of composite resin to enamel *Journal of Oral Rehabilitation* **23** 244-250.

McCracken MS & Haywood VB (1995) Effects of 10% carbamide peroxide on subsurface hardness on enamel *Quintessence International* **26** 21-24.

McCracken MS & Haywood VB (1996) Demineralization effects of 10 percent carbamide peroxide *Journal of Dentistry* **24** 395-398.

Nathoo SA, Chmielewski MB & Kirkup RE (1994) Effects of Colgate Platinum Professional Toothwhitening System on microhardness of enamel, dentin, and composite resins *Compendium* **17** S627-630.

Oliveira R, Basting RT, Rodrigues JA, Rodrigues AL Jr & Serra MC (2003) Effects of a carbamide peroxide agent and desensitizing dentifrices on enamel microhardness *American Journal of Dentistry* **16** 42-46.

Pécora JD, Cruz Filho AM, Sousa Neto MD & Silva RG (1994) *In vitro* action of various agents on the microhardness of human dentin *Brazilian Dental Journal* **5** 129-134.

Potocnik I, Kosec L & Gaspersic D (2000) Effect of 10% carbamide peroxide bleaching gel on enamel microhardness, microstructure, and mineral content *Journal of Endodontics* **26** 203-206.

Ritter AV, Leonard RH Jr, St-Georges AJ, Caplan DJ & Haywood VB (2002) Safety and stability of nightguard vital bleaching: 9 to 12 years post-treatment *Journal of Esthetic and Restorative Dentistry* **14** 275-285.

Rodrigues JA, Basting RT, Serra MC & Rodrigues AL Jr (2001) Effects of 10 percent carbamide peroxide on enamel microhardness at different bleaching times *American Journal of Dentistry* **14** 67-71

Rotstein I, Dankner E, Goldman A, Heling I, Stabholz A & Zalkind M (1996) Histochemical analysis of dental hard tissues following bleaching *Journal of Endodontics* **22** 23-25.

Seghi RR & Denry I (1992) Effects of external bleaching on indentation and abrasion characteristics of human enamel *in vitro Journal of Dental Research* **71** 1340-1344.

Smidt A, Weller D & Roman I (1998) Effect of bleaching agents on microhardness and surface morphology of tooth enamel *American Journal of Dentistry* **11** 83-85.

Van der Reijden WA, Buijs MJ, Damen JJM, Veerman ECI, ten Cate JM & Nieuw Amerongen AV (1997) Influence of polymers for use in saliva substitutes on de- and remineralization of enamel *in vitro Caries Research* **31** 216-223.

Zalkind M, Arwaz J R, Goldman A & Rotstein I (1996) Surface morphology changes in human enamel, dentin and cementum following bleaching: A scanning electron microscopy study *Endodontic Dental Traumatology* **12** 82-88.