Effect of acid etching time on nanoleakage and microtensile bond strength of the adhesive-dentin bond interface

San-jun Zhao, DDS, PhD, Ji-hua Chen, DDS, PhD, Yu Xiong, DDS, PhD, and Hui Wang DDS, PhD

Department of Prosthodontics, Stomatological College, The Fourth Military Medical University, Xi'an, P. R. China

Purpose: To observe the effect of acid etching time on nanoleakage and microtensile bond strength (μTBS) of the adhesive-dentin bond with different total-etch adhesive systems.
Materials and Methods Eighty human molars, whose occlusal enamel was removed to expose the dentin surfaces, were conditioned with phosphoric acid for 0 (control), 15, 30 or 60 s (n=20). Resin-dentin bonded specimens were produced using four one-bottle adhesives respectively; OptiBond Solo (OB), Single Bond (SB), One-Step (OS), and Prime & Bond NT (PB). Each specimen was then sectioned perpendicularly to the adhesive interface into eight beams. Four were used to measure the nanoleakage of adhesive-dentin interface by transmission electron microscope and four to conduct the μTBS test. The data was collected and statistically compared with analysis of variance and post-hoc test.
Results: Nanoleakage increased with increasing etching time (0, 15, 30, and 60 s; $p<0.05$). For the OS and PB groups, the highest μTBS were achieved after 15 s of etching, followed by 30, 60, and 0 s. For the OB and SB groups, the μTBS between 15 and 30 s etching time subgroups were not significantly different between them but significantly higher than those of 0 and 60 s etching time subgroups ($p<0.05$).
Conclusion: Increasing etching time demineralized the dentin surface to a depth greater than adhesives can penetrate, resulting in more nanoleakage and lower μTBS. **(Int Chin J Dent 2006; 6: 29-37.)**
Key Words: dentin, etching time, nanoleakage, microtensile bond strength.

Introduction

There is agreement that dentin bonding is a more unpredictable and unreliable procedure than enamel bonding,[1] what can be attributed to dentin's complex histological structure, variable composition and greater organic content.[2] Furthermore, an insufficient dentin bonding may frequently cause marginal discoloration, recurrent caries, postoperative sensitivity and the loss of restorations.

In order to achieve high bond strengths and complete marginal seal with dentin, most modern adhesive systems demineralize dentin with acidic conditioners, resulting in complex chemical and physical changes of the dentin matrix. Pashley et al.[3] defined the purpose of acid etching dentin as follows: to remove the smear layer, to expose the intertubular and peritubular dentin, to clean the dentin surface from debris, and more importantly, to demineralize the superficial dentin matrix to allow resin infiltration into the dentin surface.

Furthermore, acid-etching dentin could create voids between the collagen fibers due to the loss of hydroxyapatite crystals.[4] These crystals support the collagen fibers and prevent its denaturation. And it is difficult for adhesive monomers to penetrate a denaturated or collapsed collagen surface.[5] The longevity of composite fillings is, among other factors, dependent on the properties of the junction between the dentin matrix and filling materials. Penetration of bacterial products, various acids and even oral fluids through the voids at the interface between a restoration and tooth have been regarded as detrimental to the longevity of the restorations.[6,7]

To distinguish the leakage within demineralized dentin where disparities existed between the depths of demineralization and monomer diffusion, from the typical microleakage, Sano et al.[8,9] introduced the term "nanoleakage", which was described as porosities located within/underneath the hybrid layer in a range less than 50 nm.[10,11] These porosities features collagen-rich fibrous network created by dentin demineralization that

should be penetrated by resin to reinforce the demineralized dentin structures.[12] It has been suggested that improvement of the penetrating properties of the primer might facilitate the filling of porous demineralized collagen zone. However, reducing the depth of demineralized dentin may also be equally effective in producing a good bonding.[13]

The purpose of the present study was to observe the effect of different etching times on nanoleakage and microtensile bond strengths to dentin achieved with four adhesive systems. The null hypothesis to be tested was that the adhesive systems produce similar nanoleakage and bond strengths irrespective of etching time.

Materials and Methods

Preparation of ammoniacal silver nitrate

Silver nitrate (25 g, $AgNO_3$; XK13-201-0099-031, Jiaozuo Chemical, Henan, China) was dissolved in 25 mL of 28% aqueous ammonium hydroxide (NH_4OH; Dongda Chemical, KaiFeng, China) in the presence of ambient laboratory light, creating a suspension of black silver particles. Additional 28% NH_4OH was used to titrate the black solution, with magnetic stirring at room temperature (25±2˚C), until the solution slowly became clear as the ammonium ions combined the silver into diamine silver ions ($[Ag(NH_3)_2]^+$). This solution was diluted to 50 mL with distilled water to achieve a 50 wt% solution (pH=9.5) that was transferred to an amber bottle for storage.

Tooth preparation and bonding procedures

Eighty caries-free human molar teeth were stored in 0.5% chloramine-T trihydrate at 4˚C and used within six months of extraction. The roots of teeth were mounted in self-curing acrylic resin (Quick Resin B, Shofu Inc., Kyoto, Japan) and placed in distilled water for 24 hours prior to the experiment. The occlusal enamel was removed using a low-speed diamond disc (Isomet 11-1180, Buehler Ltd., Lake Bluff, IL, USA**)** under copious air-water spray, and superficial occlusal dentin was finished with 600-grit silicon carbide paper under running water to create a smear layer of clinically relevant thickness.[14]

Table 1. Single-bottle adhesives used for test.

Bonding system	Lot number	Composition
OptiBond Solo (OB)	108D24	Bis-GMA, HEMA, GPDM, Photoinitators, Filler[a], Ethanol
Single Bond (SB)	1105	Bis-GMA, Polyalkenoic acid co-polymer, Dimethacrylates, HEMA, Photoinitators, Ethanol, Water
One-Step (OS)	0100011728	BIS-GMA, BPDM, HEMA, Photoinitators, Filler[b], Actone
Prime&Bond NT (PB)	0009000254	PENTA, UDMA, Resin R5-62-1, T-Resin, D-Resin, Nanofillers, Photoinitators, Stabilizer, Cetylamine hydrofluoride, Actone

a, A mixture of barium aluminum borosilicate glass, fumed silica, and disodium hexafluorosilicate; b, 8.5% filled, an average particle size of 1 μm; Bis-GMA, bis-phenol A diglycidyl-methacrylate; PENTA, dipentaerythritol-pentaacrylate phosphoric acid ester; HEMA, 2-hydroxylethyl methacrylate; GPDM, glycerolphosphate dimethacrylate; BPDM, biphenyl dimethacrylate.

The 80 specimens were randomly assigned to four groups (n=20) according to the four bonding agents; OptiBond Solo (OB, Kerr Corp., Orange, CA, USA), Single Bond (SB, 3M-ESPE, St. Paul, MN, USA), One-Step (OS, Bisco, Inc., Schaumburg, IL, USA), and Prime&Bond NT (PB, Dentsply/Caulk, Milford, DE, USA). The composition of the adhesive systems used in this study is summarized in Table 1. Each group was further randomly divided into four subgroups (n=5) accordingly to the different etching times: 0 (not-etched), 15,

30 and 60 s. The 0 s subgroups served as a negative control group. All specimens were stored in distilled water at room temperature for 24 hours. The bonding procedure for each group was carried out according to the manufactures' instructions (Table 2) except for the etching time, (15 s was the manufacturers' recommended etching time for the four test adhesives). After etching each tooth surface with a 32% phosphoric acid (Uni-Etch, Lot: 0400008319, Bisco Inc.), a 2.0 mm increment of composite material (Charisma, Lot 081, Heraeus Kulzer, GmbH & Co., Hanau, Germany) was applied to the bonded surfaces and light-cured for 40 s using a light curing unit (QHL75 Curing Lite, Dentsply). A single operator, under identical room temperature conditions, performed the etching and bonding procedures. After 24 hours in water at 37˚C, the specimens were thermocycled for 500 cycles in baths kept at 5˚C and 55˚C in order to simulate oral environment according to ISO/TS 11405, 2003. Each specimen was sectioned perpendicularly to the bonded interface into eight 1.0×1.0×4.0 mm beams with a low-speed saw (KB-01, FMMU, Xi'an, China) under running water.

Table 2. Instructions recommended by manufacturers.

	OptiBond Solo (OB)	**Single Bond (SB)**
Blot dry technique	Blot dry with cotton pellet	Blot excess water using a cotton pellet or mini-sponge
Moist extent of dentin	Dry lightly. Do not desiccate.	Appearing glistening without pooling of water
Primer/Adhesive	A coat	2-3 consecutive coats
Agitation	Light brushing motion for 15 s	Gentle agitation with a fully saturated applicator for 15 s
Air-drying	Lightly air-thin for maximum 3 s	Gently air thin for 5 s
Light curing	20 s	10 s
	One-Step (OS)	**Prime&Bond NT (PB)**
Blot dry technique	Remove excess water by blotting the surface with a foam pellet, alternatively, air dry for 2-3 s	Blot dry conditioned areas with a moist cotton pellet
Moist extent of dentin	Leaving visibly moist with a shiny surface	Leaving moist
Primer/Adhesive	A minimum of two generous coats	A coat, remaining fully wet for 20 s
Agitation	Agitating slightly for 10-15 s	No
Air-drying	Gently, thoroughly	Gently, 5 s
Light curing	10 s	10 s

Etching time, 15 s for all materials.

Silver staining technique

Four of the eight beams sectioned from each specimen were coated with two layers of fast-setting nail varnish (2A075 Jet-Set, L'Oréal, Paris, France) applied 1 mm from the bonded interfaces. Without allowing these beams to be dehydrated, they were immersed immediately in the ammoniacal silver nitrate solution in the dark. We did not allow the tooth beams or varnish to dry completely so as to avoid the creation of artificial submicron hiati beneath the resin-dentin interfaces that could be mistaken for nanoleakage.[15]

After 24 hours, the silver-stained beams were rinsed thoroughly in distilled water and placed in photo-developing solution (Q/YYCX1-1999, Guanlong Photographic Equipment Co., Shanghai, China) for 8 hours under a fluorescent light to facilitate reduction of the silver ions into metallic silver particles within potential voids along the resin-dentin interface,[16,17] and then fixed in fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/L cacodylate buffer, pH=7.3) for 1 hour and rinsed thoroughly with 0.1 mol/L sodium cacodylate buffer.

Transmission electron microscopic (TEM) observation

The silver-stained beams except for the ones showing microleakage (gap between dentin and composite visible under optical microscope using 80x were demineralized in an aqueous solution of 0.1 mol/L ethylene

diamine tetra-acetic acid (EDTA-$Na_2H_2 \cdot 2H_2O$; BG/T 1401-1998; Tianjing Chemical Reagent Co., Inc., Tianjing, China), which has been buffered with sodium hydroxide to a pH of 7, for 48 hours.

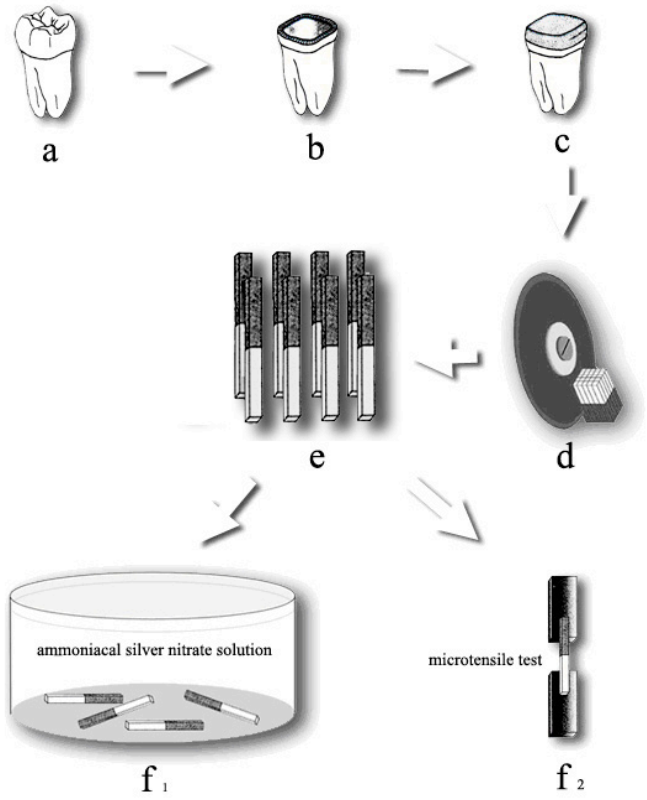

Fig. 1. Diagram detailing specimen preparation.

According to the TEM protocol of Tay et al.,[18] the beams were dehydrated in an ascending acetone series (30% to 100%), immersed in propylene oxide as a transition fluid, and embedded in epoxy resin (E-20; Hengyuan Chemical, Huangshan, China) at 60˚C for 48 hours. Each resin-embedded beam was trimmed into a 2×1 mm block that contained the silver-stained interface and re-embedded in epoxy resin. Dermineralized sections with 90 nm thickness were prepared using a microtome (Ultrotome, LKB-2188, Nova, Sweden). The sections were collected on single-slot, carbon and formvar-coated copper grids. Then, the silver-stained interfaces were examined by a transmission electron microscope (JEM-2000EX, JEOL, Tokyo, Japan). All TEM sections were examined at 100 kV without further staining. The size and percentage distribution of silver particles of the ten digitized micrograph from each of the 16 subgroups were determined as the amount of nanoleakage using image analysis software (Scion Image 4.02, Scion Corp., Frederick, MD, USA). Means and standard deviations of silver penetration areas were calculated for specimens from each time period (0, 15, 30, and 60 s).

Microtensile test

Another four of the eight beams harvested from each specimen were further trimmed by an ultra-fine diamond bur mounted in a high speed hand-piece under water coolant to produce a cross-sectional surface area of approximately 1.0 mm^2, measured using a digital caliper. The trimmed beams were attached to the grips of a custom-made holder with cyanoacrylate adhesive (Yuwang, Shandong Industrial & Commercial Co., Ltd., Jinan, China) and stressed to failure at a crosshead speed of 1 mm/minute on a universal test machine (Autograph,

AGS210, Shimadzu, Kyoto, Japan). The micro tensile bond strength (μTBS) of each beam was calculated as the force at failure divided by the bonded cross-sectional surface area and expressed in MPa. The μTBS data and nanoleakage amount of these materials were subjected to one-way ANOVA and Least Significant Difference (LSD) test at the 95% level of confidence. Fig. 1 summarizes the experimental design.

Results

Nanoleakage in the form of reticular silver deposits could be observed predominantly along the base of hybrid layers of all adhesive systems under different etching times except for the 0 s subgroups (Fig. 2). The means and standard deviations of the μTBS and nanoleakage are summarized in Table 3 (μTBS), Table 4 (nanoleakage), and Fig. 3.

Table 3. Dentin microtensile bond strength (MPa) and standard deviations (SD).

Bonding system	0 s		15 s		30 s		60 s	(etching time)
	μTBS	SD	μTBS	SD	μTBS	SD	μTBS	SD
OptiBond Solo (OB)	8.4	1.7	25.4	4.2	26.4	5.2	21.1	4.0
Single Bond (SB)	8.4	1.6	24.3	4.0	24.8	3.5	21.2	3.1
One-Step (OS)	9.3	2.7	28.7	4.9	25.0	3.7	21.3	3.9
Prime&Bond NT (PB)	8.3	1.5	27.1	4.1	23.6	3.9	20.2	3.7

For both OS and PB groups, the values of μTBS are significantly different among the four etching times. For OB and SB groups, the values of μTBS between 15 s and 30 s etching subgroups were not significantly different, which were all significantly higher than those of 0 and 60 s etching subgroups ($p<0.05$).

Table 4. Dentin nanoleakage (%) and standard deviations (SD) for the different etching times.

Bonding system	0 s		15 s		30 s		60 s	(etching time)
	%	SD	%	SD	%	SD	%	SD
OptiBond Solo (OB)	0	0	14.3	2.1	17.7	2.4	21.2	3.3
Single Bond (SB)	0	0	12.9	1.8	17.3	2.2	22.3	3.1
One-Step (OS)	0	0	11.7	2.0	13.9	1.9	18.5	2.4
Prime&Bond NT (PB)	0	0	11.5	2.2	13.6	2.2	17.5	2.3

For all groups, the values of nanoleakage are significantly different among the four etching times according to one-way ANOVA analysis ($p<0.05$).

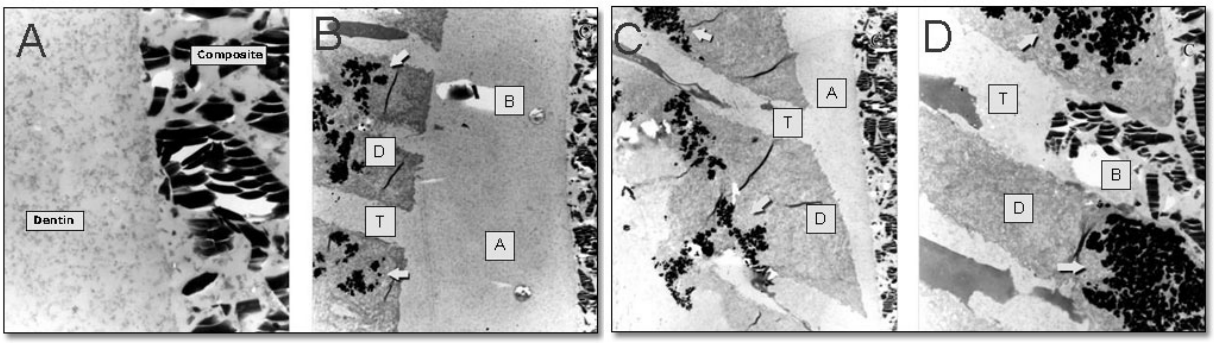

Fig. 2. Transmission electron micrographs of resin-interface achieved with Single Bond (SB).
There was no nanoleakage observed along the resin-dentin interface with 0 s etching time (not etched) (A). Nanoleakage (silver particles) in the form of reticular silver deposits could be observed predominantly along the base of hybrid layers with 15 (B), 30 (C), and 60 s (D) etching times. Open arrowhead: nanoleakage, in the form of interconnecting dendrite silver deposits, could be identified within the resin/dentin interface. A, adhesive layer; B, air bubble; C, composite resin; D, intertubular dentin; and T, dentin tubule.

To explore the interaction term, one-way ANOVA test was performed for each of the four different adhesives

as a function of etching time. For all groups, nanoleakage gradually increased in accord with increasing etching time. For both OS and PB groups, one-way ANOVA revealed significant differences among the four etching time and LSD test indicated that etching for 15 s resulted in the hightest μTBS (28.7 MPa for OS, 27.1 MPa for PB) following by 30, 60 and 0 s. For OB and SB groups, the μTBS between 15 s and 30 s etching subgroups were not significantly different, while the μTBS of 15 s and 30 s etching subgroups were significantly higher than those of 0 and 60 s etching subgroups ($p<0.05$).

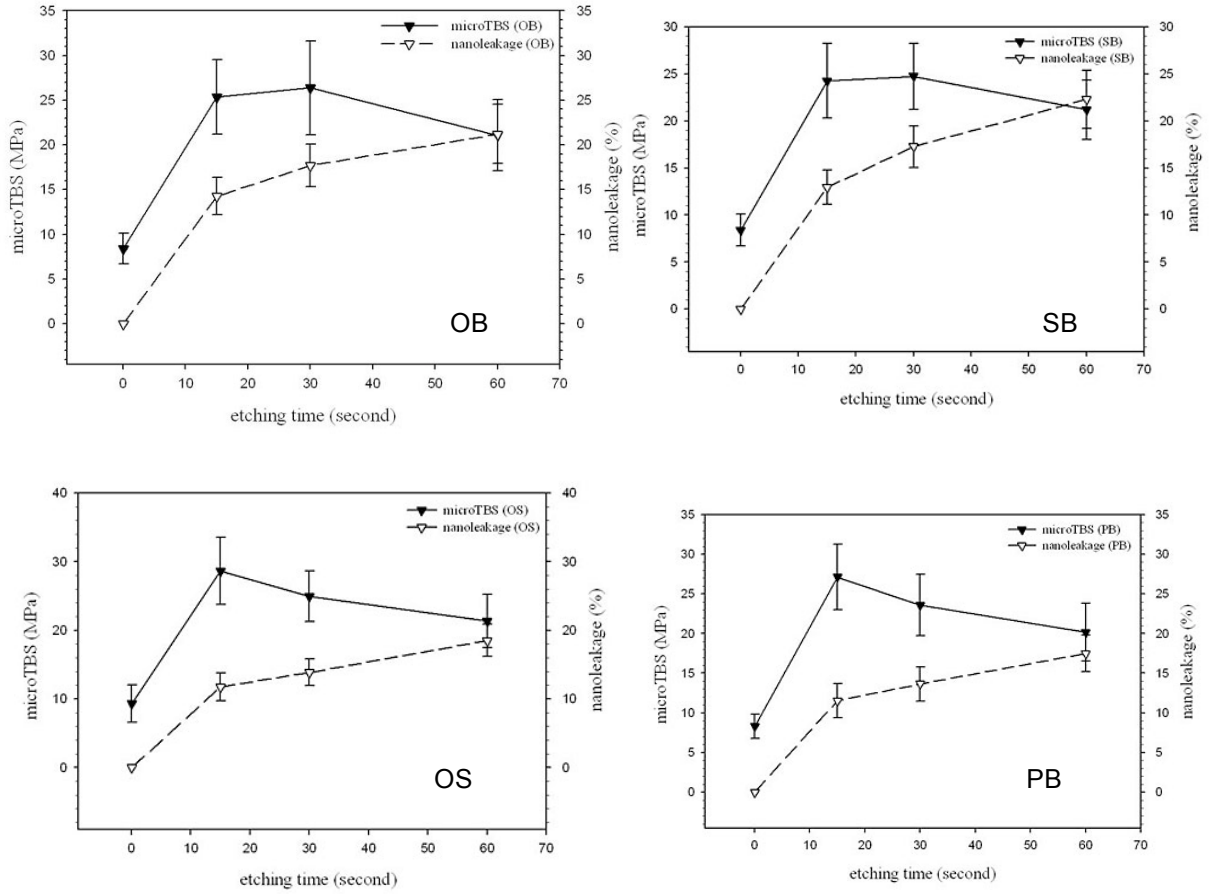

Fig. 3. Relationship between microtensile bond strengths (μTBS) and nanoleakage (silver penetration area) at each etching time period for OptiBond Solo (OB), Single Bond (SB), One-Step (OS), and Prime&Bond NT (PB).

Discussion

The bonding mechanism of resin to dentin is described as micromechanical, and is generated by monomer impregnation of exposed collagen of demineralized superficial dentin. Theoretically, in order to promote optimal bonding and sealing, the adhesive should completely diffuse across the full thickness of the etched dentin. This would encapsulate and protect the collagen fibril, fill interfibrillar spaces and permit physical interaction with the unaltered underlying dentin.[19-21] The diffusion and curing of adhesive resins through demineralized dentin are of crucial importance to create strong adhesion. However, it might be difficult to diffuse adhesive resin through demineralized dentin because of the presence of water around collagen fibers, the depth of the demineralized zone, the time allowed for the primer to penetrate, and nonuniform distribution of monomers (such as the viscosity).[20,22-24]

Most contemporary adhesives use an acid etching gel (32-37% phosphoric acid) to demineralize dentin surface. It is easier for the monomer to infiltrate a thin demineralized zone than a deeper zone. Immoderate

etching can expose collagen to depths that current dentin adhesives may not be able to penetrate, resulting in a porous collagen layer susceptible to degradation,[5,25] which results in nanoleakage. The term "nanoleakage" has been suggested to distinguish silver uptake into gap-free resin bonded interfaces from microleakage.[8] Nanoleakage was defined as the diffusion of silver ions through nanometer-sized channels that has not been fully penetrated by the adhesive, leaveing spaces for fluid penetration under the hybrid layer.

For measuring the effect of different etching time on nanoleakage, in the present study, we etched the dentin surface with 32% phosphoric acid for 0 (not etched), 15, 30 and 60 s and proceeded to bond with OB, SB, OS, and PB respectively. The statistically significant increase in the amount of penetration of silver ions in accord with increasing in etching time (0, 15, 30 and 60 s) supports that nanoleakage is located in the partially demineralized dentin, which is not completely penetrated by the adhesive systems. The longer the etching time, the deeper the expected layer of partially demineralized dentin would be. This would also explain why there were differences between adhesive systems with different compositions, as the degree of silver particles penetration not only depends on the depth of the partially demineralized dentin but also on the ability of the individual adhesive system to penetrate and reinforce this zone.

Except for nanoleakage reported recently, to a certain extent, conditioning with etching gel could considerably improve the bond strength of adhesive resins to dentin, which has been already accepted by clinicians today. Methacrylates with both hydrophobic and hydrophilic groups promoted monomer penetration and impregnation into the etched dentin, becoming entangled with the collagen fibrils, creating a hybrid layer after their polymerization,[26-30] which is essential to obtain proper adhesion of resinous materials to dentin. When etching time is increased, the hybrid layer thickness increases as a result of deeper demineralization.[31] Although the depth of demineralization needed for optimal adhesion has not been determined, Nakabayashi et al. suggested that a demineralization depth into dentin of 1.0-2.0 μm is sufficient for optimal bond strength.[21] Paul et al. reported that increasing the etching time had a negligible effect on bond strength of Single Bond, however, the silver uptake increased upon prolonged etching times.[32] In contrast, Abu-Hanna et al. correlated dentin bond strength to etching time and reported a significant decrease in bond strength values for Syntac as a result of the increased etching time.[33]

The current study evaluated the effect on dentin nanoleakage and bond strength of reducing the etching time to 0 s or increasing it to 60 s from the recommended 15 s etching time. In order to avoid the possibility of the minerals such as amorphous calcium phosphates may be dissolved in the acidic conventional silver nitrate ($AgNO_3$) solution (pH=3.4),[34] which may produce artefactual microporosities that give rise to false positive results,[35] a modified silver-staining techinique, ammoniacal silver nitrate ($[Ag(NH_3)_2]^+$) solution (pH=9.5), was used in this study.[36] For both acetone-based adhesives, OS and PB groups, the highest μTBS were achieved after 15 s of etching, followed by 30, 60 and 0 s (not-etched). For both the ethanol and water-based adhesives, OB and SB groups, the μTBS between 15 and 30 s etching subgroups were not significantly different, while the μTBS of 15 and 30 s etching subgroups were significantly higher than those of 0 and 60 s etching subgroups. These differences may indicate that the solvents used in adhesives influence their performances and that acetone-based adhesives are more technique-sensitive than the ethanol and water-based adhesives, probably because the different ability of acetone and ethanol to chase water as well as the different vapor pressure.[33,37,38] More studies are needed to elucidate the effect of different solvents on morphology and bond strength of adhesive-dentin bonding.

Undoubtedly, the existence of nanoleakage may have potential long-term consequences in dentine bonding procedures. Indeed, this kind of leakage may allow for the penetration of bacterial products and dentinal or oral fluid along the interface, which may result in hydrolytic degradation of either the adhesive resin or the exposed collagen, thereby compromising the stability of the bond,[39,40] ultimately, decreasing the longevity of the composite restoration. Consequently, from a clinical perspective, it would be advantageous to optimize dentinal bonding during clinical operation (concentration of acidic conditioners, application technique, etching time, etc.), and further studies should be performed to develop adhesive systems that could minimize the nanoleakage and achieve high bond strength on the resin-dentin interface.

Within the limitations of this in vitro study, it was concluded that different etching time resulted in significantly different dentin μTBS and nanoleakage. In addition, increased etching times demineralized the dentin surface to a depth greater than adhesives could penetrate, resulting in more nanoleakage and lower μTBS. The 15 s etching dentin (the manufacturers' recommendation) produced the highest μTBS and a moderate amount of nanoleakage for all adhesive systems tested. The results reject the null hypothesis that the adhesive systems produce similar nanoleakage and bond strengths irrespective of etching time.

Acknowledgment

This adhesives used in this study were generously sponsored by 3M-ESPE, Bisco Inc., and Kerr Co. The authors are grateful to Biao Kang for his technical support and Dr. Kai-cheng Li for his assistance in preparing this manuscript.

References

1. Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res 1955; 34: 849-53.
2. Mjor IA, Nordahl I. The density and branching of dentinal tubules in human teeth. Arch Oral Biol 1996; 41: 401-12.
3. Pashley DH, Horner JA, Brewer PD. Interactions of conditioners on the dentin surface. Oper Dent 1992; Suppl 5: 137-50.
4. Pashley DH. The effects of acid etching on the pulpodentin complex. Oper Dent 1992; 17: 229-42.
5. Nakabayashi N, Nakamura M, Yasuda N. Hybrid layer as dentin-bonding mechanism. J Esthet Dent 1991; 3: 133-8.
6. Haller B. Recent developments in dentin bonding. Am J Dent 2000; 13: 44-50.
7. Kiyomura M. Bonding strength to bovine dentin with 4META/MMA-TBB resin: Long term stability and influence of water. J Jpn Dent Mater 1987; 6: 860-72.
8. Sano H, Shono T, Takatsu T, Hosoda H. Microporous dentin zone beneath resin-impregnated layer. Oper Dent 1994; 19: 59-64.
9. Sano H, Takatsu T, Ciucchi B, Herner JA, Mattews WG, Pashly DH. Nanoleakage: leakage within the hybrid layer. Oper Dent 1995; 20: 18-25.
10. Sano H, Yoshiyama M, Ebisu S, et al. Comparative SEM and TEM observations of nanoleakage within the hybrid layer. Oper Dent 1995; 20: 160-7.
11. Van Meerbeek B, Yoshida Y, Lambrechts P, et al. A TEM study of two water-based adhesive systems bonded to dry and wet dentin. J Dent Res 1998; 77: 50-9.
12. Sano H, Takatsu T, Ciucchi B, Russell CM, Pashly DH. Tensile properties of resin-infiltrated demineralized human dentin. J Dent Res 1995; 74: 1093-102.
13. Titley K, Chernecky R, Maric B, Smith D. Penetration of a dentin bonding agent into dentin. Am J Dent 1994; 7: 190-4.
14. International Organization for Standardization. Dental materials - Testing of adhesion to tooth structure. ISO/TS 11405: 2003.
15. Agee KL, Pashley EL, Itthagarun A, Sano H, Tay FR, Pashley DH. Submicron hiati in acid-etched dentin are artifacts of desiccation. Dent Mater 2003; 19: 60-8.
16. Mair LH. An investigation into the permeability of composite materials using silver nitrate. Dent Mater 1989; 5: 109-14.
17. Wu W, Cobb E, Dermann K, Rupp NW. Detecting margin leakage of dental composite restorations. J Biomed Mater Res 1983; 17: 37-43.
18. Tay FR, Moulding KM, Pashley DH. Distribution of nanofillers from a simplified-step adhesive in acid-conditioned dentin. J Adhes Dent 1999; 1: 103-17.
19. Manabe A, Katsuno K, Itoh K, Wakumoto S, Miyasaka T. Bonding efficacy of erythritol methacrylate solutions as dentin primers. J Dent Res 1991; 70: 1294-8.
20. Van Meerbeek B, Inokoshi S, Braem M, Lambrechts P, Vanherle G. Morphological aspects the resin-dentin interdiffusion zone with different dentin adhesive systems. J Dent Res 1992; 71: 1530-40.
21. Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res 1982; 16: 265-73.
22. Gwinnett AJ. Moist versus dry dentin: Its effect on shear bond strength. Am J Dent 1992; 5: 127-9.
23. Abate PF, Rodriguez VI, Macchi RL. Evaporation of solvent in one-bottle adhesives. J Dent 2000; 28: 437-40.
24. Erickson RL. Mechanism and clinical implications of bond formation for two dentin bonding systems. Am J Dent 1989; 2: 117-23.
25. Perdigao J, May KN Jr, Wilder AD Jr, Lopes M. The effect of depth demineralization on bond strengths and morphology of the hybrid layer. Oper Dent 2000; 25: 186-94.
26. Eick JD, Cobb CM, Chappell RP, Spencer P, Robinson SJ. The dentinal surface: its influence on dentinal adhesion. Part I. Quintessence Int 1991; 22: 967-77.

27. Eick JD, Robinson SJ, Cobb CM, Chappell RP, Spencer P. The dentinal surface: its influence on dentinal adhesion. 2. Quintessence Int 1992; 23: 43-51.
28. Eick JD, Robinson SJ, Chappell RP, Cobb CM, Spencer P. The dentinal surface: its influence on dentinal adhesion. Part III. Quintessence Int 1993; 24: 571-82.
29. Nakabayashi N. The hybrid layer: a resin-dentin composite. Proc Finn Dent Soc. 1992; 88 Suppl 1: 321-9.
30. Walshaw PR, McComb D. SEM evaluation of the resin-dentin interface with proprietary bonding agents in human subjects. J Dent Res 1994; 73: 1079-87.
31. Hashimoto M, Ohno H, Endo K, Kaga M, Sano H, Oguchi H. The effect of hybrid layer thickness on bond strength: demineralized dentin zone of the hybrid layer. Dent Mater 2000; 16: 406-11.
32. Paul SJ, Welter DA, Ghazi M, Pashley D. Nanoleakage at the dentin adhesive interface vs microtensile bond strength. Oper Dent 1999; 24: 181-8.
33. Abu-Hanna A, Gordan VV. Evaluation of etching time on dentin bond strength using single bottle bonding systems. J Adhes Dent 2004; 6: 105-10.
34. Li H, Burrow M, Tyas MJ. The effect of load cycling on the nanoleakage of dentin bonding systems. Dent Mater 2002; 18: 111-9.
35. Gale MS, Darvell BW. Dentine permeability and tracer tests. J Dent 1999; 27: 1-11.
36. Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single-step adhesives. J Dent Res 2002; 81: 472-6.
37. Tay FR, Gwinnett JA, Wei SH. Relation between water content in acetone/alcohol-based primer and interfacial ultrastructure. J Dent 1998; 26: 147-56.
38. JacobsenT, Soderhold KJ. Some effects of water on dentin bonding. Dent Mater 1995; 11: 132-6.
39. Pioch T, Staehle HJ, Duschner H, García-Godoy F. Nanoleakage at the composite-dentin interface: a review. Am J Dent 2001; 14; 252-8.
40. Sano H, Yoshikawa T, Pereira PN, et al. Long-term durability of dentin bonds made with a self-etching primer in vivo. J Dent Res 1999; 78: 906-11.

Correspondence to:

Dr. Ji-hua Chen
Department of Prosthodontics, Stomatological College, The Fourth Military Medical University
Xi'an, 710032, P. R. China
Fax: +86-29-8253-4510 E-mail: jhchen@fmmu.edu.cn

Received November 29, 2005. Revised January 5, 2006. Accepted February 21, 2006.