

EFFECT OF WATER STORAGE ON MICROTENSILE BOND STRENGTH OF A TWO-STEP SELF-ETCH ADHESIVE AND A TWO-STEP ETCH-AND-RINSE ADHESIVE

Juan C. Mendez¹, Gloria E. Pabon¹, Sergio P. Hilgenberg², Eugenio J. Garcia³, Beatriz Arana-Correa¹

¹ Department of Biomaterials, Operative and Esthetic Dentistry, School of Dentistry, University of Santiago de Cali, Cali, Colombia.

² School of Dentistry, University Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil.

³ School of Dentistry, UNORP, São Jose do Rio Preto, SP, Brazil.

ABSTRACT

This study evaluated the effect of 24 h (1d) and one-year (1yr) water storage on the microtensile dentin bond strength of a two-step total-etch and a two-step self-etch adhesive system. Ten extracted human third molars were sectioned perpendicularly to their long axis to expose flat occlusal dentin surfaces. Teeth were divided into two groups (n=5) according to the adhesive used: a two-step etch-and-rinse adhesive (Adper Single Bond 2) and two-step self-etching adhesive (Adper Scotchbond SE). Composite resin (Z350) build-ups were incrementally constructed on the bonded surfaces. Specimens were sectioned into sticks (cross-sectional mean area 0.8 mm²) and after 1d and 1yr of storage in distilled water at 37°C, the sticks were stressed to failure by microtensile test (1 mm/min). Interfacial observation

of silver nanoleakage was performed using scanning electron microscopy (SEM). Data (MPa) were analyzed by two-way ANOVA and Bonferroni's test ($p = 0.05$). SB2 showed the highest bond strength values after 1d of water storage. After 1yr, SB2 values significantly decreased and were similar to ASE, independently of water storage period. Both adhesives, independently of storage time, showed silver nitrate uptake within the hybrid layer and the adhesive layer. One-year of water storage only affected the bond strength of the two-step etch-and-rinse adhesive.

Keywords: dentin bonding agent - tensile strength - scanning electron microscopy

EFFECTO DEL ALMACENAMIENTO EN AGUA EN LA RESISTENCIA ADHESIVA DE UN ADHESIVO AUTO-ACONDICIONANTE DE DOS PASOS Y DE UN ADHESIVO DE GRABADO ÁCIDO INDEPENDIENTE DE DOS PASOS

Resumen

Evaluar el efecto del almacenamiento en agua por 24 h (1d) y un año (1a) sobre la resistencia de unión de un adhesivo de grabado ácido independiente de dos pasos y un adhesivo autocondicionante de dos pasos. Diez terceros molares fueron cortados perpendiculares a su eje longitudinal para exponer superficies planas de dentina. Los dientes fueron divididos en dos grupos según el adhesivo a ser usado: uno de acondicionamiento ácido independiente de dos pasos (Adper Single Bond 2) y uno autocondicionante de dos pasos (Adper Scotchbond SE). Incrementos en resina compuesta (Z350) fueron construidas sobre las superficies tratadas con adhesivo. Los especímenes fueron cortados para obtener probetas (área de sección transversal = 0,8 mm²) y después de 1d y 1a de almacenamiento en agua destilada a 37° C, fueron sometidas a la prueba de microtracción

(1 mm/min). La captación de nitrato de plata en las interfaces fue analizada con microscopia electrónica de barrido (MEB). Los datos (MPa) fueron analizados con ANOVA de dos factores y la prueba de Bonferroni ($p = 0,05$). Después de 1d de almacenamiento SB2 presentó los mayores valores de resistencia de unión. No hubo diferencias entre los adhesivos después de 1a, y para ASE estos valores fueron similares al tiempo 1d. Independientemente del tiempo de almacenamiento, ambos adhesivos presentaron captación de nitrato de plata dentro de la capa híbrida y la capa de adhesivo. Un año de almacenamiento en agua solo afectó los valores de resistencia de unión del adhesivo de grabado ácido independiente de dos pasos.

Palabras clave: agentes de adhesión a dentina - resistencia a la tracción - microscopia electrónica de barrido

INTRODUCTION

Self-etching adhesives were developed to provide some advantages over etch-and-rinse adhesives such as simplification of the bonding technique and reduction of postoperative sensitivity. Inde-

pendently of the bonding strategy, i.e. the use or not of a preliminary separate etching step, the stability of the bonded interfaces of both approaches depends on the creation of a compact, homogeneous hybrid layer¹.

Even though most current adhesives systems have shown excellent immediate bond strength values, they do not have the same behavior in long-term studies². Several factors are involved in the reduction of the longevity of bonded interfaces, such as occlusal chewing forces, temperature, acid challenges, enzymes and microorganisms³. Water degradation may occur due to collagen or polymer hydrolysis. The former is commonly related to total-etch systems due to the poor resin infiltrated zone with naked collagen fibrils that are highly susceptible to hydrolysis. The latter was observed in both total-etch and self-etch adhesive systems and depends on factors such as the composition of the adhesive systems².

Microtensile test and aging by storage in water are two *in vitro* methodologies that provide important information about resin-tooth interface degradation after long storage periods. Therefore, the purpose of this study was to evaluate the effect of 24 h and one year of water storage on the μ TBS using a two-step etch-and-rinse system as well as a two-step self-etching system. The null hypothesis tested is that no significant difference will be detected among the different bonding strategies in the one-day and one-year water storage period.

MATERIAL AND METHODS

Tooth Preparation

After approval of the Committee of Ethics in Research from the University of Santiago de Cali, ten intact, non-carious, non-restored, human third molars cleaned of gross debris were selected for this study. They were previously disinfected in 0.5% chloramine, stored in distilled water and used within six months after extraction. Flat coronal dentin surfaces were obtained by sectioning the occlusal enam-

el of all teeth with a diamond disc under water cooling (Isomet 1000, Buehler, Lake Bluff, IL, USA). The exposed dentin surfaces were further polished on wet # 600-grit silicon-carbide paper for 60 s to standardize the smear layer and randomly assigned to two groups (n=5) according to the adhesive system (Table 1): a two-step etch-and-rinse adhesive (Single Bond 2 [SB2], 3M ESPE, St. Paul, MN, USA) and a two-step self-etching adhesive (Adper SE Plus [ASE], 3M ESPE, St. Paul, MN, USA).

Restorative Procedure

Adhesives were applied following manufacturer's instructions. Composite build-ups were made with three incremental layers (2 mm) of resin (Z350, 3M ESPE, St. Paul, MN, USA). Each layer of resin composite was light-activated for 40 s with a quartz-tungsten halogen-light unit set at 500 mW/cm² (Optilux 501, Kerr Corporation, Orange, CA, USA).

Microtensile test

After storage in distilled water for 24 h at 37 °C each tooth was sectioned into sticks with a cross-sectional area of approximately 0.8 mm² using a diamond disc under water cooling (Isomet 1000, Buehler, Lake Bluff, IL, USA). Specimens (25-30 per tooth) were randomly divided in two groups and subjected to microtensile bond strength test after 24 h (1d) or 1 year (1yr) of storage in distilled water at 37 °C. The remaining dentin thickness of specimens was not considered.

The number of premature debonded sticks (D) per tooth during specimen preparation was recorded. The cross-sectional area of each stick was measured with the digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, TYO, JAP) to the nearest 0.01 mm for calculation of the bond strength values (BS). Each

Table 1: Composition (batch number) of materials used in this study.

Materials	Composition (Batch Number)
Scotchbond™ Etchant gel	Water 55–65%, phosphoric acid 30–40% and synthetic amorphous silica 5–10%. (TS20)
Adper Single Bond 2	Adhesive: dimethacrylates, HEMA, polyalkenoid acid copolymer, 5 nm silane treated colloidal silica, ethanol, water, photoinitiator. (7NK)
Adper Scotchbond SE	Liquid A: water, HEMA, surfactant, pink colorant. (8AP) Liquid B: UDMA, TEGDMA, TMPTMA, HEMA phosphates, MHP, bonded zirconia nanofiller, initiator system based on camphorquinone. (8AP)
Filtek Z350	Bis-GMA, TEGDMA, UDMA, Bis-EMA Nanosilica fillers, Zirconia/silica nanoclusters. (N135069)

HEMA = 2-hydroxyethyl methacrylate; UDMA = urethane dimethacrylate; TEGDMA = triethylene glycol dimethacrylate; TMPTMA = trimethylolpropane trimethacrylate; MHP = methacrylated phosphates.

bonded stick was attached to a device for microtensile testing with cyanoacrylate glue and stressed to failure in a universal testing machine (Model 5565, Instron Corp., Canton, MA, USA) at a crosshead speed of 1 mm/min. The failure modes were evaluated at 400X (HMV-2, Shimadzu; Tokyo, JAP) and classified as cohesive ([C] failure exclusive within dentin or resin composite, adhesive ([A] failure at resin/dentin interface), or adhesive/mixed ([A/M] failure at resin/dentin interface that included cohesive failure of the neighboring substrates).

Table 2: Mean bond strength (MPa) and standard-deviation for each experimental condition.

Storage time	Adhesive system	
	SB2	ASE
1d	38.22 ± 3.90 ^a	31.22 ± 4.85 ^b
1yr	30.90 ± 3.12 ^b	30.95 ± 2.22 ^b

* Different letters indicate statistically significant difference between groups ($p < 0.05$)

Table 3: Number of specimens (percentage %) according to the fracture pattern* or pre-test failures (PTF) for each experimental condition.

Adhesive	Storage time	AM	CD	CR	PTF
SB2	1d	65 (92.85)	0 (0)	0 (0)	5 (7.15)
	1yr	54 (80.59)	2 (2.98)	6 (8.95)	5 (7.46)
ASE	1d	62 (91.17)	0 (0)	2 (2.94)	4 (5.88)
	1yr	53 (82.81)	1 (1.56)	8 (12.5)	2 (3.12)

*AM: adhesive/mixed fracture mode; CD: cohesive fracture in dentin; CR: cohesive fracture in resin.

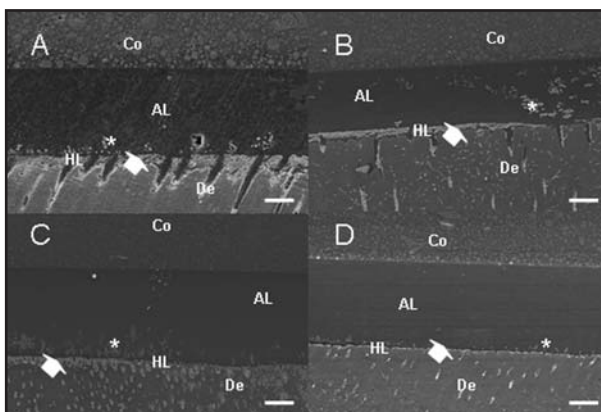


Fig. 1: Representative scanning electron micrographs (1200x) of SB2 (a and b) and ASE (c and d) after 1d (a and c) and 1y (b and d) of water storage. Both adhesives showed silver nitrate uptake within the hybrid layer (white arrow) and "water-tree like" formations in the adhesive layer (asterisk). (Bar = 10 μ m) (Co= composite resin; AL= adhesive layer; HL= hybrid layer; De= dentin).

Scanning electron microscopy for silver nitrate leakage evaluation

For scanning electron microscopy (SEM) analysis of the interface leakage two bonded sticks of each tooth were randomly selected and immersed in ammoniacal silver nitrate solution for 24 h. The silver-stained sticks were rinsed thoroughly in distilled water and placed in photodeveloping solution for 8 h under a fluorescent light to reduce the diamine silver ions into metallic silver grains within potential voids along the bonded interfaces. Then, stained sticks were embedded in epoxy resin (Epon-Thin™, Buheler Ltd., Illinois, USA) and polished to high gloss with SiC papers and diamond pastes of decreasing abrasiveness (Metadi II®, Buehler Ltd, Lake Bluff, IL, USA) using polish cloths. After sonication in distilled water for 5 min they were mounted on aluminum stubs, then carbon coated (BAL-TEC SCD 050 Sputter Coater, Balzers, Liechtenstein) and observed by SEM (SS 440, LEO Electron Microscopy Ltd; Cambridge, UK).

Statistical analysis

Microtensile bond strength values were analyzed by two-way ANOVA, with the adhesive system and storage time as main factors. Bonferroni test for multiple comparisons was used when the *F*-factor was significant ($\alpha = 0.05$). Representative SEM images at the resin–dentin interfaces were only evaluated qualitatively.

RESULTS

Microtensile bond strength

After one day of water storage, SB2 showed the highest bond strength values (38.22±3.90) ($p < 0.05$). However, after one year, SB2 mean (30.90±3.12) showed a statistically significant decrease ($p < 0.05$) and was statistically similar ($p > 0.05$) to ASE after 1d (31.22±4.85) and 1yr (30.95±2.22) of water storage. ASE mean was not affected by water storage ($p > 0.05$) (Table 2). The overall failure modes of this study are depicted in Table 3, showing that the majority of failures were adhesive. The number of pre-testing failures was similar among groups.

Scanning electron microscopy

The resin–dentin interfaces of all groups are shown in Fig. 1. Hybrid layer and resin tag formation were observed for both adhesives. Silver uptake within

the hybrid layer and water tree formation within adhesive layer were observed for both adhesives independently of water storage period.

DISCUSSION

Although clinical studies are preferred for predicting the behavior of adhesive systems, *in vitro* studies can provide results more quickly and inexpensively⁴.

One year of water storage did not affect the bond strength of ASE. Although it has two bottles, it only reacts with dental surfaces once both liquids, A and B, are mixed into cavities. Hydrophilic and hydrophobic components are separated to avoid phase separation and premature degradation in the bottle⁵. The high water content of liquid A (80-90%) is necessary for ionization of the acidic functional monomers of liquid B, but had no deleterious effect on bond strength even after 1yr of water storage. Hydrolysis of unprotected collagen is unexpected since demineralization and monomer infiltration occurs at the same time and depth, resulting in the formation of a thin but uniform hybrid layer.

Our results disagree with those obtained by Sauro et al⁶. The lack of differences between 1d and 1y results could be explained because they used stored whole teeth, whereas our study used stored bonded sticks. As mentioned above, indirect water storage, i.e. storage of entire teeth, showed significantly better bond strength values than direct water storage because the surrounding enamel-resin bond warrants protection against degradation⁶⁻⁸. The bond strength values obtained in our study for ASE are almost three times greater than those obtained by Belli et al.⁹, who attributed this to the low capacity of ASE to dissolve the smear layer as consequence of the formation of an uneven layer of liquid A resulting in an inhomogeneous etching of the Class I cavities. As mentioned above, our study was performed on a flat surface that lacks angles facilitating the application of the adhesive⁷. A previous study showed that the decrease in bond strength of self-etch adhesives was material-dependant. A low pH value produces complete smear layer and smear plug dissolution, facilitating the contact between water and hybrid and adhesive layers¹⁰. The major content of more hydrophilic monomers increases water sorption and water entrapment, affecting adhesive polymerization and contributing to hydrolytic degradation^{7,11-13}. The bond strength values of SB2 after 1d of storage were significantly higher than those of ASE. Never-

theless, one year of water storage produced a significant decrease in SB2. Our results are in agreement with the values obtained by previous studies^{14,15}. Simplified adhesives have more hydrophilic monomers that can lead to water sorption, decreasing the mechanical properties of the resin and making the polymer swell^{8,16}. Another factor mentioned is the discrepancy between the depth of demineralized dentin and resin infiltration, which left unprotected collagen fibrils beneath the hybrid layer more prone to hydrolysis¹³. According to Osorio et al.⁷, when resin-dentin bonds are protected by surrounding resin-infiltrating enamel, no decrease in bond strength was observed for SB after one-year aging. Metalloproteinases (MMPs) are endogenous enzymes released from the dentin matrix once the conditioning step is performed and have been shown to participate in the degradation of the hybrid layer¹⁶. Phosphoric acid is a strong acid (pH= 0.03-0.05) used as a conditioning step for SB2, and ASE reaches a pH of approximately 1.5 when liquids A and B are mixed. Both are sufficiently acidic to activate gelatinolytic and collagenolytic activity, but only phosphoric acid was also able to denature the MMP activity¹⁶. However, as shown by De Munck et al.¹⁷, this anticolagenolytic activity is not enough to prevent degradation for longer periods, even after the adhesives were modified with MMP inhibitors such as chlorhexidine (CHX). This agrees with the abovementioned results, poor monomer infiltration left unprotected collagen fibrils beneath hybrid layer that can be affected by hydrolytic or enzymatic activity¹⁸.

In contrast to phosphoric acid, the pH of ASE was insufficient to destroy the MMPs¹⁶. Previous studies have shown that modification of the primer of a two-step self-etching adhesive with an MMP inhibitor (chlorhexidine) depends on the concentration of the inhibitor^{19,20}. Nevertheless, our results suggested that demineralized collagen fibrils were not affected by ASE because most of them were covered by resin and thus protected from enzymatic and hydrolytic action. Longer periods of water storage are also suggested for a better understanding of the behavior of the current dental adhesives.

CONCLUSION

One year of water storage promoted a decrease in bond strength of the two-step etch-and-rinse adhesive system.

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CORRESPONDENCE

Eugenio Jose Garcia
Departamento de Materiais Dentários, Faculdade de Odontologia, Universidade de São Paulo.
Av. Prof. Lineu Prestes 2227
São Paulo, SP, Brasil
e-mail: eugarcia@usp.br

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