

LONG-TERM BOND STRENGTH OF ADHESIVE SYSTEMS APPLIED TO ETCHED AND DEPROTEINIZED DENTIN

Ninoshka UCEDA-GÓMEZ¹, Alessandro Dourado LOGUERCIO², Sandra Kiss MOURA³, Rosa Helena Miranda GRANDE⁴, Margareth ODA⁵, Alessandra REIS²

1- MSc, PhD student, Department of Operative Dentistry, School of Dentistry, University of São Paulo, São Paulo, SP, Brazil.

2- PhD, Assistant Professor, Department of Dental Materials and Operative Dentistry, University of Oeste Santa Catarina, Joaçaba, SC, Brazil.

3- MSc, PhD student, Department of Dental Materials, School of Dentistry, University of São Paulo, São Paulo, SP, Brazil.

4- PhD, Associate Professor, Department of Dental Materials, School of Dentistry, University of São Paulo, São Paulo, SP, Brazil.

5- PhD, Associate Professor, Department of Operative Dentistry, School of Dentistry, University of São Paulo, São Paulo, SP, Brazil.

Corresponding address: Dr. Alessandro Dourado Loguercio - Departamento de Materiais Dentários e Dentística - Faculdade de Odontologia - UNOESC - R. Getúlio Vargas, 2125 - 89600-000 - Joaçaba, SC, Brazil - Phone/ Fax: +55-49-3551-2004 - e-mail:aloguercio@hotmail.com

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ABSTRACT

The aim of this study was to evaluate the early and 12-month bond strength of two adhesive systems (Single Bond-SB and One Step-OS) applied to demineralized dentin (WH) and demineralized/NaOCl-treated dentin (H). Twenty flat dentin surfaces were exposed, etched, rinsed and slightly dried. For the H groups, a solution of 10% NaOCl was applied for 60 s, rinsed (15 s) and slightly dried. The adhesives were applied according to the manufacturer's instructions and composite resin crowns were incrementally constructed. After 24 h (water-37°C), the specimens were sectioned in order to obtain resin-dentin sticks (0.8 mm²). The specimens were tested in microtensile (0.5 mm/min) immediately (IM) or after 12 months of water storage (12M). The data (MPa) were subjected to ANOVA and Tukey's test ($\alpha=0.05$). Only the main factors adhesive and time were significant ($p=0.004$ and $p=0.003$, respectively). SB (42.3±9.1) showed higher bond strengths than OS (33.6±11.6). The mean bond strength for IM-group (42.5±8.7) was statistically superior to 12M (33.3±11.8). The use of 10% NaOCl, after acid etching, did not improve the immediate and the long-term resin-dentin bond strength.

Uniterms: Dentin-bonding agents; Dentin; Tensile strength.

INTRODUCTION

The retention and sealing provided by current adhesive systems rely on hybrid layer formation. However, there are still difficulties concerning the monomer infiltration within the collagen network. When demineralized dentin is air-dried, collagen fibrils are brought closer together, resulting in the collapse of the organic matrix that reduces the permeability of the demineralized zone¹². This has been shown to cause marked reduction of the bond strengths to dentin²¹. Excess of water, on the other hand, causes the dilution of resin monomers and phase separation of the hydrophobic and hydrophilic moieties which ultimately compromises resin infiltration²⁵ and avoids an adequate polymerization of resin monomers¹⁴.

The above mentioned issues highlight that the maintenance of demineralized collagen network, exposed by acid etching is critical. In addition, the total depth of dentin demineralization is not fully infiltrated by monomers which leaves an exposed collagen network, close to the unaffected dentin, susceptible to hydrolytic degradation over time^{2,21}. Based on that, the use of sodium hypochlorite

(NaOCl), as a nonspecific proteolytic agent, could be useful because it eliminates the collagen network exposed by acid etching⁶ reducing the technique sensitivity related to wet bonding²⁰.

When deproteinization is performed, reports on early bond strength are controversial^{6,23}. However, there is no report about the longevity of such adhesive interface after water storage. When adhesives are applied to dentin in a conventional manner, significant decreases in the resin-dentin bond strengths and higher nanoleakage occur after prolonged water storage (6 to 36 months)^{2,7,24}. Essentially two main components of the hybrid layer are susceptible to degradation over time: the organic constituent, represented by the naked collagen fibrils, not encapsulated by resin monomers, or the polymer formed among the collagen fibrils, after light activation²¹. If the collagen fibrils are more prone to degradation, as suggested by some authors^{7,8}, it is likely that the bonding interfaces formed after deproteinization with sodium hypochlorite are more resistant to water storage than those bonds produced conventionally.

A recent benefit of NaOCl treatment of demineralized dentin was reported by Pioch, et al.¹⁸ and Chersoni, et al.³

Pioch, et al.¹⁸ showed that the application of sodium hypochlorite for 60 s, reduced silver penetration in the porosities located close to unaltered mineralized dentin and within the superficial collagen-rich fibrous network. Chersoni, et al.³ showed that the dentin pre-treatment reduces the water movement through hybrid layer reducing the long-term degradation of resin bonds.

Hence, the purpose of this *in vitro* study was to evaluate the early and 12-month microtensile bond strength of two-step etch-&-rinse adhesive systems applied to etched or etched and deproteinized dentin. The hypothesis to be tested is that the bond strength of adhesive systems applied to etched and deproteinized dentin is not affected by 12 months of water storage.

MATERIAL AND METHODS

Two different solvent-based, etch-&-rinse adhesive systems were tested: Single Bond (SB - 3M/ESPE, St. Paul, MN, USA), an ethanol/water-based system and One-Step (OS - Bisco, Schaumburg, IL, USA), an acetone-based system. Their composition, application mode and batch number are displayed in Table 1.

Twenty extracted, caries-free human third molars were used. The teeth were collected after granting the patient's informed consent under a protocol approved by the University of São Paulo Institutional Review Board. The teeth were disinfected in 0.5% chloramine, stored in distilled water and used within six months after extraction. The teeth were allocated in four experimental groups of five teeth for each tested condition.

A flat dentin surface was exposed after wet grinding the occlusal enamel on 180-grit SiC paper. The enamel-free, exposed dentin surfaces were further polished on wet 600-grit silicon-carbide paper for 60 s to create a standardized smear layer.

The adhesive systems were applied according to the

manufacturers' instructions in half of the sample (Table 1). The other half was acid etched with 37% phosphoric acid, rinsed and then conditioned with 10% sodium hypochlorite solution for 60 s (Table 1). The dentin surfaces were washed thoroughly (15 s) and air-dried. Then, they were rewetted using a micropipette (Pipetman, Gilson, NY, USA). An amount of 3.5 and 1.5 mL of water was used for OS and SB, respectively, before the application of adhesive systems.²¹

The adhesives were light activated for 10 s using a VIP light unit (Bisco, Schaumburg, IL, USA) with a light intensity of 600 mW/cm². The time elapsed between rewetting and adhesive application was the time required to open the bottles, pour the adhesive on the microbrush and take it to the tooth surface. Resin composite build-ups (Z-250; 3M/ESPE, St. Paul, MN, USA) were constructed on the bonded surfaces in 1-mm increments that were individually light activated for 30 s using the same light unit and light intensity. All bonding procedures were carried out by a single operator at a room temperature of 24°C and 75% relative humidity.

After storage of the bonded teeth in distilled water at 37°C for 24 h, they were longitudinally sectioned in both "x" and "y" directions across the bonded interface with a diamond saw in a Labcut 1010 machine (Extec Corp., Enfield, CT, USA) to obtain sticks with a cross-sectional area of approximately 0.8 mm².

The number of premature debonded sticks (D) *per* tooth during specimen preparation was recorded. Specimens originated from the areas immediately above the pulp chamber had their remaining dentin thickness (RDT) measured with a digital caliper and recorded to verify the dentin depth (Absolute Digimatic, Mitutoyo, Tokyo, Japan). The cross-sectional area of each stick was measured with a digital caliper to the nearest 0.01 mm and recorded for posterior calculation of the bond strength.

The bonded sticks originated from the same teeth were randomly divided and assigned to be tested immediately (IM) or after 12 months of distilled water storage (12 M) containing 0.4% sodium azide² at 37°C. The storage solution

TABLE 1- Materials, manufacturers and corresponding batch numbers

Adhesive systems	Composition	Application mode	Batch number
Single Bond (3M ESPE)	1. Scotchbond – 37% phosphoric acid 2. Adhesive – Bis-GMA, HEMA, dimethacrylates, polyalquenoic acid copolymer, initiators, water and ethanol	Without NaOCl treatment:	OEH
		a, b, c, e1, f, g, h	
One Step (Bisco)	1. Uni-Etch – 32% phosphoric acid 2. Adhesive – Bis-GMA, BPDm, HEMA, initiator and acetone	With NaOCl treatment:	01007932
		a, b, c, d, b, c, e1, f, g, h	
One Step (Bisco)	1. Uni-Etch – 32% phosphoric acid 2. Adhesive – Bis-GMA, BPDm, HEMA, initiator and acetone	Without NaOCl treatment:	01007932
		a, b, c, e2, f, g, h	
One Step (Bisco)	1. Uni-Etch – 32% phosphoric acid 2. Adhesive – Bis-GMA, BPDm, HEMA, initiator and acetone	With NaOCl treatment:	01007932
		a, b, c, d, b, c, e2, f, g, h	

a – acid-etch (15s); **b** – rinse (15s); **c** – air-dry (30s); **d** – application of 10% sodium hypochlorite for 60s; **e1** - dentin rewetted with 1.5ml of water; **e2**- dentin rewetted with 3.5ml of water; **f** – two coats of adhesive systems, brushed for 10s each; **g** – air-dry for 10s at 20 cm; **h** – light-cure (10s - 600 mW/cm²)

was not changed because it maximizes degradation. The pH was monitored monthly.

At each storage time period, individual bonded sticks were attached to a microtensile testing with cyanoacrylate resin (Zapit, Dental Ventures of North America, Corona, CA, USA) and subjected to a tensile force in a universal testing machine (EMIC, São José dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm/min.

The failure modes were evaluated at 400X (HMV-2, Shimadzu, Tokio, Japan) and classified as cohesive (failure exclusive within dentin or resin composite, C), adhesive (failure at resin/dentin interface – A), or adhesive/mixed (failure at resin/dentin interface that included cohesive failure of the neighboring substrates, A/M).

The average bond strength of each group was calculated and expressed as an index that assumes the relative contribution of the different failure modes to the overall performance of that specific group²¹.

A three-way repeated measures ANOVA (material vs. surface treatment vs. time) and Tukey’s multiple-comparison test were used to analyze the data at $\alpha = 0.05$. The statistical model took into consideration the multiple specimens obtained from the same tooth.

RESULTS

Table 2 shows the averages for all experimental groups. Three-way ANOVA did not detect any statistically significant effects for the triple and double interactions. The use of sodium hypochlorite has not improved the mean bond strength for both adhesives, as this factor was not significant ($p > 0.05$).

A significant effect was detected for the factors adhesive system and time on the bond strength to dentin ($p = 0.004$ and $p = 0.003$, respectively). Table 3 shows the averages for each adhesive system and each period of time. Lower bond strengths were detected for OS ($p < 0.05$). Regarding the long-term bond strengths, Table 3 shows a significant drop on bond strengths after a period of 12 months for both systems ($p < 0.05$).

DISCUSSION

While the acid etching alone removes hydroxyapatite and exposes the hydrate intertubular collagen network, the posterior deproteinization of this demineralized dentin layer,

removes the collagen and exposes several lateral secondary tubules that was not observed on etched dentin surfaces⁹. Thus, dentin turns into a porous structure with multiple irregularities, rich in hydroxyapatite crystals and higher wettability similar to etched enamel²⁶. The results of this study demonstrated that both substrates can lead to similar bond strengths, as these values were unaffected by the surface treatment after acid etching.

The literature on this matter is rather controversial. The findings of this study are in accordance with the outcomes from other authors, reporting unaltered bond strengths to dentin when 10% sodium hypochlorite was used^{6,10,20}, even when employing different solvent-based adhesive systems. Others researchers reported that bond strengths to dentin is reduced^{15,28}, regardless of the adhesive system employed. However, other groups found that the treatment of demineralized dentin with NaOCl is dependent on the adhesive system employed^{4,19}. These inconsistencies in the literature avoid the widespread use of this technique, as its performance is erratic.

Yamauti, et al.²⁹ and Yoshida, et al.³⁰ have demonstrated a deterioration on the resin-dentin bond strengths when a total-etch adhesive was stored in 10% NaOCl for 1 to 5 hours. According to the authors, this bond deterioration was likely due to the deproteinization effect of NaOCl on the collagen-rich layer, not encapsulated by resin, beyond the hybrid layer.

Based on that, these naked collagen fibrils started being blamed for the reported degradation of resin-dentin bonds^{7,8}

TABLE 3- Bond strength means (MPa) and standard deviation for each adhesive system and testing period*

Adhesive Systems	
SB	OS
42.3 ± 9.14a	33.6 ± 11.6b
Testing period	
Immediate	12 months
42.5 ± 8.7c	33.3 ± 11.7c

*Different letters indicate statistically significant difference in each row.

TABLE 2- Bond strength means (MPa) and standard deviation for adhesive systems applied conventionally or after deproteinization

Sodium Hypochlorite Adhesive/ Time	Without		With	
	IM	12M	IM	12M
SB	47.7 ± 7.2	39.4 ± 14.1	41.0 ± 4.9	36.0 ± 8.5
OS	36.5 ± 9.2	29.4 ± 6.9	45.9 ± 6.8	23.5 ± 9.0

and recently Pashley, et al.¹³ showed that collagen degradation occurred over time, *via* host-derived matrix metalloproteinases (MMPs) that are released slowly over time, even in absence of bacteria.

In fact, this can explain the bond strength degradation that occurred to the conventionally bonded groups, but cannot explain the degradation of NaOCl-treated groups after 12 months of water storage. A significant resin-dentin bond strength reduction occurred with NaOCl, as also demonstrated in a previous study¹⁷. Although we have not conducted SEM and TEM studies, it can be postulated, from literature findings, that the NaOCl-treated surfaces seem to be free of loose collagen fibrils^{10,16}. The absence of naked collagen fibrils suggests that the polymer formed by light curing of adhesives could be degraded over the testing period. Hydrophilic resins, such as those present in the current adhesives, are highly prone to absorb water¹. Since all adhesives used in the present study contain significant amounts of hydrophilic monomers, water sorption over time can also be regarded as a contributor to the observed reduction in bond strengths. In addition, failure in removing all residual water entrapped in the deepest regions of demineralized and demineralized and deproteinized dentin induces the formation of poorly polymerized polymer chains¹⁴, that would be weaker and less stable over time than those formed in water-free regions.

The use of NaOCl represents an extra step among several crucial others on dentin bonding and one may not assure a superior performance when the dentin deproteinization is performed after acid etching^{5,22,27}. For instance, Ferrari, et al.⁵ conducted an *in vivo/in vitro* study, and evaluated the efficacy of NaOCl application on microleakage, both in occlusal and cervical margins of the restorations, after a clinical service of 60-90 days. The authors observed a better marginal sealing in cavities not treated with NaOCl. Additionally, the presence of reactive residual free-radicals as a result of the oxidizing action of NaOCl, may compete with the propagation vinyl free-radicals generated during light-activation of the adhesive, resulting in premature chain termination and incomplete polymerization¹¹. Therefore, the use of this technique should be avoided in a clinical basis, as there is little evidence of its effectiveness.

CONCLUSIONS

The use of 10% NaOCl for 1 min after dentin demineralization did not improved the bond strength to dentin either immediately or after a 1-year period.

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