Effects of two 10% peroxide carbamide bleaching agents on dentin microhardness at different time intervals

Patricia Moreira de Freitas, DDS
Roberta Tarkany Basting, DDS, MS, ScD
Antonio Luiz Rodrigues, Jr, DDS, MS, ScD
Mônica Campos Serra, DDS, MS, ScD

Objective: The purpose of this in vitro study was to evaluate the microhardness of human dentin exposed to two 10% carbamide peroxide agents at different bleaching times. Method and materials: Opalescence 10% and Rembrandt 10% were tested. A placebo agent was used as a control group. The bleaching and placebo agents were applied to the surface of human dentin fragments for 8 hours and then stored in individual receptacles with artificial saliva for the remaining 16 hours each day. Microhardness testing was performed at baseline, after 8 hours, 7, 14, 21, 28, 35, and 42 days of treatment, and 7 and 14 days posttreatment. Results: Analysis of Variance and the Tukey test revealed significant differences in microhardness values for dentin treated with the agents within each time interval. There was a decrease in the microhardness values of dentin for both bleaching agents after 8 hours of treatment. Fourteen days after the completion of treatment, the microhardness values for dentinal surfaces treated with either Opalescence or Rembrandt reached the baseline values; the dentinal surfaces treated with a placebo exhibited an increase in microhardness values posttreatment. Conclusion: Ten percent carbamide peroxide bleaching agents decreased dentinal microhardness over time, but after 14 days in artificial saliva storage at the completion of treatment, the baseline microhardness values were recovered. (Quintessence Int 2002;33:370-375)

Key words: bleaching agent, carbamide peroxide, dentin, microhardness, vital tooth bleaching

CLINICAL RELEVANCE: Because 10% carbamide peroxide bleaching agents alter dentinal microhardness, the concomitant use of methods to inhibit this demineralization might be useful; in this in vitro study, 14 days' storage in artificial saliva had a remineralizing effect.

Nightguard vital bleaching has been suggested as an efficient and simple procedure for removing intrinsic and extrinsic stains from the teeth. Different products and systems have appeared on the market for in-office use, such as 35% hydrogen peroxide, or as over-the-counter products. However, 10% carbamide peroxide bleaching agents are still the most utilized at-home bleaching technique, supported by several reports of their safety and effectiveness and by American Dental Association approval.

Because the bleaching of vital teeth involves direct contact of the whitening agent on the outer enamel surface for an extensive period of time, and the agent reaches the dentin in areas of enamel defects or abrasions, exposed root surfaces, and the marginal areas between dentin and restorations, many studies have evaluated the potential adverse effects of these carbamide peroxide agents. Scanning electron microscopic evaluations have revealed changes in enamel and in dentinal surface morphology.

Because of the acidic property of bleaching agents, changes in the mineral content of the teeth can be expected. Loss of mineral content or demineralization alters enamel and dentinal microhardness, although saliva, fluorides, or other remineralizing solutions can maintain the balance between the phenomena of demineralization and remineralization.

Some in vitro studies have indicated that there are no significant changes in enamel microhardness when 10% carbamide peroxide agents are used.
although others have observed a decrease in micro-hardness values or mineral loss after application of these bleaching agents.\textsuperscript{15,20,24-27} In a combined in vitro and in vivo study, a decrease in the initial microhardness of enamel was followed by an increase in enamel microhardness, which may have resulted from remineralization by saliva.\textsuperscript{14}

In dentin, Nathoo et al.\textsuperscript{22} found no microhardness changes, although Pécora et al.\textsuperscript{28} and Rotstein et al.\textsuperscript{20} showed significant alterations in the microhardness and mineral content when they used 10% carbamide peroxide agents.

Because the observations in dentin are still controversial, there is a need for additional research on the effects of 10% carbamide peroxide bleaching agents on dentinal microhardness at different time intervals. The aim of this study was to evaluate in vitro the microhardness of dentin after exposure to two 10% carbamide peroxide bleaching materials and a placebo agent for 42 days of treatment. The microhardness was also measured 7 and 14 days post-treatment.

**METHOD AND MATERIALS**

**Experimental design**

The factors under study were:

1. Treatment agents (in three levels): Opalescence 10% (Ultradent), Rembrandt 10% (Den-Mat), and a placebo agent as a control.
2. Time (in ten levels): baseline, 8 hours, 7, 14, 21, 28, 35, and 42 days of treatment, and 7 and 14 days posttreatment period (corresponding to 49 and 56 days after the beginning of the treatment).

The experimental units consisted of 60 sound human dentinal fragments, randomly and evenly assigned to the three different treatment agents (20 dentinal fragments per group). Knoop microhardness response was evaluated by quantitative methods. Repeated measurements of Knoop microhardness were taken on the surface of each specimen at each time interval in a split-plot design.

**Dentin preparation**

Thirty freshly extracted, nonerupted third molars were used. Immediately after extraction, the teeth were stored in 10% formaldehyde (pH 7.00). The crowns were removed approximately to the cementoenamel junction, and the roots were longitudinally sectioned with double-faced diamond disks (Sorensen) used at a low motor speed (Kavo) to obtain 60 dentinal fragments. The apical third was discarded, and only the cervical region was used. Care was taken not to leave the dentinal fragments dehydrated for long periods. After sectioning was completed, specimens were soaked in distilled and deionized water at 37°C.

The dentinal fragments were required to be larger than 4 x 4 x 3 mm. Those fragments that were observed to have stains or cracks under a stereomicroscope used at X30 (Meiji Techno EMZ series) were not used. The 60 dentinal fragments were embedded individually in a self-curing polyester resin in a polyvinylchloride ring mold 2.0 cm in diameter so that the external surface of the dentin was exposed. The resin was left to polymerize for 24 hours.

The molds were removed, and the external surfaces of the dentinal fragments were leveled with a water-cooling mechanical grinder (Maxgrind, Solotest). Aluminum oxide disks were used in a sequential granulation of 600, 1,000, and 1,200 grit (Carborundum, 3M Dental) cooled with water. These procedures were conducted to form parallel planar surfaces for the Knoop microhardness tests.

A standardized area of 9 mm² (3 x 3 mm) of exposed dentin was created on the specimens by covering the remaining dentinal fragment with two coatings of nail varnish (Colorama, CEIL).

**Bleaching and placebo materials**

Two 10% carbamide peroxide bleaching agents were evaluated: Opalescence (pH 6.68) and Rembrandt (pH 6.19). Their compositions are presented in Table 1. The control group consisted of a placebo agent prepared with carboxymethylcellulose and glycerin. The color and consistency of the placebo agent were similar to those of Opalescence, but the placebo was pH neutral and had no carbamide peroxide.

**Bleaching treatment**

The dentinal fragments were exposed to the treatment agents (experimental and control) for 8 hours a day for a period of 42 days.
An individual tray was manufactured for each specimen from a 0.4-mm-thick flexible ethyl vinyl acetate polymer (Bio-Art Equipment) placed in a vacuum-forming machine (P7, Bio-Art Equipment).

For the application of the treatment agents, a syringe was used to apply 0.02 mL of each agent to each specimen. The specimens were individually covered with the tray and soaked in individual closed containers with 13.5 mL of artificial saliva (pH 7.00) at 37°C.

After 8 hours, the specimens were taken out of the storage media, and the trays were removed. The treatment agents were washed from the surface of the dentinal fragments under running distilled and deionized water for 5 seconds.

During the remaining daily time (16 hours per day), the fragments were maintained in individual receptacles with 13.5 mL of artificial saliva (pH 7.00) at 37°C. The artificial saliva was changed daily. The artificial saliva consisted of a remineralization solution proposed by Featherstone et al. and modified by Serra and Cury.

Posttreatment phase

After the 42-day treatment period, the specimens were maintained at 37°C in their individual containers with 13.5 mL of artificial saliva (pH 7.00), which was changed daily.

Microhardness testing

Microhardness measurements were performed before the initial exposure to the treatments (baseline), after 8 hours, 7, 14, 21, 28, 35, and 42 days, and 7 and 14 days posttreatment (corresponding to 49 and 56 days after the 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). Three indentations were made on each specimen. A load of 25 g was applied for 5 seconds each time.

Statistical analysis

The average of the three Knoop Hardness Numbers was taken. Statistical analysis involved a parametric method using analysis of variance in a split-plot design (repeated measurements at the same experimental unit). The Tukey test (α = .005) was used to compare the differences among treatment agents at each time interval to a 5% level of significance.

RESULTS

Figure 1 and Table 2 show the mean Knoop microhardness values for each treatment agent at different time intervals and the results of the Tukey test.

The analysis of variance and Tukey test revealed significant differences for each treatment agent within each time interval (P < .0001). Because the Tukey test verified significant differences in microhardness values among the three dentinal specimen groups at baseline, comparisons between placebo and Opalescence, Opalescence and Rembrandt, and placebo and Rembrandt within each time interval were not carried out.

There was a decrease in the mean microhardness values of dentin treated with Opalescence and Rembrandt during the treatment period, although the values had returned to baseline 14 days after the completion of treatment. For the group treated with the placebo, the microhardness values for dentin remained the same during the treatment period; values increased, to greater than baseline values, in the posttreatment period.

DISCUSSION

The chemistry of the carbamide peroxide bleaching agents is based on its ability to generate free radicals, which are highly reactive. These free radicals (the hydrogen peroxide) are nonspecific, extremely unstable, and can potentially react not only with the pigmented carbon rings but also with other organic molecules to achieve stability. Other radicals can be generated, and breakdown of the organic matrix can occur.

Several studies have been conducted to evaluate the potential effects of 10% carbamide bleaching agents on enamel. In this in vitro investigation, human dentin exposed to two 10% carbamide peroxide bleaching agents exhibited a decrease in the microhardness values over time. Pécora et al. also showed a decrease in microhardness values when they applied a 10% carbamide peroxide agent on dentin for 72 hours, and Rotstein et al. observed a significant reduction in the mineral content after immersing dentin in the three 10% carbamide peroxide agents and incubating for 7 days.

In the present study, immediately after 8-hour application of Opalescence and Rembrandt, there was a significant decrease in microhardness values that could be related to some mineral loss. Although the dentinal fragments were immersed in artificial saliva during the application of the bleaching agents, it was not enough to allow a remineralizing effect during the first 8 hours of treatment. However, the results
TABLE 2 Mean Knoop microhardness values for each treatment agent at different time intervals

<table>
<thead>
<tr>
<th>Time</th>
<th>Placebo</th>
<th>Opalescence</th>
<th>Rembrandt</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
</tr>
<tr>
<td>Baseline</td>
<td>9.97</td>
<td>4.06</td>
<td>20</td>
</tr>
<tr>
<td>8 H</td>
<td>6.00</td>
<td>1.47</td>
<td>20</td>
</tr>
<tr>
<td>7 D</td>
<td>9.36</td>
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<tr>
<td>14 D</td>
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<td>21 D</td>
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<td>19</td>
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<tr>
<td>28 D</td>
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<td>2.39</td>
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<tr>
<td>35 D</td>
<td>10.93</td>
<td>3.63</td>
<td>20</td>
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<tr>
<td>42 D</td>
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<td>49 D</td>
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<tr>
<td>56 D</td>
<td>14.80</td>
<td>4.60</td>
<td>20</td>
</tr>
</tbody>
</table>

SD = standard deviation. Sig = significance. The Tukey test compared the differences among time intervals at the 5% level of significance. Equal letters indicate mean values that are not significantly different (time comparisons for the same treatment agent).

Fig 1 Mean Knoop microhardness of dentin fragments treated with different bleaching agents at different time intervals.

Obtained in this experiment indicate that artificial saliva should be enough to provide the dissociation of the bleaching agents. The remineralizing effect was verified in the posttreatment period, when there was a significant increase in the microhardness values to baseline values.

Comparisons among the three treatment agents within each time interval could not be carried out, probably because of the methodology employed in this experiment. Differences in the diameter of the dentinal ducts along the extension of the root and the procedures of leveling and polishing the dentinal surface could have been responsible for the statistically significant differences in microhardness values at baseline.

The pH of 10% carbamide peroxide bleaching agents is reported to range from 4.60 to 7.40. In the present study, the pH of the bleaching agents was 6.68 for Opalescence and 6.19 for Rembrandt; both products...
have acidic properties that could affect the physical and chemical structure of dentin. Of major concern is the possibility of dentinal demineralization, which occurs at a pH lower than 6.50; enamel demineralization occurs at a pH lower than 5.50.\textsuperscript{9,20}

The acidic properties of the bleaching agents could be responsible for decreases in the dentinal microhardness during the treatment period. Leonard et al\textsuperscript{17} pointed out that a moderately low-pH bleaching solution in vivo reduces the pH of saliva in the mouth during the first 5 minutes. After 15 minutes of treatment, the pH increases to greater than baseline, probably because of chemical reactions to neutralization of acidic carbamide peroxide by saliva. Although Opalescence and Rembrandt did not present a very low pH level, there was a significant decrease in dentin microhardness values during treatment.

When a placebo agent was used during the same period, there were no differences in dentinal microhardness until the 42nd day of treatment. The placebo agent consisted of a neutral-pH glycerin and carboxy-polymethylene polymer solution and was considered a better choice as an adequate control group, providing equal hydration of the samples. Glycerin and carboxymethylcellulose are inactive ingredients, and the manufacturers do not make any claims about the action of those products. However, in an enamel microhardness evaluation comparing two 10% carbamide peroxide bleaching agents with and without carboxypolymethylcellulose, McCracken and Haywood\textsuperscript{24} found a significant decrease in microhardness in the outer 25 mm of the enamel surface after treatment with the product containing carbopol. This difference was related not only to the pH level of the products but also to the presence of carbopol. Although Basting et al\textsuperscript{25} also observed a decrease in microhardness values for enamel at different treatment times when using a placebo agent, in the present study there was no decrease in dentinal microhardness over time in the placebo-treated group.

The use of a remineralizing solution or fluorides could inhibit the demineralization caused by the acidic pH of the bleaching agents. Remineralization potential exists in saliva substitutes that contain calcium and phosphate ions,\textsuperscript{19,29} such as the artificial saliva used in this study. The remineralization of the dentinal surface was only observed in the postbleaching period, when the microhardness values for Rembrandt returned to baseline levels. For Opalescence, an increase in microhardness values was observed at the 7th day and from the 28th day of treatment on. This recovery toward baseline microhardness values also might be expected in vivo because of some important factors, such as salivary flow, the buffering capacity of saliva, oral hygiene,\textsuperscript{6} and the use of topical fluo-

rides,\textsuperscript{11} which may increase the remineralization of bleached enamel and dentin.

The decrease in dentinal microhardness values during the treatment with two 10% carbamide peroxide bleaching agents seems to injure the dentin structure. However, a postbleaching period allowed remineralization and an increase in the microhardness to baseline values. The concomitant use of methods to inhibit the demineralization during tooth bleaching might be useful. The results of this study emphasize that at-home whitening agents require professional supervision to ensure the proper application of bleaching agents, the use of the recommended amount of gel or paste, the correct length of treatment, and the prevention of adverse reactions.

**CONCLUSION**

1. Exposure to 10% carbamide peroxide bleaching agents decreased dentinal microhardness during the treatment period.

2. Fourteen days after the completion of treatment, there was a recovery of the baseline microhardness values because of the remineralizing effect of artificial saliva.

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