Evaluation of shear bond strength and microleakage of deproteinized dentin bonded with three total-etch adhesive systems

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Purpose: To evaluate the effect of NaOCl conditioning on shear bond strength and marginal sealing of resin bonded dentin with three total-etching adhesive systems.
Materials and Methods: Flat dentin surfaces were prepared and received the following treatments: Group I, 35% phosphoric acid for 15 s; Group II, 35% phosphoric acid for 15 s followed by 10% NaOCl for 60 s; Group III received no additional conditioning and served as control. Each group was applied with three adhesive systems: One Step plus, Prime&Bond NT and Single Bond. Resin restorations were built up and then submitted to bond strength test. For microleakage evaluation, 72 class V cavities were prepared and grouped as described above and then restored with composite resin, dye penetration along the bonding interface was assessed. All data were subjected to statistical analysis.
Results: All specimens conditioned with phosphoric acid showed similar shear bond strength without statistical difference among adhesive systems used ($p>0.05$). Compared with that of the acid-etched groups, One Step plus demonstrated statistically increased bond strength following NaOCl application ($p<0.05$), while that of Prime&Bond NT remains unaffected though obviously decreased values were obtained ($p>0.05$). Single Bond yielded significantly lower bond strength ($p<0.05$). No statistical difference in microleakage scores was shown among dentin pretreatments or adhesive systems used ($p>0.05$). For all groups, the microleakage at the gingival margin was much severer than that at the occlusal margin ($p<0.05$).
Conclusion: The efficacy of resin bonding to deproteinized dentin was systems specific, which was decided by the composition of adhesive systems. **(Int Chin J Dent 2006; 6: 82-88.)**
Key Words: dentin bonding, sodium hypochlorite, microleakage, shear bond strength.

Introduction

The introduction of phosphoric acid etching technique into enamel bonding by Buonocore in 1955 resulted in a strong micromechanical bond.[1] Since then, surface conditioning by different procedures has been extensively used in adhesive dentistry, in order to modify the dental bonding substrates favorable for resin adhesion. As for dentin bonding, acid etching was a mostly used technique, by which the dentin surface was demineralized, exposing collagen matrix for resin infiltration to form a resin dentin interdiffusion zone. The creation of resin dentin interdiffusion zone, also named as hybrid layer, has been considered as the essential mechanism of dentin bonding.[2]

Due to the difference in the nature, bonding to dentin was not as reliable as bonding to enamel. Although the collagen matrix exposed by acid-etching is crucial to the formation of hybrid layer, it plays a negative role in dentin adhesion by presenting a delicate bonding substance susceptible to collapse.[3] Without the presence of water, the demineralized collagen network would collapse and shrink to prevent resin monomers from penetrating in. On the other hand, when dentin surface was too hydrated, "hybridoid layer" containing voids and porosities would be created within the bonding interface. Greatly decreased bond strength was shown both to over-dried and over-wet dentin.[4] Therefore, bonding to etched dentin is technique sensitive due to the difficulty in finding the correct balance of residual moisture. Additionally, a demineralized dentin zone termed nanoleakage could be detected within the interface,[5] which was created by the discrepancy between the depth of demineralization and that of resin infiltration, leaving collagen matrix at the base of hybrid layer uncoated. Even the most current self-etching primer systems, which were known as demineralizing and priming dentin simultaneously, showed distinct nanoleakage.[6] It was believed these nanoleakage channels could provide pathway for water, enzymes, acid and bacterial products to enter into the bonded interface, resulting in degradation of uncoated collagen fibrils and elusion of incompletely polymerized resin monomers, and lead to premature failure of dentin bonding.[7]

Considering the negative role of collagen fibrils in dentin adhesion, removal of the exposed organic matrix would possibly minimize these problems. Therefore, a combined use of phosphoric acid and sodium hypochlorite (NaOCl) was adopted to produce a porous mineralized surface similar to the etched enamel by deproteinizing the demineralized dentin.[8] Theoretically, due to the lack of uncoated collagen fibrils, the technical sensitivity could be eliminated. Something most important is that resin infiltrated into the mineralized substrate could develop a more reliable resin-dentin bond without the occurrence of nanoleakage,[9] thus enhanced durability could be expected.

Although some researchers doubted the existence of potential oxide influence of residual NaOCl on resin polymerization and reported decreased bond strength in their studies,[10] most of the previous studies showed positive results[8,11,12] with the application of NaOCl on demineralized dentin. It has also been reported that the higher the concentration of NaOCl, the greater the dentin bond strength until a plateau is reached at a concentration of 10%, for an application time of 60 s.[11]

However, most of the previous studies have been focused on the concentration and application time of NaOCl,[11-13] few researches have been conducted to investigate the influence of deproteinization on dentin bonding with different adhesive systems. Therefore, the purpose of the current study was to evaluate the bonding efficacy of three total-etching bonding systems to the dentin deproteinized with NaOCl, by shear bond strength (SBS) test and microleakage evaluation.

Materials and Methods

Materials

Human permanent posterior teeth extracted for severe periodontitis treatment were collected. All of the teeth were free of caries and fillings. After hand-scaled and cleaned, they were stored in distilled water at 4˚C for up to one month following extraction. Three adhesive systems used in this study, One Step plus (OS, Bisco, Schaumburg, IL, USA), Prime&Bond NT (PB, Densply Caulk, Milford, DE, USA), and Single Bond (SB, 3M ESPE, St Paul, MN, USA), are detailed in Table 1.

Table 1. Composition of the total-etch adhesive systems used in this study.

Adhesive	Lot number	Composition
One-Step plus (OS)	0500002934	Bis-GMA, BPDM, HEMA, Initiator, Glass filler, Acetone
Prime&Bond NT (PB)	0011000963	PENTA, UDMA, Butylated hydroxytoluene, 4-ethyl dimethyl aminobenzoate, Cetylamine hydrofluoride, Silica nanofiller, Acetone
Single Bond (SB)	4BK	Bis-GMA, HEMA, Dimethacrylates, Alkenoic acid co-polymer, Initiator, Ethanol, Water

Bis-GMA, Bisphenol A diglycidyl methacrylate; BPDM, Adduct of 2-hydroxyethyl methacrylate and 3,4,4',5'-biphenyl tetra-carboxylic anhydride; HEMA, 2-hydroxylethyl methacrylate; PENTA, Dipentaerythritol pentaacrylate phosphoric acid ester; UDMA, Urethane dimethacrylate.

Shear bond strength (SBS) test

Thirty-six intact molars were selected for SBS test. Each molar was sectioned longitudinally into two pieces and ground with #360 and #600 grit silicon carbide abrasive papers serially under running water to create a uniform smear layer on the exposed dentin surface. Following this, an adhesive tape with a punched hole, which measured 2 mm in diameter, was placed on the finished dentin surface to demarcate the bonding area. The prepared specimens were then randomly assigned to three groups according to the following pretreatments. Group I, Acid-etched group. Dentin was etched with 35% phosphoric acid for 15 s and then rinsed with tap water for 10 s. Excess water was removed by blotting with an absorbent pellet, leaving dentin visibly moist. Group II, Deproteinized group. Dentin was treated with 10% NaOCl for 60 s following the acid-etching procedures described in Group I. Rinsed with copious water for 60 s, dentin was thoroughly air-dried. Group III, Control group. The specimens were ground and received no additional conditioning before the application of dentin adhesive systems.

For each group, the prepared specimens were bonded with one of the three total-etch adhesives: OS, PB, and SB. Eight specimens were used for each adhesive. Two continuous layers of dentin adhesives were applied and light cured according to the manufacturers' directions with a light-curing system (Spectrum 800, Densply). Following adhesive application, a brass ring measured 3 mm in inner diameter and 2 mm in height was then placed on the treated surface. A composite resin (Charisma, shade A3, 067, Heraeus Kulzer, GmbH & Co., Hanau, Germany) was filled into it in three increments and light activated for 20 s each.

After storing in distilled water for 24 hours at room temperature, the root of dentin slabs were embedded with the bonding interface perpendicular to the ground (Fig. 1). SBS evaluation was conducted using a universal testing machine (Autograph AG500-A, Shimadzu, Kyoto, Japan) at a cross-head speed of 0.5 mm/minute.

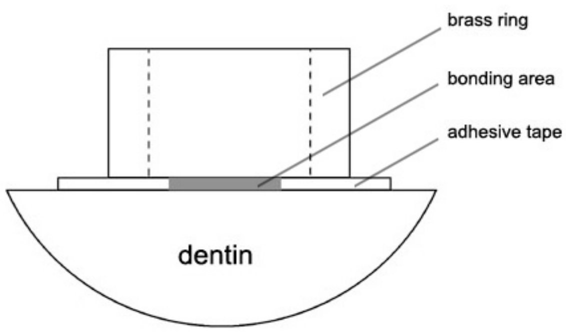

Fig. 1. Bonded specimen for shear bond strength test.

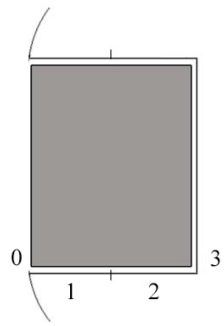

Fig. 2. Graded criteria for microleakage scores.

Microleakge evaluation

Another 36 freshly extracted premolars and molars were used for microleakage evaluation. Box-shaped class V cavities were prepared at the cemento-enamel junction on the buccal and lingual surfaces of each tooth. Each cavity was measured 3 mm in mesio-distal width, 2 mm in occluso-gingival length and 2 mm in depth, with a 0.5 mm and 45º enamel bevel. The occlusal margins of the preparations were in enamel and the gingival margins were in dentin or cementum.

The prepared cavities were randomly grouped by the treatments and adhesives as described previously with eight in each. Following surface conditioning, the adhesives were applied to the cavities according to the manufacturer's instructions and light cured. The composite resin was then placed in two increments. Each increment was cured for 20 s, and the final restoration was cured for another 20 s. Excessive materials were removed and polished after water storage for 24 hours at room temperature. The apices of the teeth were sealed with sticky wax, and the teeth were covered with two coats of nail vanish except at 1 mm around the margins of restorations. The prepared specimens were then immersed in 0.5% water solution of basic fuchsin for dye penetration for 24 hours at room temperature.

Following this, the restorations were serially multi-sectioned longitudinally into four pieces for microleakage evaluation. For each slab, the bonded interface was examined at the occlusal and gingival margins with a stereomicroscope (SZ1145, Olympus, Tokyo, Japan) at ×20 magnification. Blind evaluations were carried out by two independent observes, consensus were obtained between observers if there were conflicts in scores. After the evaluation of the dye penetration in all the cut surfaces, the section with the maximum leakage value recorded for each cavity was selected for statistical analysis. Scoring was based upon the following criteria:[14] 0, no dye penetration; 1, dye penetration along the interface to 1/2 the depth of the cavity wall; 2, dye penetration to the full depth of the cavity wall, but not including the axial wall; 3, penetration to and along the axial wall (Fig. 2).

Statistical analyses

The shear bond strength was calculated by dividing the failure load by the bonded area and expressed in MPa. The data were analyzed using Student-Newman-Keuls (SNK) test. For microleakage evaluation, occlusal and gingival scores for each group of restorations were compared by the Kruskal-Wallis nonparametric analysis. The Mann-Whitney U test was used to disclose a statistically significant difference between occlusal and gingival margins. Significance was considered at the 0.05 level.

Results

SBS results are summarized in Table 2. SBS was greatly enhanced following acid-etching for all the three adhesive systems compared with the untreated control group. However, no statistical difference was shown among the three adhesive systems when the dentin surface was treated with phosphoric acid ($p>0.05$), though the highest bond strength was obtained with SB. Compared with the acid-etched groups, OS demonstrated statistically increased bond strength following the additional NaOCl treatment ($p<0.05$), while that of PB remains unaffected though distinctly decreased values were obtained ($p>0.05$). However, SB yielded a significant lower bond strength ($p<0.05$), which similar to that of the untreated control group.

Table 2. Shear bond strength (SBS), standard deviations in MPa, and statistical categories.

Group	Conditioning	One Step plus (OS)			Prime&Bond (PB)			Single Bond (SB)		
		Mean	SD	SC	Mean	SD	SC	Mean	SD	SC
I	Acid-etched	14.3	4.8	b	13.6	5.3	b, c	17.3	9.7	a, b
II	Deproteinized	19.5	3.3	a	9.3	2.5	c, d	4.3	1.2	d
III	Control	4.5	1.4	d	5.8	2.2	d	4.0	1.4	d

Subgroups with the same letter are not statistically significant ($p>0.05$). Specimen size, n=8. SD, Standard deviation. SC, Statistical category.

Summaries of the distribution of the dye penetration scores at the occlusal and gingival margins are shown in Table 3. None of the dentin treatments or adhesive systems tested in this study could completely eliminate microleakage at resin-teeth interfaces. With each adhesive tested, much severer microleakage was demonstrated at the gingival margins than at the occlusal margin ($p<0.05$). All of the adhesive systems tested showed similar marginal sealing ability at both the enamel margins and dentin margins when conventional wet-bonding procedures were performed. As dentin treatments were concerned, no statistical differences in leakage scores was shown between the acid-etched group and the deproteinized group for each adhesive, at both the occlusal margins and gingival margins. However, microleakage of control groups showed significant higher scores than that of the treated groups (p<0.05).

Table 3. Microleakage scores at the occlusal and gingival margins.

Group	Conditioning	Margin	One Step plus (OS)					Prime&Bond (PB)					Single Bond (SB)				
			0	1	2	3	Total	0	1	2	3	Total	0	1	2	3	Total
I	Acid-etched	Occulusal	5	3	0	0	3	4	4	0	0	4	6	1	1	0	3
		Gingival	1	1	4	2	15	0	2	3	3	17	1	2	3	2	14
II	Deproteinized	Occulusal	6	1	1	0	3	3	5	0	0	5	3	4	1	0	6
		Gingival	0	1	5	2	17	0	1	3	4	19	0	1	4	3	18
III	Control	Occulusal	3	1	4	0	9	3	0	5	0	10	1	3	3	1	12
		Gingival	0	0	2	6	22	0	1	2	5	20	0	1	3	4	19

Specimen size, n=8. No statistical difference was shown between adhesive systems used (p>0.05).

Discussion

Adhesion of dental material to enamel is a well-known reliable procedure. However, bonding to dentin with current dentin adhesive systems, including both the total-etch systems and self-etching primer systems, is less reliable than that to enamel. Due to the potential of surface modification by NaOCl treatment following acid etching, by providing an enamel-like porous mineralized bonding substrate, dentin deproteinization has been occasionally proposed as an alternative bonding procedure to achieve an enhanced resin-dentin bond.[12]

In the present study, OS resulted in increased shear bond strength when bonding to deproteinized dentin, compared with the acid-etched group. This could be probably attribute to the increased roughness and enhanced permeability of the deproteinized dentin. The combined steps of etching and NaOCl treatment lead to an altered "moth eaten" appearance with larger dentinal tubule openings,[15] which would not shown by either treatment alone.[16] Meanwhile, a hydrophilic mineralized surface with higher energy characterized by enhanced wettability would also be achieved due to the removal of residual collagen matrix.[17] Hence, the penetration of resin into the modified dentin is improved and results in the formation of uniform resin tags with anastomoses in lateral branches. The enhanced micromechanical interlocking between composite resin and dentin was considered to contribute to the increased bond strength. Additionally, it was suggested that the potential chemical interaction at the resin-dentin interface would also strengthen the bond.[18]

Although increased bond strength was shown with the used of OS, PB and SB demonstrated neutral or negative results following the application of NaOCl on etched dentin. These findings indicated that the effect of NaOCl treatment on dentin bond strength was system specific. In a previous study, Saboia et al.[19] reported increased bond strength with Prime&Bond 2.1 following NaOCl conditioning, while that obtained with Single Bond decreased significantly. They explained it by the difference in the solvents of the adhesives, which acted as a hydrophilic carrier to enhance the penetration of resin monomers into dentin substrate. Being an acetone-based adhesive, Prime&Bond 2.1 showed stronger penetration of resin monomer into the deproteinized dentin than that of the ethanol-based Single Bond. In contrast with it, the current study showed a distinct decreasing tendency in bond strength with the use of PB, the descendant of Prime&Bond 2.1, although no statistical difference was found.

As inconstant influence of NaOCl conditioning on dentin bonding was shown with different adhesives in this study, it was speculated that bonding to deproteinized dentin relies on the diffusion ability of resin monomers of adhesives. When NaOCl was applied to the etched dentin surface, it would not only remove the exposed collagen fibrils, but also solubilize the fibrils down into the underlying mineralized matrix, leaving nano-sized porosities within the conditioned substrate. Adhesives with litter monomers could easily penetrate into the submicron porosities previously occupied by collagen fibrils, to create an enhanced micromechanical interlocking. However, the relatively larger molecular weight of polyalkenoic acid copolymer, which was the functional monomer of SB, prevent it from effectively diffusing into the mineralized dentin,[17] although it was high diffusive in the collagen matrix of etched dentin. This could probably explain why SB yields decreased bond strength to deproteinized dentin, while that of OS was increased. Additionally, it was also suggested that some adhesives such as OS contained acidic monomers could act as self-etchant, result in improved penetration to the bonding substrate.[8]

Good marginal adaptation to the tooth structure is believed to reduce marginal discoloration, secondary caries, postoperative sensitivity, and pulpal irritation related to microleakage. Dye penetration has been widely used to test the microleakage in vitro. Conventionally, the scoring of microleakage was simply based on the medial section of the restorations,[14] that is, the results were greatly influenced by the location and angle of the incision. In the present study, serially multi-sectioned slabs were obtained and evaluated under stereomicroscopy, resulted in a more sensitive and comprehensive detection throughout the interface.

Thermocycling has been commonly used in microleakage evaluation to simulate the long-term degradation of dental bonding in vitro. However, it was doubted that if 500 cycles of thermocycling, as proposed by the ISO standard, could effectively mimicking long-term bonding effectiveness;[20] the validity and clinical significance of it were also remains equivocal, since the temperatures and dwell time used to stress restorations may not be the real temperatures of cold and hot food/beverage tolerated by patients.[21] In order to minimize the number of variables that may affect microleakage, thermocycling was omitted and the immediate marginal sealability was evaluated in this study.

The formation of marginal gaps in direct resin restorations is dependent on the polymerization shrinkage stresses of the restorative materials, the quality and strength of the adhesive bond and the configuration of the cavity, among which the contraction forces of polymerization are considered the most important cause of bond failure and consequently microleakage. When polymerized, the resin composite shrinks towards the stronger bond at the occlusal margin and so pulls away from the weaker bond at the gingival margin, creating micro gaps that allow microleakage to occur. As shown in the current study, all groups showed higher leakage scores at the gingival than at the occlusal walls. Compared with the occlusal margins, the severer microleakage shown at the gingival margins in the deproteinized groups indicated that bonding to NaOCl modified dentin surface was still inferior to the acid-etched enamel.

As no significant difference in the leakage scores at both the enamel and gingival margins among different pretreatments and materials were observed, bonding to deproteinized dentin was not superior to acid-etched dentin in marginal sealing. Even without the presence of the stress produced by thermocycling, the current adhesive systems failed to provide good sealing of resin-dentin interface. Since no materials and bonding procedures could completely prevent microleakage at tooth-resin interface, adhesive materials incorporated with anti-bacterial agents is advocated to minimize the occurrence of secondary caries.[22]

Since both bond strength and microleakage aspects are related to the quality of bonded interface, some correlation between data provided by these two tests would be expected. It is also suggested that high bond strength could better compensate the polymerization shrinkage of composite resin. However, as no consistent influence was shown on the two aspects following NaOCl application as a surface conditioner, a lack of relationship between leakage and bond strength was observed. Leakage and bond strength are independent parameters to evaluate the quality of adhesion between restorative materials and dental tissues.[23]

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