FACULDADE DE MEDICINA
UNIVERSIDADE D
COIMBRA

MESTRADO INTEGRADO EM MEDICINA DENTÁRIA

Artigo Científico Original

Effect of long-term water aging on dentin bond strength of different adhesive systems

Efeito do envelhecimento em água a longo prazo nas forças de adesão à dentina de diferentes sistemas adesivos

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Coimbra, 2019

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Summary:

Abstract

Introduction: The durability and stability of dentin adhesion in long term and after aging still remains questionable. *In vitro* models can simulate the aging of restorations and predict their durability. The goal of this study was to evaluate the effect of water aging on dentin bond strength comparing different adhesive systems after six years of storage.

Materials and methods: Samples were prepared six years ago from 25 non-carious human molars. Dentin flat surfaces were obtained from these teeth which were sanded with sequence of silicon-carbide sandpaper to create a uniform smear layer. The teeth were randomly divided into five groups according to the adhesive systems used: Xeno® V+ (Dentsply DeTrey, Konstanz, Germany); Xeno® III (Dentsply DeTrey, Konstanz, Germany), Clearfil™ SE Bond (Kuraray Medical Inc., Okayama, Japan), OptiBond™ FL (Kerr, Orange, CA, USA) and Prime&Bond® NT (Dentsply DeTrey, Konstanz, Germany). The adhesive systems were applied according to the manufacturers' instructions and resin-composite build-ups were then made . After being stored in distilled water at 37ºC, the samples were cut to obtain sticks. The samples were stored in water according to ISO/TS 11405:2015. Six years later, the sticks were tested in a tensile mode on a universal test machine at 0.5 mm/min. The data were analyzed by Kruskal Wallis test and a post-hoc pairwise comparison test using Bonferroni correction ($p<0.05$). The failure mode was also analyzed with an optical microscope.

Results: Bond strength values increased from the Xeno® V+ (3.74±4.45 MPa), to Xeno® III (17.54±11.11 MPa), to Clearfil™ SE Bond (26.20±8.19 MPa), to Prime&Bond® NT (26.65±8.55 MPa) and to OptiBond™ FL (30.06±5.47 MPa), in this order. Etch-and-rinse and two-step self-etching adhesive systems registered higher bond strength, without statistically significant differences between them. Xeno® V+ generated very low bond strength with significant differences from all other groups. Adhesive failures were related with the lower bond strength values and cohesive failures with higher microtensile bond strengths.

Conclusion: Etch-and-rinse and two-step self-etching adhesive systems presented high levels of dentin bond strength, after six years of water aging.

Keywords: Dentin-bonding agents, water aging, microtensile bond strength, adhesive systems, bond durability.

Introduction

Dental adhesion implies a permutation process in which some inorganic material is removed, followed by resin monomers impregnation that become micro-mechanically retained in the created porosities.[1-4] In addition, some lower acidity self-etch adhesives are also capable of chemically interaction with hydroxyapatite calcium.[5]

Immediate dentin bond strength values do not always correlate well with long-term bond stability, since bonding to dentin, unlike bonding to enamel, has been shown to be less durable, particularly due to morphological and physical variations, especially the distribution of the tubular architecture, the high organic content with increased collagen concentration and the higher water content.[1-3,6-8]

Clinically, marginal deterioration and microleakage have been described as the main factors involved in the longevity and durability of the adhesive/dentin interface, since its degradation weakens adhesion and has been related with postoperative sensitivity, marginal staining, secondary caries and subsequent partial or total loss of restoration retention.[1,2,9-11]

The degradation of dentin/adhesive interface can involve deterioration of both the polymeric constituents of the adhesive systems and the collagen matrix present in the dentin-resin junction.[5,11,12] The instability of the adhesive interface is mainly related to the fact that the hybrid layer behaves as a permeable membrane, even after polymerization, allowing the circulation of water throughout the interface.[1,3,5,11] These permeable regions can be identified when infiltrated with silver nitrate, being considered the expression of the hydrolytic process and corresponding to areas of nanoinfiltration.[1,3,5] Despite different manifestations and degrees of impact, this phenomenon occurs for any type of adhesives.[1,13,14] After long periods of storage in water, nanoinfiltration patterns tend to exacerbate and to expand and can take varied morphologies.[15] This implies the degradation of the adhesive interfaces, with consequent decrease of the mechanical properties and bond strength to dentine, over time.[15]

The effects of any of the nanoinfiltration patterns result from the sequential occurrence of different phenomena: the absorption of water by the polymers and the progressive hydrolytic and enzymatic degradation of the unprotected collagen by matrix metalloproteinases (MMPs).[1]

Water absorption has been claimed as a major cause of degradation of collagen and resin and may cause a significant decrease in the modulus of elasticity of the hybrid layer.[1,16] The hydrolysis consists of a chemical process that breaks the covalent bonds between the polymers, by the addition of water to the ester bonds, with consequent formation of oligomers and monomers.[1,10,11] All this contributed to the weakening of the physical properties of the

adhesive bond and to the reduction of the adhesion forces to dentin, affecting its longevity.[1,10,13]

Regardless of the type of adhesive used, the result of the dentin-resin interface is often an incomplete hybridization of the dentin surface, leaving collagen fibrils unprotected and vulnerable to hydrolytic degradation,[1,11,17,18] but also to metalloproteinases-induced deterioration.[11,12,19] The inhibition/inactivation of these enzymes is considered a very important strategy for the preservation of the hybrid layer and the increase of the stability of the adhesion over time, and can be achieved through the use of several biomaterials, such as chlorhexidine. [5,10-12]

The adhesive interfaces are subjected to mechanical, chemical and/or thermal stimulus, which can compromise their stability and durability. The action of occlusal forces resulting, for the most part, from the masticatory cycles may induce tensions that can determine a site for initiation of a failure, which can progress and propagate.[2,11,19] Temperature oscillations in the oral cavity can also induce repetitive contractions and expansions, at the tooth/resin interface, due to the differences between the coefficients of thermal expansion between these structures, which may exacerbate the occurrence of interfacial slits.[19,20] Finally, the tensions developed during polymerization, caused by the contraction of the resins, can also affect the breakdown of the adhesive interfaces, enhancing the occurrence of marginal microleakage.[1,11,19]

The oral cavity, due to the complexity and diversity of its conditions, appears as the definitive test environment to predict the behaviour of the restorations. However, *in vitro* models may be important in providing information about the fundamental mechanisms of resin/tooth degradation, since they can simulate the aging of restorations and predict the durability of this bond.[9,21] Various artificial aging techniques may be used, depending on the specific types of degradation of the adhesive bonds that are being evaluated.[2] However, most of these methods mimic only one of the factors involved in the degradation of interfaces *in vivo*, where all generally operate simultaneously.

In aging by aqueous storage, specimens are stored in fluids for a certain period of time (ranging from a few months, up to 4 to 5 years or more).[2,8,9] There is no consensus as to the solutions used to immerse the samples, the size of the specimens that were stored (sticks or restored teeth), the temperature, the pH, and the time interval to change the solution.[12] According to ISO/TS 11405:2015,[22] in long term tests (more than six months of storage), the medium should be replaced every seven days to avoid contamination. To prevent bacterial growth, during the storage period, and thus maintain pH stability, it is recommended to add a specific solution, such as sodium azide, chloramine or even

antibiotics.[2,9] Nevertheless, these solutions may interfere differently in resin-dentin degradation.[12] A controlled storage temperature may also play an important role, being generally set at 37°C, although room temperature is also used to mimic the intraoral temperature.[2,12]

In thermo-cycling which, in an attempt to reproduce the thermal changes occurring in the oral cavity, samples are subjected to cyclic exposures at hot and cold temperatures, in water baths.[9,21] The ISO standard TS 11405:2015[22] indicates that a regime consisting of 500 cycles in water, between 5 and 55 ºC, is appropriate. However, through a review of the existing literature, it was concluded that the 500 cycles are minimal in mimicking the effectiveness of long-term adherence,[2,21,23] and although there is no consensus in the literature,[9,21] it is proposed to use 10000 cycles, since it is estimated that it corresponds to about one year of clinical function.[2,9,21,24,25] Regarding the temperature of the baths, their amplitude varies greatly from study to study, however, temperatures between 5-55ºC cover the range of temperatures that occur in the oral cavity.[9,21,25] The time period in which the sample is immersed in a bath, at a given temperature, is also a point of debate.[21] According to ISO/TS 11405:2015,[22] the immersion time should be at least 20 seconds, because corresponds to the time it takes for the oral cavity to reach its normal temperature again after consuming hot or cold food and drink. [9,21]

Mechanical loading tests can also be used to predict the influence of mechanical factors, involved in the oral cavity, on resin adhesion to dental structure.[2,9] In thermomechanical fatigue, the samples are submitted to concerted thermal and mechanical fatigue protocols.[9,21,25] In addition to these techniques, we can also refer aging by pH cycling, consisting of immersing specimens in an acid solution (pH 4.3, for 6 hours at 37ºC); and also, degradation by chemical substances like food-simulating solutions.[9]

The objective of this study was to evaluate the effect of water aging on dentin bond strength comparing different adhesive systems, after six years of storage.

The null hypothesis was that there are no significant differences in the dentin bond strength, after six years water aging, between five adhesive systems.

Materials and methods

Specimens preparation

For this study, the specimens were prepared six years ago, as described below. Twenty-five non-carious human molars were partially included in blocks of acrylic resin (Orthocryl, Dentaurum) and the occlusal surfaces were cut perpendicular to the long axis of the tooth (Accutom 5, Struers, Ballerup, Denmark) under water-cooling, thus, exhibiting a flat dentin surface without residual enamel. To achieve a standardized smear layer, all surfaces were sanded using silicon-carbide sandpaper (sequence of 240-, 400- and 600-grit) in circular motion for 60 seconds each.

Bonding and restorative procedures

The teeth were randomly distributed into five groups, according to the adhesive systems tested (Table 1).

Adhesion procedures were performed as recommended by each manufacturer (Table 2), and resin-composite build-ups were applied with Esthet.X® HD A2 (DentsplyDeTrey, Konstanz, Germany) light-curing microhybrid composite resin (Table 3). Each layer was light-cured for 10 seconds, followed by a final polymerization of 60 seconds (Bluephase®, Ivoclar Vivadent, Lichenstein). For seven days, the teeth were stored in distilled water at 37°C (Heraeus BK 6160, Kelvitron® Kp, Wehrheim, Germany).

Cutting method

Afterwards, several cuts were made along the long axis of the tooth with a low speed saw (Accutom 5, Struers, Ballerup, Denmark), under refrigeration at 300 rpm and 0.300 mm/s, as described by Sano. *et al* [26]. The free space between the various cuts was filled with light-bodied silicone Aquasil Ultra XLV (Dentsply, DeTrey, Konstanz, Germany), after the first cut in the x-axis direction. Finally, a final cut was made approximately 3 mm below the cement-enamel junction separating the various sticks which were then checked on an optical microscope (M300, Leica, Switzerland) with 40-fold magnification to exclude faulty specimens.

For each tooth, the top of adjacent sticks were identified with two colors (Fig. 1) so that half of them were tested immediately and the other ones were stored in water at 37ºC for six years. According to ISO/TS 11405:2015, the medium was replaced every seven days to avoid contamination.[22]

Table 1: Adhesive systems studied, manufacturers, chemical composition, pH values and batch numbers.

Adhesive	Manufacturer	Chemical Composition	$pH_{(ref)}$	Batch no.
Group I **Xeno® V+** **1-step/ 1 bottle** **Self-etch Adhesive**	Dentsply DeTrey, Konstanz, Germany	-Bifunctional acrylate -Acidic acrylate - Functionalized phosphoric acid ester -Water -Tertiary butanol -Initiator - Stabilizer	1.3[19]	1203000016
Group II **Xeno® III** **1-step/ 2 bottles** **Self-etch Adhesive**	Dentsply DeTrey, Konstanz, Germany	Liquid A: HEMA; purified water; ethanol; BHT; highly disperse silicone dioxide Liquid B: Pyro-EMA; PEM-F; urethane dimethacrylate; BHT; camphorquinone; ethyl-4-dimethylaminobenzoate	1.4[2]	1302000019
Group III **OptiBond™ FL** **3-step** **Etch-and-rinse adhesive**	Kerr, Orange, CA, USA	Etchant: 37.5% phosphoric acid Primer:HEMA;GPDM; PAMM; ethanol; water; photo initiator Adhesive:TEGDMA; UDMA; GPDM; HEMA; bis-GMA; filler; photo initiator	1.8[2]	4677483
Group IV **Prime&Bond® NT** **2-step** **Etch-and-rinse adhesive**	Dentsply DeTrey, Konstanz, Germany	Di-and trimethacrylate resins PENTA Photoinitiators Stabilizers Acetone Nanofillers	2.2 [2]	1206000730
Group V **Clearfil™ SE Bond** **2-step** **Self-etch Adhesive**	Kuraray, Okayama, Japan	Primer: 10-MDP; HEMA; hydrophilic aliphatic dimethacrylate; dl camphorquinone; N,N-Diethanol-p-toluidine; water Adhesive: Bis-GMA; 10-MDP; HEMA; hydrophobic aliphatic dimethacrylate; dl-camphorquinone; N,N-Diethanol-p toluidine; colloidal silica	1.9[2]	041931

Legend: BHT - Butylated hydroxyl toluene; Bis-GMA - Bisphenol A diglyciyl methacrylated; GPDM - glycerol phosphate dimethacrylate; HEMA - 2-hydroxyethl methacrylate; PAAM - Phthalic acid monoethyl methacrylated; PEM-F - Mono fluoro phosphazene modified methacrylate; PENTA - Dipentaerythritol pentaacrylate phosphate; PYRO-EMA - Phosporic acid modified methacrylate; MDP - methacryloyloxydecyl; TEGDMA - triethylene glycol dimethacrylate; UDMA - Urethane dimethacrylate.

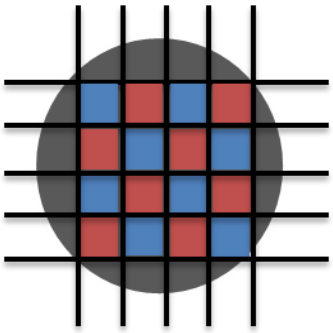

Figure 1: Representative image of identification of specimens for measurement of immediate (red) and after water storage (blue) bond strengths.

For the water aged specimens, the number of samples obtained per group was: Group I (Xeno® V+) n = 26; Group II (Xeno® III) n = 25; Group III (OptiBond™ FL) n = 36; Group IV (Prime & Bond® NT) n = 38 and Group V (Clearfil™ SE Bond) n = 38.

Microtensile bond strength testing

Six years later, each stick was bonded to a microtensile sample holder with cyanoacrylate adhesive (CE10Flex®, Ce Chem Limited, Derbyshire, UK) and then fixed on the microtensile device (Od04-Plus; Odeme Dental Research, Luzerna, Brasil). Specimens were fractured in tensile mode in a universal testing machine (Model AG-I, Shimadzu Corporation, Kyoto, Japan) at a 0,5 mm/min speed and the maximum load was recorded in Newtons and microtensile bond strength was calculated according to the following equation: μTBS = F/A=N/mm^2 = MPa, where F is the load at fracture (N) and A is the bonded area (mm^2).

The failure mode was analysed under an optical microscope (Leica CLS 150 MR, Switzerland) with a x40 magnification. The fracture pattern was classified as follow: adhesive, if the failure occurred entirely within the adhesive interface; cohesive, if it occurred completely in the composite resin (cohesive in the resin) or in the dentin (cohesive in the dentin); and finally, mixed, when both adhesive and cohesive failure occurred (Fig. 2)

Table 2: Application procedure of adhesive systems according to the respective manufacturers

Group/ Adhesive system	Application procedure
I - Xeno® V+	Apply actively adhesive for 20 sec; air-drying for 5 sec; light-curing for 10 sec.
II - Xeno® III	Mixing equal amount of Liquid A and B for 5 sec; apply actively for at least 20 sec; air-drying; light-curing for 10 sec.
III - OptiBond™ FL	Apply 37.5% phosphoric acid (Kerr Gel Etchant®) for 15 sec; rinse for 15 sec; gently air-dry; apply primer actively for 15 sec; gently air-dry for 5 sec; apply the adhesive for 15 sec; air-dry for 3 sec; light-curing for 20 sec.
IV - Prime & Bond® NT	Apply 36% phosphoric acid for 15 sec; spray and rinse with water for 15 sec; blot dry conditioned areas; apply adhesive and leave the surface wet for 20 sec; gently air-dry for at least 5 sec; polymerize for 10 sec; apply a second layer of adhesive in similar way.
V - Clearfil™ SE Bond	Apply primer for 20 sec; mild air stream; apply bond; gentle air stream; light-curing for 10 sec.

Table 3: Composite resin, manufacturers, chemical composition and batch numbers

Composite	Manufacturer	Composition	Filler	Batch no.
Esthet•X® HD A2 Microhybrid	Dentsply DeTrey, Konstanz, Germany	Bis-GMA adduct Bis-EMA adduct TEGDMA	Ba-F-Al-B-Si-glass Nanofiller sílica (77wt%; 60 vol%)	1006292

Legend: Bis-GMA - Bisphenol A dimethacrylate; Bis-EMA - Bisphenol A polyethylene glycol diether dimethacrylate; TEGDMA - Triethyleneglycol dimethacrylate

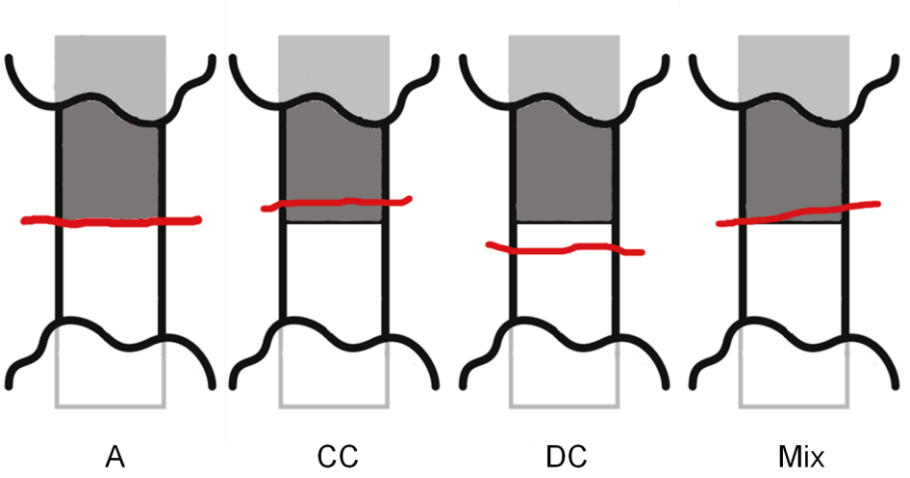

Figure 2: Representative images of the different failure modes: **A -** Adhesive failure; **CC -** Composite cohesive failure; **DC -** Dentin cohesive failure; **Mix -** Mixed failure.

Statistical Analysis

Statistical analysis was performed with IBM SPSS 23.0® software (SPSS; Chicago, IL, USA). For all analysis the significance level was set at $\alpha = 0.05$. The Kruskal Wallis test was used to calculate the bond strength distribution across the groups. In order to compare the bond strength between the different groups, Kruskal Wallis pairwise comparisons were performed using Bonferroni correction. To compare the distribution of the failure modes between groups, the chi-square test was used.

Results

The results of the microtensile bond strength test for all groups are described in figure 3 and in table 4.

The group of the OptiBond™ FL adhesive presented the better performance and lower dispersion of results.

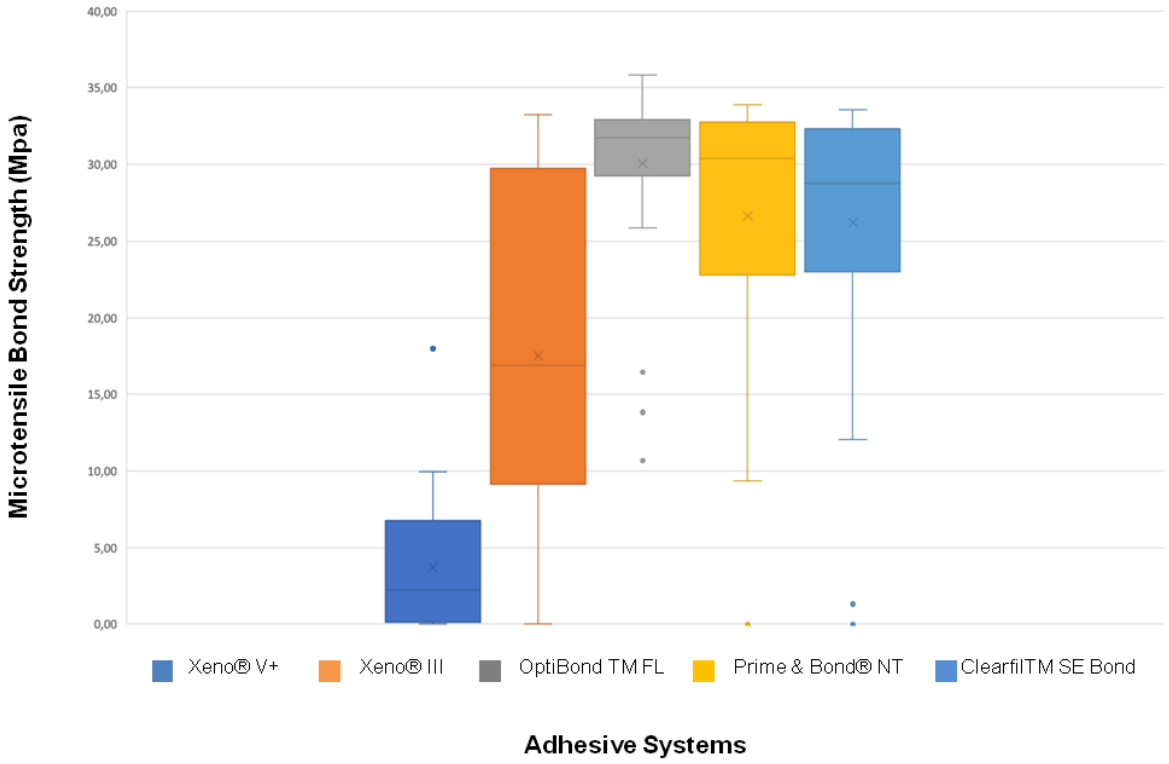

Figure 3: Box plot graphic for microtensile bond strength values distribution within groups.

Table 4: Descriptive statistics for microtensile bond strength values of the five groups.

Group	Adhesive Systems	n	Mean±SD	Min	Max	95% CI
I	Xeno®V+	26	3.74±4.45	0.00	17.98	[1.94,5.53]
II	Xeno®III	25	17.54±11.11	0.00	33.23	[12.95,22.12]
III	OptiBond™FL	36	30.06±5.47	10.68	35.85	[28.21,31.91]
IV	Prime&Bond®NT	38	26.65±8.55	0.00	33.88	[23.84,29.46]
V	Clearfil™ SE Bond	38	26.20±8.19	0.00	33.55	[23.50,28.89]

As assessed by box plot and Kolmogorov-Smirnov test, the data were not normally distributed, except for the Xeno® III group ($p > 0.05$).

Kruskal Wallis test revealed statistically significant differences among groups, considering zero as the value for pre-test failures and setting the significance level at $\alpha=0.05$. This test showed that there is an effect of the group on dentin bond strength. Thus we can reject the null hypothesis.

Bond strength values increased from the Xeno® V+ group (3.74±4.45 MPa), to Xeno® III (17.54±11.11 MPa), to Clearfil™ SE Bond (26.20±8.19 MPa), to Prime&Bond® NT (26.65±8.55 MPa) and to OptiBond™ FL (30.06±5.47 MPa), in this order.

Post-hoc pairwise comparisons were performed using the Bonferroni correction (Table 5).

Table 5: Post-hoc pairwise comparisons performed using the Bonferroni correction.

Sample1-Sample2	Test. Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.
I – II	-40.731	13.220	-3.081	0.002	0.021
I – V	-71.757	12.012	-5.974	0.000	0.000
I – IV	-77.599	12.012	-6.460	0.000	0.000
I – III	-93.564	12.147	-7.703	0.000	0.000
II – V	-31.026	12.154	-2.553	0.011	0.107
II – IV	-36.868	12.154	-3.034	0.002	0.024
II – III	-52.833	12.287	-4.300	0.000	0.000
V – IV	5.842	10.827	0.540	0.589	1.000
V – III	21.807	10.977	1.987	0.047	0.470
IV - III	15.965	10.977	1.454	0.146	1.000

This data revealed that Xeno® V+ had significant differences from all other groups ($p<0.05$). This adhesive system generated very low bond strength values. Xeno® III showed statistically significant differences from etch-and-rinse adhesives, but not with Clearfil™ SE Bond ($p<0.05$). The etch-and-rinse adhesive systems (OptiBond™ FL, Prime&Bond® NT) and the two-step self-etching (Clearfil™ SE Bond) obtained higher values of microtensile bond strength, without statistically significant difference between them (Table 5).

Pre-test failures occurred in 4 cases in the Xeno® V+ group, in 2 samples in the Xeno® III group and in 1 sample in the Prime&Bond® NT and the Clearfil™ SE Bond groups.

The Table 6 shows the distribution and frequency of the failure modes by experimental groups. As evidenced by the Chi-square test, there are statistically significant differences in the distribution of the failure mode among groups [$X2 (16)= 71.212$, $p<0.05$]. For Xeno® V+ and Xeno® III adhesive failure mode was more often detected. Also, in Prime&Bond® NT the frequency of adhesive failures was higher than the other types of failure. OptiBond™ FL and Clearfil™ SE Bond followed a similar tendency, in which composite cohesive failure were more often observed.

Table 6: Distribution of the failure patterns of the experimental groups in absolute number of specimens (percentage).

		Group				
		Xeno® V+	Xeno® III	OptiBond™ FL	Prime & Bond® NT	Clearfil™ SE Bond
Failure Mode	Adhesive	22 (84.6)	19 (76.0)	8 (22.2)	17 (44.7)	11 (28.9)
	Composite Cohesive	0 (0.0)	3 (12.0)	17 (47.2)	14 (36.8)	18 (47.4)
	Dentin Cohesive	0 (0.0)	0 (0.0)	7 (19.4)	4 (10.5)	5 (13.2)
	Mixed	0 (0.0)	1 (4.0)	4 (11.1)	2 (5.3)	3 (7.9)
	Pre-test	4 (15.4)	2 (8.0)	0 (0.0)	1 (2.6)	1 (2.6)

Discussion

Most current dental adhesives show excellent immediate and short-term adhesion efficacy, but the durability and stability of dentin adhesion in long term and after aging still remains questionable.[1,14,27]

Adhesive systems can be classified according to their mode of application in etch-and-rinse and self-etching adhesives. In dentin adhesion, two-step self-etch adhesive systems along with three-step etch-and-rinse adhesives are considered the gold standards.[8]

According to the ISO/TS 11405:2015,[22] "bond strength is the force per unit area required to break a bonded assembly with failure occurring in or near the adhesive interface". To assess the ability of an adhesive or restorative material bond to the dental substrate,[2] we can use macro or micro bond strength tests, depending on the size of the samples used.[23] These in vitro tests will help us to predict the clinical performance of the adhesive systems.

Microtensile bond strength is a test that determines the tensile load at failure divided by the cross-sectional area of the adhesive interface.[28] This test allows several specimens to be prepared from the same tooth[23,29] and makes possible to measure very small surfaces, about 1mm^2 of area.[23] It allows the measurement of the bond strength at critical areas,[30] with a more uniform distribution of tension in the reaction zone[23] and exhibits greater correlation with the loss of clinical retention.[30] Moreover, more adhesive rather than cohesive failures usually occur,[28] which is an important aspect since cohesive failures do not reflect the true adhesive strength.[31] Microtensile bond strength allows that means and variances can be calculated for each tooth, a more uniform stress distribution and facilitates scanning electron microscopy examination.[28] However, there are some flaws linked to this test, namely the technical requirement,[23] work intensity[23] and potential dehydration of the samples.[28] In addition, attachment of the specimen to the microtensile sample holder involves extreme care in handling as large losses of specimens may occur.[32]

The performance of adhesive systems is dependent on their composition. The recognition of the specific functionalities of the different compounds of each adhesive and how they may interact with the substrates may be critical for the interpretation of the results.

Clinically, adhesive interfaces fail even more frequently, due to a cumulative process of daily and cyclic stresses. The literature suggests that water storage and thermal fatigue are the most widely used artificial aging methods for evaluating adhesive interfaces. Currently, water storage is the most validated technique because it is more simple, low-cost and has a more consensual protocol. Mechanical tests combined with different aging methods, such as water storage and/or thermocycling, may provide information that is closer

to reality.[2] Nevertheless, these methods are time consuming and require considerable technological investment and a standard agreement. [2,23,33]

It is worth mentioning that, in literature, the studies with the longest aging time is with four-years water storage.[34,35] In this study, we were able to evaluate the effect of six-years water storage on dentin bond strength of different adhesive systems. Despite 6-years of storage, three adhesive systems are still commercialized. Interestingly, the ones that showed better bond strength values, OptibBond™ FL (30.06±5.47), Prime&Bond® NT (26.65±8.55) and Clearfill™ SE Bond (26.20±8.19), and the more antique. Nevertheless, null hypothesis must be rejected since there were statistically significant differences in dentin bond strength, after six years of water aging, between adhesive systems studied.

The OptiBond™ FL adhesive has in its composition bisphenol A giglyciyl methacrylated (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), and urethane dimethacrylate (UDMA) which may have a great influence on the viscosity of the uncured adhesive resin, and consequently, on the mechanical properties of the cured resin.[36] Its high filler load and high mechanical strength result in higher bond strengths.[37] Of all adhesive systems studied, this was the one with the lowest dispersion of results which were not unexpected, since this adhesive performed repeatedly favorably in several laboratory and clinical trials.[6,23] According to Munck *et al.*, several studies shows that the effectiveness of this type of adhesive is not very affected by storage in water.[2,34]

Prime&Bond® NT showed good bond strength results, despite being a two-step etch-and-rinse adhesive. Several laboratory studies have corroborated that this type of adhesive has a moderate performance due to its higher hydrophilicity and reduced hybridization potential.[6] However, the Prime&Bond® NT contains an acidic monomer, dipentaerythritol pentaacrylate phosphate (PENTA), which can interact chemically with calcium ions left on dentin surface, enhancing the quality of adhesion.[38] This adhesive system is also filled with nanoparticles that may help to establish a thicker and more uniform resin film thickness that stabilizes the hybrid layer.[39] Although, the dispersion of results was still considerable, as can be seen in figure 3.

Clearfil™ SE Bond is the self-etch adhesive that has achieved the best bond strength results. It is a “mild” self-etching adhesive that only causes superficially demineralization of dentin, keeping residual hydroxyapatite crystals attached to the collagen and forming a submicron hybrid layer that may be essential for additional chemical bonding.[6,40] The presence of 10-methacryloyloxydecyl (10-MDP) in its composition allows to establish strong ionic bonds with the calcium of the hydroxyapatite, due to the low solubility of the resulting calcium salts.[23,41] It has been proven that Clearfil™ SE Bond has good results in terms of

bonding effectiveness and durability when compared to other self-etch adhesives available.[6,40]

The one-step self-etch adhesive systems (Xeno® V+ and Xeno® III) are more unstable, the chemistry incompatibility leads to phase separation of the adhesive compounds which does not provide the formation of a high quality hybrid layer.[41] These two adhesives had the lowest bond strength values because they behave as permeable membranes and absorb great amounts of water.[6,9]

Xeno® V+ includes a high proportion of solvent and low hydrophobicity, responsible for the highly hydrophilic behavior.[42] In addition, no 2-hydroxyethl methacrylate (HEMA) is included in its constitution, which is a hydrophilic monomer that helps promoting adhesion and contributes to bond strength.[42] This fact predisposes to phase separation, with possible entrapment of water in the adhesive layer, which could be prevented by strong air-drying of the adhesive prior to light-curing.[6,23,40,42] Such procedure can lead to a reduction of the thickness of the adhesive interface, reducing adhesive effectiveness.[42]

For clinical use of Xeno® III it is necessary to mix two components prior to its application, which can lead to a greater technical sensitivity.[43] The presence of HEMA, water and ethanol gives it an hydrophilic nature, deteriorating its mechanical properties and not optimizing its degree of conversion by a greater probability of solvent retention at the adhesive interface.[44]

Pre-test failures were recorded mainly for this type of adhesives (Xeno® V+ and Xeno® III) with lower mean bond strength. Pre-test failures were considered as 0 MPa, as reported in the literature.[37] When this value is assumed, there will be an increase in the standard deviation in the test groups and therefore the quality of the results may decrease. For these two adhesive systems, however, this fact is not so much of a problem because they already demonstrate very low bond strengths.

Also, the rather low bonding effectiveness recorded for the one-step self-etch adhesives was associated with a high number of interfacial failures. In general, high bond strength was correlated with a higher tendency to fail cohesively within dentin or composite (in particular, for OptiBond™ FL and Clearfil™ SE Bond). This indicates that the actual bonding effectiveness of this two adhesives was probably not assessed,[31] because the cohesive strength of the resin material or dentin itself appeared lower than or at least as low as the interfacial bond strength.[32]

The results of this study are in conformity with the study by Loguercio *et al*.,[45] who studied the effect of three-years water storage on the performance of one-step self-etch

adhesives. For this type of adhesives mainly adhesive or mixed failures were recorded, as well as a large number of pre-test failures.

Several factors may have contributed to the variability of bond strength results between the different adhesive systems, namely, different structural characteristics between the different teeth and incoherence or failure to reproduce the sample preparation protocols.[30] According to the literature, in dentin adhesion, when adhesive interfaces are directly exposed to water aging, the obtained bond strength is significantly diminished for most of the adhesive systems tested.[8,23] This reduction tends to increase with time of storage.[8,30] The bond interface is subjected to hydrolysis and resin is subjected to water uptake with subsequent plasticization.[8] However, for adhesives like Xeno® V+ and Xeno® III no significant decrease was observed, primarily because of the low values recorded at 24 hours.[44]

De Munck et al.[34] demonstrated that direct exposure to four-year water storage did not significantly affect the bond strength of three-step total-etch adhesive systems. However, the microtensile bond strength of the two-step total-etch adhesives significantly decreased and showed a greater dispersion of results. The specimens were stored four-years either as intact composite crowns with enamel-bonded borders or sectioned in sticks. None of the systems tested had significant dentin bond strength decay when stored at full composite crowns.[34] Also, Abdalla *et al*.[35] showed that dentin bond strengths, after four-years of direct water storage, decreased significantly compared to the results at 24-hours and four-years of indirect storage. Besides, Clearfil™ SE Bond adhesive system recorded mean values of bond strength similar to this study (21 ± 2.9 MPa), after aging.[35]

Comparing to immediate bond strength, for Clearfil™ SE Bond it would be expected to have higher bond strength values compared to Prime&Bond® NT but this was not verified, although this difference was not statistically significant. However, the Prime&Bond® NT group had a much higher dispersion of results, with a higher standard deviation and presented a greater number of adhesive failures.

The results of this study are in agreement with the values obtained by Armstrong *et al.*,[46] who observed that the total-etch adhesive systems and the two-step self-etch adhesive systems, after 15-months of water storage, did not show statistically significant differences between them. In addition, one-step self-etch adhesive system showed to be significantly weaker than all other adhesives, which is demonstrated by almost 90% of pre-test failures and the greatest number of adhesive failures.[46] For Clearfil™ SE Bond, mean microtensile bond strength of 21.6 MPa was recorded after 15-months of direct storage in water, which is similar to that recorded by this study.

In the study by De Munck *et al.*,[47] when bonded to class I cavity bottom dentin and after one-year of direct water storage, the performance of OptiBond™ FL appeared stable and the highest values of bond strength were observed for this adhesive. In addition, the Clearfil™ SE Bond had a worse performance, however with still reliable values, which is in agreement with this study. This adhesive was more affected by direct water exposure and the failure mode shifted for more interfacial failures.[47] One possible explanation for these results may be given by recent findings that demonstrate that the durability of resin-dentin bonds in 10-MDP based adhesives has been wrongly attributed to the presence of nano-layered structures of 10-MDP resulting calcium salts.[48] In the study of Tian *et al.*[48], after one year of water aging, the nanolayering features were identified in the 10-MDP primer-treated dentin interface but its bond strength decreased significantly. This may be associated with a weak connection between the 10-MDP resulting calcium salts and the dentin surface.[48] In resin composites, glass fillers and silica are silanized with methacryloxy silanes to allow them to bond to the methacrylate resin matrix. In the case of 10-MDP-Ca salts, the inward facing of the methacrylate groups of two 10-MDP molecules may drastically reduce the number of freely available methacryloxy functionalities for coupling to the resin matrix.[48] The presence of HEMA can also reduce nanolayering, inhibiting MDP from interacting chemically with hydroxyapatite.[49]

In the systematic review of Masarwa *et al.*,[8] no significant difference was found between two-step self-etch adhesives when compared to total-etch adhesives although microtensile bond strengths were higher for total-etch than two-step self-etch at all testing times. The included studies showed that one-step self-etch adhesive systems provide less bonding strength if compared to the others.[8]

Conclusion

Within the limitations of this in vitro study, it can be concluded that:

- Etch-and-rinse adhesives and two-step self-etch adhesives showed superior microtensile bond strength values, after six years of water aging.

- Adhesive failure mode was correlated to lower bond strength results, while the cohesive failures are more common with higher adhesion values.

Acknowledgements

First, I would like to thank my thesis advisors, Doctor Alexandra Vinagre and Prof. Doctor João Carlos Ramos, for all the support, sharing of knowledge, motivation and constant availability, and for all precious guidance and advice in the accomplishment of this work.

To Doctor Ana Messias for help in the statistical analysis of the results.

To Mara, Alfredo and Mariana, the best teammates, for your help, support and company during the last few months.

To Manuela, Silvia and all my friends for their patience, motivation and constant friendship.

And, finally, my family and particularly my parents and brothers for the support and unconditional love throughout all this years.

This study was carried out using the means provided by the laboratory of mechanical tests and preparation of samples of the area of Dental Medicine.

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