High Levels of Hydrogen Peroxide in Overnight Tooth-Whitening Formulas: Effects on Enamel and Pulp

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ABSTRACT

Purpose: Limited data are available to assess the safety of high levels of hydrogen peroxide in overnight tooth-whitening formulas. The purpose of this study was to assess the effects of hydrogen peroxide on enamel microhardness, pulp penetration, and enamel morphology.

Materials and Methods: Colgate Platinum Professional Overnight Whitening System (Colgate Oral Pharmaceuticals, Inc., Canton, MA, USA) (10% carbamide peroxide, equivalent to 3.5% hydrogen peroxide) was compared with two prototype formulations containing either 7.0% or 12.0% hydrogen peroxide. In the pulp chamber studies, human extracted teeth were exposed to 3.5%, 7.0%, or 12.0% hydrogen peroxide for 30 minutes, 4 hours, or 7 hours. Microhardness, electron spectroscopy for chemical analysis, and atomic force microscopy evaluations were made from enamel blocks cut from human extracted molars. The enamel blocks were evaluated following 14 7-hour treatments (98 h total).

Results: At 7 hours' post-treatment, hydrogen peroxide penetrated the pulp chamber at 23.12 ± 10.09, 24.58 ± 6.90, and 26.39 ± 5.43 μg for 3.5%, 7.0%, and 12.0% hydrogen peroxide, respectively. With regard to enamel morphology, pulp penetration, microhardness, and elemental composition, no statistically significant differences were observed between treatment groups following 98 hours of treatment.

Conclusions: Hydrogen peroxide does not adversely affect enamel morphology or microhardness. The levels recovered in pulp indicate that hydrogen peroxide is not expected to inhibit pulpal enzymes.

CLINICAL SIGNIFICANCE

Overnight tray products containing levels of hydrogen peroxide of 3.5%, 7.0%, and 12.0% are not expected to adversely affect the enamel or pulpal enzymes. Additional safety studies are needed to assess the potential for tooth sensitivity and gingival irritation.

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Tooth-whitening continues to be a rapidly growing area in esthetic dentistry. The demand increases each year, particularly since tooth-whiteners can be tailored to fit the individual needs of the consumer. There are a variety of delivery options, including custom-fit mouth trays, paint-on products, and film technologies. The most common active ingredient in tooth-whiteners is carbamide

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peroxide, which, when present at 10%, releases 3.5% hydrogen peroxide. Carbamide peroxide has been recognized by the US Food and Drug Administration as a category I (generally recognized as safe) oral antiseptic. Professional "in-office" products typically contain high concentrations of hydrogen peroxide (30–35%), whereas professional "take-home" products usually contain up to 10% hydrogen peroxide or 22% carbamide peroxide. Other uses of peroxides in dentistry include treatment against plaque, periodontal disease, and gingivitis.[1–3]

The safety of hydrogen peroxide in oral care products is well documented, and its use in cosmetic dentistry has existed for over 70 years.[4] Despite this long history of safe use, patients have reported mild, transitory effects including dentinal sensitivity, oral irritation, oral sloughing, and swollen oral tissues. Owing to the increasing levels of peroxide used in tooth-whitening products today, clinicians have expressed concerns about the effects of these products on dental enamel and pulp. The majority of published in vitro safety studies have focused on the effects of 10% carbamide peroxide on enamel, dentin, and pulp. Several authors have shown that carbamide peroxide (10%) does not adversely affect the pulp, enamel morphology, or enamel microhardness.[5–12] Similar observations were made in studies with composite resins, in which 10% carbamide peroxide did not affect the microhardness of enamel or dentin.[12] Some investigators have shown adverse effects of peroxide on enamel at these same concentrations; however, these data were no different than observations made with fruit juices or cola.[13]

Limited data are available to assess the safety of high peroxide concentrations in tooth-whitening formulas intended for extended use. The majority of published literature has reported such studies using 10% carbamide peroxide or less. Slezak and colleagues demonstrated that there were no adverse effects on enamel microhardness when a paint-on tooth whitener (25% carbamide peroxide) was applied for 83 hours.[14] However, the design of that study does not mimic consumer exposure to carbamide peroxide when delivered in a custom-fit tray. Generally, consumers are instructed to wear mouth trays for up to 7 hours (overnight) for 7 to 14 days. As a result, it is not known whether these products contribute to adverse effects on enamel or pulp.

To evaluate the effects of tooth whiteners on enamel microhardness, morphology, and pulpal penetration, we have conducted a series of in vitro studies to assess each of these parameters. The duration of treatment was 98 hours. This study design is consistent with the maximum prescribed treatment regimen of the product: overnight treatment (7 h) for 2 weeks.

MATERIALS AND METHODS

Pulp Chamber Penetration by Hydrogen Peroxide

Penetration of hydrogen peroxide into the pulp chamber of human extracted teeth treated with the test products was measured using a leukocrystal violet/horseradish peroxidase colorimetric analysis. Teeth were prepared as previously described.[15] Twenty teeth were sectioned above the furcation of the roots or 3 mm below the cementoenamel junction. The pulp tissue was removed, and any external root surface was covered with nail polish. The crown was left uncovered 3 mm gingival to the marginal ridges.

The 20 teeth were divided into four groups of five teeth, with one group serving as a control group. The pulp chambers were filled with 2M acetate buffer, which served to stabilize any peroxide that diffused into the chamber during treatment. The crowns of the control group were exposed to distilled water rather than product. The crowns of the remaining three groups were treated with Colgate Platinum Professional Overnight Whitening System (Colgate Oral Pharmaceuticals, Inc., Canton, MA, USA), which contains 10% carbamide peroxide, equivalent to 3.5% hydrogen peroxide; the 7% hydrogen peroxide paste; or the 12% hydrogen peroxide

paste. After 30 minutes of treatment at 37°C, the acetate buffer solution was removed from the pulp chamber and tested for hydrogen peroxide using the leukocrystal violet/horseradish peroxidase colorimetric assay. This process was repeated using new teeth for 4 and 7 hours of treatment.

Preparation and Treatment of Enamel Blocks

Enamel blocks used for evaluations of microhardness, electron spectroscopy for chemical analysis (ESCA), and atomic force microscopy (AFM) were cut from human extracted molars and polished to a high luster using a method described by Nathoo and colleagues.[12] The enamel surface was free from decay and physical defects. The blocks were cut from the teeth using a diamond disk to a thickness of approximately 2.5 mm. The dentin side of the block was flattened with a tapered bur. The blocks underwent a flattening regimen on the enamel side using a Minimet Polisher (Buehler, Lake Bluff, IL, USA) with a thinning attachment and a 30 μm diamond polishing disk. To remove scratches and provide a high luster, the enamel blocks were polished again with a Minimet Polisher, using a series of successively finer grades of diamond pastes (6, 3, and 1 μm) on a micropolishing cloth.

Twelve enamel blocks were prepared for each study (microhardness, ESCA, and AFM). For each study the twelve blocks were divided into groups of three, with one group serving as a control group. All blocks were equilibrated for 7 hours at 37°C in human stimulated whole saliva. The enamel side of the blocks was then treated for 7 hours at 37°C with Colgate Platinum Professional Overnight (10% carbamide peroxide, equivalent to 3.5% hydrogen peroxide), the 7% hydrogen peroxide paste, or 12% hydrogen peroxide paste. The control group remained in human stimulated whole saliva. After treatment the whitening paste was removed under running water and the blocks were incubated in saliva at 37°C overnight. The 7-hour treatment procedure and overnight equilibration in saliva was repeated for a total of 14 7-hour treatments (98 h of total exposure). This procedure simulates in vivo conditions that would be used by a patient for 2 weeks of overnight whitening treatments. Treated blocks were then sonicated in distilled water and evaluated for surface changes by microhardness, ESCA, and AFM analysis as described below.

Microhardness Measurements

Microhardness measurements were made using a Leitz Miniload Microhardness Tester (Brown & Sharpe, North Kingstown, RI, USA) equipped with a Knoop diamond indentor and a 50 g load. Six indentations were made at random locations on each enamel block, with a dwell time of 15 seconds for each. Knoop hardness numbers (KHNs) were recorded using a Boeckeler Video Hardness Measurement System (Boeckeler Instruments, Inc, Tucson, AZ, USA).

Electron Spectroscopy for Chemical Analysis

ESCA was used to determine the surface elemental composition of the enamel blocks both before and after whitening treatment. ESCA analyses were conducted using a Physical Electronics (PHI) model 5600 ESCA spectrometer (Physical Electronics, Eden Prairie, MN USA). A monochromatic x-ray source equipped with an aluminum anode (Al Kα = 1486.7 eV) operated at 400 W was used to excite photoemission. Emitted photoelectrons were analyzed using a spherical capacitor electron energy analyzer. To determine the elements present on the enamel surfaces, ESCA survey spectra were obtained at an analyzer pass energy of 187.85 eV. For quantification purposes, high-resolution spectra of the elements detected were obtained at a pass energy of 29.35 eV. A 2.5 × 0.8 mm area of the surface was analyzed. An electron flood gun operated at 20 mA emission current and 10 eV electron energy was used to neutralize sample charging during analysis. Data acquisition and storage were accomplished using an HP Vectra work station running PHI-PC Access software (Physical Electronics, Eden Prairie, MN, USA). The atomic percentages of the elements present on the enamel surfaces were calculated using software and

atomic sensitivity factors included with the instrument data system.

Atomic Force Microscopy

AFM was used to determine the surface morphology of each enamel block before and after whitening treatment. The images were obtained using a Topometrix TMX 2000 Discoverer AFM instrument (Veeco Metrology Group, Sunnyvale, CA, USA). The instrument was operated in the contact mode for all samples. AFM images were obtained over a 50 × 50 μm area. To ensure representative areas of the surface had been studied, five separate images were obtained for each sample surface. The average roughness (Ra) was calculated for each image using the AFM system software. The mean values of Ra for all images for each block before and after whitening treatment were compared to determine whether the treatment caused an increase in roughness for the enamel surface. An increase in the mean Ra for the enamel surface after treatment indicates that a roughening of the surface has occurred.

Statistical Analysis

One-way analysis of variance was used to compare the three products. Statistical significance was declared if the p value was .05 or less.

RESULTS

Pulp Chamber Penetration by Hydrogen Peroxide

The level of peroxide that penetrated into the pulp chamber at 0.5, 4, and 7 hours is shown in Table 1. The control samples showed < 1 μg of peroxide in the pulp chamber. In the samples treated with product, peroxide penetration into the pulp increased with longer treatment times, reaching similar levels for all products after 7 hours. At 4 and 7 hours, there was no statistical difference between the three products.

TABLE 1. PULP CHAMBER PENETRATION BY HYDROGEN PEROXIDE.*

Test Group	After 30 min (μg)	After 4 h (μg)	After 7 h (μg)
Control	0.09 ± 0.15[a]	0.52 ± 0.13[a]	0.62 ± 0.10[a]
Colgate Platinum	0.52 ± 0.10[b]	7.11 ± 2.60[b]	23.12 ± 10.09[b]
7% test product	1.79 ± 0.45[c]	6.42 ± 3.65[b]	24.58 ± 6.90[b]
12% test product	2.12 ± 0.69[d]	14.65 ± 2.63[b]	26.39 ± 5.43[b]

*Superscript letters denote statistical difference among products at each time point ($p < .05$).

Microhardness

Microhardness testing revealed that there was no significant change in enamel hardness after 98 hours' exposure to any of the three whitening products compared with the untreated saliva controls ($p > .90$). KHNs are shown in Table 2.

Electron Spectroscopy for Chemical Analysis and Atomic Force Microscopy

ESCA and AFM were used to characterize the surfaces of the treated polished enamel blocks. ESCA was used to determine the compositions of the surfaces pre- and post-treatment, whereas AFM was used to image and quantify any changes in surface roughness caused by the treatments. The ESCA and AFM results are shown in Table 3. The ESCA results are presented as the atomic percentages of the elements detected. The AFM results are presented as Ra values in nanometers. The Ra values are the mean of five different 50 μm square areas sampled on each block surface.

TABLE 2. MICROHARDNESS OF ENAMEL AFTER TREATMENT WITH TEST PRODUCTS FOR 98 HOURS.

Product	Average Microhardness Pretreatment (KHN)	Average Microhardness Post-treatment (KHN)
Saliva control	330.90 ± 34.79	338.46 ± 39.42
Colgate Platinum	329.26 ± 33.98	337.64 ± 54.20
7% test product	327.18 ± 18.25	334.79 ± 18.07
12% test product	332.59 ± 43.22	343.48 ± 43.16

KHN = Knoop hardness number.

TABLE 3. ESCA AND AFM ANALYSIS OF ENAMEL AFTER TREATMENT WITH TEST PRODUCTS FOR 98 HOURS.*

Group	ESCA Analysis: Calcium (Atomic %)	ESCA Analysis: Phosphorous (Atomic %)	AFM Analysis of Roughness (nm)
Colgate Platinum			
Untreated	9.43 ± 0.90	7.36 ± 0.61	4.27 ± 0.34
Treated	10.05 ± 0.71	8.43 ± 0.30	4.95 ± 0.59
7% test product			
Untreated	10.54 ± 1.94	7.56 ± 1.70	5.30 ± 1.13
Treated	9.45 ± 2.00	7.78 ± 1.46	4.96 ± 0.74
12% test product			
Untreated	10.97 ± 0.49	8.24 ± 0.61	4.89 ± 0.71
Treated	9.98 ± 0.61	8.32 ± 0.48	4.86 ± 0.80

AFM = atomic force microscopy; ESCA = electron spectroscopy for chemical analysis.
*Values represent the mean of three blocks ± SD.

The ESCA data for all the blocks showed no significant change in phosphorus or calcium levels, comparing pre- and post-treatment values for each product. The AFM data indicate that the Ra was not changed as a result of treatment with any of the whitening formulas. Also, there was no evidence of surface roughening or etching in any of the images for the treated enamel surfaces.

DISCUSSION

Depending on its concentration, carbamide peroxide in tooth-whitening products has the potential to adversely affect the enamel, dentin, and pulp. Therefore, it is important for the safety assessment to consider both the method of delivery and the treatment time employed. Patients using Colgate Platinum Professional Overnight typically use the product overnight for a period of 5 to 7 nights. However, this duration may be as long as 14 days. For comparison, we determined the effects of 7% and 12% hydrogen peroxide on various in vitro parameters using a 2-week treatment regimen. Each tooth-whitening formula is formulated with Polyox (Dow/Union Carbide, Piscataway, NJ, USA), which increases the formula viscosity and prolongs the release of hydrogen peroxide. Therefore, it is possible for hard and soft tissue damage to occur as a result of increased contact time with peroxide.

Several investigators have shown that hydrogen peroxide readily penetrates through dental hard tissues.[15–17] Furthermore, an inhibition of pulpal enzymes in the presence or absence of heat has been shown.[17] In the present study, only microgram quantities of peroxide were recovered in the pulp chamber following the overnight, 14-day treatment with 3.5%, 7.0%, and 12.0% hydrogen peroxide. According to Bowles and Ugwuneri, milligram quantities are required to inhibit pulpal enzymes.[7] Interestingly, there were no significant differences in the levels of peroxide found in the pulp chamber after 4 and 7 hours with any of the test formulas. This finding may be attributed to the slow release of the whitening agent from the formulation as well as peroxide degradation before reaching the pulp chamber.

Owing to its acidic pH, hydrogen peroxide has the ability to adversely affect the enamel. In the present study, none of the Colgate Platinum Professional Overnight, 7%, or 12% hydrogen peroxide test formulations (all above pH 5.5) altered enamel microhardness, composition, or roughness. The findings with Colgate Platinum Professional Overnight are not surprising since the majority of in vitro studies evaluating 10% carbamide peroxide under this treatment regimen have failed to demonstrate adverse effects. Adverse effects on enamel have been reported when teeth were treated with 10% carbamide peroxide for 70 hours (10 h/d for 7 d).[18] Conversely, other authors have reported no detrimental effects on dental enamel when carbamide peroxide (10% and 15%) was applied for 336 hours (8 h/d for 6 wk).[19]

Similar findings have been reported for 10% carbamide peroxide under comparable test conditions.[20,21] Although a loss of calcium from enamel surfaces has been reported following treatment with 10% carbamide peroxide for 6 hours, this loss is equivalent to the loss that occurs upon drinking fruit juices and soft drinks.[13] The absence of enamel damage in the present studies may be due to the fact that the samples were soaked in saliva, which has remineralizing abilities. These conditions better mimic enamel exposure during in vivo treatment. In addition, Colgate Platinum Professional Overnight formulations contain calcium pyrophosphate and calcium phosphate, which also promote remineralization.

CONCLUSIONS

Repeated application of formulations containing 3.5% (equivalent), 7.0%, and 12.0% hydrogen peroxide do not alter enamel morphology, microhardness, or roughness. In addition, the levels recovered in the pulp chamber indicate that hydrogen peroxide is not expected to inhibit pulpal enzymes. Although these findings support the safety of such formulations in professional tooth-whitening products, additional studies are needed to address the effects of these concentrations and treatment regimens on tooth sensitivity and gingival irritation.

DISCLOSURE

The authors work for Colgate-Palmolive Company.

REFERENCES

1. Zinner DD, Duany LF, Llorente M. Effects of urea peroxide in anhydrous glycerol on gingivitis and dental plaque. J Prevent Dent 1978; 5:38–40.
2. Shapiro WB, Kaslick RS, Chasens AI. The influence of urea peroxide gel on plaque, calculus, and chronic gingival inflammation. J Periodontol 1973; 44:636–639.
3. Keyes PH. Microbiologically monitored and modulated periodontal therapy. Gen Dent 1978; 33:105–114.
4. Walsh LJ. Safety issues relating to the use of hydrogen peroxide in dentistry. Aust Dent J 2000; 45:257–269.
5. Cohen SC. Human pulpal response to bleaching procedures on vital teeth. J Endod 1979; 5:134–138.
6. Schulte JR, Morrissette DB, Gasior EJ, Czajewski MV. The effects of bleaching application time on the dental pulp. J Am Dent Assoc 1994; 125:1330–1335.
7. Bowles WH, Ugwuneri Z. Pulp chamber penetration by hydrogen peroxide following vital bleaching procedures. J Endod 1987; 13:375–377.
8. Haywood VB, Leech T, Heymann HO, Crumpler D, Brugger K. Nightguard vital bleaching: effects on enamel surface texture and diffusion. Quintessence Int 1990; 21:801–804.
9. Haywood VB, Houck V, Heymann HO. Nightguard vital bleaching: effects of varying pH solutions on enamel surface texture and color change. Quintessence Int 1991; 22:775–782.
10. Seghi RR, Denry I. Effects of external bleaching on indentation and abrasion characteristics of human enamel in vitro. J Dent Res 1992; 71:1340–1344.
11. Murchison DF, Charlton DG, Moore BK. Carbamide peroxide bleaching: effects on enamel surface hardness and bonding. Oper Dent 1992; 17:181–185.
12. Nathoo SA, Chmielewski MB, Kirkup RE. Effects of Colgate Platinum Professional toothwhitening system on microhardness of enamel, dentin, and composite resins. Comp Contin Educ Dent 1994; Suppl 17: S627–S630.
13. McCracken MS, Haywood VB. Demineralization effects of 10% carbamide peroxide. J Dent 1996; 24:395–398.
14. Slezak B, Santarpia P, Xu T, et al. Safety profile of a new liquid whitening gel. Compend Contin Educ Dent 2002; 23(11 Suppl 1):4–11.
15. Thitinanthapan W, Satamanont P, Vongsavan N. In vitro penetration of the pulp chamber by three brands of carbamide peroxide. J Esthet Dent 1999; 11:259–264.
16. Bowles WH, Burns H. Catalase/peroxidase activity in dental pulp. J Endod 1992; 18:527–529.
17. Bowles WH, Thompson LR. Vital bleaching: the effects of heat and hydrogen peroxide on pulpal enzymes. J Endod 1986; 12:108–113.
18. Josey AL, Meyers IA, Romaniuk K, Symons AL. The effect of a vital bleaching technique on enamel surface morphology and the bonding of composite resin to enamel. J Oral Rehabil 1996; 23:244–250.
19. Oltu U, Gurgan S. Effects of three concentrations of carbamide peroxide on the structure of enamel. J Oral Rehabil 2000; 27:332–340.
20. Lopes GC, Bonissoni L, Baratieri LN, Vieira LC, Monteiro S. Effect of bleaching agents on the hardness and morphology of enamel. J Esthet Restor Dent 2002; 14:24–30.
21. Spalding M, Taveira LA, de Assis GF. Scanning electron microscopy study of dental enamel surface exposed to 35% hydrogen peroxide: alone, with saliva, and with 10% carbamide peroxide. J Esthet Restor Dent 2003; 15:154–164.

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