Wet or dry, normal or deproteinized dentin surfaces as substrate for dentin adhesives

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Bond strength between resin composite and dentin mediated by several dentin adhesives applied to dry or wet acid-etched dentin or to dry or wet acid-etched and deproteinized dentin were measured and analyzed. Human dentin were A) acid-etched and blot-dried for 1 sec (= wet), B) as A but dried with air for 10 sec (= dry), C) acid-etched and treated with hypochlorite and then dried for 1 sec, and D) as C but dried with air for 10 sec. Eight dentin adhesives were used in each group for bond strength measurements. The results were compared by means of ANOVA and the Tukey test. Collagen removal increased significantly the strength of the bond by 10–18 MPa for five adhesives when tested dry (D versus A). When tested wet, collagen removal increased the strength by 10–12 MPa for three adhesives (C versus A). Normal etched dentin showed a reduction in strength of 14–15 MPa for three of the adhesives when tested dry instead of wet (B versus A). For one dentin adhesive no significant change in bond strength due to collagen removal and/or drying conditions was observed. It was hypothesized that low technique sensitivity of an adhesive may be linked to its ability to wet and adhere to collapsed collagen fibers and to the surface of the underlying mineralized tissue. Comparisons of bond strengths obtained by using dried or wet acid-etched dentin and dried or wet acid-etched and deproteinized dentin may be useful for evaluating the efficiencies of dentin adhesives. □ *Adhesives; dentin bonding; hypochlorite; wetting*

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Several studies (1–10) describe the micromorphological structure of the surface obtained after dentin has been acid-etched. A surface layer of demineralized collagen is seen, which under wet conditions has an open, voluminous structure maintained by hydrogen bonding between the chains involving water molecules. Below, a partly demineralized zone is located above the normal dentin (9, 11). In the absence of water—for example, after drying the surface with a stream of air—the collagen structure collapses, covering the porous, partly demineralized zone.

According to the above studies, a dentin adhesive system applied to wet, acid-etched dentin will occupy the spaces between the demineralized collagen and, after polymerization, form a hybrid layer composed of collagen fibers maintained in a polymer. The polymer may be in contact with the underlying partly demineralized dentin. In cases where the demineralized collagen layer is collapsed as a result of drying, some adhesives will still be able to penetrate and occupy the spaces between the demineralized collagen because of their ability to restore the collapsed collagen layer, whereas other adhesives do not possess this ability (12, 13).

Even though valuable information on the nature of the entanglement between adhesives and the surface substructure has been obtained by means of the above micromorphological studies, it is not possible, on basis of pictures, to predict the magnitude of the bond between dentin and a composite resin established by an adhesive. In this respect, one has to depend on other types of measurements, such as bond strength measurements.

The ability of an adhesive to penetrate through the collagen layer to make a bond may be linked to its solubility characteristics and polarity (14), its molecular mobility in combination with the permeability coefficient (15), and the free surface energy of the surface to be bonded (16). An adhesive should be hydrophilic to a certain extent so as not to be repelled by the water-containing collagen layer. Furthermore, an adhesive should displace or be miscible with the water around the demineralized collagen fibers, in order to penetrate and adhere sufficiently to the collagen and to the underlying partly demineralized dentin. In addition, it is advantageous if the adhesive contains a sufficiently high concentration of water and water-miscible methacrylates, giving the capability of restoring the structure of the collagen in case of collapse (1, 17). This may be established with, for example, aqueous 2-hydroxyethylmethacrylate (HEMA) solutions. Results support the assumption that the structure will be restored most extensively when the concentration of HEMA is approximately 35% (18).

A limited ability for some adhesives to completely fill out the spaces between the demineralized collagen has been shown by detecting silver deposits in that layer formed after penetration (6, 7). The finding by Spencer & Swafford (19) of a protein layer not embedded in the

Table 1. Composition of dentin adhesives. The compositions of the commercial products are in accordance with information from the manufacturer

Name (batch no.)	Abbreviation	Manufacturer	Composition
Clearfil Liner Bond 2v (0044A)	CL	Kuraray Co., Osaka, Japan	1. MDP, HEMA, hydrophilic DMA, CQ, amine, water 2. MDP, BisGMA, HEMA, hydrophilic DMA, silica, BPO
Excite (556608)	EX	Ivoclar-Vivadent, Schaan, Liechtenstein	Phosphonic acid acrylate, HEMA, BisGMA, DMA, silica, catalysts, ethanol
Optibond FL (1588-1/-2)	OF	Kerr Co., Orange, Calif., USA	1. HEMA, GPDM, PAMM, CQ, ethanol, water 2. HEMA, BisGMA, GDM, filler, CQ
Optibond Solo Plus (011727)	OS	Kerr Co.	BisGMA, HEMA, GDM, GPDM, filler, ethanol
PQ-1 (64101)	PQ	Ultradent Products, Inc., Jordan, Utah, USA	HEMA, monomers, natural resins, fluoride, filler, CQ, ethanol
Prime&Bond NT (600.67.2.56)	PB	Dentsply DeTrey, Konstanz, Germany	PENTA, urethane-BisGMA, elastomeric resins, cetylamine hydrofluoride, silica, CQ, acetone
Scotchbond 1 (4242) A01	SB	3M Co., Minneapolis, Minn., USA	BisGMA, HEMA, diacrylate, MA-PAA, CQ, ethanol, water
	A0	Experimental	47% HEMA, 6% MA-PAA, 4% UEDMA, 5% glutaraldehyde, 1% maleic acid, 36.7% water, 0.3% CQ

BisGMA = bis-phenol-A-glycidyl dimethacrylate; CQ = camphor quinone; DABE = *N,N*-dimethyl-*p*-aminobenzoicacid ethyl ester; DMA = dimethacrylate; GDM = glyceroldimethacrylate; GPDM = glycerophosphate dimethacrylate; HEMA = 2-hydroxyethylmethacrylate; MA-PAA = methacrylate-modified polyacrylic acid; MDP = 10-methacryloyloxydecyl dihydrogenphosphate; PAMM = mono (2-methacryloxy-ethyl)phthalate; PENTA = phosphonated penta-acrylate ester; TEGDMA = triethyleneglycol dimethacrylate; UEDMA = urethane dimethacrylate.
The components in the experimental product A01 were obtained from Sigma-Aldrich, Vallensbæk Strand, Denmark, except for MA-PAA, which was a gift from Heraeus Kulzer, Wehrheim, Germany. All concentrations are w/w%.

bonding resin between the hybrid zone and the normal dentin supported this observation.

A tensile strength of around 8–10 MPa has been reported for the collagen structure obtained by decalcifying dentin specimens (20). This corresponds to about 20% of the tensile bond strength between dentin and composite mediated by adhesives to moist dentin, which exceeded 50 MPa in one study (21). That study was performed by some of the same authors as in the former study (20) and with a technique that seems to justify the comparison. Therefore, in case of a hybrid layer in which the dentin adhesive does not reach the partly demineralized layer above the mineralized dentin, one may expect modest bond strength, because it might depend on the tensile strength of collagen.

The importance of the presence of the hybrid layer for the bond has been investigated by comparing the bond strength of normal acid-etched dentin with that of acid-etched dentin that has had the demineralized collagen removed by deproteinizing agents such as collagenase or hypochlorite (3, 22–32). Inconsistent results were obtained by these studies, perhaps due to differences in dryness of the etched surface before bonding.

On the basis of the above descriptions it can be hypothesized that low technique sensitivity of an adhesive may be linked to its ability to wet and adhere to collapsed collagen fibers and to the surface of the underlying mineralized tissue.

The purpose of this study was to investigate the technique sensitivity of several dentin adhesives by measuring and comparing the bond strength between a resin composite and dentin mediated by the adhesives applied on dried or wet acid-etched dentin, or on dried or wet acid-etched and deproteinized dentin.

Materials and methods

Extracted, caries-free human molars stored in 0.5 wt/wt% chloramine-T were embedded in an auto-curing resin (Durofix-2; Struers, Copenhagen, Denmark) and stored in water until use. The specimens were randomly distributed into 4×8 groups ($n = 6$), ground on wet silicon carbide paper (final step, 1000 grit) until a flat approximal dentin surface appeared, and then pretreated in four ways before application of one of eight dentin adhesives (Table 1).

Group A: 32 specimens were acid-etched with 35 w/w% phosphoric acid (diluted from 85% orthophosphoric acid obtained from E. Merck, Darmstadt, Germany) for 20 sec, rinsed with water for 15 sec, and blot-dried by pressing a piece of tissue paper (Kimcare, Kimberly-Clark Co., Bagsværd, Denmark) to the surface for 1 sec.

Group B: The same as group A, but the surfaces were dried with a stream of air for 10 sec instead of blot drying. The stream of air was in this group applied at a distance of approximately 1 cm and in a manner mimicking application performed by a dental unit.

Group C: Thirty-two specimens were acid-etched with 35 w/w% phosphoric acid for 20 sec, rinsed with water for 15 sec, and treated with 0.5 vol.% Na-hypochlorite (pH ~ 10.3; DanDental A/S, Vallensbæk, Denmark) for 1 h while stirring. The specimens were rinsed with water for 20 sec, stored in water, and then blot-dried by pressing a piece of tissue paper to the surface for 1 sec.

Group D: As for group C, but the surfaces were dried with a stream of air in 10 sec instead of blot drying.

The commercial adhesives were used in accordance with the manufacturer's instructions except that acid

Table 2. Mean bond strengths (s) between a resin composite and dentin, pretreated in accordance with one of the conditions A, B, C, or D. For these results, letters in superscript designate means within a row which are not significantly different, such as an evaluation of differences for each adhesive due to treatment. The mean bond strength (*s*) shown to the right represents the combined results for each adhesive

	Etched and dried for		Etched, hypochlorite-treated, and dried for		
Adhesive	1 sec, A, MPa (*s*)	10 sec, B, MPa (*s*)	1 sec, C, MPa (*s*)	10 sec, D, MPa (*s*)	A + B + C + D, MPa (*s*)
CL	22.2^{a} (6.06)	14.9^{a} (4.83)	19.1^{a} (2.31)	21.1^{a} (1.34)	19.3 (4.77)
EX	8.3^{a} (2.16)	7.0^{a} (4.84)	20.3^{b} (2.91)	21.2^{b} (2.75)	14.2 (7.39)
OF	21.8^{a} (3.35)	21.0^{a} (2.85)	32.1^{b} (2.52)	38.9^{b} (4.71)	28.4 (8.28)
OS	32.6^{b} (7.72)	$28.6^{a,b}$ (4.12)	$25.0^{a,b}$ (2.02)	21.6^{a} (4.58)	27.0 (6.28)
PQ	$23.1^{b,c}$ (5.89)	8.4^{a} (6.07)	29.2^{c} (7.24)	18.8^{b} (5.60)	19.9 (9.66)
PB	20.0^{b} (4.46)	6.1^{a} (1.95)	24.4^{b} (3.18)	21.7^{b} (5.05)	18.1 (8.06)
SB	16.8^{b} (4.44)	2.7^{a} (1.54)	13.1^{b} (1.90)	13.3^{b} (2.63)	11.5 (6.02)
A0	25.1^{a} (4.26)	20.6^{a} (2.66)	36.4^{b} (6.87)	$30.2^{a,b}$ (5.94)	28.1 (7.72)

etching was performed as described above. The instructions describing application method, number of layers, and curing method were followed closely. Light-curing of the adhesive and the resin composite (see below) was performed with a halogen light source (XL 3000, 3M Co., St. Paul, Minn., USA) operating at 480 mW/cm^2. The experimental adhesive, A01, was applied twice, each time for 10 sec, and then light-cured for 10 sec. A split cylindrical Teflon mold with a diameter of 3.6 mm and a height of 2.8 mm was clamped to the dentin surface, and the mold was filled with a resin composite to a height of approximately 2 mm as one portion and light-cured for 40 sec. This resin composite was made by mixing a hybrid filler with an experimental light-curable resin to 55 vol% as described previously (33). The hybrid filler was identical to that found in the resin composite Herculite and supplied by Kerr Co., Orange, Calif., USA. The mold was removed after at least 10 min, and specimens were stored in water at 37°C for 1–3 days before shear-bond testing. The shear test was performed with a wire loop around the composite cylinder placed in close contact with the dentin surface, at a crosshead speed of 1 mm/min in an Instron Universal Testing Machine (Instron, High Wycombe, UK). The means and standard deviations of the results representing the 32 groups were calculated. Statistical analysis was performed using one-way ANOVA and the Tukey test with $P = 0.05$ as the level of significance (34).

Results

The results are presented in Table 2, which shows the mean bond strengths (standard deviations (*s*)) between a resin composite and dentin specimens, pretreated in accordance with one of the conditions A, B, C, or D. For each row, letters in superscript adjacent to values designate means within a row that are not significantly different—an evaluation of differences for each adhesive due to treatment (ANOVA, $F = 23.5$, $P = 7.5\ 10^{-45}$, and the Tukey test). The mean bond strength (*s*) shown to the right represents the combined results for each adhesive.

All adhesives showed relatively high bond strength to acid-etched, hypochlorite-treated dentin when applied under both wet and dry conditions (groups C and D). Relatively low bond strength (<10 MPa) to acid-etched dentin under both wet and dry condition was observed with EX (groups A and B).

Lower bond strength to acid-etched and dry dentin compared with wet dentin was observed with PQ, PB, and SB (Table 2, groups A and B).

Lower bond strength to acid-etched, hypochlorite-treated, and dry dentin compared with wet dentin was observed with PQ (Table 2, groups C and D).

Removal of collagen gave higher bond strength under wet condition with EX, OF, and A0 (Table 2, groups A and C), whereas removal of collagen under the dry

Table 3. Evaluation of differences in the mean bond strengths (Table 2) between the eight adhesives. The results are given as symbols for the adhesives with the mean bond strengths in parentheses. For each treatment and for the combined results (A + B + C + D), means that are not significantly different are marked with a vertical line. Each column is in order of increasing mean bond strength

A	B	C	D	A + B + C + D
EX (8.3)	SB (2.7)	SB (13.1)	SB (13.3)	SB (11.5)
SB (16.8)	PB (6.1)	CL (19.1)	PQ (18.8)	EX (14.2)
PB (20.0)	EX (7.0)	EX (20.3)	CL (21.1)	PB (18.1)
OF (21.8)	PQ (8.4)	PB (24.4)	EX (21.2)	CL (19.3)
CL (22.2)	CL (14.9)	OS (25.0)	OS (21.6)	PQ (19.9)
PQ (23.1)	A0 (20.6)	PQ (29.2)	PB (21.7)	OS (27.0)
A0 (25.1)	OF (21.0)	OF (32.1)	A0 (30.2)	A0 (28.1)
OS (32.6)	OS (28.6)	A0 (36.4)	OF (38.9)	OF (28.4)

condition increased the bond strength with EX, OF, PQ, PB, and SB (Table 2, groups B and D).

Table 3 represents an evaluation of differences in the mean bond strengths between the eight adhesives and the combined results shown to the right. The results are given as symbols for the adhesives, with the mean bond strengths in parentheses. For each treatment and for the combined results (A + B + C + D) a vertical line designates means that are not significantly different according to the Tukey test. ANOVA for the combined results showed $F = 23.4$, $P = 7.5\ 10^{-45}$. Each column is given in order of increasing mean bond strength.

Discussion

The test in this study made use of the same type of resin composite and the same type and concentration of phosphoric acid. This was chosen to minimize the number of variables, making comparisons of the adhesives more acceptable. A 1-h treatment with a weak solution of hypochlorite was chosen on the basis of results previously obtained from scanning electron microscope studies. A 1-h treatment is not clinically relevant, but the results of this treatment served to obtain further information about the various agents' bonding capabilities.

Table 2 shows that all adhesives had either higher or unaltered bond strength to hypochlorite-treated (deproteinized) dentin as compared with normal etched dentin. This is in accordance with results from various authors (22, 23, 25, 30–32), reporting, for several adhesives, either unaltered or higher bond strength to deproteinized dentin than to normal etched dentin. This is not in accordance with reports (24, 26–28) showing in some cases lower strength depending on type of agent. These discrepancies are not easy to explain but may be linked to differences in methods. Two reports shows for the same dentin adhesive (Single Bond = Scotchbond 1) opposite results. In one study (25) unaltered or increasing bond strength was seen with increasing application time for a hypochlorite solution and for a collagenase solution. The other study (26) reported the opposite: decreasing bond strength with increasing application time for both hypochlorite and collagenase. It can be concluded that in most of the studies higher or unaltered strength was observed when adhesives were tested on deproteinized dentin compared with normal etched dentin. This may be explained by a higher lipophilicity of the deproteinized surface, which might better match that of the bonding agents and the resin composite.

Tests with deproteinized dentin may be of interest in judging the clinical implication of making bonds to dentin that are altered due to collagenase-producing bacteria. In addition, tests performed with dry and wet dentin surfaces may be of special importance, since it is difficult in a clinical situation to control the humidity of prepared dentin. It is well established that some adhesives must be applied on a wet surface to give the bond a substantially high strength (12). This is in accordance with the results shown in Table 2, indicating that the adhesives PQ, PB, and SB show higher bond strength under wet than under dry conditions.

These adhesives' content of lipophilic ingredients including organic solvents (Table 1) may explain why the adhesives are not capable of restoring collapsed collagen. It has been advocated (35) that dentin adhesives containing solvents, especially acetone, better facilitate the infiltration of the adhesive components and thus mediate a stronger bond to dentin. The results obtained with the experimental adhesive A01 are not in accordance with this statement, since this adhesive is capable of inducing relatively high bond strength both under wet and dry conditions. The adhesive does not contain organic solvents but contains a substantial amount of water.

The statistical analysis shown in Table 3 is an attempt to evaluate the efficiency of the adhesives. The adhesive giving the highest bond strength varies depending on the method used. In the case of method A, it was OS, A0, and PQ. In the case of method B, OS, OF, and A0 gave the highest strength. By method C, the adhesives were A0, OF, and PQ. Finally, by method D the adhesives were OF and A0. Since adhesives preferentially should induce high bond strength under various clinical situations—they should, for example, not be technique-sensitive—an evaluation by comparing the combined results (A + B + C + D in Table 3) might be useful. According to this, the adhesives OF, A0, and OS might be a proper choice.

Conclusions

Comparisons of bond strengths obtained by using dried or wet acid-etched dentin and dried or wet acid-etched and deproteinized dentin may be useful for evaluating the efficiencies of dentin adhesives.

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