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Critical Analysis of Aging Protocols on the Adhesive Interface

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ABSTRACT

The aim of this study was to evaluate the influence of different aging protocols on the microtensile Bond Strength (μTBS) and nanoleakage between dentin and resin composite, using three adhesive systems. The enamel of vestibular surface of 150 bovine incisors was ground to expose the dentin. Teeth were randomly distributed in accordance with the adhesive systems (Adper Scotchbond Multi-Purpose -S, Clearfil SE bond -C, Scotchbond Universal Adhesive -U) and the aging protocols (storage for 24 hours, storage for 6 months, storage for 12 months, mechanical cycling and thermocycling), totaling 15 experimental groups (n=10). After construction of composite blocks over the hybridized dentin, teeth were sectioned, and beams obtained. Microtensile and nanoleakage tests were performed. The percentage of silver nitrate was recorded under energy dispersive spectroscopy. Data were submitted to Kruskal-Wallis' test and Mann-Whitney U for post hoc comparisons (5%). Storage for 12 months resulted in lower μTBS values ($p<0.05$) for C and S, but none aging condition was significantly harmful to U adhesive. S showed the highest μTBS values. C and U had more adhesive failures than S, which had more cohesive failures. The storage for 12 and 6 months and the mechanical cycling resulted in higher nanoleakage ($p<0.05$) for all adhesives. For mechanical cycling, it was found differences in nanoleakage between adhesives (U<S=C, $p<0.05$) and also for 12 months storage (C<U=S, $p<0.05$). Storage for 12 months was the aging protocol that most caused damage to the adhesive interface with the tested adhesives systems.

INTRODUCTION

In dental restorations, adhesive stability is related to the effective coupling between the co-monomers of the adhesive system and the dental substrate (1). Most current adhesives show a favorable immediate bonding effectiveness (2-4), but the maintenance of the bond over time seems to be more of a challenge, largely because the oral cavity is an inhospitable environment for adhesion.

Two main reasons have been cited to explain the degradation of the dentin-adhesive interface over time. First, the polymer adhesive surrounding the collagen fibrils, a result of the hybridization process, can suffer hydrolytic breakdown associated with the sorption of water by the polymer (1,5-6). Second, the exposed collagen fibrils can suffer degradation by the action of MMPs (matrix methalloproteinases), due to their being left exposed because of inadequate infiltration of the adhesive monomers within the demineralized dentin (6-7). Etch-and-rinse adhesives would be expected to suffer collagen degradation more than self-etching adhesives, as the presence of exposed collagen fibrils is less likely for the latter (8).

Of additional concern is the fact that thermal and mechanical loading of the restorations are common clinical situations which can also contribute to the degradation of the adhesive interface. These factors are associated with the differences between the linear coefficients of thermal expansion (5) and elastic modulus (9) of the polymers and the tooth structures. Also, water can accelerate the interface degradation process as the diffusion of water into the interface may be accelerated by the loading (10). Consequently, when the adhesive interface is exposed to the oral cavity, problems such as marginal discoloration, poor marginal adaptation and subsequent loss of retention can occur, mainly when dentin is involved (1,10). Indeed, the most frequent reason cited for the clinical failure of adhesive restorations is the development of secondary caries (11). Certainly, there is at least some evidence for a correlation between microtensile bond strength results after 6 months of water storage and clinical success of class V restorations (12).

In order to diminish the differences between *in vivo* and *in vitro* conditions, some studies have challenged the adhesive interface through aging mechanisms in an attempt to better simulate oral conditions. Currently, the main aging protocols used in laboratory studies are thermocycling, mechanical cycling and water storage over time. Although these are all widely used, one issue to consider is identifying the aging protocol that can effectively challenge the adhesive interface.

Therefore, the aim of this study was to evaluate the influence of different aging protocols on the µTBS of three different adhesive systems. Nanoleakage was also determined to correlate changes in the bond strength with a physical reputation of the tightness of the adhesive/tooth interfacial seal. The hypothesis to be tested was that certain accelerated aging protocols can be used to stress the adhesive interface, leading to reductions in bonding that can at least in part be explained by fluid migration through the interface.

MATERIALS AND METHODS

The enamel of the vestibular surface of 150 bovine incisors, disinfected for one week in 0.5% aqueous chloramine solution, was ground on 120- grit silicon carbide paper under water cooling, and then on 320- grit silicon carbide paper for 30 seconds to create a standardized smear layer. Teeth were randomly distributed into three groups in accordance with the adhesive systems used: S = Adper Scotchbond Multi-Purpose (3M ESPE, Saint Paul, MN, USA), C = Clearfil SE bond (Kuraray Noritake Dental, Kurashiki, Okayama, Japan), and U = Scotchbond Universal Adhesive (3M ESPE). The compositions of the adhesive systems and their mode of application to the tooth surface are shown in Table 1.

Table No. 1: Adhesive systems

Adhesive	Classification	Composition	Application procedure*
Adper Scotchbond Multi-Purpose	ER 3 steps	Primer: HEMA, Polyalkenoic acid, Ethanol Bond: BisGMA, HEMA	• Acid Etching - 15s • Rinse - 15s • Drying - filter paper • Primer application • Air-drying - 5s • Bond application

Clearfil SE bond	SE 2 steps	Primer: 10-MPD, HEMA, Hydrophilic aliphatic dimethacrylate, dl-Camphorquinone, N,N-Diethanol-p-toluidine, Water Bond: 10-MDP, BisGMA, HEMA, Hydrophobic aliphatic dimethacrylate, dl-Camphorquinone, N,N-Diethanol-p-toluidine, Colloidal silica	• Primer application - 20s on dentin • Air-drying • Bond application • Air-drying
Scotchbond Universal Adhesive	SE 1 step	BisGMA, HEMA, Silica, Ethanol, Dimethacrylate, Water, 10-MDP, Copolymer of acrylic and itaconic acids, Camphorquinone	• Application - 20s on dentin • Air drying - 5s

Abbreviations: BisGMA, bisphenol A diglycidyl ether dimethacrylate; HEMA, 2-hydroxymethyl methacrylate; 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate. ER, etch and rinse adhesive; SE, self-etch adhesive.

*Information provided by the respective manufacturers

Adhesive systems were applied according to the manufacturer's recommendation. All them were light-cured for 10s using a LED light-curing unit (Demi L.E.D. Curing Light; Demetron Kerr, Middleton, WI, USA) with direct irradiation of 1000 mW/cm^2. The irradiance was checked with a radiometer (Demetron Kerr) after every five irradiations, without variation. Composite blocks with 4 mm in thickness were built up freehand on the dentin surface with an incremental technique, using 2 mm thick increments of dental composites (Filtek Z350

XT, 3M ESPE). Each increment was light-cured for 20 s.

The composite-tooth units (n=10) were cut perpendicularly and vertically in the “x” and “y” directions, through the bonded interface on a low speed saw with diamond-coated disk under water cooling (Isomet 1000; Buehler, Lake Bluff, IL, USA) to produce beams with 1.0 ± 0.2 mm^2 cross-section. The composite-tooth units were sectioned prior to the application of the aging protocol, except for the mechanical cycling portion, which required the specimen to be intact and for which the load was applied over the whole composite surface.

The following aging protocols were applied:

C - Storage in distilled water at 37°C for 24 h (24) – Control group.

6 - Storage in distilled water at 37°C for 6 months.

12 - Storage in distilled water at 37°C for 12 months.

M - Mechanical cycling (Figure 1): 250,000 cycles at a load of 98N and applied at a frequency of 2.5 Hz. The load was applied through a cylindrical stainless-steel plunger (5mm diameter) in a mechanical loading machine (ER-37000NG; Erios, São Paulo, SP, Brazil).

T - Thermocycling: 1,000 cycles between water baths at 5°C, 37°C and 55°C respectively, with a dwell time of 20 seconds in each bath, using a thermal cycler (OMC 220b; Odeme, Luzerna, SC, Brazil).

Thus, there were 15 experimental groups representing the combinations of the three composites and five aging protocols: S24, S6, S12, SM, ST, C24, C6, C12, CM, CT, U24, U6, U12, UM, UT.

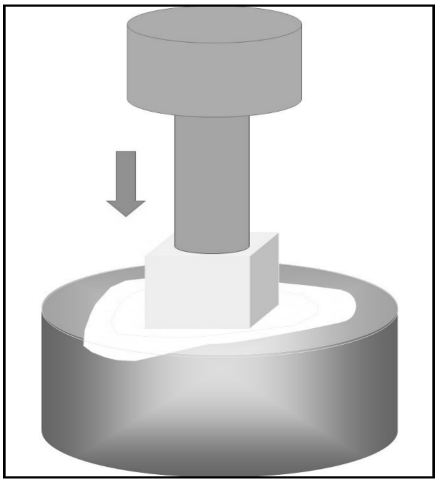

Figure No. 1: Mechanical load being applied to the composite block

Microtensile Bond Strength (μTBS)

Prior to testing the μTBS, the cross-sectional area of each beam was measured with a digital caliper (Mitutoyo, Tokyo, Japan). Each beam was then attached to a stainless steel jig (Odeme, Luzerna, SC, Brazil) using a cyanoacrylate adhesive (Super-Bonder; Loctite, São Paulo, SP, Brazil) and subjected to tensile loading in a universal testing machine (EMIC DL 2000; EMIC, São Paulo, SP, Brazil) at a crosshead speed of 1 mm/min until fracture. Very few pre-test failures occurred. The number of these were noted, but they were excluded from the statistical analysis. Ten beams were used for each tooth and the tooth was the experimental unit.

Failure mode analysis was performed with a stereomicroscope (Olympus SZ40; Olympus, Tokyo, Japan) at 120X magnification. The failures were categorized as adhesive, cohesive in dentin, cohesive in composite or mixed.

Nanoleakage Evaluation

Two beams of each tooth were selected for nanoleakage evaluation. They were coated with two layers of nail varnish up to 1 mm from the bonding interface on both ends. The specimens were rehydrated for 10 minutes in distilled water, immersed in an ammoniacal silver nitrate solution (50% w/v) and kept in total darkness for 24 hours. Then they were rinsed with distilled water and immersed in photo developing solution (Kodak, São Paulo, SP, Brazil) and exposed to fluorescent light for 8 hours.

After polishing the surface with silicon carbide papers of decreasing abrasiveness (600-, 1200-, 4000-grit SiC paper), the specimens were ultrasonically cleaned for 10 minutes and dried for 48 hours in a desiccator at 37°C. The specimens were fixed with a carbon adhesive on a stub and a sample holder for low vacuum was used. The composite/dentin interface was observed in a Scanning Electron Microscope (SEM) (Phenom ProX; Phenom-world BV, Hurk, Eindhoven, Netherlands) at 2000X magnification, 15kV accelerating voltage and backscattered mode. Three images were registered for each beam: two of both ends (right and left sides) and one of the center, by a technician who was blinded to the study conditions. The silver nitrate uptake in the hybrid was registered as a percentage of the total area scanned (137µm x 137µm), by means of energy dispersive spectroscopy (Software Phenon Pro Suite; Phenom-world BV) (13).

Statistical Analysis

A mean value for both tests was calculated for each tooth (n=10). Data were submitted to Shapiro-Wilk's and Levene's tests to determine their normality and homogeneity, respectively. As neither normality of data distribution nor homogeneity of variances were verified, the non-parametric Kruskal-Wallis test was applied, followed by the Mann-Whitney U for post hoc comparisons at a significance level of $p<0.05$. All analyses were performed with Statgraphics 5.1 software (Manugistics, Rockville, MD, USA).

RESULT

The storage for 12 months diminished the µTBS values for Scotchbond and Clearfil adhesives when compared to 24 hours of storage (Table 2). No aging protocol affected the bond strength of Universal. For most of the aging conditions, Scotchbond showed higher µTBS values than the other adhesives systems. Clearfil was most affected by 12 months of storage, having the lowest µTBS values after this aging protocol.

Table No. 2: Median values for μTBS (MPa)

Adhesive System	Aging protocols				
	24 (Control)	6	12	M	T
S	31.62 $^{a\ A}$	31.83 $^{a\ A}$	26.81 $^{b\ A}$	37.69 $^{a\ A}$	28.70 $^{a,b\ A}$
C	26.70 $^{a,c\ B}$	24.39 $^{a\ B}$	14.83 $^{b\ B}$	30.92 $^{c\ B}$	23.69 $^{a\ A}$
U	27.64 $^{a\ B}$	23.46 $^{a\ B}$	24.86 $^{a\ A}$	26.99 $^{a\ B}$	25.42 $^{a\ A}$

Same superscript lowercase letters indicate no statistically significant difference in the same line. Same superscript capital letters indicate no statistically significant difference in the same the same column.

Clearfil and Universal adhesives had a higher percentage of failures classified as adhesive, whereas Scotchbond adhesive showed mostly cohesive failures in the resin (Figure 2).

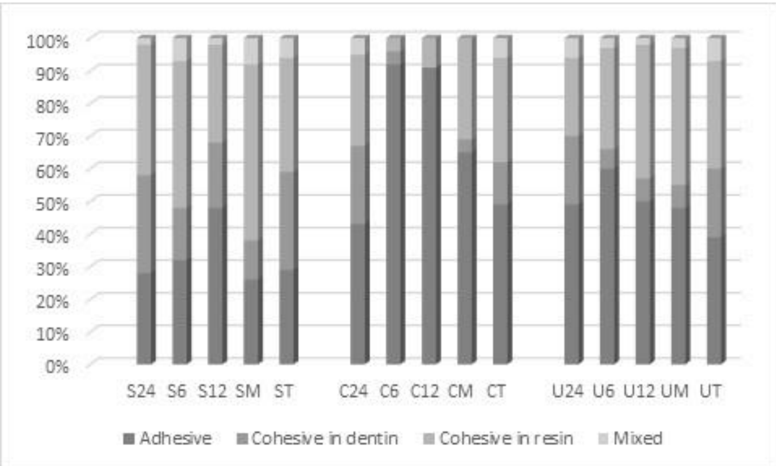

Figure No. 2: Mode of failure (%)

The percent of the overall bonding area that demonstrated nanoleakage of the silver nitrate stain was very low overall, ranging between 0.6 and 2.53% for all conditions (Table 3). The nanoleakage was the same for the controls and the specimens subjected to thermocycling. However, the storage for 6 and 12 months and the mechanical cycling increased the values of silver nitrate leakage for all the adhesives when compared with the control group (Figure 3). Scotchbond and Universal were the most affected by 12 months storage, presenting higher values of nanoleakage than Clearfil.

Table No. 3: Median values for nanoleakage (%)

Adhesive System	Aging protocols				
	24	6	12	M	T
S	$0.62^{a\,A}$	$1.89^{b\,A}$	$2.53^{c\,A}$	$1.32^{b\,A}$	$0.61^{a\,A}$
C	$0.70^{a\,A}$	$1.80^{b\,A}$	$1.80^{b\,B}$	$1.29^{b\,A}$	$0.80^{a\,A}$
U	$0.60^{a\,A}$	$1.05^{b\,A}$	$2.53^{c\,A}$	$1.00^{b\,B}$	$0.71^{a,b\,A}$

Same superscript lowercase letters indicate no statistically significant difference in the same line. Same superscript capital letters indicate no statistically significant difference in the same the same column.

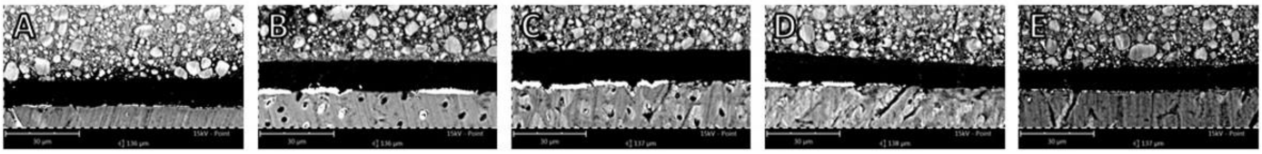

Figure 3- SEM images (Phenom ProX; 2000X), representing the composite/resin interface impregnated by silver nitrate in the different aging protocols.: (A) control group - 24 hours. (B) 6 months of storage. (C) 12 months of storage. (D) mechanical cycling. (E) thermocycling.

DISCUSSION

The purpose of the present study was to investigate how different aging protocols, commonly used to challenge the adhesive interface in laboratory studies, can influence the composite-dentin interface. The number of cycles performed in the present study, for the thermal and mechanical cycling, was based on a period of 12 months of aging in oral environment, making fairer the comparison with the storage for 12 months (14).

The null hypothesis of the present study was partially rejected, i.e., only some aging protocols influenced the µTBS and nanoleakage for the adhesives. For the µTBS test, thermocycling did not affect the adhesive interface, irrespective of the adhesive systems. This lack of an effect was also seen in the nanoleakage, where the specimens subjected to thermocycling showed the same silver nitrate penetration as the controls. This result is also consistent with that of Sezinando *et al.* (15), who showed that thermocycling did not also reduce µTBS, though the trend in their study and this study were for the µTBS values after this aging protocol to be lower than those found for storing for 24 hours. Another study (16) found a negative influence of the thermocycling on the µTBS for both one-step self-etching and etch-and-rinse adhesives. In this study, a higher number of thermal cycles was used, 5000 vs. 1000 in the present study, and the specimens were sectioned into beams after the thermal cycling, in contrast with the present study. Despite the differences in the linear thermal expansion coefficient between dentine and the restorative material (5), it could be speculated that due to the low rate of thermal diffusion in the composite (17), coupled with the short dwell time, minimal stress was experience by the interface, especially for the beams removed from the most internal areas, and thus the marginal seal and consequently the leakage was not affected (18). Another study (3) also found no statistical difference evaluated for the nanoleakage even after 10,000 thermocycles, when self-etching adhesives were used.

In the present study, µTBS values after storing for 6 months were similar to the controls for all of the adhesives. In another study (15), storage for 6 months diminished the µTBS only for a one-step self-etching adhesive. In that study, beams with a cross section of 0.5 mm^2 were tested, instead of $1mm^2$ as used in the present study As the aqueous medium storage is related to the hydrolysis of the collagen and/or the resin, which are components of the adhesive interface (10), a smaller cross section area could have suffered more consequences of the degradation, leading to lower values of µTBS, even with only 6 months of storage. Another study (19) also found significantly lower µTBS values for the Clearfil SE Bond after storing for 6 months.

However, it can be noted that there was a tendency to decrease the µTBS values after storing for 6 months, except for the Scotchbond Multi-Purpose. This could be explained by the degradation of the adhesive interface via hydrolysis, which could be taking place. One proof of this was that the storage for longer time (1 year) impaired the µTBS values, for all tested adhesive systems when compared to the control groups, and the difference was statistically significant for Clearfil and Scotchbond Multi-Purpose. The lack of effect for Universal may be related to the efficiency of the monomers in infiltrating the smear layer and producing resin tags (20).

The mechanical cycling did not produce significant reductions in bond strength, which is in accordance with another study (21) that tested one-step self-etching adhesives. It could be speculated that, in the present study, all adhesives formed a similar hybrid layer, which was capable of uniformly distributing the stresses generated at the interface (21). In contrast, Montagner (4) found that mechanical loading had a negative influence on both conventional and self-etching adhesives, though this may be explained by the higher number of cycles applied (750,000 cycles) in their study compare to that used in this study (250,000 cycles).

Though the bond strengths were not always affected, the action of the aging protocols, except for thermocycling, caused increased nanoleakage as compared with the controls for all the adhesives. It may be noted that the one-step self-etch adhesive showed lower nanoleakage after mechanical cycling when compared with the other adhesives. It could be speculated that the thin hybrid layer promoted for these adhesives (16), make them less susceptible to the degradation caused by the mechanical stress.

The longer storage time was most deleterious for Scotchbond Multi-Purpose and Universal adhesives, producing greater nanoleakage. Similar results were reported in another study when an etch-and-rinse adhesive was used and tested after 6 months storage (22). Anchieta *et al.* (23) also evaluated the influence of 12 months of water storage on the dentin–adhesive interfaces and found a higher nanoleakage, except for the Clearfil SE Bond, which was considered a more stable adhesive. The 10-MDP, a functional monomer presented in the composition of Clearfil, can interact with residual hydroxyapatite within the hybrid layer, forming a stable MDP-Ca salt deposition and a strong nanolayer at the adhesive interface (24). This chemical interaction may have been responsible for its enhanced bond stability over time as shown in that study. However, comparing the difference in specimen size of the two studies, the smaller size of the samples of the present study could impair even more the adhesive interface, irrespective of the kind of adhesive tested.

On the other hand, the Clearfil adhesive showed lower nanoleakage values at the time of 12 months of storage, despite the lower µTBS values, when compared with the other adhesives. Maybe, even with a lower leakage, the degradation for this adhesive could have caused more drastic effects on the adhesive interface, reducing the adhesive resistance.

It is also important to comment that, in general, the etch-and-rinse adhesive showed the highest bond strength values. These findings are in accordance with previous studies that reported a better bonding effectiveness of these adhesives when compared with the self-etching ones (2,16). The Scotchbond Multi-Purpose promotes a well-impregnated hybrid layer, covered with a uniform layer of adhesive (2). On the other hand, the self-etching adhesives systems may not form a uniform adhesive layer, which may lead to an incomplete polymerization, contributing to the lower bond strengths observed with these materials (25).

With regards to the mode of failure, the self-etching adhesives showed a higher number of adhesive failures. According to another study (24), higher percentages of adhesive failures were associated with low bond strengths and the number of adhesive failures increased with the aging methods. Thus, the higher number of cohesive failures for the etch-and-rinse adhesive can be associated with their better bonding efficiency. Also, there was a tendency for the number of adhesive failures to increase with the aging protocols, indicating some possible degradation of the adhesive interface.

CONCLUSION

Taking into account the limitations of the present study, the previous discussion suggests that the adhesive systems tested were quite stable for all aging protocols. In general, the nanoleakage was low, which would be related to the bond strengths not changing significantly, except for two adhesives at 12 months. The storage for 12 months was an aging protocol that challenge the adhesive interface for Adper Scotchbond Multi-Purpose and Clearfil SE bond. Additional studies, with different experimental conditions, should be conducted, including even others adhesives, to confirm the results obtained, as a higher number of cycles could be employed to see differences.

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