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Reversal of Compromised Bonding to Oxidized Etched Dentin

ABSTRACT

The mechanism responsible for hydrogen-peroxide- or sodium-hypochlorite-induced reductions in dentin bond strength is unknown. This *in vitro* study tested the hypothesis that these oxidizing agents were responsible by attempting to reverse the effect with sodium ascorbate, a reducing agent. Human dentin was treated with these oxidants before or after being acid-etched and with or without post-treatment with sodium ascorbate. They were bonded with either Single Bond or Excite. Hydrogen peroxide reduced the bond strengths of both adhesives, while sodium hypochlorite produced reduction in adhesion of only Single Bond ($p < 0.05$). Following treatment with sodium ascorbate, reductions in bond strength were reversed. Transmission and scanning electron microscopy showed partial removal of the demineralized collagen matrix only by sodium hypochlorite. The observed compromised bond strengths cannot be attributed to incomplete deproteinization and may be related to changes in the redox potential of the bonding substrates.

KEY WORDS: sodium ascorbate, sodium hypochlorite, hydrogen peroxide, microtensile bond strength, ultrastructure.

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INTRODUCTION

Sodium hypochlorite and hydrogen peroxide are common endodontic irrigants that are used for the debridement and deproteinization of mechanically prepared, smear-layer-covered radicular dentin (Heling and Chandler, 1998). Sodium hypochlorite is also frequently used for chemomechanical caries removal (Nordbø *et al.*, 1996; Haak *et al.*, 2000) and the arrest of hemorrhage in pulpal exposures before bonding to coronal dentin occurs (Cox *et al.*, 1998). Recent studies showed that bond strengths of some adhesives were compromised by the use of these reagents on root (Nikaido *et al.*, 1999) and crown dentin (Inai *et al.*, 1998; Pioch *et al.*, 1999; Frankenberger *et al.*, 2000; Prati *et al.*, 2000), as well as enamel (Titley *et al.*, 1993).

The incomplete removal of the partially denatured or destabilized collagen matrix has been proposed as a possible reason for compromised bond strength in sodium-hypochlorite-treated, acid-etched dentin (Perdigão *et al.*, 2000). This, however, does not explain why significant bond strength reduction was also observed when sodium hypochlorite or hydrogen peroxide was used before dentin was etched (Nikaido *et al.*, 1999). Sodium hypochlorite, apart from being an effective deproteinizing agent (Hawkins and Davies, 1998a), is similar to hydrogen peroxide in that it is also a potent biological oxidant (Daumer *et al.*, 2000). If the decreased bond strength observed in sodium-hypochlorite- and hydrogen-peroxide-treated etched dentin is the result of the oxidizing action of these chemicals, it may be possible for the compromised bond strength to be reversed by a reduction of the oxidized surfaces with a neutral, biocompatible anti-oxidant such as sodium ascorbate (Rose and Bode, 1993) before resin bonding occurs.

This study examined the effects of sodium hypochlorite, hydrogen peroxide, and sodium ascorbate on bonding to acid-etched dentin. These chemicals were applied both before and after acid-etching occurred. The former treatment sequence is often used in bonding to endodontically treated teeth, while the latter is used in hemorrhage control of pulpal exposures in deeply acid-etched dentin. The null hypothesis tested was that the use of sodium ascorbate has no effect on the bonding of two single-bottle adhesives, Single Bond (3M ESPE, St. Paul, MN, USA) and Excite (Vivadent, Schaan, Liechtenstein), to sodium-hypochlorite- or hydrogen-peroxide-treated, etched coronal dentin.

MATERIALS & METHODS

Bonding was performed on the occlusal surfaces of deep coronal dentin from extracted human third molars. The teeth were collected after each patient's informed consent was obtained under a protocol reviewed and approved by the institutional review board of the Medical College of Georgia, USA. The teeth were used within one month following extraction. The occlusal enamel was removed by means of a slow-speed saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water lubrication.

Table. Application of Adhesives to Acid-etched, Deep, Coronal Dentin before or after Treatment with 5% Sodium Hypochlorite, 10% Hydrogen Peroxide, and/or 10% Sodium Ascorbate

Treatment Sequence	Treatment Protocol	Single Bond Bond Strength[c] (MPa)	Excite Bond Strength[c] (MPa)
After acid-etching[a]			
	Distilled water for 1 min	46.5 ± 5.6 (15)[1]	48.3 ± 12.5 (15)[1,2]
	Sodium ascorbate for 1 min	31.9 ± 5.5 (14)[2]	36.4 ± 10.0 (13)[2,3]
	Sodium hypochlorite for 1 min	38.6 ± 4.9 (14)[2]	58.1 ± 10.7 (15)[1]
	Sodium hypochlorite for 1 min, rinse, sodium ascorbate for 1 min	45.8 ± 7.3 (14)[1]	51.0 ± 8.7 (15)[1]
	Hydrogen peroxide for 1 min	29.2 ± 4.6 (14)[2]	32.3 ± 12.3 (14)[3]
	Hydrogen peroxide for 1 min, rinse, sodium ascorbate for 1 min	46.3 ± 5.2 (14)[1]	48.9 ± 10.4 (14)[1,2]
Before acid-etching[b]			
	Sodium hypochlorite for 10 min	35.0 ± 10.0 (15)[2]	47.8 ± 9.2 (14)[1,2]
	Sodium hypochlorite for 10 min, rinse, sodium ascorbate for 10 min	50.2 ± 7.4 (16)[1]	46.6 ± 10.6 (14)[1,2]
	Hydrogen peroxide for 10 min	30.4 ± 6.8 (14)[2]	30.1 ± 10.4 (16)[3]
	Hydrogen peroxide for 10 min, rinse, sodium ascorbate for 10 min	46.1 ± 8.5 (14)[1]	51.8 ± 11.4 (16)[1]

[a] Specimens were acid-etched with 32% phosphoric acid for 15 sec, treated with the different solutions, and thoroughly rinsed. Bonding was then performed by means of a moist bonding technique. Rinsing was performed with distilled water for 20 sec.

[b] Specimens were treated with the different solutions and thoroughly rinsed. They were then acid-etched with 32% phosphoric acid for 15 sec, and bonded by means of a moist bonding technique. Rinsing was performed with distilled water for 20 sec.

[c] Values are means ± standard deviation. Number of specimens tested is included in brackets. For each adhesive, bond strength results were analyzed by one-way analysis of variance and Student-Newman-Keuls multiple-comparison tests. Groups identified by different superscript numerals are significantly different for each adhesive system used ($p < 0.05$).

Experimental Design

Two adhesives were used, each consisting of 10 experimental groups with 4 teeth each. Three restored teeth were used for bond strength evaluation by the microtensile bond test, and failure mode analysis by scanning electron microscopy. The fourth was prepared for ultrastructural examination by transmission electron microscopy.

For each adhesive, group designations and the treatment regimes are listed in the Table. All teeth were etched with a 32% phosphoric acid gel (Uni-Etch, Bisco, Schaumburg, IL, USA) for 15 sec and rinsed for 20 sec. Each group of teeth was treated with different chemical solutions or their combination for 60 sec each under constant agitation, and then rinsed for 20 sec. The solutions used were distilled water (positive control), 5.25% sodium hypochlorite (Riedel-de Haën, Seelze, Germany), 10% hydrogen peroxide (Riedel-de Haën), and 10% sodium ascorbate (negative control). In the four groups that were treated with different solutions, bonding surfaces were first treated with either sodium hypochlorite or hydrogen peroxide before or after being acid-etched. They were rinsed with distilled water for 20 sec before sodium ascorbate was applied. After treatment, the teeth were bonded visibly moist with two coats of the adhesive, briefly air-dried, and then light-cured for 10 sec. Composite build-ups were performed with a light-cured composite (Spectrum, Dentsply Caulk, Milford, DE, USA) in five 1-mm increments. The teeth were stored in distilled water at 37°C for 24 hrs.

Ultrastructural Examination of Intact Resin-Dentin Interfaces

For Single Bond, both undemineralized and demineralized ultrathin sections of the bonded specimens were prepared according to the transmission electron microscopy protocol described in Tay *et al.* (1999). Sections were double-stained with uranyl acetate and Reynold's lead citrate and examined with a transmission electron microscope (Philips EM208S, Eindhoven, The Netherlands) operating at 80 kV. Undemineralized sections were also examined unstained.

Tensile Bond Strength and Failure Mode Evaluation

Specimens from the 20 groups were sectioned into serial slabs by means of an Isomet saw under water lubrication, and then hand-trimmed into dumbbell-shaped specimens according to the technique for the microtensile bond test reported by Sano *et al.* (1994). Three teeth from each group yielded from 13 to 16 specimens for bond strength evaluation. Specimens were stressed to failure under tension in a universal testing machine (Model 4440; Instron Inc., Canton, MA, USA) at a crosshead speed of 1 mm *per* min.

The dentin side of each fractured specimen was air-dried, coated with gold/palladium, and examined with a scanning electron microscope (Cambridge Stereoscan 440, Cambridge, UK) operating at 10-20 kV. The exact area of each fractured specimen was derived from image analysis of the digitized micrographs, from which the tensile bond strength was calculated. Failure modes were recorded as adhesive, mixed, or cohesive failures in either dentin or resin.

For each adhesive, bond strength data from the 10 experimental groups were statistically analyzed with the use of SigmaStat Version 2.03 (SPSS, Chicago, IL, USA). Using a one-way analysis of variance, we set statistical significance in advance at the 0.05 probability level. Multiple comparisons were done by the Student-Newman-Keuls test at $\alpha = 0.05$.

RESULTS

Transmission electron microscopy of resin-dentin interfaces bonded with Single Bond after treatment with distilled water, 10% sodium ascorbate, 10% hydrogen peroxide, and hydrogen peroxide followed by sodium ascorbate showed similar results, in that 4- to 5-μm-thick hybrid layers were present with intact, banded collagen fibrils that were about 100 nm in diameter (Fig. 1A). Infiltration of the electron-dense polyalkenoic acid copolymer from the adhesive was limited to the surface 0.5 μm of the hybrid layer, and around the periphery of the dentinal tubules (Fig. 1B). Deproteinization was incomplete in groups treated with sodium hypochlorite alone or sodium hypochlorite followed by sodium ascorbate, with remnant hybrid layers between 0.3 and 1.5 μm thick. Sparsely distributed, darkly stained collagen fibrils within the hybrid layer were segregated

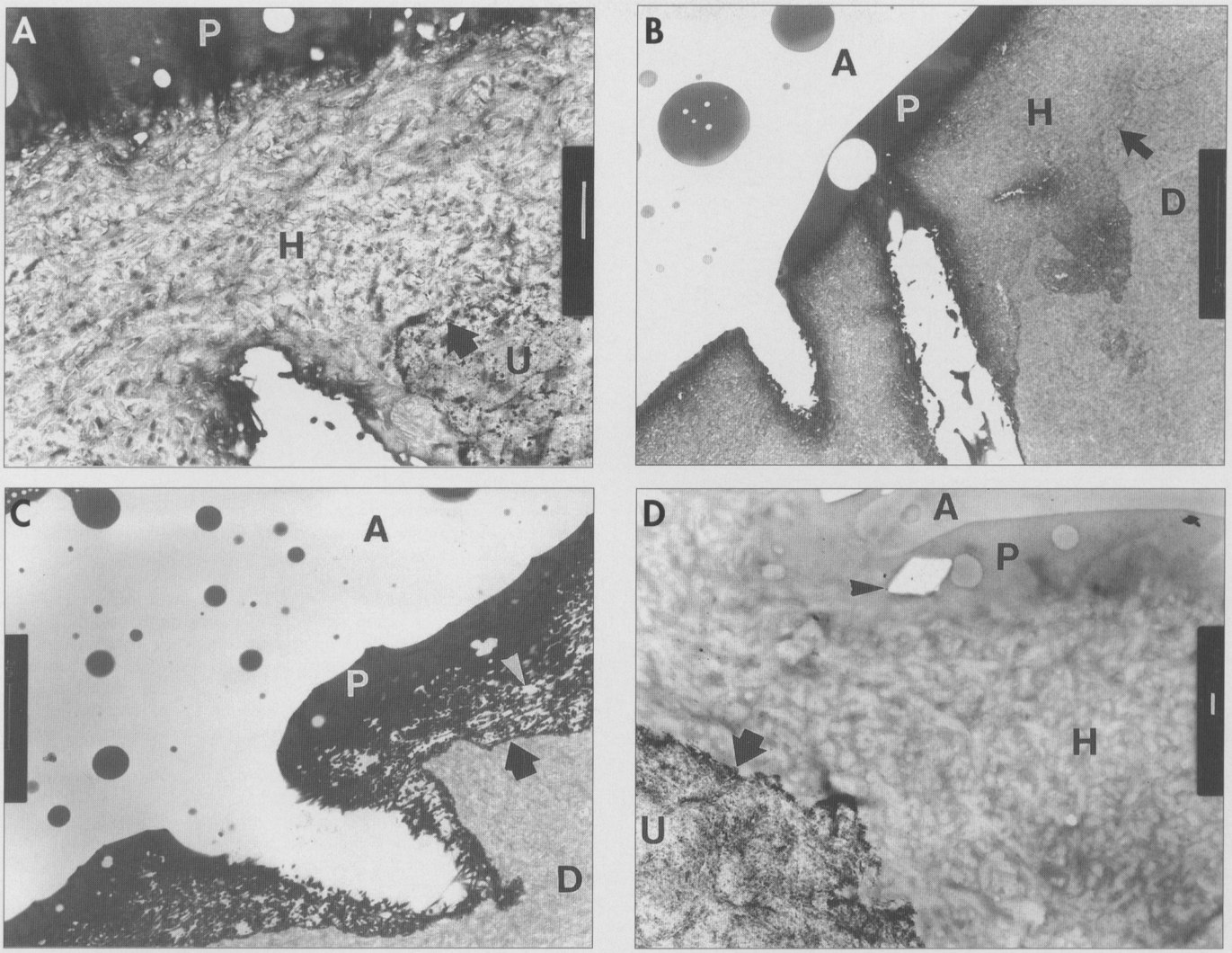

Figure 1. Transmission electron microscopy micrographs of the resin-dentin interfaces in different experimental groups bonded with Single Bond. **(A)** Stained, undemineralized section from the group treated with distilled water (positive control) showing the presence of banded collagen fibrils within the hybrid layer. Staining resulted in partial dissolution of the minerals from the underlying mineralized dentin. Bar = 1 μm. **(B)** Stained, demineralized section from the group treated with hydrogen peroxide. The surface of the hybrid layer (H) was electron-dense due to the binding of heavy metal stains to the infiltrated polyalkenoic acid copolymer. The rest of the hybrid layer was only palely stained. Bar = 5 μm (the group treated with hydrogen peroxide followed by sodium ascorbate was similar in ultrastructural appearance). **(C)** Stained, demineralized section from the group treated with sodium hypochlorite. The hybrid layer contained sparsely distributed, electron-dense collagen fibrils that were incompletely deproteinated. Numerous electron-lucent spaces devoid of collagen fibrils (arrowhead) were evident. Bar = 2 μm (the group treated with sodium hypochlorite followed by sodium ascorbate has a similar ultrastructural appearance). **(D)** Unstained, undemineralized section from the group treated with sodium ascorbate (negative control) showing the presence of the silhouette of a diamond-shaped sodium ascorbate crystal (arrowhead) that was trapped within the adhesive resin along the tubular orifice. Bar = 300 nm. A, adhesive layer; P, polyalkenoic acid copolymer; H, hybrid layer; U, undemineralized intertubular dentin; D, laboratory demineralized intertubular dentin; arrow, demineralization front.

by wide, electron-lucent spaces (Fig. 1C). These collagen fibrils were reduced to 60 to 80 nm in diameter but still retained their banding characteristics (not shown). Silhouettes of diamond-shaped crystals, entrapped by the adhesive resin, could be identified from undemineralized sections, in groups that were pre-treated with sodium ascorbate (Fig. 1D).

Mean tensile bond strengths for the 10 experimental groups of each adhesive are listed in the Table. For Single Bond, sodium hypochlorite, hydrogen peroxide, or sodium ascorbate (negative control), when used alone, produced significant ($p < 0.05$) reductions in resin-dentin bond strength. When sodium ascorbate was used after sodium hypochlorite or hydrogen peroxide, the compromised bond strengths were effectively reversed and were not significantly different ($p > 0.05$) from that of the positive control. For Excite, there was no significant decrease in bond strength both before and after sodium hypochlorite treatment, and sodium ascorbate did not produce any significant increase in bond strength ($p > 0.05$). However, bond strengths decreased significantly both before and after hydrogen peroxide treatment, and were reversed with the use of sodium ascorbate ($p < 0.05$).

Mixed failures were predominantly observed in all groups under scanning electron microscopy examination. There were minimal cohesive failures in resin composites, and no cohesive failures in dentin were observed. Differences among various groups could be discerned by the variation in extent of resin infiltration and resin tag integrity along fractured hybrid layers. For Single Bond (Fig. 2A), the hybrid layer was better infiltrated in the distilled water control group, although isolated areas with incompletely infiltrated collagen could be identified

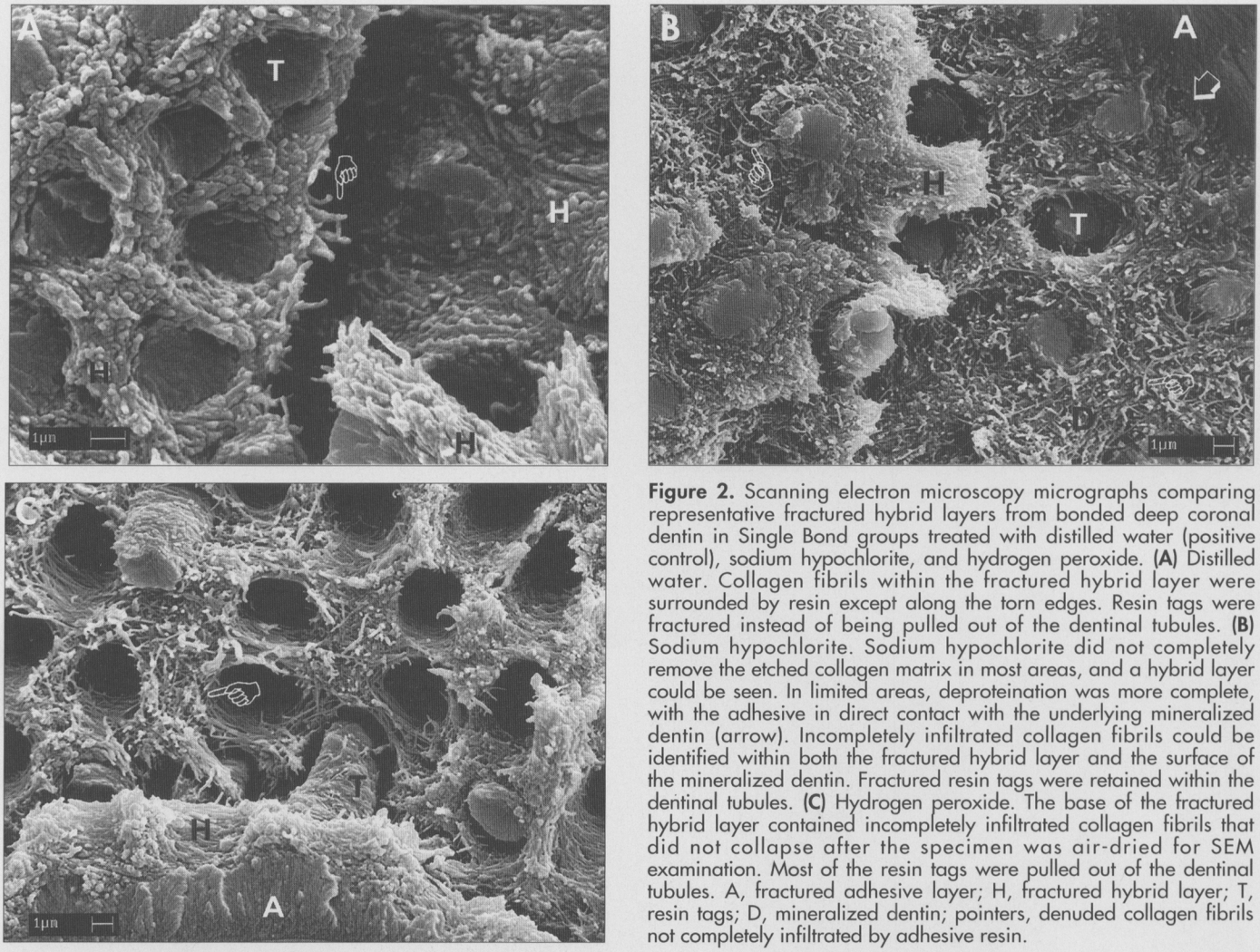

Figure 2. Scanning electron microscopy micrographs comparing representative fractured hybrid layers from bonded deep coronal dentin in Single Bond groups treated with distilled water (positive control), sodium hypochlorite, and hydrogen peroxide. **(A)** Distilled water. Collagen fibrils within the fractured hybrid layer were surrounded by resin except along the torn edges. Resin tags were fractured instead of being pulled out of the dentinal tubules. **(B)** Sodium hypochlorite. Sodium hypochlorite did not completely remove the etched collagen matrix in most areas, and a hybrid layer could be seen. In limited areas, deproteination was more complete, with the adhesive in direct contact with the underlying mineralized dentin (arrow). Incompletely infiltrated collagen fibrils could be identified within both the fractured hybrid layer and the surface of the mineralized dentin. Fractured resin tags were retained within the dentinal tubules. **(C)** Hydrogen peroxide. The base of the fractured hybrid layer contained incompletely infiltrated collagen fibrils that did not collapse after the specimen was air-dried for SEM examination. Most of the resin tags were pulled out of the dentinal tubules. A, fractured adhesive layer; H, fractured hybrid layer; T, resin tags; D, mineralized dentin; pointers, denuded collagen fibrils not completely infiltrated by adhesive resin.

(not shown). In contrast, uncollapsed, denuded collagen fibrils were ubiquitous within the hybrid layer and adjacent to the demineralization front when dentin was treated either with sodium hypochlorite (Fig. 2B) or hydrogen peroxide (Fig. 2C). In addition, most of the resin tags were pulled out of the tubules together with the fractured hybrid and adhesive layers in the group treated with hydrogen peroxide.

Rhombohedral crystals were observed within incomplete resin tags in etched dentin that was treated with sodium ascorbate only (Fig. 3A). These crystals were also present when sodium ascorbate was applied after sodium hypochlorite (Fig. 3B) or hydrogen peroxide treatment (Fig. 3D). Fractured resin tags were attached to the dentinal tubules *via* the peripheral extensions of the hybrid layer. The difference in hybrid layer thickness in these two groups could also be readily discerned.

DISCUSSION

Our transmission electron microscopy results consistently demonstrated the presence of a remnant hybrid layer when 5.25% sodium hypochlorite treatment was used for 60 sec. This phenomenon was also observed with the use of a commercial 10% sodium hypochlorite gel on etched dentin (Perdigão *et al.*, 2000). For Single Bond, there is also an increase in electron density of the stained collagen fibrils in hybrid layers in groups treated with sodium hypochlorite and sodium hypochlorite followed by sodium ascorbate. Removal of interfibrillar proteoglycans by sodium hypochlorite (Schiller *et al.*, 1997; Hawkins and Davies, 1998b) may enhance the interaction of the carboxylic moieties of the polyalkenoic acid copolymer with amide linkages of the collagen fibrils (Ikemura *et al.*, 1998).

Retention of a partially denatured, remnant collagen matrix could not be solely responsible for compromised bonding to sodium-hypochlorite-treated dentin, since tensile bond strength was not affected in Excite, and was effectively reversed after sodium ascorbate treatment in Single Bond. Reversal of compromised bond strength in both Single Bond and Excite was also observed when sodium ascorbate was used on hydrogen-peroxide-treated dentin either before or after acid-etching occurred. Although oxidative damage by the application of hydrogen peroxide to reconstituted and acid-soluble collagen can result in the latter's thermal destabilization (Komsa-Penkova *et al.*, 2000) and susceptibility to fragmentation (Kato *et al.*, 1992; Hawkins and Davies, 1997), the dentin collagen matrix is highly cross-linked. Pyridinoline cross-links that occur in collagen Types I and II were found to be disrupted by sodium hypochlorite but not by hydrogen peroxide (Daumer *et al.*, 2000), with the formation of chloramines and protein-derived radical intermediates (Hawkins and Davies, 1999). The presence of these reactive residual free-radicals in sodium-hypochlorite-treated dentin may compete with the propagating vinyl free-

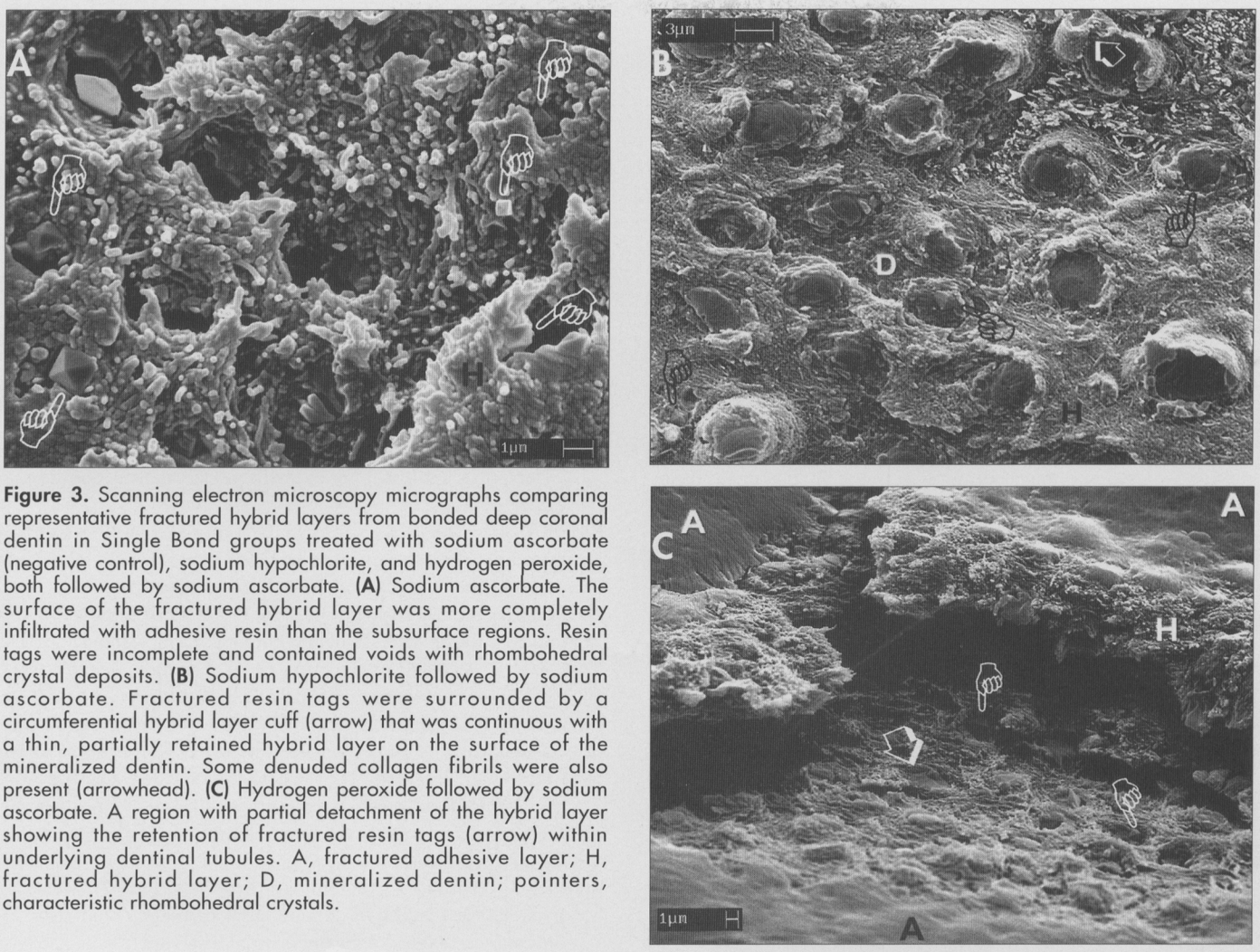

Figure 3. Scanning electron microscopy micrographs comparing representative fractured hybrid layers from bonded deep coronal dentin in Single Bond groups treated with sodium ascorbate (negative control), sodium hypochlorite, and hydrogen peroxide, both followed by sodium ascorbate. **(A)** Sodium ascorbate. The surface of the fractured hybrid layer was more completely infiltrated with adhesive resin than the subsurface regions. Resin tags were incomplete and contained voids with rhombohedral crystal deposits. **(B)** Sodium hypochlorite followed by sodium ascorbate. Fractured resin tags were surrounded by a circumferential hybrid layer cuff (arrow) that was continuous with a thin, partially retained hybrid layer on the surface of the mineralized dentin. Some denuded collagen fibrils were also present (arrowhead). **(C)** Hydrogen peroxide followed by sodium ascorbate. A region with partial detachment of the hybrid layer showing the retention of fractured resin tags (arrow) within underlying dentinal tubules. A, fractured adhesive layer; H, fractured hybrid layer; D, mineralized dentin; pointers, characteristic rhombohedral crystals.

radicals generated during light-activation of the adhesive, resulting in premature chain termination and incomplete polymerization. Conversely, reduction in bond strength in hydrogen-peroxide-treated dentin could be caused by residual solution in the collagen matrix and dentinal tubules that eventually broke down to oxygen and water (Nikaido *et al.*, 1999). Liberation of oxygen could either interfere with resin infiltration into etched dentin (Torneck *et al.*, 1990), or inhibit polymerization of resins that cure *via* a free-radical mechanism (Rueggeberg and Margeson, 1990). This may have been responsible for the presence of pulled-out resin tags in hydrogen-peroxide-treated etched dentin (Fig. 2C).

Application of sodium ascorbate alone did not improve the bond strengths of both Single Bond and Excite to etched dentin. It is unlikely that the characteristic crystals observed along the fractured interfaces were responsible for the decreased bond strength, since they were found in all groups that were treated with sodium ascorbate. Ascorbic acid and its sodium salt are potent anti-oxidants that are capable of quenching reactive free-radicals in biological systems (Gutteridge, 1994). In this study, we did not use ascorbic acid to avoid the potential double-etching effect of this mild acid on etched dentin. The observed drop in bond strength in ascorbate-treated dentin may be explained by the ability of this reducing agent to donate two high-energy electrons to scavenge the free-radicals (VanDuijn *et al.*, 2000) that are formed during resin polymerization. The anti-oxidant ability of sodium ascorbate can help to neutralize and reverse the oxidizing effects of sodium hypochlorite or hydrogen peroxide in biological systems (Smit and Anderson, 1992; Hawkins and Davies, 1999; Carr *et al.*, 2000). In the present context, it is possible that by restoring the altered redox potential of the oxidized bonding substrate, sodium ascorbate allows free-radical polymerization of the adhesive to proceed without premature termination, and hence reverses the compromised bonding in sodium-hypochlorite- or hydrogen-peroxide-treated acid-etched dentin.

The results require rejection of the null hypothesis. Although the use of sodium ascorbate reverses the compromised bond strength of Single Bond to oxidized dentin, we realize that this phenomenon may be system-specific. The clinical implication of this study is that with the use of an anti-oxidant such as sodium ascorbate, clinicians can acid-etch and bond immediately to endodontically treated teeth that were irrigated with sodium hypochlorite or hydrogen peroxide, without compromising the clinical performance or longevity of these restorations (Nikaido *et al.*, 1999). 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 use on dentin will create any adverse biological effect or clinical hazard. More work has to be done to elucidate the mechanism of this reversal process by chemical analytical methods.

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