The effects of seven carbamide peroxide bleaching agents on enamel microhardness over time


Since its introduction by Haywood and Heymann,1 nightguard vital bleaching has been suggested as an efficient and simple procedure for removing intrinsic and extrinsic stains from teeth.2,3 Many products and systems have appeared on the market for in-office use, such as 35 percent hydrogen peroxide, as well as for over-the-counter use. However, a 10 percent carbamide peroxide bleaching agent is the most commonly used at-home bleaching product,2,4 owing to its safety and effectiveness. Variations of this technique have been introduced, including the use of higher concentrations of carbamide peroxide agents (from 10 to 22 percent), with carboxy-polymethylene polymer used as a thickening agent to improve tissue adherence and to result in a timed or sustained release of the whitening agent.5

In the presence of saliva, 10 percent carbamide peroxide releases 3 percent hydrogen peroxide and 7 percent urea. Researchers believe that peroxide-containing bleaching agents remove tooth discolorations through oxidation.6 Although dental hard tissues are highly mineralized, their organic content also can play an important role in the bleaching process. In the presence of decomposition catalysts, enzymes and saliva, the hydrogen peroxide ionization process occurs and the free radicals diffuse through the interprismatic substance of the enamel, opening the highly pigmented carbon rings

ABSTRACT

Background. Different concentrations of carbamide peroxide (10 to 22 percent) have been used successfully as bleaching agents, but the adverse effects on enamel microhardness at different times are unknown.

Methods. The authors analyzed seven bleaching agents and a placebo. The agents were applied on the surface of human dental fragments for eight hours per day for 42 days and stored in vials containing artificial saliva. Microhardness measurements were performed at baseline, eight hours and seven, 14, 21, 28, 35 and 42 days, as well as at seven and 14 days after treatment had ended.

Results. Enamel treated with different bleaching agents or a placebo experienced a similar decrease in microhardness values over time, with the exception of fragments exposed to Opalescence PF 20 percent (Ultradent Products, South Jordan, Utah), or OPA20. Until the 49th day, the enamel exposed to OPA20 exhibited the lowest differences from baseline values. After 14 posttreatment days, enamel treated with placebo, Nite White 10 percent Excel (Discus Dental, Los Angeles), Nite White 16 percent Excel and Opalescence 10 percent exhibited the greatest differences from baseline values. An increase in enamel microhardness occurred at the end of the posttreatment period, although baseline values were not reached.

Conclusions. Different concentrations of carbamide peroxide agents result in decreases in enamel microhardness. A post-bleaching period in artificial saliva resulted in recovery of baseline microhardness values (one agent) or an increase in values, although baseline values were not reached for these products.

Clinical Implications. Higher concentrations of carbamide peroxide containing 0.11 percent ion fluoride can bleach teeth in a shorter period, with fewer hazardous effects on the enamel mineral content.
Changes in the inorganic/organic ratio in enamel caused by the breakdown of the organic matrix. Furthermore, because the clinical bleaching procedure with carbamide peroxide agents requires repeated and prolonged exposure times, it also is important to evaluate the effects of higher concentrations of these agents at different bleaching times.

The aim of this study was to evaluate the microhardness of the enamel exposed to different concentrations of carbamide peroxide agents at different bleaching times.

**MATERIALS AND METHODS**

**Experimental design.** The treatment agents studied included the following:

- Nite White 10 percent Excel, or NW10 (Discus Dental, Los Angeles);
- Nite White 16 percent Excel, or NW16 (Discus Dental);
- Nite White 22 percent Excel, or NW22 (Discus Dental);
- Opalescence 10 percent, or OPA10 (Ultradent Products, South Jordan, Utah);
- Opalescence PF 20 percent, or OPA20 (Ultradent Products);
- Rembrandt 15 percent, or REM15 (Den-Mat, Santa Maria, Calif.);
- Nupro Gold, or NG10 (Dentsply Preventive Care, York, Pa.);
- placebo agent (mixed formula, Proderma-Pharmacy, Piracicaba, Brazil), used as a control.

We measured the microhardness of the agents at the following treatment times: baseline; eight hours; and seven, 14, 21, 28, 35 and 42 days, as well as seven and 14 days after the treatment ended (that is, days 49 and 56).

The experimental units consisted of 120 sound human enamel dental fragments, randomly and evenly assigned to the eight agents (15 dental fragments per group). The Knoop microhardness response variable was evaluated via quantitative methods. We took three measurements of Knoop microhardness on the surface of each specimen at each time interval for a nested design (that is, hierarchical correlations are taken into account).

This study was approved by the ethical committee of the Dentistry School of Piracicaba, University of Campinas, Brazil, in agreement with Brazil’s National Health Council.

**Preparation of the dental fragments.** We used 50 nonerupted freshly extracted third molars for this study. Immediately after extrac-
tion, the teeth were kept in 10 percent formaldehyde (pH 7.0). Using double-faced diamond disks and a low-speed motor, we removed the roots approximately 2 to 3 millimeters apical to the cementoenamel junction and sectioned the crowns longitudinally to obtain 120 dental fragments. We took care not to leave the dental fragments out in the air for a long period to prevent dehydration.

We immersed the fragments in distilled and deionized water at 37°C. The dental fragments had to be larger than 4 mm × 4 mm × 3 mm to be included in the study. Fragments that contained stains or cracks on visualization with a stereomicroscope at ×30 magnification were not used. The fragments were embedded individually in self-curing polyester resin in a polyvinyl chloride ring mold that was 2.0 centimeters in diameter. The external surface of the enamel was exposed and the resin was left to polymerize for 24 hours.

The molds were removed and the external surfaces of the dental fragments were leveled with a water-cooling mechanical grinder. Aluminum oxide disks of 400, 600 and 1,000 grit were used sequentially. We performed the polishing using polishing cloths and diamond pastes (6, 3, 1 and 0.25 micrometers), along with mineral oil.

These procedures were conducted to form parallel planar surfaces for the Knoop microhardness tests. We created a standardized 9 mm² area (3 mm × 3 mm) of exposed enamel (size of the window for testing) on the specimens by covering the remaining dental fragment (that is, the part that was not exposed) with two coatings of nail varnish. Afterward, we randomly distributed the 120 specimens to the treatment agent groups (15 dental fragments per group) and stored them in distilled and deionized water at 37°C for two days, at which time they were exposed to the treatment agents.

**Specification of the materials.** In this study, we evaluated seven carbamide peroxide bleaching agents. The control group received a placebo agent that was prepared with carbopol and glycerin. The color and consistency of the placebo agent were similar to those of one of the bleaching agents (OPA10). We used a pH meter to measure the pH levels of the bleaching agents at the beginning of the study. Table 1 presents the basic composition, lot number, pH level and manufacturer of each agent.

**Exposure of dental fragments to treatment agents.** The enamel fragments were exposed to the treatment agents (experimental and control) for eight hours per day for 42 days. Before the treatment period, we had 120 trays manufactured for each specimen (to simulate intraoral conditions), consisting of a 0.4-mm-thick flexible ethyl vinyl acetate polymer made in a vacuum-forming machine.

Using a syringe, we applied 0.02 milliliter of the assigned agent to each specimen every day for 42 days. Each specimen was covered with a tray, which was immersed in a closed vial containing 13.5 mL of artificial saliva (pH = 7.0) at 37°C. After eight hours, we removed the specimens from the storage media and the trays from the specimens. The treatment agents were washed from the surface of the enamel fragments under running distilled and deionized water for five seconds. During the remaining 16 hours per day, the fragments were immersed in individual closed vials (that were washed under running distilled and deionized water) containing 13.5 mL of artificial saliva (pH = 7.0) at 37°C. We changed the artificial saliva in the containers daily. The artificial saliva consisted of a remineralization solution that was developed by Featherstone and colleagues and modified by Serra and Cury. After the 42nd day of treatment, the specimens were retained in the individual vials and immersed in 13.5 mL of artificial saliva (pH = 7.0) at 37°C; again, the saliva was changed daily.

**Microhardness measurements.** We performed microhardness measurements before the initial exposure to the bleaching agents (baseline values) and after eight hours and seven, 14, 21, 28, 35 and 42 days, as well as after seven and 14 posttreatment days (corresponding to 49 and 56 days after the initial application of the bleaching agents). We used a Knoop indenter, keeping the long axis of the diamond parallel to the outer enamel surface in a microhardness testing machine (Future Tech-FM-1e, Tokyo, Japan). The Knoop microhardness test was used because it can measure both hardened and softened materials. We made three indentations on each specimen, using a load of 25 grams applied for five seconds each time.

We evaluated the data with a classical linear
model—analysis of variance, or ANOVA—in a nested design into a hierarchical structure of fixed effects. The time trend of microhardness (that is, the correlation between time and microhardness) was adjusted via polynomial regression, and it was used to verify the variations in microhardness related to the different measurement times for each treatment agent. The use of baseline differences is widely recommended for a repeated-measures design, because of intraunit variation in the dental fragments. Such strategy plays a central role in experimental designs, because it serves to equalize the error, especially when there is great variation at baseline.24 We used the Tukey test to compare differences among agents at each time, at a 5 percent level of significance.24

RESULTS

Table 2 shows the mean Knoop microhardness values at baseline, the differences from baseline values, the standard error and the Tukey test clusters for each treatment agent at each time. The figure (page 1340) shows estimated Knoop microhardness differences at each time. The ANOVA showed statistical differences for treatment agents, time and treatment agents–time interaction ($P < .0001$).

We found significant differences in mean microhardness values between treatment agents at each time ($P < .0001$). The Tukey test verified that, after eight hours, the microhardness of the enamel exposed to placebo did not differ from the microhardness of the enamel exposed to other bleaching agents, except for NW10 and OPA20. Over time (from the seventh to the 35th day), microhardness values were similar for all materials, but differences were exhibited among them at each measurement time. On the 42nd day, no differences in microhardness values were exhibited among NW22, NG10 and REM15. Until the 49th day (that is, the seventh post-treatment day), the enamel surface exposed to OPA20 exhibited the lowest microhardness differences from baseline values compared with enamel treated with other agents; on the 56th day, however, surfaces treated with OPA20 did not differ from surfaces treated with REM15. After 14 post-treatment days, enamel treated with placebo, NW10, NW16 and OPA10 exhibited the greatest declines in microhardness from baseline values compared with enamel treated with other agents.

Polynomial regression showed that, with the

<table>
<thead>
<tr>
<th>BLEACHING AGENT</th>
<th>COMPOSITION</th>
<th>pH</th>
<th>MANUFACTURER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nite White 10% Excel (Peppermint) (lot no. 9FK)</td>
<td>10% carbamide peroxide, polyethylene glycol, propylene glycol, hydroxypropilcellulose, carbopol, flavor, sodium hydride</td>
<td>7.49</td>
<td>Discus Dental (Los Angeles)</td>
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<tr>
<td>Nite White 16% Excel (Peppermint) (lot no. 8HJ)</td>
<td>16% carbamide peroxide, polyethylene glycol, propylene glycol, hydroxypropilcellulose, carbopol, flavor, sodium hydride</td>
<td>7.46</td>
<td>Discus Dental</td>
</tr>
<tr>
<td>Nite White 22% Excel (Peppermint) (lot no. 9KD)</td>
<td>22% carbamide peroxide, polyethylene glycol, propylene glycol, hydroxypropilcellulose, carbopol, flavor, sodium hydride</td>
<td>7.84</td>
<td>Discus Dental</td>
</tr>
<tr>
<td>Opalescence 10% (Regular) (lot no. 3MPL)</td>
<td>10% carbamide peroxide, carbopol, glycerin, flavoring</td>
<td>6.68</td>
<td>Ultradent Products (South Jordan, Utah)</td>
</tr>
<tr>
<td>Opalescence PF 20% (Regular) (lot no. 3MTJ)</td>
<td>20% carbamide peroxide, carbopol, glycerin, flavoring, 3% potassium nitrate, 0.11% ion fluoride</td>
<td>6.70</td>
<td>Ultradent Products</td>
</tr>
<tr>
<td>Rembrandt 15% (Regular) (lot no. 030371545)</td>
<td>15% carbamide peroxide, glycerin, sodium citrate, carbopol, flavor, triethanolamine</td>
<td>6.22</td>
<td>Den-Mat (Santa Maria, Calif.)</td>
</tr>
<tr>
<td>Nupro Gold (Regular) (lot no. 991021)</td>
<td>10% carbamide peroxide, glycerin, carbopol, flavor</td>
<td>6.24</td>
<td>Dentsply Preventive Care (York, Pa.)</td>
</tr>
<tr>
<td>Placebo</td>
<td>5% glycerin, 1.2% carbopol</td>
<td>7.0</td>
<td>Proderma-Pharmacy (Piracicaba, Brazil)</td>
</tr>
</tbody>
</table>
exception of OPA20, the enamel microhardness of fragments exposed to a particular treatment agent did not change much during the bleaching period. The enamel treated with various concentrations of carbamide peroxide or a placebo exhibited a decrease in microhardness values. However, during the treatment period and after seven or 14 posttreatment days, we observed a decrease in enamel microhardness differences from baseline values for all treatment agents. For OPA20, the enamel microhardness values surpassed the baseline values at the 49th and 56th days (that is, during the posttreatment period).

DISCUSSION

Changes in the chemical or morphological structure of enamel must be of concern when bleaching is used as a treatment for whitening teeth. Although some studies have reported that there were no significant changes in enamel microhardness when using short-term regimens of carbamide peroxide,11,16,25,26 other studies have found a decrease in enamel microhardness when using these bleaching agents for two weeks or more,12,18,27 even with the additional use of concentrated fluoride solutions.17

Pinheiro Junior and colleagues19 showed that a 16 percent carbamide peroxide agent significantly decreased the enamel microhardness values when compared with three 10 percent carbamide peroxide agents, suggesting that a higher concentration of carbamide peroxide could lead to deleterious effects on the dental structure. Until now, no other reports have been published on the effects of different concentrations of carbamide peroxide bleaching agents (15, 16, 20 and 22 percent) on enamel microhardness.

The findings of this in vitro investigation show that human enamel exposed to different concentrations of carbamide peroxide or a placebo agent result in a decrease in microhardness values. Eight hours after the bleaching agents were applied, we observed a significant decrease in microhardness values. Although the dental frag-
ments were immersed in artificial saliva during application of the bleaching agents, this did not allow recovery of the microhardness values during the first eight hours of treatment.

**pH values.** One possible explanation for the rapid decrease in microhardness values could be the pH of the 10 percent carbamide peroxide bleaching agents. Some studies reported that the pH of these agents ranges from 4.6 to 7.4,⁴,⁸,¹²-¹⁴ which can affect the physical and chemical structure of the enamel.⁶ In our study, the pH of the bleaching agents ranged from 6.22 to 7.84, which means that the products did not exhibit acidic properties. None of the products had a pH lower than 5.5 and, therefore, would not contribute to the enamel demineralization.²²,²⁸

In addition, Leonard and colleagues⁴ pointed out that a moderately low-pH bleaching solution in vivo reduces the pH of saliva in the mouth during the first five minutes, but an increase above baseline is expected after 15 minutes of treatment, probably owing to the chemical reactions of the neutralization of acidic carbamide peroxide by saliva. One also might expect that the tray used during the application of the agents did not allow for this remineralization during the eight hours of treatment, since it acted as a barrier with regard to the direct contact and flow of the artificial saliva with the dental fragments. In a clinical setting, the pH of saliva, plaque and bleaching agent in the mouth may rise as a result of conditions in the oral environment, and the presence of peroxidases, enzymes and saliva may neutralize the harmful effects of hydrogen peroxide.

**Placebo agent.** An intriguing result of this study was that there were no differences in enamel microhardness between placebo and some of the bleaching agents evaluated at different times. The placebo agent consisted of a neutral-pH glycerin and a carbopol gel, and we selected it for the control group because it provided hydration of the samples equal to that of samples treated with a bleaching agent. Manufacturers have made no claims about the action of glycerin and carbopol; thus, these agents generally have been considered to be inactive ingredients.

However, when comparing two 10 percent carbamide peroxide bleaching agents, with and without carbopol, McCracken and Haywood²⁷ found a significant decrease in microhardness in the outer 25 mm of the enamel surface that had been treated with the product containing carbopol. This finding relates both to the pH level of the products and to the presence of carbopol. This also suggests that glycerin or carbopol could act as a demineralizing agent²⁷ or as an impermeable barrier, inhibiting the penetration of artificial saliva solution through the enamel surface, and resulting in a lack of recovery of baseline microhardness values.

**Remineralization.** Despite the decrease in enamel microhardness values, we could verify a remineralization effect during the treatment period and at the end of the posttreatment period, when a decrease in the microhardness differences from baseline values occurred. The use of remineralization solutions or fluorides could inhibit the decrease in microhardness caused by the bleaching agents. Remineralization potential exists in saliva substitutes that contain calcium and phosphate ions,²²,²⁸ such as the artificial saliva used in this study.

When using 10 percent carbamide peroxide agents, Rodrigues and colleagues¹⁸ demonstrated the remineralization potential of this artificial saliva. Even though the baseline microhardness
values were higher than the posttreatment values for all of the agents except OPA20, the differences in microhardness values obtained on the seventh and 14th posttreatment days were lower than the differences obtained during the treatment period.

It is possible that storing the dental fragments in the artificial saliva for a longer period would have allowed a recovery of baseline microhardness values. This recovery toward baseline microhardness values also might be expected in in vivo conditions because of some important factors, such as salivary flow, buffering capacity of saliva, oral hygiene and the use of topical fluorides that may increase the remineralization of bleached enamel.

In our study, OPA20 exhibited the best results in terms of the microhardness profile over time. Because of some ingredients in OPA20, such as potassium nitrate and fluoride, the microhardness differences from baseline values during the treatment period (eight hours to 42nd day) were not significant compared with those of the other agents evaluated. During the posttreatment period, dental fragments treated with OPA20 exhibited an increase in enamel microhardness that surpassed baseline values. The presence of fluoride in OPA20 could act as a remineralizing agent, by forming a calcium fluoride layer on enamel that inhibits demineralization or a decrease in microhardness values. Potassium nitrate also could be responsible for an increase in enamel microhardness as a result of deposition of mineral, although its benefits typically are related to reduced dentin hypersensitivity by occluding dentin tubules or by blocking nerve conduction.

On the 56th day, the enamel exposed to REM15 also showed an increase in microhardness values that was not different from the results achieved on enamel treated with OPA20. Even though the microhardness profile of the enamel exposed to REM15 was similar to that of the other agents evaluated, there was a rapid increase in microhardness on the 56th day. REM15 contains sodium citrate, which is effective in controlling tooth hypersensitivity. Sodium citrate probably also can increase enamel microhardness over time, by reacting with the subproducts of the carbamide peroxide.

Because the enamel microhardness of tooth fragments exposed to all of the bleaching agents (except for OPA20) was similar during the bleaching period, one might expect that higher concentrations of carbamide peroxide are not more deleterious than 10 percent carbamide peroxide agents. Therefore, when one wants to achieve faster whitening of teeth, higher concentrations of properly selected materials might be chosen. With 10, 15, 16 or 22 percent carbamide peroxide concentrations, the same esthetic results can be achieved in a shorter time, compared with the results for lower concentrations of carbamide peroxide agents, even though an increase in tooth sensitivity can occur. When other ingredients are added to the bleaching agent, as is the case for OPA20 and REM15, a reduction in tooth sensitivity can be observed as a result of the presence of fluoride, potassium nitrate and/or sodium citrate, and an increase in microhardness values over time can be demonstrated.

The decrease in microhardness values observed during the treatment period with carbamide peroxide bleaching agents seems to be the result of damage to the enamel structure, regardless of the concentration. A postbleaching period allowed for recovery of baseline values (for OPA20) or an increase in the microhardness values (for placebo, NW10, NW16, NW22, OPA10, REM15 and NG10), although the baseline values were not reached for these products.

It is likely that the storage of dental fragments in artificial saliva for more than two weeks after bleaching resulted in a continuous increase in mineral gain and the return of the microhardness values to baseline or near baseline. Clinically, this mineral intake would be faster because of the presence of saliva, plaque control and the use of fluorides and other methods to achieve the remineralization. However, these results illustrate the need for at-home whitening agents to be used with professional supervision, to ensure proper application of the bleaching agents, use of the recommended amount of gel or paste, proper length of treatment and appropriate steps to take to prevent adverse reactions.

CONCLUSION

The results of this study show that different concentrations of carbamide peroxide bleaching agents and a placebo agent result in decreases in enamel microhardness from baseline values. However, products containing fluoride may result in fewer hazardous effects on enamel mineral content.
ment of Restorative Dentistry, Ribeirão Preto Dentistry School, University of São Paulo, Ribeirão Preto, Brazil.

Dr. Rodrigues is a professor, Department of Social Medicine, Clinical Hospital of the School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil.

Dr. Serra is a professor, Department of Restorative Dentistry, Ribeirão Preto Dentistry School, University of São Paulo, Ribeirão Preto, Brazil. Address reprint requests to Dr. Serra, Faculdade de Odontologia de Ribeirão Preto-USP, Departamento de Odontologia Restauradora-Dentística Avenida do Café, s/n, CEP: 14040-904 Ribeirão Preto-SP Brazil, e-mail “mcserra@forp.usp.br”.

The authors received the financial support of Foundation for Research Support of São Paulo State (FAPESP) grants 99/11735-2 and 98/14425-1.