Effects of Peroxide Gels on Enamel and Dentin *In Vitro*

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ABSTRACT

The effects of bleaching gels on the physical and ultrastructural characteristics of enamel, dentin and pulp were examined. Ten tooth blocks (each) were prepared in acrylate mounts and placed into treatment groups including: Control (WHSCTR), 5.3 % H_2O_2 Gel - 14 hours treatment (5.3PER), 6.5 % H_2O_2 Gel - 70 hr. (6.5 PER), Placebo to 5.3 % Gel - 14 hr. (5.3PL), Placebo to 6.5 % Gel - 70 hr. (6.5PL), Opalescence® 10 % Carbamide Peroxide (CP) Gel - 70 hr. (OP10) and Opalescence® 20 % CP Gel - 70 hr. (OP20). The 5.3 and 6.5 % H_2O_2 gels (and placebos) were prepared in Crest Whitestrips™ base. Treatments included a cycling regimen of 2 hr. bleaching (0.5 gm. gel/spec. 2x/day) with saliva/buffer immersion in between (and 48 hr. saliva post trmt.). Post bleaching specimens were cross-sectioned, and ° of specimens were mounted with sectioned faces perpendicular and polished to 0.3 mm finish. Hardness (VHN) measurements were carried out on surface enamel (pre/post trmt.) and on subsurface enamel and dentin (post-trmt.) (Buehler, 500 gm. ld.) Ultrastructure was examined by Confocal Laser Scanning Microscopy (reflection mode) and SEM. VHN measured: Surface enamel: Pre:Post = 5.3PL 355 ±19:368 ±16(nsd); 6.5PL 359 ±24:353 ±25(nsd); WHSCTR 337 ± 31:352 ±11(nsd); 5.3PER 349 ±26:368 ±20(sig. p <0.05 stud.paired t); OP10 356 ±27:351 ±16(nsd); 6.5PER 349 ±6:360 ±17(nsd); OP20 343 ±24:341 ±18(nsd); Subsurface enamel: 6.5PL 337 ± 27 a; OP20 333 ± 14 ab; 6.5PER 321 ±20 ab; 5.3PER 316 ± 8 ab; OP10 309 ± 7 ab; WHSCTR 306 ± 27 ab; 5.3PL 305 ± 222 b; Subsurface dentin: 5.3PL 61 ± 5 a; WHSCTR 58 ± 3 ab; OP10 58 ± 5 ab; OP20 58 ± 3 ab; 6.5PER 56 ± 7 ab; 5.3PER 55 ± 12 ab; 6.5PL 52 ± 3 b {ab ≠ ANOVA Tukey Kramer HSD p < 0.05}. **VHN and microscopy measures support that bleaching produced no softening or ultrastructural changes of surface/subsurface enamel or subsurface dentin.**

INTRODUCTION

Vital tooth whitening continues to gain in popularity in the United States. Experience suggests that vital tooth bleaching is indeed a safe procedure. However researchers and the American Dental Association recommend studies to ensure the safety of new whitening formulations to both hard and soft tissues.

A more complete understanding of the mechanism of tooth bleaching associated with non-etching oxidizing agents is necessary to establish the benefits and limitations of consumer-safe whitening procedures. This study examined the effects of hydrogen peroxide bleaching on surface and subsurface enamel and subsurface dentin, hardness and ultrastructure.

MATERIALS AND METHODS

General Study Design: The effects of bleaching on tooth structure and hardness were examined by evaluating bleaching effects on enamel surface hardness, enamel subsurface hardness, dentin subsurface hardness and surface and subsurface ultrastructure as assessed by Confocal Laser Microscopy.

The approach to testing included the selection of treatment groups which would bracket the clinical range of peroxide exposure with respect to most dentist applied and OTC gel systems. Treatments thus included bleach compositions providing from 3.3 - 6.7 % hydrogen peroxide with applications for periods ranging from 14-70 hours tooth exposure. Control treatments included placebo gels and a negative control.

Tooth Preparation: Rectangular enamel sections approximately 3-4 mm in diameter were prepared under a water cooled saw and mounted in methacrylate polymer (Durabase) with surface polishing to 0.3 mm finish.

Enamel specimens were pre-measured for tooth color (Fuji digital Camera) (to confirm whitening took place in the study) on L*a*b* scale with internal standards serving as controls. Specimens were pre-evaluated for surface microhardness using a Buehler hardness tester (Vickers 500 gr.).

Enamel specimens (10/group) were cycled through a treatment regimen simulating bleach and saliva exposure which 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 (WHS).

To establish an initial salivary conditioning film, specimens were individually exposed to WHS for 2 hours at 37°C - 2 ml per specimen. Following this, specimens were placed in 0.5 grams of test gel (bulk gel) face down in a clean cell culture cluster for 2 hours at 37°C. Following 2 hour bleaching, specimens were washed with tap water and a wet tooth brush and reimmersed into WHS at 37°C for an additional 2-3 hours, after which a second 2 hour bleaching was carried out. Following the second bleaching, specimens were again individually washed with water and reimmersed overnight in fresh WHS kept at 37°C. This bleaching/saliva cycling was carried out for the entire duration of treatments. Following the specified time period of bleaching, specimens were post soaked for 48-72 hours in WHS (changed 2x per day) to re-establish specimen hydration equilibria.

Following treatments, the color and surface microhardness of enamel specimens were measured as described above. Following surface measures specimens were separated into different post treatment analysis group as follows. A subset of specimens was transverse cross sectioned vertically. A subset of these was (n=4) mounted in methacrylate - transverse section side up and polished to 0.3 mm finish. Three microhardness indents were taken within mid enamel and within mid dentin to assess subsurface enamel and dentin structural integrity. These same samples were saved for post treatment evaluation of bleaching effects on subsurface enamel ultrastructure and subsurface dentin ultrastructure using Confocal Laser Scanning Microscopy. CLSM was carried out under oil immersion on polished transverse sectioned faces on a Leica Diaplan CLSM with illumination provided by a mixed He-Ar laser at 488 nm. Transverse scans were carried out at 5-15 mm subsurface depth at power levels ranging from 300-600 mW.

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RESULTS

A subset of enamel specimens (n=4 from cross sections) were remounted surface side up and examined for surface morphology changes by Scanning Electron Microscopy according to American Dental Association guidelines including 200x and 2000x magnification. SEM's were collected on a Joel SEM/EDAX under both high vacuum (gold sputtered coating) and variable pressure (non-coated) mode.

A subset of enamel specimens (n=4 from cross sections) were remounted surface side up and examined for surface morphology changes by Confocal Laser Scanning Microscopy.

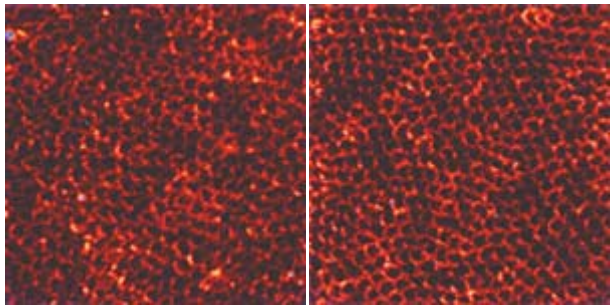
Bleaching Treatments included:

- 5.3% H_2O_2 Gel - 14 hours treating {5.3PER}
- Placebo gel to 5.3 % H_2O_2 - 14 hours treating {5.3PL}
- 6.5 % H_2O_2 Gel - 70 hours treating {6.5PER}
- Placebo gel to 6.5 % H_2O_2 - 70 hours treating {6.5PL}
- 10 % Carbamide Peroxide (20 % water added) Opalescence Gel - 70 hours treating {OP10}
- 20 % Carbamide Peroxide (20 % water added) Opalescence Gel - 70 hours treating {OP20}
- Whole Human Saliva Only Control {WHSCTR}

Peroxide gels were prepared in Crest Whitestrips peroxide gel base.

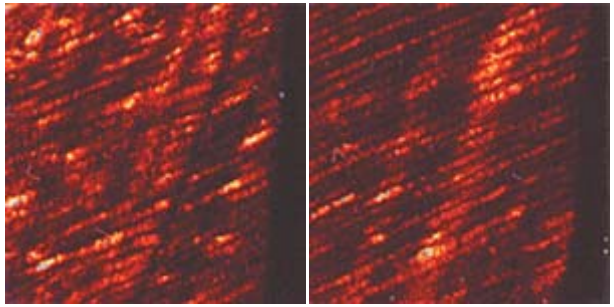
Surface microhardness assessments were analyzed by Students paired t analyses ($p < 0.05$) between pre and post bleached specimens in each treatment group with the reading per specimen derived from five indents used as an average for the analyzed variable. SEM and CLSM measures were qualitatively evaluated with documentation herein of standard images.

Confocal Laser Microscopy of Bleached and Control Specimens



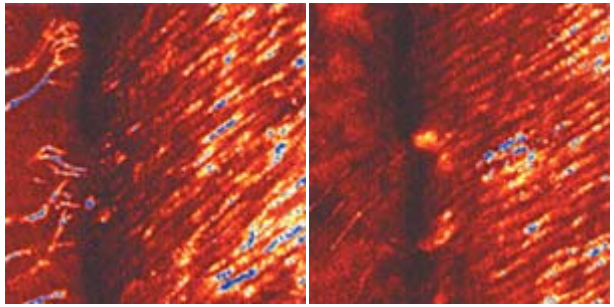
5.3% Peroxide Saliva Control

Surface CLSM View 5-15 mm Subsurface Focal Plane



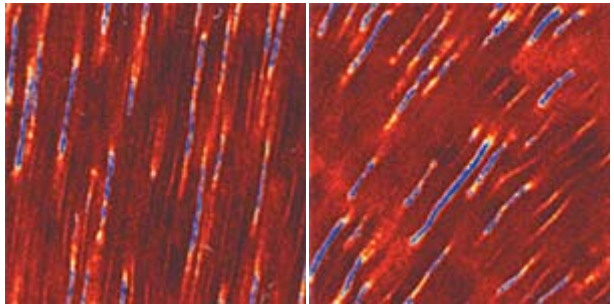
5.3% Peroxide Saliva Control

CLSM Cross Section View of Enamel 5-15 mm Subsurface Focal Plan



5.3% Peroxide Saliva Control

CLSM Cross Section View of DEJ 5-15 mm Subsurface Focal Plane



5.3% Peroxide Saliva Control

CLSM Cross Section View of dentin 5-15 mm Subsurface Focal Plane

Surface Microhardness Changes During Bleaching

Treatment	VHN Initial ± SD	Students t $p < 0.05$	VHN Final ± SD
5.3PL	355 ± 19	ns	368 ± 16
6.5PL	359 ± 24	ns	353 ± 25
WHSCTR	337 ± 31	ns	352 ± 11
5.3PER	349 ± 26	sig.	368 ± 20
OP10	356 ± 27	ns	351 ± 16
6.5PER	349 ± 26	ns	360 ± 17
OP20	343 ± 24	ns	341 ± 18
Avg. for groups	349 ± 7.4		356 ± 9.8

Subsurface Microhardness Changes on Transverse Sectioned Surfaces Post Bleaching

Treatment	VHN Enamel Subsurface ± SD	VHN Dentin Subsurface ± SD
5.3PL	305 ± 22 b	61 ± 5a
6.5PL	337 ± 27a	52 ± 3 b
WHSCTR	306 ± 27 b	58 ± 3 ab
5.3PER	316 ± 8a b	55 ± 12 ab
OP10	309 ± 7a b	58 ± 5 ab
6.5PER	321 ± 20a b	56 ± 7 ab
OP20	333 ± 14a b	58 ± 3 ab

$p < 0.05$ Anova a≠b

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CONCLUSION

-In vitro bleaching did not affect surface enamel or subsurface enamel and dentin hardness or ultrastructure

-There were no differences in the effects of various bleach gel formulations - with peroxide formulated in Crest Whitestrips gel base reacting similarly to benchmark control commercial gels

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