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## ABSTRACT

Oxygen inhibits polymerization of resin-based materials. We hypothesized that compromised bonding to bleached enamel can be reversed with sodium ascorbate, an anti-oxidant. Sandblasted human enamel specimens were treated with distilled water (control) and 10% carbamide peroxide gel with or without further treatment with 10% sodium ascorbate. They were bonded with Single Bond (3M-ESPE) or Prime&Bond NT (Dentsply DeTrey) and restored with a composite. Specimens were prepared for microtensile bond testing and transmission electron microscopy after immersion in ammoniacal silver nitrate for nanoleakage evaluation. Bond strengths of both adhesives were reduced after bleaching but were reversed following sodium ascorbate treatment ( $P < 0.001$ ). Resin-enamel interfaces in bleached enamel exhibited more extensive nanoleakage in the form of isolated silver grains and bubble-like silver deposits. Reduction of resin-enamel bond strength in bleached etched enamel is likely to be caused by a delayed release of oxygen that affects the polymerization of resin components.

**KEY WORDS:** carbamide peroxide, sodium ascorbate, microtensile bond strength, ultrastructure.

# Reversal of Compromised Bonding in Bleached Enamel

## INTRODUCTION

Previous studies have shown that carbamide peroxide bleaching agents in the range of 10-35% adversely affect the bond strength of composite to acid-etched enamel when bonding is performed immediately after the bleaching procedure (Titley *et al.*, 1992; Garcia-Godoy *et al.*, 1993; Miles *et al.*, 1994). Reduction in bond strengths was reported to be more pronounced with the use of acetone-based adhesives (Sung *et al.*, 1999). This is a concern in cosmetic dentistry and orthodontics, particularly with the popularity of “in-office” bleaching techniques (Swift, 1997; Spyrides *et al.*, 2000), since a period of up to three weeks is required before resin-enamel bond strengths return to values obtained for unbleached enamel (Cavalli *et al.*, 2001).

Compromised bond strengths that were observed for some single-bottle adhesives when dentin was treated with hydrogen peroxide before acid-etching could be effectively reversed with an anti-oxidant such as sodium ascorbate, when it was used for at least one-third of the time of application of the oxidizing bleaching agent (Lai *et al.*, 2001). We hypothesized that compromised bonding to carbamide-peroxide-bleached enamel may be similarly reversed with sodium ascorbate before resin bonding, since polymerization inhibition of the adhesive resins is a likely mechanism for the adverse effects of bleaching on enamel bonding (Dishman *et al.*, 1994). Since retention of surface and subsurface residual peroxide or peroxide-related substances (Torneck *et al.*, 1990) may be responsible for the time-dependent reduction in the quality of resin-enamel bonds, we anticipated that these subtle changes may be elucidated by transmission electron microscopy examination of the distribution of nanoleakage patterns (Sano *et al.*, 1995) within the bonded interfaces of oxidized acid-etched enamel and those that were subsequently neutralized with the anti-oxidant. Thus, the null hypothesis of this study was that there is no difference in the microtensile bond strengths and distribution of nanoleakage patterns of single-bottle adhesives bonded to carbamide-peroxide-bleached, acid-etched enamel and those that were further neutralized with sodium ascorbate.

## MATERIALS & METHODS

Extracted human third molars were collected after the patients' informed consent had been obtained under a protocol reviewed and approved by the institutional review board of the Medical College of Georgia, USA. The mesial and distal surfaces of these teeth were cleaned with pumice and sandblasted with 50- $\mu$ m alumina to provide bonding surfaces that were devoid of the surface aprismatic enamel.

### Experimental Design

Single Bond (3M-ESPE, St. Paul, MN, USA), an ethanol-based, and Prime&Bond NT (Dentsply DeTrey, Konstanz, Germany), an acetone-based single-bottle adhesive were used, each consisting of 3 experimental groups with 5 teeth each. Four teeth were used for bond strength evaluation, and the fifth

tooth was prepared for nanoleakage evaluation by transmission electron microscopy. The 3 experimental groups were as follows:

(I) Control group. The teeth were placed in distilled water for 8 hrs. The bonding surfaces were etched with a 32% phosphoric acid gel (Uni-Etch, Bisco, Inc., Schaumburg, IL, USA) for 15 sec and rinsed with water for 20 sec before bonding.

(II) Bleached group. The teeth were bleached by the placement of 10% carbamide peroxide (NuproGold, Dentsply DeTrey; pH = 6.4) around the enamel at 100% relative humidity for 8 hrs. They were rinsed and immersed in distilled water for 10 min and then etched with phosphoric acid as previously described.

(III) Ascorbate group. After the teeth were bleached and rinsed as previously described, they were immersed in 10% sodium ascorbate (Sigma Chemical Co., St. Louis, MO, USA) for 3 hrs (*i.e.*, at least one-third of the bleaching time) to neutralize the oxidizing effect of carbamide peroxide, according to the method described in Lai *et al.* (2001). Before being etched with phosphoric acid, the treated teeth were immersed in distilled water for 10 min to dissolve the sodium ascorbate crystals that were deposited on the bonding surfaces.

The treated teeth were bonded with two coats of either Single Bond or Prime&Bond NT. Bonded surfaces were air-dried and then light-cured for 10 sec. Composite buildups were performed in 5 1-mm increments with either a hybrid composite (Renamel Sculpt, Cosmedent, Inc., Chicago, IL, USA) for bond strength testing, or a microfilled lining composite (Protect Liner F, Kuraray Medical Inc., Tokyo, Japan) for transmission electron microscopy. The teeth were stored in distilled water at 37°C for 24 hrs.

### Microtensile Bond Strength Evaluation

Bonded teeth were sectioned occluso-gingivally into serial slabs, and further sectioned into 0.9 x 0.9 mm composite-enamel beams, according to the “non-trimming” technique of the microtensile test for enamel bond-testing reported by Pashley and Tay (2001). Specimens were stressed to failure under tension in a Bencor Multi-T device (Danville Engineering, San Ramon, CA, USA) with the use of a universal testing machine, Model 4440 (Instron, Inc., Canton, MA, USA) at a crosshead speed of 1 mm *per min.* The results were analyzed by a two-way analysis of variance (treatment regimen *vs.* adhesives), and multiple comparisons were done by Tukey’s test at  $\alpha = 0.05$ .

### Nanoleakage Evaluation and Transmission Electron Microscopy

A modified silver staining technique (Pashley *et al.*, 2002) was used with basic 50 wt% ammoniacal silver nitrate (pH = 9.5) to avoid the possibility of artifactual dissolution of enamel apatites. The solution

was prepared by the dissolution of 25 g of silver nitrate crystals (Sigma) in 25 mL of distilled water. Concentrated (28%) ammonium hydroxide (Sigma) was used to titrate the black solution until it became clear as ammonium ions complexed the silver into diamine silver ( $[Ag(NH_3)_2]^+$ ) ions. We diluted this solution to 50 mL with distilled water to achieve a 50 wt% solution.

Two 0.9-mm slabs from each group were prepared from the bonded teeth overlaid with the lining composite. They were coated with two layers of fast-setting nail varnish applied 1 mm from the bonded interfaces. They were immersed in ammoniacal silver nitrate for 24 hrs. The silver-stained slabs were rinsed thoroughly in distilled water and placed in photodeveloping solution for 8 hrs under a fluorescent light to reduce the diamine silver ions into metallic silver grains within potential voids along the bonded interfaces. Undemineralized, epoxy-resin-embedded, 90-nm-thick ultrathin sections were prepared according to the transmission electron microscopy protocol of Tay *et al.* (1999). The unstained sections were examined by means of a transmission electron microscope (Philips EM208S, Philips, Eindhoven, The Netherlands) operating at 80 kV.

### RESULTS

Microtensile bond strength results are shown in the Table. There were significant differences among the three treatment regimes ( $P < 0.001$ ) but not between the adhesives ( $P = 0.196$ ). There was no significant interaction between the two factors ( $P = 0.822$ ). For both adhesives, bond strengths were reduced by about 25% when bonding to carbamide-peroxide-bleached enamel. The compromised bond strengths were effectively reversed when the bleached enamel was treated with 10% sodium ascorbate prior to being acid-etched and adhesive application.

Transmission electron microscopy revealed that acid-etching of the sandblasted enamel resulted in the complete removal of the superficial layer of aprismatic enamel. The etching pattern was mild in some regions of the control group (Fig. 1A). A baseline nanoleakage pattern consisting of isolated silver grains could be seen (Figs. 1B, 1C). In the bleached enamel, a more extensive etching pattern was seen (Fig. 2A). Dense aggregation of silver grains could be observed along the resin-enamel interface as well as within the basal part of the adhesive layer (Figs. 2B, 2C). In addition, bubble-like structures with peripheral silver deposits were ubiquitously identified (Figs. 2B, 2C). In the bleached but ascorbate-treated group, a mild etching pattern similar to the control was observed (Fig. 3A). The abnormal bubble-like structures were absent. However, the baseline silver grain deposition could still be seen within the acid-etched prismatic enamel (Fig. 3B).

The distribution of nanoleakage patterns in Single Bond in the 3 experimental groups was similar to that in Prime&Bond NT (not shown).

### DISCUSSION

Microtensile bond strengths and nanoleakage distribution of both the ethanol-based and the acetone-based single-bottle adhesives were different when the adhesives were bonded to carbamide-peroxide-bleached, acid-etched enamel, compared with those that were further neutralized with sodium ascorbate. Hence, the null hypothesis is rejected.

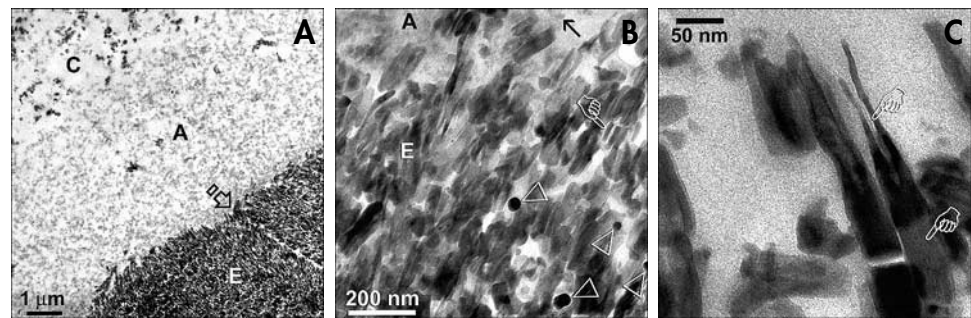
**Table.** Microtensile Bond Strengths of Adhesives to Acid-etched, Bleached Enamel

Treatment Regime	Single Bond Bond Strength <sup>a</sup> (MPa)	Prime & Bond NT Bond Strength <sup>a</sup> (MPa)
Distilled water (control)	32.0 ± 6.0 (18) <sup>1</sup>	33.1 ± 4.2 (20) <sup>1</sup>
10% carbamide peroxide	24.0 ± 5.1 (16) <sup>2</sup>	23.7 ± 7.9 (21) <sup>2</sup>
10% carbamide peroxide, then 10% sodium ascorbate	33.5 ± 8.7 (20) <sup>1</sup>	36.2 ± 7.3 (20) <sup>1</sup>

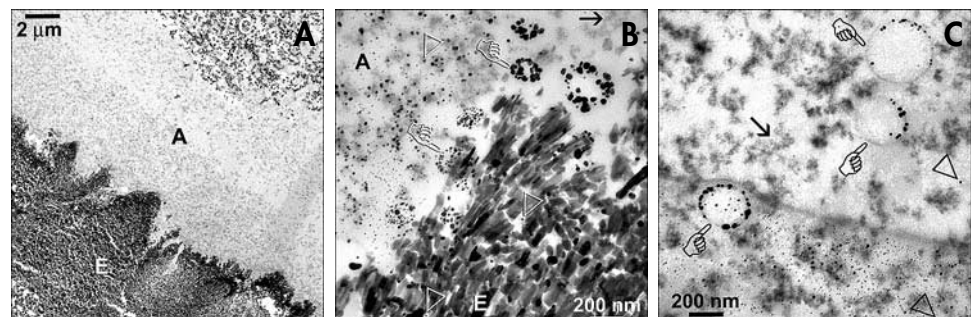
<sup>a</sup> Values are means ± standard deviation. The number of specimens tested is included in brackets. Bond strength results were analyzed by two-way analysis of variance and Tukey’s multiple-comparison tests. Groups identified by different superscript numbers are significantly different ( $P < 0.05$ ).

We understand that hybridization of aprismatic enamel can occur *via* resin penetration into subsurface microporosities created by phosphoric-acid-etching (Pashley and Tay, 2001) and do not advocate the removal of this surface layer clinically. In this study, we removed the surface aprismatic enamel layer by sandblasting only to create a more uniform surface for comparison of etching effects. This is based on our previous study that the aprismatic layer was inconsistently observed and was present only in some regions of the enamel surface after acid-etching (Pashley and Tay, 2001), making it difficult for comparisons to be made between bleached and unbleached etched enamel. Using this protocol, we did not find any difference in the etching effect between bleached and unbleached etched enamel at the ultrastructural level. Although carbamide peroxide bleaching produced enamel surface morphological alterations (Perdigão *et al.*, 1998; Cimilli and Pameijer, 2001), these changes were slight with the use of 10-16% compared with 35% carbamide peroxide (Oltu and Gurgan, 2000). Moreover, the etching effect of 10% carbamide peroxide is system-specific (Rodrigues *et al.*, 2001) and is likely to be pH-dependent (Shannon *et al.*, 1993). Thus, it can be expected that the demineralization effect of NuproGold, with a pH value of 6.4, is relatively mild (McCracken and Haywood, 1996), and any surface and subsurface alterations would probably have been masked by the more aggressive phosphoric-acid-etching (Ernst *et al.*, 1996; Potocnik *et al.*, 2000).

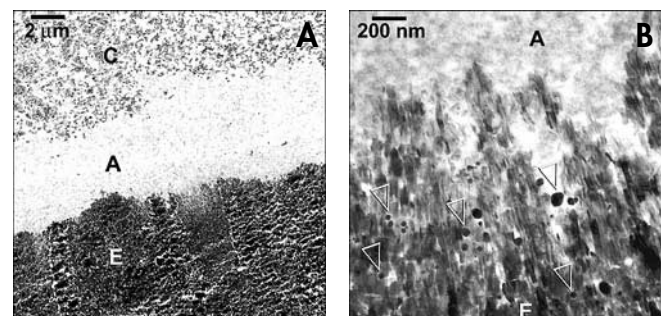
To date, all nanoleakage studies were performed on resin-dentin bonds, with the assumption that the high-energy enamel surfaces created by acid-etching are optimized for resin infiltration (Pioch *et al.*, 2001). In this study, a baseline nanoleakage pattern, in the form of isolated silver grains, could be observed within the etched enamel in all treatment groups. Since a basic version of ammoniacal silver nitrate was used (pH = 9.5), it is unlikely that the observed results were artifacts produced by laboratory demineralization of enamel apatites that can occur with the use of acidic, conventional 50 wt% silver nitrate solutions (pH = 3.4). Without the use of demineralized sections with special staining for enamel proteins (Pashley and Tay, 2001), we could not see the extent of resin-infiltration within the hybridized enamel. The electron-dense, almost



**Figure 1.** Transmission electron micrographs showing the nanoleakage in phosphoric-acid-etched enamel (control) that was bonded with Prime&Bond NT. (A) A low-magnification view of the resin-enamel interface. C, resin composite; A, adhesive containing nanofiller particles; E, prismatic enamel; arrow, interprismatic sheath. (B) A high-magnification view showing the presence of isolated, electron-dense silver grains (open arrowheads) within the etched, resin-infiltrated enamel (E). Apatite crystallites were partially dissolved and exhibit central hole regions (pointer). Arrow: nanofiller clusters within the adhesive layer (A). (C) A very high magnification of (B), showing the presence of the central dark line (pointers) within a partially dissolved apatite crystallite.



**Figure 2.** Transmission electron micrographs showing the nanoleakage in carbamide-peroxide-bleached, acid-etched enamel that was bonded with Prime&Bond NT. (A) A low-magnification view of the resin-enamel interface. C, resin composite; A, adhesive containing nanofiller particles; E, prismatic enamel. A region with more extensive etching is depicted, although areas with etching effect similar to that in Fig. 1A were commonly observed. (B) A high-magnification view of the resin-enamel interface, showing more extensive silver grain deposition (open arrowheads) within the etched enamel (E) as well as the adhesive layer (A). Additional bubble-like structures with peripheral silver deposits (pointers) were also evident. Arrow: less-electron-dense nanofiller clusters in the adhesive. (C) A high-magnification view showing a dense aggregation of isolated silver grains (open arrowheads) and almost spherical, bubble-like structures with incomplete peripheral silver deposits (pointers) in the adhesive layer. Arrow: nanofiller clusters.



**Figure 3.** Transmission electron micrographs showing the nanoleakage in carbamide-peroxide-bleached enamel that was treated with sodium ascorbate prior to being acid-etched and the application of the Prime&Bond NT adhesive. (A) A low-magnification view of the resin-enamel interface. C, resin composite; A, adhesive containing nanofiller particles; E, prismatic enamel. (B) A high-magnification view showing the presence of isolated, electron-dense silver grains (open arrowheads) within the etched, resin-infiltrated enamel (E). Bubble-like structures that were previously observed in the bleached enamel were absent after sodium ascorbate treatment.

spherical isolated silver grains within the etched enamel could be easily differentiated from the adjacent angular apatite crystallites or the less electron-dense nanofiller clusters in the adhesive (Tay *et al.*, 1999). Their presence suggested that there is a possibility of over-etching and incomplete resin infiltration at the base of phosphoric-acid-etched enamel, unlike the use of self-etch adhesives (Shimada and Tagami, personal communication). Nevertheless, such a phenomenon was extremely mild in view of the very low density of the silver grains observed.

This nanoleakage pattern became more dense along the resin-enamel interface of carbamide-peroxide-bleached enamel. In addition, bubble-like structures with incomplete peripheral silver deposits were also observed. These features were present even after the bleached enamel was rinsed and immersed in water for 10 min prior to being acid-etched and the adhesive application. It is known that hydrogen peroxide released from carbamide peroxide, due to its low molecular weight, can penetrate enamel to reach the dental pulp (Gokay *et al.*, 2000), and that there is a continuous leaching of the hydrogen peroxide that is retained in the bleached enamel (Adibfar *et al.*, 1992). Since dental adhesives polymerize by a free radical polymerization mechanism that involves the generation of free radicals through light-activated redox initiators (Monroe *et al.*, 1968), the hydrogen peroxide may break down to release oxygen that is trapped within the adhesive during light-activation. This may account for the preponderance of the almost spherical bubble-like structures along the resin-enamel junction and close to the base of the adhesive layer (Figs. 2B, 2C). Release of oxygen from the bleached enamel probably results in incomplete polymerization of the adhesive in these regions (Torneck *et al.*, 1990; Dishman *et al.*, 1994). This could account for the observation of an increased density of voids along the acid-etched, bleached enamel interface (McGuckin *et al.*, 1992). Similar to previous studies (Torneck *et al.*, 1990; McGuckin *et al.*, 1992; García-Godoy *et al.*, 1993), our failure mode results (not shown) indicated predominant adhesive failures along the resin-enamel interface in bonded bleached enamel, compared with more mixed and cohesive failures in the other two treatment regimes.

It is interesting that compromised bonding to acid-etched bleached enamel was reversed with sodium ascorbate, an anti-oxidant. Previous studies suggested the subsurface enamel organic matrix was altered by the oxidizing effect of hydrogen peroxide (Seghi and Denry, 1992; Hegedus *et al.*, 1999). Based on our present findings, it is possible that these are not permanent structural alterations, but reversible changes in redox potential of the organic components. It is also speculated that the peroxide ions may have temporarily substituted the hydroxy radicals in the apatite lattice (Zhao *et al.*, 2000). Since these lattice substitutions are thermodynamically unfavorable, such a process may be reversed by an anti-oxidant. Such a hypothesis remains speculative and has to be further investigated by chemico-analytical methods. Unlike our previous study, we immersed our sodium-ascorbate-treated specimens in water for an additional 10 min to dissolve completely the rhombohedral crystal depositions that are present after sodium ascorbate treatment (Lai *et al.*, 2001). This may prevent the dissolution of the crystalline deposits and the generation of voids along the resin-enamel interfaces as resins eventually absorb water and leach. Understandably, the use of sodium ascorbate to reverse the oxidizing effect of a bleaching agent involves a substantially lengthy period, which may not be

clinically acceptable. However, in light of the fact that a post-bleaching period of 2-3 wks is required for enamel bonding to return to normal, it may be possible to incorporate the sodium ascorbate into a gel to be placed by patients themselves in the bleaching tray before bonding. Since vitamin C and its salts are non-toxic and are widely used in the food industry as anti-oxidants, it is unlikely that their intra-oral use will create any adverse biological effect or clinical hazard. The potential clinical use of a sodium ascorbate gel to reverse the oxidizing effect of home and "in-office" bleaching with carbamide peroxide must be further investigated.

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