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The Effect of Whitening Agents on Caries Susceptibility of Human Enamel

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Clinical Relevance

The results of this study provide support for the concept that vital tooth whitening does not produce caries susceptibility in human enamel.

SUMMARY

This *in vitro* study evaluated whether the treatment of human enamel with whitening agents containing different concentrations of carbamide or hydrogen peroxide changes the susceptibility of enamel to caries. Twenty-four sound human incisors were selected for this study. For each tooth, the crown was sectioned into two halves in the cervical-incisal direction. One half of the sectioned tooth was treated and the other half was used as a control specimen. Each half was randomly divided into three treatment groups (eight two-halves/group). The whitening agents were 10% carbamide peroxide, 20% carbamide peroxide with fluoride and 35% hydrogen peroxide. Following pretreatment, the specimens were demineralized for four days in an *in vitro* microbial caries model and then analyzed using a confocal laser scanning microscope (CLSM). Results showed that there were no significant differences between the treated and controlled specimens for teeth treated with 10% carbamide peroxide or 35% hydrogen peroxide. However, specimens treated with 20% carbamide peroxide with FP (0.11% fluoride and potassium nitrate) were less susceptible to caries than their controls at $p \leq 0.05$. In conclusion, application of bleaching agents does not increase the caries susceptibility of human enamel.

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INTRODUCTION

At-home and in-office bleaching procedures have been recognized as successfully treating discolored teeth in esthetic dentistry. When vital teeth are bleached, direct contact is established between the bleaching agent and the outer enamel surface (Ernst, Marroquin & Willershausen-Zonnchen, 1996). Clinical observations have shown no important clinically adverse effects as a result of tooth whitening with moderate concentrations of hydrogen peroxide or carbamide peroxide (Haywood, 1994; Goldstein, 1995). Most laboratory studies have confirmed the clinical observation that whitening gels containing carbamide or hydrogen peroxide have not demonstrated a significant effect on hard tissue morphology (Ernst & others, 1996; Oltu & Gurgan, 2000; Gultz & others, 1999; McCracken & Haywood, 1996).

Clinically, however, it is not unusual to see a white spot develop during the whitening process, which disappears after discontinuing the procedure. The concern has been raised whether or not these white spots are precarious lesions. Therefore, this *in vitro* study was conducted to evaluate whether treating human enamel with whitening agents containing different concentrations of carbamide or hydrogen peroxide changes the susceptibility of enamel to caries.

METHODS AND MATERIALS

Three groups of extracted teeth were used in this study. Two groups of teeth were pretreated for eight hours with 10% or 20% carbamide peroxide (Opalescence). The 20% carbamide peroxide product also contained 0.11% fluoride and potassium nitrate (FP). The 10% whitening agent is accepted by the American Dental Association (ADA) as a "safe" and "effective" product to be used at home, and the 20% agent was also recommended for home use. The third group was pretreated with 35% hydrogen peroxide (Opalescence Xtra). This agent is approved for professional in-office use and was applied three different times with three 10-minute applications, for a total of nine applications.

Twenty-four sound human incisors were selected for this study. For each tooth, the root was removed and the crown sectioned into two halves in the cervical-incisal direction. One half of the sectioned tooth was treated and other half was used as a control specimen. Each half was attached with cyanoacrylate adhesive to one end of a plexiglass rod. The cut side of the specimen was sealed with nail varnish to prevent bacterial penetration into opened tubules when the specimens were placed in the demineralizing model. Each half was randomly divided into three treatment groups (eight groups of two-halves).

The control halves were placed in deionized water. The eight tooth halves in Group 1 were treated with 10% carbamide peroxide (Opalescence 10%, Ultradent Products, Inc, South Jordan, UT, USA) for eight hours on 14 consecutive days by placing the specimen upright in a holder and applying the whitening agent to the labial surface. The halves in Group 2 were similarly treated with 20% carbamide peroxide with FP (Opalescence 20% PF, Ultradent Products, Inc). This whitening agent contains 0.11% sodium fluoride and 3% potassium nitrate. The third group had 35% hydrogen peroxide placed on the labial surfaces. A curing light was then placed approximately 0.25 inches from the specimen surfaces, with the specimens being exposed for 30 seconds and the gel left in place for a total of 10 minutes. Hydrogen peroxide was then rinsed from the surface of each tooth. This procedure was repeated two more times during each treatment session. A total of three treatment sessions, each involving three individual treatments, separated by a minimum of three days, were applied to the specimens in Group 3 during a 14-day treatment period. During non-treatment times, all teeth were placed in a highly humid environment at 4°C.

Following pretreatment, the specimens were demineralized for four days an *in vitro* microbial caries model (Fontana & others, 2000). The eight specimens in each treatment group and their controls were mounted onto acrylic plates that fit tightly on the stirring magnet of four caries-forming vessels. All the specimens were gas sterilized, then placed in the microbial demineralizing model to generate carious lesions.

All the groups were inoculated once at the beginning of the experiment with a mid-log phase culture of *S mutans* TH16. Following inoculation, the specimens were incubated at 37°C for two hours to allow the bacteria to adhere to tooth surfaces before beginning treatment. The specimens were then exposed at 37°C to circulating trypticase soy broth supplemented with 5% sucrose (TSBS) for 30 minutes three times a day and to a mineral washing solution (MW) for total of 22.5 hours per day. The circulating fluids were delivered to and removed from the treatment vessels by time-regulated peristaltic pumps. The TSBS solution reproduced nutrient intake three times/day and the MW was used as an artificial saliva buffering solution. Each treatment group had its own TSBS bottle. The specimens were connected to two MW bottles. On days one and three, the drainage containers for all three groups were changed and the drainage fluid was monitored for pH, bacterial viability and the absence of contamination. After four days of demineralizing treatment in the model, the tooth specimens were removed and monitored for the development of primary caries in all specimens, including halves treated and non-treated with whiteners.

Confocal Laser Scanning Microscope (CLSM) Analysis

These analyses were done blindly, without any knowledge of the specimen groups' assignment or pretreatment. The specimens were analyzed in numerical order, thereby randomizing the treatment groups. Each specimen half was cut in half, perpendicular to the cervical incisal axis, using a Silverstone Taylor hard tissue microtome. One-half of each cut was stained overnight with an 0.1 mM solution of Rhodamine B fluorescent stain. The cut stained surface was then allowed to dry before being analyzed with a CLSM to determine the presence and extent of lesions. The samples were examined with a CLSM using Metamorph software (Universal Image Corp, West Chester, PA, USA). After being brought into focus using a 10x Nikon objective, NA 0.25, the specimens were illuminated with the argon laser using a 488 nm excitation wavelength. Confocal slits were set at 25 μm 100%. For collection of the images, the samples were frame-averaged using 100 frames per image. Lesion measurements were made in both the whitener-pretreated half and the non-pretreated half, and the following parameters were measured: area of the fluorescent lesion, fluorescence (total gray) of the lesion and depth of the lesion. The differences in lesion parameters between the pretreated and non-treated halves of the tooth were calculated.

Mean CLSM parameter data (± SD) was determined for the pretreated and non-pretreated lesions in each treatment group and analyzed for significant intra-group differences using a paired *t*-test. For each CLSM measured parameter, differences between the lesion in whitener-pretreated and non-pretreated enamel were calculated for each specimen and mean differences (± SD) were determined for each group. Multiple inter-group comparisons were then conducted using one-way analysis of variance (ANOVA). Multiple comparisons of the three groups were done using Tukey's procedure to determine significant differences among the group means when significant F-values were found. The level of significance accepted for all parameters measured in this study was $p \leq 0.05$.

Table 1: *Paired* t-*test Group 1 (10% CP)*

Parameters	Specimen	N	Mean	SD+/-	Sig
Total area (μm)²	Treated	8	8603.4	1727.5	
	Controlled	8	7152.5	2604.3	0.301
Total gray value	Treated	8	109.25	51.36	
	Controlled	8	74.67	30.76	0.178
Lesion depth (μm)	Treated	8	37.1	5.58	
	Controlled	8	39.97	7.56	0.451

Table 2: *Paired* t-*test Group 3 (35% HP)*

Parameters	Specimen	N	Mean	SD+/-	Sig
Total area (μm)²	Treated	8	8295.4	2438.82	
	Controlled	8	10667.0	3018.16	0.590
Total gray value	Treated	8	78.08	19.68	
	Controlled	8	120.42	62.89	0.200
Lesion depth (μm)	Treated	8	40.44	5.41	
	Controlled	8	48.34	10.2	0.087

Table 3: *Paired* t-*test Group 2 (20% CP with FP)*

Parameters	Specimen	N	Mean	SD+/-	Sig
Total area (μm)²	Treated	8	6060.8	2151.7	
	Controlled	8	8887.4	2302.5	0.027
Total gray value	Treated	8	69.7	36.37	
	Controlled	8	108.6	50.11	0.027
Lesion depth (μm)	Treated	8	30.13	9.2	
	Controlled	8	42.18	9.26	0.034

RESULTS

The paired *t*-test showed that there were no significant differences between the treated and controlled specimens for teeth treated with 10% carbamide peroxide or 35% hydrogen peroxide (Tables 1 and 2). However, specimens treated with 20% carbamide peroxide with FP were less susceptible to caries than their controls at $p \leq 0.05$ (Table 3). Figures 1, 2 and 3 illustrate the CLSM images from representative specimens in the study. ANOVA and Tukey's tests revealed that the total lesion area of the first group was significantly higher than the third group at $p \leq 0.05$.

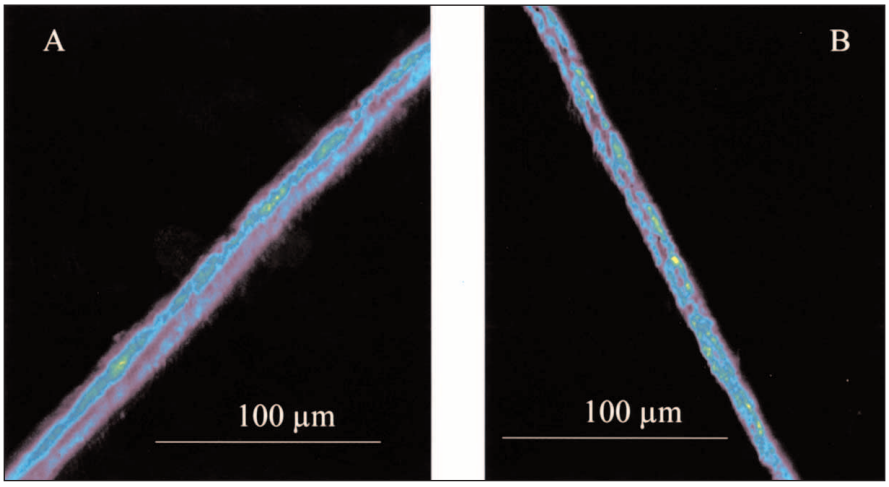

Figure 1. *(A) image of specimen treated with 10% CP, (B) control specimen of image (A). The pseudocolor shows the caries lesion in enamel.*

DISCUSSION

The CLSM images permit non-destructive examination of surface and subsurface hard tissue ultrastructural changes and provide evidence of structural changes within tissues (Duschner, Sonju-Clason & Ogaard, 1996).

In CLSM, laser light from out of focus planes is eliminated by the confocal pinhole technique, hence, only light remitted from the exact focus plane is recorded. The analog output of the photomultiplier detector is coordinated three-dimensionally with the scan position of the laser and the position of the object table. With computer assistance, this data is transferred into a three-dimensional matrix of digital values of light

intensity. For visualization, this information is transformed into an arbitrary scale of pseudocolors, then presented as three-dimensional images (White & others, 2000).

Hydrogen peroxide has a low molecular weight. Therefore, it can readily penetrate into enamel. Thus, inner oxidative effects are likely to occur in the subsurface enamel where more organic material is present and where oxidation is capable of altering the outer enamel and surface (Hegedus & others, 1999).

Haywood, Houck and Heymann (1991) studied, *in vitro,* the effect of three commercially available 10% carbamide peroxide solutions and a 1.5% hydrogen peroxide solution on enamel surface and color. No significant differences in enamel surface texture were detected between the treated and control enamel surfaces of the teeth in all groups. Leonard and others (1999) compared maxillary teeth after being bleached every night for six months with mandibular teeth that had not been bleached. They reported that there was "no discernable difference in surface texture between the maxillary and mandibular teeth as viewed under the Scanning Electron Microscope (SEM) at 200 or 2000 times magnification." Covington and others (1990) also looked at the effect of carbamide peroxide gel on the structure of human enamel in a SEM study. They reported the development of focal areas of very shallow erosion rather than pitting. Analysis of the outer 100 nm indicated a distinct loss of organic component from treated surfaces. He concluded that there was "… a controlled oxidation process in which the organic phase of the enamel is mobilized, without producing grossly unacceptable enamel surface topography."

Bitter (1998) reported that a 14-day exposure to whitening agents caused an alteration of the enamel surface, exposing the enamel prismatic layer and possibly dentin. This effect was seen up to 90 days post-treatment; however, no control was reported in this study for comparison. Also, the patients practiced poor oral hygiene with the teeth that were whitened, because the teeth were scheduled for extraction. Duschner and others (2000) used confocal laser scanning microscopy to examine the effects of bleaching on enamel and dentin.

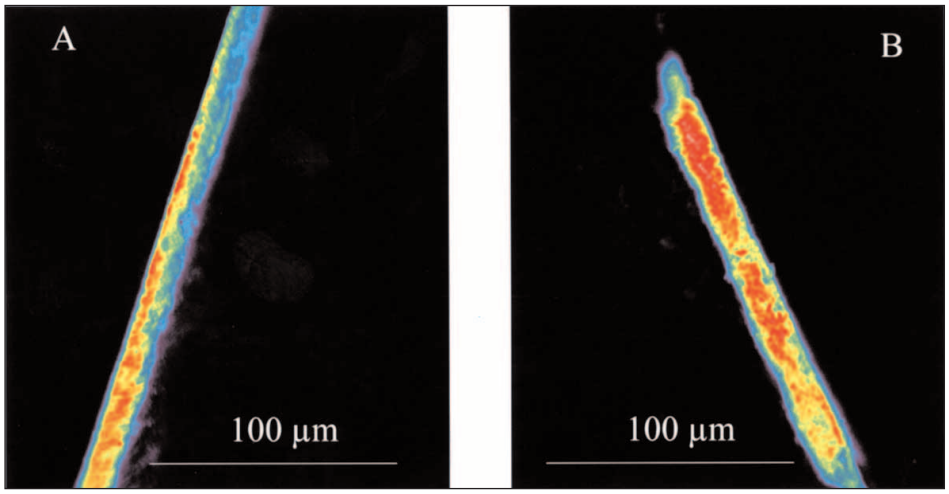

Figure 2. *(A) image of specimen treated with 20% CP, (B) control specimen of image (A). The pseudocolor shows the caries lesion in enamel.*

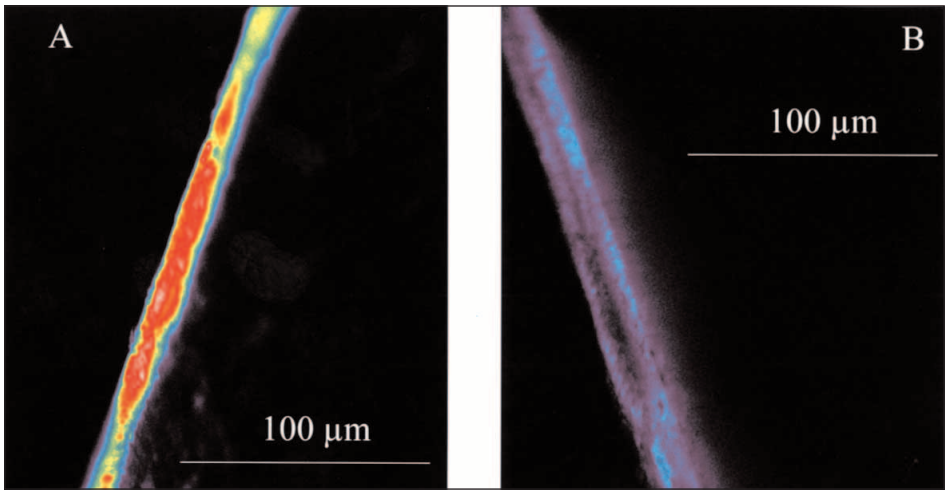

Figure 3. *(A) image of specimen treated with 35% HP, (B) control specimen of image (A). The pseudocolor shows the caries lesion in enamel.*

The results showed that no significant micromorphological changes were found in subsurface enamel. Potocnik, Kosec and Gaspersic (2000) studied the effect of 10% carbamide peroxide on enamel microhardness, microstructure and mineral content. They found local microstructural and chemical changes that were not clinically significant. Araujo and others (2003), in an *in situ* study, evaluated bleaching with 10% CP for one hour vs seven hours per day compared with a control and reported no difference in microhardness among the different regimens. White and others (2000) studied the effect of tooth-whitening gels on enamel ultrastructure by using confocal laser scanning microscopy. They found no significant micromorphological changes associated with the whitening process in subsurface enamel. Recently, a SEM study of dental enamel surfaces exposed to 35% hydrogen peroxide and 10% carbamide peroxide considered these gels safe for enamel

(Spalding, Taveira & de Assis, 2003). Basting, Rodrigues and Serra (2003) reported on the effects of seven carbamide peroxide bleaching agents on enamel hardness over time. His study showed a decrease in microhardness only after the first eight hours of a 45-day bleaching study.

In this study, no significant differences in caries susceptibility were observed between the untreated control specimens and those specimens treated with 10% carbamide peroxide or 35% hydrogen peroxide. On the other hand, specimens treated with gel containing 20% carbamide peroxide were significantly reduced in caries susceptibility when compared with their untreated controls. This is probably related to fluoride incorporation in 20% FP carbamide peroxide gels. This is in agreement with laboratory studies with fluoride enhanced enamel remineralization. A CLSM study by González-Cabezas and others (1998) revealed that remineralization is significantly greater in specimens treated with fluoride-containing dentifrices. Attin and others (1997) observed that remineralization of bleached enamel was improved by the application of fluoride. Future studies comparing the effect of 10% carbamide peroxide-containing fluoride and 20% carbamide peroxide-containing fluoride are recommended.

CONCLUSIONS

Application of bleaching agents does not increase caries susceptibility of human enamel. A bleaching agent-containing fluoride reduced caries susceptibility.

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