

Effect of self-etching primers containing *N*-acryloyl aspartic acid on dentin adhesion

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Abstract: The purpose of this study was to evaluate the feasibility of aspartic acid derivative, *N*-acryloyl aspartic acid (*N*-AAsp) when used as a self-etching primer prior to the application of a commercial bonding agent containing 10-methacryloxydecyl dihydrogen phosphate. *N*-AAsp was synthesized by the reaction of DL-aspartic acid and acryloyl chloride. *N*-AAsp can be dissolved in water up to 25 wt % and can adequately remove the smear layer. When 20 wt % *N*-AAsp was applied as a self-etching primer, the tensile bonding strength of resin composite to dentin doubled to 16.0 MPa compared with that of the nonetching group or the 40 wt % H₃PO₄ etching/water rinse group. Scanning electron microscopic observations revealed that the formation of

the hybrid layer at the interface between the resin composite and dentin and the thickness of the hybrid layer increased with *N*-AAsp concentration. The hybrid layer in the specimens treated with 20 wt % *N*-AAsp aqueous solution presented a uniform structure, whereas those treated with 5 wt % *N*-AAsp solution showed a porous structure. We conclude that 20 wt % *N*-AAsp aqueous solution has good potential value as a self-etching primer. © 2000 John Wiley & Sons, Inc. *J Biomed Mater Res*, 51, 569–574, 2000.

Key words: dentin adhesion; self-etching primer; *N*-acryloyl aspartic acid; tensile bond strength; hybrid layer

INTRODUCTION

In 1982, Nakabayashi et al.¹ proposed that the adhesion of resin to dentin is produced by the creation of a hybrid layer. Today, that theory is widely acknowledged and the creation of the hybrid layer is considered as a major mechanism of adhesion between resin and dentin promoted by current adhesive systems.

The permeability of dentin substrates and the diffusing ability of monomers are both essential in creating a hybrid layer at the resin–dentin interface.² To improve dentin permeability by decalcification of the smear layer and underlying dentin, some adhesive systems use phosphoric acid etching. For example, a commercial bonding agent containing 10-methacryloxydecyl dihydrogen phosphate (MDP) was reported to have good diffusing ability into decalcified dentin and to create a hybrid layer when applied after phosphoric acid etching used as a pretreatment.³ However, phosphoric acid denatures exposed collagen fibrils

during smear layer removal.⁴ It was also reported that the bonding agent could not infiltrate the whole of the dentin layer decalcified by an etchant, thus nonresin-impregnated dentin remains between the hybrid layer and the intact dentin. Kiyomura⁵ reported that a 5-year storage of a bonded specimen in water gradually decreased the bond strength of resin to acid-etched dentin. He noted that the collagen fibrils left unprotected with resin in nonresin-impregnated dentin might be hydrolyzed. Judging from these reports, it is considered that phosphoric acid etching is not always effective for dentin adhesion. Therefore, a new pretreatment, as a substitute for acid etching, is required to eliminate the formation of the nonresin-impregnated dentin and to obtain tight adhesion between resin and dentin. The self-etching priming system has been developed mainly in Japan to serve as the new pretreatment for dentin adhesion.^{6–10} Watanabe et al.¹⁰ reported that methacryloxyethyl hydrogen phenyl phosphate diluted in 2-hydroxy ethyl methacrylate (HEMA)/water is effective as a self-etching primer prior to the application of a bonding agent.

On the other hand, it has been reported that amino acid derivatives can promote adhesion between resin and dentin when applied as a primer on acid-etched dentin.^{11–14} Suzuki et al.¹² reported that *N*-meth-

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acryloyl amino acids dissolved in water promoted diffusion of the bonding agent into the acid-etched dentin and creation of a hybrid layer exhibiting tight adhesion. In their report, the pH values of the aqueous solutions containing various *N*-methacryloyl amino acids ranged from 1.5 to 2.5 which are low enough to decalcify the dentin. Amino acid derivatives are expected to have better biocompatibility and a high affinity with collagen, because amino acid is a unit component of collagen.^{11,12}

There were two hypotheses in this study. The one was that treatment with an aqueous solution of an amino acid derivative as a self-etching primer instead of phosphoric acid etching prior to the application of the bonding agent could improve the adhesion between resin to dentin. The other was that two kinds of acids in a molecule would be more effective in the self-etching primers like *N*-acryloyl aspartic acid (*N*-AAsp).

The purpose of the present study was first to evaluate the effect of the self-etching primer containing *N*-acryloyl aspartic acid on the adhesion between resin and dentin promoted by the bonding agent, in comparison to phosphoric acid, and second to investigate the effect of the self-etching primer on the quality of the hybrid layer created at the resin–dentin interface.

MATERIALS AND METHODS

Synthesis of *N*-AAsp

N-AAsp was synthesized by a reaction of DL-aspartic acid and acryloyl chloride according to previous reports.^{15,16} DL-aspartic acid (Tokyo Kasei, Tokyo, Japan) was obtained commercially and used without further purification. Acryloyl chloride (Tokyo Kasei) was purified by distillation under reduced pressure before use. The structure of the synthesized product was confirmed by elemental analysis and ¹H, ¹³C nuclear magnetic resonance (NMR) analyses. For NMR spectra measurement, 100 mg of the product was dissolved in 1.0 mL of DMSO or D₂O solution, and ¹H and ¹³C NMR spectra were obtained using an EX-270 spectrometer (JEOL, Tokyo, Japan) operated at 67.80 MHz and 270.05 MHz, respectively.

To determine the pKa values, 1.0 mL of D₂O solution containing 1.5 mmol % of *N*-AAsp was prepared. The pH of the solution was then changed in the range from 0.5 to 8.0 by the addition of NaOH or HCl. ¹³C NMR spectra of the solutions with various pH values were obtained and the pKa values of the carboxyl groups in the *N*-AAsp molecule were determined by simulation of the ¹³C chemical shifts as a function of pH based on the Henderson–Hasselbalch equation.¹¹

Experimental self-etching priming solutions

The priming solutions were prepared by dissolving *N*-AAsp in distilled water at room temperature (24°C) in con-

centrations ranging from 5 to 25% by weight (5, 10, 15, 20, and 25wt %). The pH value of each primer solution was measured using a pH meter (F-23; Horiba, Kyoto, Japan) at room temperature.

Bond strength measurement

The labial surfaces of freshly extracted bovine teeth were ground using 600-grit abrasive paper under wet conditions to expose the flat dentin surfaces. The dentin surfaces were treated with 50 µL of a priming solution containing various concentrations of *N*-AAsp for 60 s and air-dried. A polyethylene ring with an inner diameter of 3.8 mm and a height of 2.0 mm was mounted onto the dentin surface using sticky wax. A light-cured bonding agent (Photobond®; Kuraray, Osaka, Japan) containing MDP, HEMA, dimethacrylates, and ethanol was coated onto the specimen surface. After 20 s, the surface was gently air-blown and light-irradiated for 20 s according to the manufacture's instructions. Then, a light-cured resin composite (Clearfil AP-X®; Kuraray) was filled and irradiated for 40 s, after which the polyethylene ring was removed. After the bonded specimens were stored in distilled water at 37°C for 24 h, a stainless steel rod was attached to the surface of the cured resin composite using a cyanoacrylate adhesive (Aronα; Tohokagaku, Japan) and the assembly was set in the tensile test jig. Tensile bond strength (TBS) was measured using a universal-testing machine (Autograph; Shimadzu, Kyoto, Japan) at a cross-head speed of 2.0 mm/min. The specimens without etching, or etched with 40 wt % H₃PO₄ for 30 s were made for comparison. In the case of 40 wt % H₃PO₄ etching, the specimens were rinsed with distilled water and air-dried. TBSs were measured in the manner described above. Ten specimens were tested for each group.

Scanning electron microscopic (SEM) observation

The morphological changes of the dentin surface due to the treatment with the priming solutions were observed using a SEM (DS-720; Topcon, Tokyo, Japan). The dentin specimens treated with priming solution for 60 s and air-dried were fixed with 4% glutaraldehyde for 24 h, dehydrated in ethanol and subjected to critical point drying. They were then gold coated (IB-3; Eiko Engineering, Tokyo, Japan) prior to the SEM observation. In the case of 40 wt % H₃PO₄ etched specimens, a rinsing procedure with distilled water was added before the air-dry procedure.

For observation of the resin–dentin interface, the bonded specimens using Photobond and Clearfil AP-X were sectioned through the resin–dentin interfaces using a low-speed rotary cutting machine (Isomet; Buehler Ltd., Lake Bluff, IL). After the cut surface of the each specimen was polished with 2000-grit abrasive carbide paper, it was etched with 40 wt % H₃PO₄ for 15 s to remove the inorganic substrate and then rinsed under a stream of water. The specimen was then immersed in a sodium hypochlorite solution (Neo Cleaner®; Neo Chemical Industry, Tokyo, Japan) for 4

h to dissolve the organic substrate and was rinsed with water to observe the resin–dentin hybrid layer. The specimens were fixed, dried, and gold-coated as described above and subjected to SEM observation.

Statistic analysis of the data

The data of tensile bond strength were statistically analyzed using one-way factorial ANOVA and Fisher's PLSD methods at a significance level of 0.05 using the Stat View 4.02 program (Abacus Concepts, Inc., Berkeley, CA).

RESULTS

Identification of synthesized monomer and pKa value of carboxyl groups of *N*-AAsp

Elemental analysis of *N*-AAsp. Found: C, 44.7; H, 4.7; N, 7.6. Calculated for $C_7H_9NO_5$: C, 44.9; H, 4.8; N, 7.5%.

The 1H -NMR and ^{13}C -NMR spectra of *N*-AAsp are shown in Figure 1. These results confirmed that the monomer synthesized was *N*-AAsp. The pKa values of carboxyl groups in the main and side chains of *N*-AAsp were 3.67 and 4.19, respectively.

TBS value and pH of the priming solution

Table I summarizes the TBS and pH values of each specimen and priming solution. The pH of the solutions decreased with the increase in *N*-AAsp concentration. For example, 5 wt % *N*-AAsp solution showed pH 2.13 and 25 wt % *N*-AAsp solution showed pH 1.60. On the other hand, TBS increased with *N*-AAsp concentration except the specimens treated with 25 wt % *N*-AAsp, which showed lower TBS values than those treated with 20 wt % *N*-AAsp solution.

The TBS value of the specimen treated with 20 wt % *N*-AAsp was 16.0 MPa. This value is more than twice that of the nonetched specimens, 6.9 MPa, or the specimens etched with 40 wt % H_3PO_4 , 7.6 MPa, and is significantly higher than any of the other experimental groups ($p < 0.05$). Specimens treated with 10, 15, and 25 wt % *N*-AAsp solutions also showed significantly higher TBS values than those of nonetched specimens or those etched with 40 wt % H_3PO_4 ($p < 0.05$). In the case of specimens treated with 5 wt % *N*-AAsp solution, we found no significant difference compared with nonetched or 40 wt % H_3PO_4 etched specimens even though the average TBS value in itself was higher than in the nonetched or 40 wt % H_3PO_4 etched specimens.

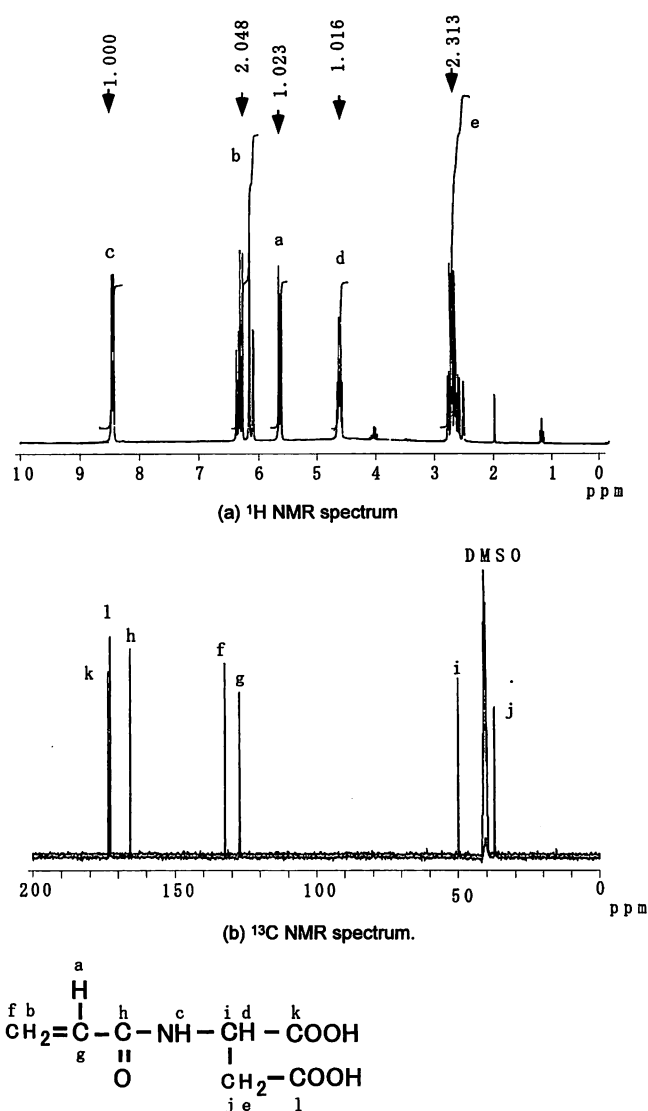


Figure 1. 1H and ^{13}C NMR spectra of *N*-AAsp. (a) 1H NMR spectrum, (b) ^{13}C NMR spectrum. The symbols attached to the chemical structure correspond to those in the spectra.

SEM analysis

Figure 2 shows typical SEM images of the dentin surfaces before and after treatment with 40 wt % H_3PO_4 or *N*-AAsp solutions. The smear layer found before etching or priming procedure [Fig. 2(A)] was effectively removed and the decalcified intertubular dentin surface was exposed except when the dentin surface was treated with 5 wt % *N*-AAsp, in which case the smear layer was not completely removed and the dentinal tubules orifices were still plugged with the smear layer. We found that 40 wt % H_3PO_4 acid showed the highest decalcification ability. In other words, the dentinal tubule orifices were less widened when treated with *N*-AAsp solution, even though its concentration was higher than 10 wt %, and that dentinal plugs were observed to be still partially remain-

TABLE I
Tensile Bond Strengths of the Dentin Treated with
Various *N*-Acryloyl Aspartic Acid
(*N*-AAsp)/Water Mixtures

	Concentration of <i>N</i> -AAsp in Water (wt %)	Average Tensile Bond Strength (MPa)	SD (MPa)	pH
No etching ^a	—	6.9	2.8	—
Phosphoric acid etching ^b	—	7.6	3.0	—
5 wt % <i>N</i> -AAsp/ water ^c	5	7.9	2.4	2.13
10 wt % <i>N</i> -AAsp/ water ^c	10	10.4	3.9	1.91
15 wt % <i>N</i> -AAsp/ water ^c	15	11.0	4.6	1.78
25 wt % <i>N</i> -AAsp/ water ^c	25	12.4	3.0	1.60
20 wt % <i>N</i> -AAsp/ water ^c	20	16.0	5.9	1.73

^aA bonding agent was applied on the untreated dentin surface.

^bA bonding agent was applied on the dentin surface etched with 40 wt % phosphoric acid for 60 s.

^cThe dentin surface was treated with *N*-AAsp/water mixtures for 60 s.

^dValues connected by vertical bars indicate no significant difference.

ing in the tubule orifices. In addition, we found that a membrane-like deposit covered the dentin surface when treated with *N*-AAsp. Particularly, the dentin surface treated with 25 wt % *N*-AAsp aqueous solution was coated with a thick layer of deposit [Fig. 2(G)].

Figure 3 represents the resin–dentin interfaces in the cross-sectioned specimens treated with priming solutions containing various concentrations of *N*-AAsp. Hybrid layers were created in all specimens. The thickness of the layer increased with the *N*-AAsp concentration as shown in Figure 4. The thickness of the hybrid layer in specimens treated with 5 wt % and 20 wt % *N*-AAsp aqueous solutions were approximately 0.7 μ m and 1.8 μ m, respectively.

Figure 5 shows high-magnification photographs of the hybrid layers created in the specimens treated with 5 wt % and 20 wt % *N*-AAsp aqueous solutions. The hybrid layer in the specimens treated with 5 wt % *N*-AAsp aqueous solution shows a porous structure, whereas that treated with 20 wt % *N*-AAsp aqueous solution appears relatively uniform.

DISCUSSION

The results obtained in this study clearly demonstrate that *N*-AAsp has good potential value as a self-

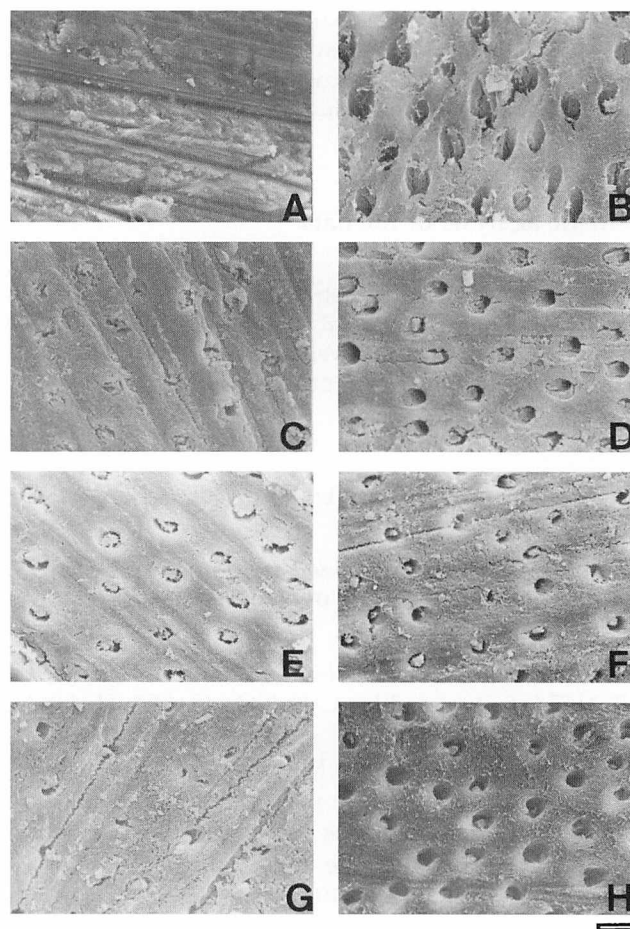


Figure 2. SEM images of the treated dentin surfaces: The original magnification was $\times 2500$. (A) ground dentin surface; surface was covered with smear layer. (B) 40 wt % H_3PO_4 for 30 s, water-rinse, air-dried; smear layer was removed and dentin tubules were widely opened. (C) 5 wt % *N*-AAsp aqueous solution for 60 s, air-dried; smear layer remained. (D) 10 wt % *N*-AAsp aqueous solution; (E) 15 wt % *N*-AAsp aqueous solution; (F) 20 wt % *N*-AAsp aqueous solution; (G) 25 wt % *N*-AAsp aqueous solution, dentin surface was covered with membrane-like deposition; (H) dentin surface treated with 25 wt % *N*-AAsp was washed with acetone. Bars = 4.0 μ m.

etching primer. It was prepared by condensing aspartic acid and acryloyl chloride. And favorably, it is highly soluble in water. The bond strength promoted by the treatment with *N*-AAsp solution prior to the application of an adhesive bonding agent, Photobond containing MDP and HEMA etc., was significantly higher than in the nonetching group or phosphoric acid etching/water rinse group, and *N*-AAsp facilitated the formation of the hybrid layer at the resin–dentin interface. It was shown that *N*-AAsp not only removed the smear layer and decalcified the underlying dentin but also primed the collagen fibrils exposed after decalcification of the inorganic parts of dentin and facilitated the infiltration and diffusion of the bonding agent into the decalcified dentin layer. *N*-AAsp also has many advantages in addition to its

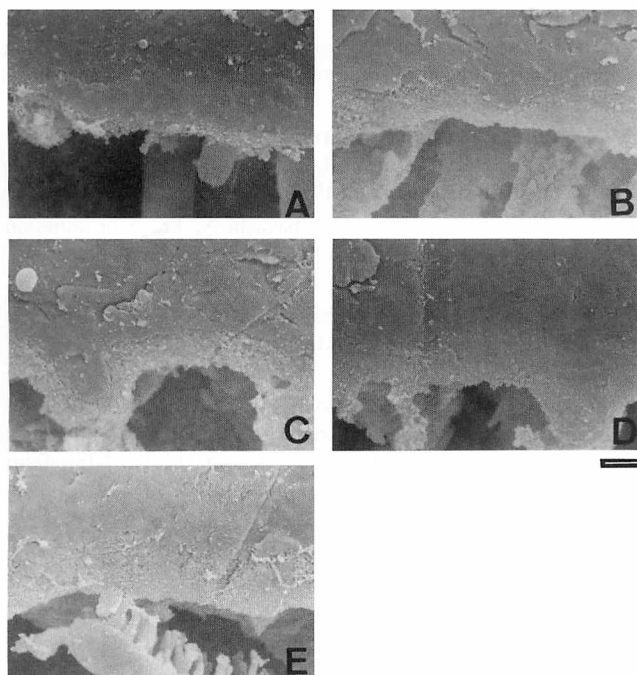


Figure 3. SEM images of resin-dentin interfaces in cross-sectioned specimens. The original magnification was $\times 10,000$. Hybrid layers were created at the resin-dentin interfaces. (A) 5 wt % *N*-AAsp aqueous solution for 60 s, air-dried; thin hybrid layer was recognized. (B) 10 wt % *N*-AAsp aqueous solution; (C) 15 wt % *N*-AAsp aqueous solution; (D) 20 wt % *N*-AAsp aqueous solution, thick and well-created hybrid layer was observed. (E) 25 wt % *N*-AAsp aqueous solution. Bars = 1.0 μm .

bonding efficacy. First, it can be prepared relatively easy in very high purity. Second, it can be dissolved in water without adding any other organic solvent and has high solubility at approximately 25 wt %. The self-etching primer could eliminate a water rinsing process that could simplify the bonding procedures.

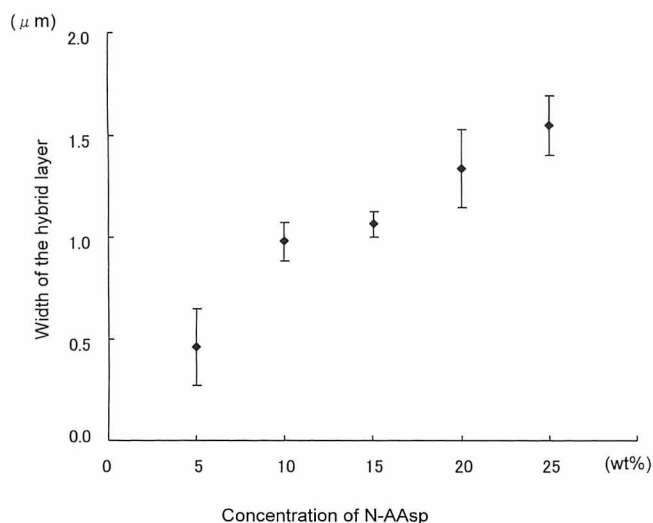


Figure 4. Effect of *N*-AAsp concentration on the width of the hybrid layer.

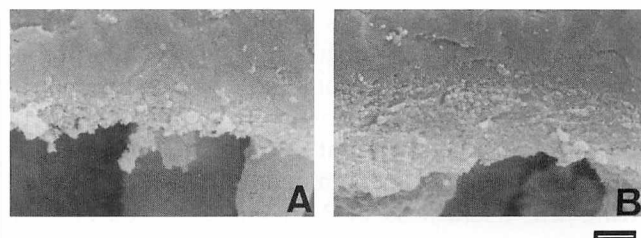


Figure 5. High magnification SEM images of resin-dentin interfaces in the specimens treated with 5 wt % and 20 wt % *N*-AAsp aqueous solutions. The original magnification was $\times 20,000$. Hybrid layers were created at the resin-dentin interfaces but their structures were different. (A) 5 wt % *N*-AAsp aqueous solution for 60 s, air-dried; poorly created hybrid layer was recognized. (B) 20 wt % *N*-AAsp aqueous solution; hybrid layer appeared to be more uniformly created. Bars = 0.5 μm .

We recommend the use of 20 wt % *N*-AAsp solution as a self-etching primer because in our studies it yielded the highest bonding strength. It is easy to understand that the bond strength of resin to dentin increases with the *N*-AAsp concentration because a higher *N*-AAsp concentration results in a lower pH, higher decalcifying ability, and higher affinity with collagen. However, the TBS value increased with *N*-AAsp concentration up to 20 wt % and decreased when 25 wt % *N*-AAsp solution was used. The pH value of 25 wt % *N*-AAsp solution was the lowest among the *N*-AAsp solutions tested. In fact, it removed the smear layer completely and widened the dentinal tubules. In addition, it gave the widest hybrid layer at the resin-dentin interface. Suzuki et al.¹² reported that the thickness of the hybrid layer bears no relationship to the bond strength between resin and dentin. They concluded that formation of the hybrid layer was a necessary but insufficient condition for higher bond strength. When 25 wt % *N*-AAsp solution was applied to the dentin surface and air-dried, the decalcified surface appeared to be covered with a thick deposit. The deposit layer should consist of *N*-AAsp monomer and calcium phosphate mineral dissolved from the smear layer and dentin. This layer would not prevent penetration of the bonding agent into the dentin surface. For example, the deposit layer was removed completely and the dentin tubules were exposed when rinsed with acetone [Fig. 2(H)].

With respect to the quality of the hybrid layer, we found different morphologies between the hybrid layer formed using 5 wt % *N*-AAsp and that formed using 20 wt % *N*-AAsp. As stated previously, the former appeared relatively porous whereas the latter was apparently more uniformly created. To observe the hybrid layer, the specimen was exposed to 40 wt % H_3PO_4 and sodium hypochlorite solutions. In this treatment, hydroxyapatite, an inorganic mineral of

dentin, and collagen fibrils unprotected by the bonding resin were dissolved during the specimen preparation. Therefore, the porous structure of the hybrid layer may have been due to insufficient penetration of a bonding resin component into the dentin surface.

Although the results obtained in this study demonstrate the usefulness of *N*-AAsp as a self-etching primer, we have not clarified the detailed mechanism of why *N*-AAsp can be used as an efficient self-etching primer. As stated previously, a self-etching primer is required not only to remove the smear layer but also to allow penetration of the bonding agent into the modified dentin surface. One of the characteristics of *N*-AAsp is that it has two carboxylic acid groups in its chemical structure. Of course, carboxylic acid plays an important role in the decalcification of the smear layer and the underlying intact dentin. For the carboxylic acid to act as an acid it has to ionize to release hydrogen ions. However, it was reported that ionized carboxylic acid lost its chemical affinity with collagen fibrils.¹⁷ Fortunately, the *N*-AAsp molecule has two carboxyl groups having different pKa in its chemical structure. The pKa values of the carboxyl groups in the main and side chains of the *N*-AAsp molecule were 3.67 and 4.19, respectively. It is considered that the former carboxyl group plays a greater role in the decalcification of the smear layer and that the latter carboxyl group plays a greater role in the interaction with dentinal collagen. This consideration was the reason for designing and preparing *N*-AAsp in this study. Further investigation is necessary based on these initial findings.

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