

Peroxide Interactions With Hard Tissues: Effects on Surface Hardness and Surface/Subsurface Ultrastructural Properties

Donald J. White, PhD
Research Fellow

Kathy M. Kozak, BS
Senior Research Associate

James R. Zoladz, AAS
Senior Laboratory Assistant
The Procter & Gamble Company
Mason, Ohio

Heinz Duschner, PhD
Professor

Hermann Götz, BS
Senior Research Associate

Applied Structure and Microanalysis
Medical Faculty
The University of Mainz
Mainz, Germany

Learning Objectives

After reading this article, the reader should be able to:

- describe an in vitro methodology for evaluating the effect of peroxide bleaching on enamel surface and subsurface properties.
- compare the effects of bleaching with carbamide peroxide, hydrogen peroxide, and placebo gels on the enamel structure.
- discuss why confocal laser scanning microscopy (CLSM) is a useful measurement tool for investigating the effects of bleaching on enamel subsurface structures.
- describe the differences seen in CLSM images between enamel that has been demineralized and enamel that has undergone bleaching.

Abstract: Laboratory studies were performed to assess the impact of peroxide bleaching on enamel surface and subsurface physical and ultrastructural properties. Human enamel blocks were prepared, polished, and measured for native color. Cyclic bleaching treatments were carried out with soaks in whole stimulated saliva interspersed with bleaching treatments using bulk bleaching gels from commercial bleaching systems including Opalescence® (20% and 10% carbamide peroxide systems) and Crest® Whitestrips, a hydrogen peroxide gel formula, at doses of 5.3% and 6.5% hydrogen peroxide. Treatments ranged from conditions of normal use (14 hours as recommended for Crest® Whitestrips) to excessive bleaching (70 hours). Controls included nontreated as well as treatments with placebo (not containing peroxide) gels. Surface hardness and confocal laser scanning microscopy (CLSM) techniques were used to characterize the effects of bleaching on the physical properties and ultrastructure of the teeth. Tooth color measurements revealed dose-response bleaching in vitro with the increases in L* and decreases in b* normally expected with effective bleaching. Placebo control treatments did not bleach. Surface hardness measurements showed no decreases associated with tooth bleaching. CLSM measurements also showed no effects from tooth bleaches on the surface or subsurface prism architecture of enamel. This was opposed to significant changes seen with even moderate levels of demineralization associated with the caries process. These studies support: (1) the safety of Crest® Whitestrips formulas for enamel surfaces and tooth subsurfaces; and (2) the generic safety of peroxide bleaching of hard tissues associated with conditions of both recommended use and overuse.

The cosmetic dentistry industry continues to grow at an accelerated pace, as does patient interest in tooth-whitening procedures. Today, patients may access multiple routes toward improving tooth appearance, including improved dentifrices, in-office or home-prescribed professional bleaching kits, and, increasingly, over-the-counter (OTC) bleaching products.¹⁻³ Modern bleaching systems are most effective in producing improvements in tooth appearance, removing both exterior (extrinsic) dental stains as well as interior (intrinsic) sources of discoloration within the tooth structures.^{4,5} The whitening efficacy of bleaching systems is produced through the chemical oxidation actions of peroxides. While it is generally acknowledged that modern bleaching gels are safe for both hard and soft tissues,^{6,7} a more complete understanding of the mechanism of tooth bleaching may be useful to more fully establish the benefits and limitations of consumer-safe whitening procedures.

Our laboratories have undertaken extensive research to more fully understand the effects of modern oxidative whitening on tooth structure and function, including detailed examination of peroxide chemistry in both surface and subsurface enamel and dentin. The experiments reported here examined the effects of hydrogen peroxide gels used in the Crest® Whitestrips^a system as well

^a The Procter & Gamble Co, Cincinnati, OH 45202; 800-492-7378

as other commercial bleaching gels on the surface physical properties and surface/subsurface ultrastructural characteristics of dental enamel.

Materials and Methods

General Study Design

The effects of bleaching on tooth structure and hardness were examined by evaluating treatment effects on enamel surface hardness and surface/subsurface ultrastructure as assessed by confocal laser scanning microscopy (CLSM). The testing approach included the selection of treatment groups that would bracket the clinical range of peroxide exposure with respect to most dentist-applied and OTC peroxide gel systems. Treatments included gel compositions with peroxides at concentrations equivalent to 3.3% to 6.7% hydrogen peroxide, with application periods ranging from 14 hours to 70 hours. Control treatments included placebo gels and a negative (nontreated) control.

Treatment Groups

Bleaching treatments included those outlined below:

- 5.3% hydrogen peroxide gel—14 hours of treatment
- Placebo gel to 5.3% hydrogen peroxide—14 hours of treatment
- 6.5% hydrogen peroxide gel—70 hours of treatment
- Placebo gel to 6.5% hydrogen peroxide—70 hours of treatment
- 10% carbamide peroxide (Opalescence^{®b} gel) (20% water added in the laboratory to activate the carbamide peroxide gel)—70 hours of treatment
- 20% carbamide peroxide (Opalescence^{®b} gel) (20% water added in the laboratory to activate the carbamide peroxide gel)—70 hours of treatment
- Whole human saliva only—control

Note: Hydrogen peroxide gels were prepared in Crest[®] Professional Whitestrips gel base.

Tooth Preparation

Rectangular enamel sections approximately 3 mm to 4 mm in diameter were prepared under a water-cooled saw and mounted in a methacry-

late polymer, Durabase[®], with surface polishing to a 0.3- μ m finish. Enamel specimens were premeasured for tooth color (Fuji Fujix X-2000 digital camera^d) with color values recorded as averaged areas in 2-mm circular centers of specimens using the Commission Internationale de l'Eclairage (CIE) L* (light-dark) and b* (yellow-blue) scales with internal standards serving as controls. Specimens were evaluated for surface microhardness using a Buehler hardness tester^e with a Vickers diamond applied at 500-g loading.

Cycling Treatment of Tooth Specimens

Enamel specimens (10 per group) were cycled through a treatment regimen simulating the bleach and saliva exposures that are encountered under in situ conditions. Cycling treatments were carried out with specimens in individual cells of a 12-well polystyrene cell-culture plate. The cycling regimen was initiated by immersing specimens into 2 mL of wax-stimulated, whole human saliva at 37°C to establish an initial salivary conditioning film. Then, specimens were placed in 0.5 g of test gel (bulk gel) face down in a clean cell-culture cluster for 2 hours at 37°C. After 2 hours of bleaching, specimens were washed with tap water and a wet toothbrush and reimmersed into whole human saliva at 37°C for an additional 2 to 3 hours, after which a second 2-hour bleaching period was carried out. After the second bleaching, specimens were again individually washed with water and reimmersed overnight in fresh whole human saliva kept at 37°C. This bleaching/saliva cycling was carried out for the entire duration of treatments. After the specified time period of bleaching, specimens were postsoaked for 48 hours to 72 hours in whole human saliva (changed twice per day) to reestablish specimen hydration equilibria.

Evaluation of Treatment Effects

After treatments, the color and surface microhardness of enamel specimens were measured as described above. The ultrastructural effects of the bleaches on enamel were determined by CLSM. CLSM records were carried out under oil immersion in the reflection mode on surfaces of enamel specimens

^b Ultradent Products, Inc, South Jordan, UT 84095; 800-552-5512

^c Reliance Dental Mfg Co, Worth, IL 60482; 708-597-6694

^d Fuji Inc, Tokyo, Japan; 800-800-FUJI

^e Buehler Ltd, Lake Bluff, IL 60044; 800-BUEHLER

^f Leica Imaging Systems, Ltd, Cambridge, England

Table 1—Color Changes of Enamel: L* and b*

Treatment	ΔL^* From Baseline \pm SD	Δb^* From Baseline \pm SD
5.3 PL	-0.51 ± 0.62 d	$+0.87 \pm 0.62$ d
6.5 PL	-0.29 ± 0.58 d	$+1.30 \pm 0.61$ d
WH SCTR	-0.18 ± 1.02 d	$+1.32 \pm 0.60$ d
5.3 PER	$+2.23 \pm 1.18$ c	-2.90 ± 1.45 c
OP10	$+4.60 \pm 2.15$ b	-4.73 ± 2.09 b
6.5 PER	$+5.35 \pm 1.64$ b	-3.84 ± 1.33 bc
OP20	$+7.56 \pm 1.62$ a	-6.62 ± 2.40 a

Letters denote treatment significance: $P < 0.05$ ANOVA where $a \neq b$

PL = placebo; OP = Opalescence[®]; WH SCTR = whole human saliva control; PER = hydrogen peroxide; SD = standard deviation

using a Leica Aristoplan^f CLSM with illumination provided by a mixed-gas helium-argon laser at a wavelength of 488 nm. The use of CLSM in the reflection mode at this wavelength has been established for providing a useful means for nondestructive microscopic histotomography of surface and subsurface areas of naturally wet oral hard tissues down to about 150 μm .⁸ Transverse scans were carried out at lesion surfaces and in subsurface enamel progressing in 0.3- μm increments. For inter-comparisons of individual images, the parameters of the system (ie, photomultiplier voltage, laser power, etc) were kept constant during the experiment. Images were recorded both as individual confocal planes (for surface visualization) and as three-dimensional reconstructions to provide three-dimensional images of the dental tissues below the enamel surface. These combined measurements permitted the detailed ultrastructural evaluation of bleaching effects both on and within dental structures.

Statistics

Surface color and surface microhardness assessments were analyzed by the Student *t* test ($P < 0.05$). For color assessments, the averages of the 10 specimens were compared. For hardness, each specimen had an average hardness obtained with the reading of five indents. Specimen averages were then combined and compared. CLSM measures were qualitatively evaluated with documentation herein of standard representative images.

Control Enamel Samples

The interpretation of CLSM results was helped by comparison of measurements

obtained in a separate set of experiments examining the caries process. A series of artificial caries lesions were developed in polished enamel surfaces by immersing specimens in synthetic demineralization media (0.10 mol/L lactic acid, 50% saturated hydroxyapatite, and 0.2% Carbopol^{®g} polyacrylic acid, pH 5.0) for periods of 24, 48, and 96 hours as described in detail previously.⁹ Half of the surfaces were covered with varnish during demineralization to serve as an internal control to each specimen (because of lateral bleach diffusion, this is not possible in bleach specimens). This is the standard methodology of the Procter & Gamble laboratories for the simulation of the early caries process used in the authors' fluoride research. Control lesions of various sizes were examined by CLSM techniques for comparison to bleached samples as described previously.

Results

Color changes associated with bleaching are shown in Table 1. Bleaching under these cycling conditions was highly successful; bleaching treatments showed significant effects in increasing L* (specimens became lighter) with ΔL^* showing positive increases ranging from +2.23 to +7.56 units. As anticipated, the higher-dose peroxide treatments (6.5% hydrogen peroxide and 20% carbamide peroxide) showed stronger bleaching reactivity. Similar trends were seen on analysis of the color b* parameter (yellow to blue), with Δb^* decreasing (lower b* means less yellow, more blue) for all bleaching treatments and showing the largest decrease for the highest peroxide dose (20% carbamide peroxide). The effective

^g Noveon North America, Cleveland, OH 44141; 800-379-5389

Table 2—Surface Microhardness Changes During Bleaching

Treatment	Vickers Hardness Initial Number ± SD	Student <i>t</i> Test <i>P</i> < 0.05	Vickers Hardness Final Number ± SD
5.3 PL	355 ± 19	ns	368 ± 16
6.5 PL	359 ± 24	ns	353 ± 25
WHSCTR	337 ± 31	ns	352 ± 11
5.3 PER	349 ± 26	sig.	368 ± 20
OP 10	356 ± 27	ns	351 ± 16
6.5 PER	349 ± 26	ns	360 ± 17
OP 20	343 ± 24	ns	341 ± 18
Average for groups	349 ± 7.4	ns	356 ± 9.8

PL = placebo; OP = Opalescence®; WHSCTR = whole human saliva control; PER = hydrogen peroxide; SD = standard deviation

bleaching shown for specimens under these laboratory conditions verified their applicability for assessments of effects on surface physical properties and ultrastructure.

Table 2 shows the results of surface-hardness evaluations of treatment groups. Bleaching did not significantly soften enamel; not a single treatment exhibited a significant decline in the Vickers hardness number after cyclic bleaching and salivary exposure. In fact,

the 5.3% hydrogen peroxide and 6.5% hydrogen peroxide bleaching treatments appeared to harden enamel with cycling, with the 5.3% hydrogen peroxide producing significant increases.

Figure 1 shows CLSM images of a treatment series observed with a surface orientation. In this orientation, CLSM images are revealed as prism peripheries shown as the bright circular reflections. Here, we see no

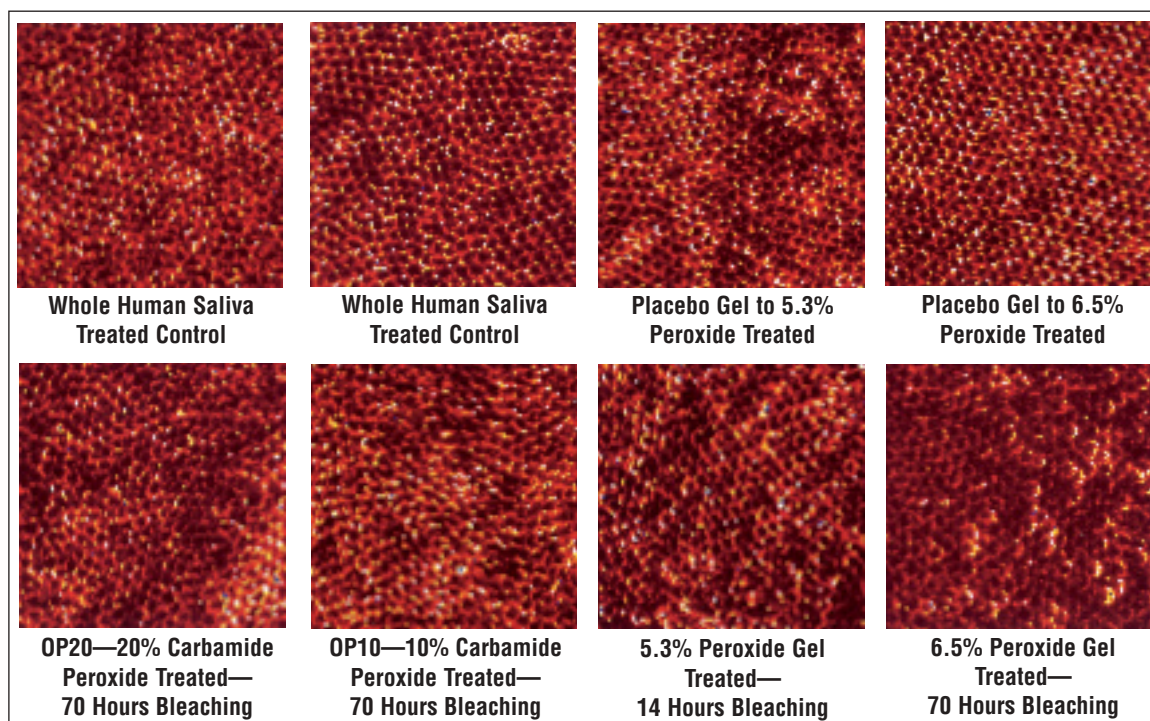


Figure 1—CLSMs of enamel surfaces—comparison of bleached specimens to controls. Reflections from interprismatic enamel produce more vivid circular patterns. Within prisms, the enamel acts translucent and light is not reflected back. Bleached specimens show no evidence of structural changes with images consistent with specimen-to-specimen variability.

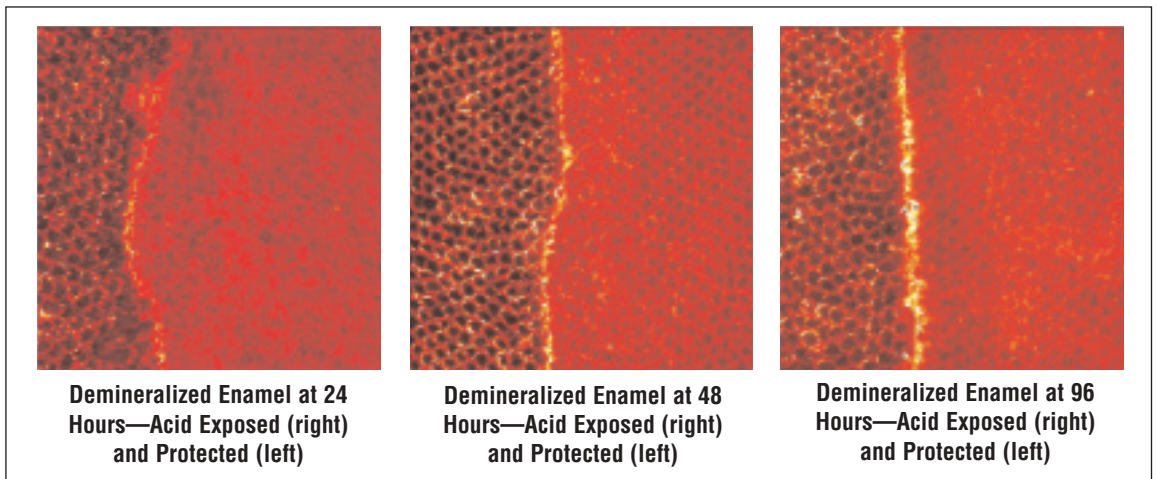


Figure 2—Surfaces exposed to laboratory demineralization for various time periods. Caries process produces loss of interprismatic reflection resulting in a dulling and diffusion of image. Clear demarcation of the varnish control for images. Extended demineralization returns some features—possibly through surface remineralization, but a more diffuse image is maintained.

changes in the apparent ultrastructure of the enamel associated with bleaching (compare bleached and control specimens of Figure 1). The sensitivity of the CLSM technique to ultrastructural changes in this viewing range is illustrated in Figure 2, where changes associated with the caries process are shown. Note how the initial caries process results in diffuse elimination of strong peripheral prism reflections. The comparison of these effects to the bleaching images supports the passivity of the bleaching process to surface enamel ultrastructure.

Figure 3 illustrates the three-dimensional reconstruction of bleached and control specimens viewed with the CLSM technique. These three-dimensional reconstructions are derived from single-plane images (such as

those shown in Figure 1) constructed together with successive scans in optical planes within the teeth. In the direct-reflection mode, the “tubular” structures are actually the periphery of the enamel prisms clearly outlined as they progress through the tissue. Of importance, the similarity of control and bleached specimens observed in Figure 3 supports the passivity of the bleaching process to hard tissue ultrastructure. This is particularly evident in contrast to Figure 4, where the three-dimensional CLSM assessment of the demineralization process associated with the caries process is revealed.

Discussion

These laboratory studies were directed toward the development of a more complete understanding of the mechanism of tooth

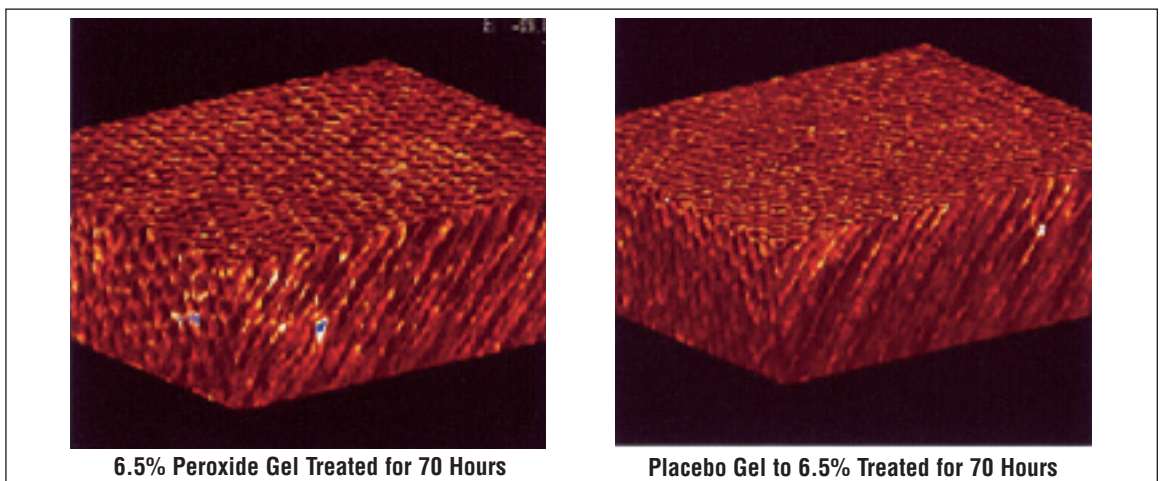


Figure 3—CLSMs of bleached and control enamel—three-dimensional reconstruction of CLSM ultrastructure prepared by progressive collection of two-dimensional image planes in 0.3- μm increments. The prismatic enamel is shown as columns in normal reflection; again the result of interprismatic reflection as opposed to translucency in prism interior. Bleaching with 6.5% hydrogen peroxide produced no apparent ultrastructural changes in enamel.

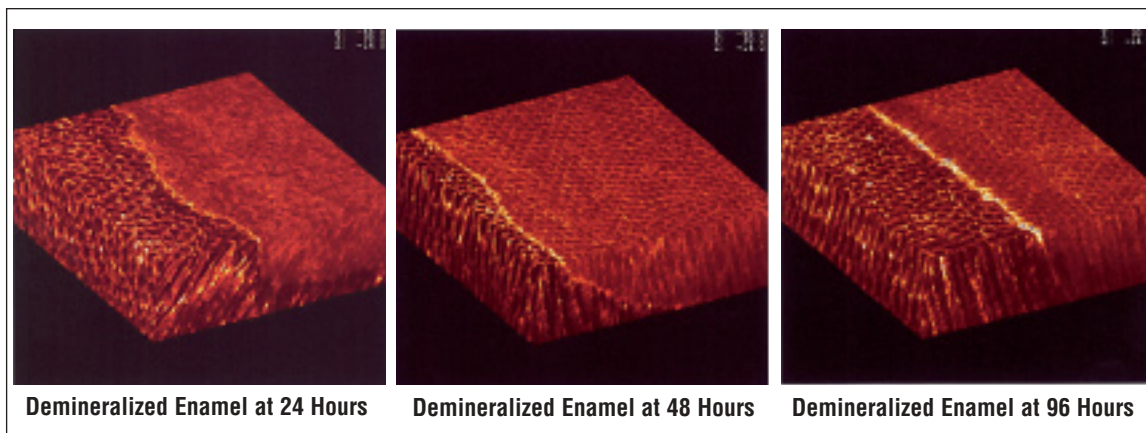


Figure 4—CLSMs of demineralized and control enamel—three-dimensional reconstruction of CLSM ultrastructure prepared by progressive collection of two-dimensional image planes in 0.3- μm increments. The prismatic enamel is shown as columns in normal reflection; again the result of interprismatic reflection as opposed to translucency in prism interior. Demineralization process simply visualized in direct reflection mode, with depth of damage and ultrastructural changes in interprismatic enamel evident. Line of demarcation to varnish exclusion is clearly evident (varnish removed before analysis). Changes are in stark contrast to bleaching images, where bleaching process is shown as benign.

bleaching in order to more fully establish the benefits and limitations of consumer-safe whitening procedures. In these experiments, bleaching procedures encompassed a wide range of exposure. In terms of the time of applied bleaching, the exposures ranged from periods matching those expected clinically (ie, 14 hours of tooth bleaching for the Crest® Whitestrips system) to conditions extending far beyond those experienced by patients/consumers or prescribed by professionals (ie, 70 total hours of bleaching). In terms of the quantity of applied bleach, all of these experiments can be considered to be in excess of clinical applications, because the bleaches herein were applied in bulk gels. The authors' laboratory tests revealed that these treatments as bulk gels supply fixed and constant peroxide concentrations, with virtually no depletion of the active agents during application. This is in contrast to clinical bleaching procedures, where the peroxide concentrations underneath strips or within trays is rapidly depleted in situ.¹⁰ The conditions of bleaching carried out herein were in excess of what is anticipated clinically, and can be used as confirmation of the safety of these applications under conditions of rigorous use.

In addition to the simulation of bleach concentrations and time of exposure, these experiments involved bleaching of tooth surfaces, with surface and subsurface ultrastructural effects assessed after treatment. The authors' previous studies had focused on bleaching of cross-sections of teeth.¹¹ Importantly, the color analysis of treated teeth in this study con-

firmed that bleaching had indeed occurred, rendering later measurements of the effects of bleach on enamel properties more relevant. Many literature studies of the effects of bleach on enamel do not confirm or report actual tooth bleaching, rendering their conclusions suspect. The authors' experience is that non-aqueous gels do not bleach well in vitro without saliva or water added to facilitate diffusion. This is why the nonaqueous carbamide peroxide treatments were doped with water to facilitate conversion to hydrogen peroxide to hasten bleaching actions. The color changes reported here were substantial, and in excess of typical L^* and b^* changes expected in the clinical situation,¹² again supporting the applicability of these tests to support general safety of bleaching procedures.

The hardness analysis of treated specimens revealed no softening effects associated with extensive bleaching. This is in agreement with previous literature observations on the passivity of bleaching oxidation with respect to mineral components of the teeth and corresponding ultrastructure associated with their physical properties. Within the cycling protocol reported here, whitening of the 5.3% hydrogen peroxide and 6.5% hydrogen peroxide groups actually showed some hardening, which can be reasoned to result from salivary cycling associated with the remineralization phenomena. It is conceivable that bleach interactions with pellicle proteins and enamel surface organics might produce increased reactivity of these surfaces with sali-

va soaks promoting mineralization.

Duschner and colleagues^{8,11} have described in detail the principles of the CLSM technique and characteristic images obtained with hard tissue specimens when the CLSM is applied in the reflection mode. The confocal images shown in Figures 1 through 4 give information about the intensity of light remitted from the microstructures of surface and subsurface hard tissues. For enamel visualized perpendicular to the surface, Duschner et al⁸ suggest that the images present as remittance of the interprismatic enamel, with prismatic enamel appearing as dark zones of poor light remittance (translucent). The structural dimensions and patterns of these observations, both in dentin and enamel, follow ultrastructural observations by other techniques and hence validate interpretation of the CLSM observations. The three-dimensional reconstructions permit the visualization of the prism boundaries of the enamel as the prisms extend from the surface toward the dentin, illustrated clearly in these studies.

Nondestructive CLSM histotomographic images can provide sensitive evidence of structural changes within hard tissues. Even a slight caries acid challenge, or the presence of hypermineralized enamel, can eliminate ultrastructure from enamel and radically change the CLSM image. Figures 2 and 4 illustrate the sensitivity of CLSM in reflection mode in observing the ultrastructural changes associated with the caries process produced under mild laboratory conditions. The images in Figures 1 and 3 present no evidence of detectable ultrastructural changes associated with the bleaching process, particularly when we compare these to the major effects that even minor demineralization produces, as highlighted in the control images reported.

As mentioned, a more complete understanding of the mechanism behind tooth whitening associated with nonetching oxidizing agents, including the development of a detailed understanding of the ultrastructural effects throughout the tooth, is worthwhile to understand the benefits and limits of safe tooth whitening procedures. These results demonstrate no significant ultrastructural effects within enamel or dentin associated with tooth bleaching of various intensity, including con-

ditions of demonstrably excessive treatment. These observations provide support for the authors' clinical experience that vital tooth bleaching produces no effects on the structure or function of the teeth; the only meaningful adverse effect being reports of mild and transient episodes of dentinal hypersensitivity. Further studies are evaluating ultrastructural effects on teeth bleached in a conventional fashion, including detailed examination of processes responsible for tooth whitening and, in particular, characterization of nonfunctional proteins and stains undergoing oxidation in the bleaching process.

Disclosure

The research conducted at The University of Mainz was supported by Procter & Gamble.

References

1. Gerlach RW: Shifting paradigms in whitening: introduction of a novel system for vital tooth bleaching. *Compend Contin Educ Dent* 21(suppl 29):S4-S9, 2000.
2. Goldstein RE: In-office bleaching: where we came from, where we are today. *J Am Dent Assoc* 128(suppl):11S-15S, 1997.
3. Clinical Research Associates: At-home tooth bleaching, state-of-art 2001. *CRA Newsletter* 25(2):1-4, 2001.
4. Goldstein R, Garber D: *Complete Dental Bleaching*. Chicago, IL: Quintessence; 1995.
5. Leonard RH Jr: Efficacy, longevity, side effects and patient perceptions of nightguard vital bleaching. *Compend Contin Educ Dent* 19(8):766-774, 1998.
6. Haywood VB: Nightguard vital bleaching: current concepts and research. *J Am Dent Assoc* 128(suppl):19S-25S, 1997.
7. Haywood VB: Considerations and variations of dentist-prescribed, home-applied vital tooth-bleaching techniques. *Compend Contin Educ Dent* 15(suppl 17):S616-S621, 1994.
8. Duschner H, Sonju-Clasen A, Ogaard B: Detection of early caries by confocal laser scanning microscopy. In: *Early Detection of Dental Caries: Proceedings of the First Annual Indiana Conference, Indiana School of Dentistry, Indianapolis, Indiana, 1996*. Indianapolis, IN: Indiana School of Dentistry; 1996:145-156.
9. White DJ: Use of synthetic polymer gels for artificial carious lesion preparation. *Caries Res* 21(3):228-242, 1987.
10. Sagel PA, Odioso LL, McMillan DA, et al: Vital tooth whitening with a novel hydrogen peroxide strip system: design, kinetics, and clinical response. *Compend Contin Educ Dent* 21(suppl 29):S10-S15, 2000.
11. White DJ, Kozak KM, Zoladz JR, et al: Effects of tooth-whitening gels on enamel and dentin ultrastructure—a confocal laser scanning microscopy study. *Compend Contin Educ Dent* 21(suppl 29):S29-S34, 2000.
12. Gerlach RW, Gibb RD, Sagel PA: A randomized clinical trial comparing a novel 5.3% hydrogen peroxide whitening strip to 10%, 15%, and 20% carbamide peroxide tray-based bleaching systems. *Compend Contin Educ Dent* 21(suppl 29):S22-S28, 2000.