EFFECT OF WATER STORAGE ON MICROSHEAR BOND STRENGTH OF FOUR DENTAL ADHESIVE SYSTEMS TO DENTIN

ABSTRACT

AIM: To evaluate the microshear bond strength of 4 dental adhesive systems after 1 year-water storage. **MATERIAL AND METHODS:** The sample consisted of 120 cylinders of composite, obtained from 24 bovine incisors, which were divided into four experimental groups: G1-Scotchbond Multi-Purpose, G2-Single Bond 2, G3-Clearfil SE Bond, G4-Adper Easy One and two storage times in distilled water: T0 - 24h and T1 - 1 year. A bivariate analysis was performed using the ANOVA and Tukey test (α=0.05). Results were evaluated inter- and intra-group in both times. **RESULTS:** After 24 hours, G1T0 (39.68A ± 11.55) showed higher bond strength (MPa) followed by G2T0 (22.71B ± 4.07), G3T0 (18.94BC ± 7.29) and G4T0 (13.30C ± 3.94). After 1 year of storage only G1T1 (33.95A ± 6.35) and G3T1 (13.59BC ± 2.63) maintained the bond strength values ($p \geq 0.05$), while G2T1 (10.62C ± 4.32) and G4T1 (4.49D ± 2.49) presented a decrease in this values. **CONCLUSION:** The hydrophobic two-step adhesive systems maintained the bond strength after 1-year water storage.

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KEYWORDS

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INTRODUCTION

Advances in dentistry have led to the emergence of techniques and materials innovations. The improvement of esthetic restorative materials makes the adhesive systems essential products in various clinical applications, responsible for uniting the dental restorative material structure. In dental materials market several types of adhesive systems are available, making the choice of the "ideal" adhesive difficult, since different indications and application protocols need to be observed during their clinical application care.[1]

With the aim of simplifying the technique, adhesive systems more hydrophilic and compatible with dentine were developed, being necessary that research be performed to confirm that these adhesive systems, besides the reduction of working time, have also and efficacy of bond strength. For this purpose, different artificial aging techniques were used in order to predict the clinical performance of tested materials. The most commonly used artificial aging technique is water storage. In this technique, the bonded specimens are stored in fluid at 37º C for a specific period of time. This period may vary from a few months[2] up to four-to-five years or longer.[3]

The basic mechanism for the adhesion of dentin and enamel to restorative products occurs through replacement by resin monomers of minerals extracted by acid etch. These resin monomers penetrate the porosities created and through this interface generate micromechanical adhesion.[4]

Some studies have confirmed that simplified bonding procedures do not necessarily provide improved bonding performance, especially at long term but rather suggest that the resistance to degradation of dentin-resin bonding depends of the material.

It has been speculated that hydrolytic degradation within the hybrid layer gradually increases due to water penetration through nanoleakage channels, resulting in lower bond strengths and interfacial failure after as little as nine months. It has also been suggested that the bond strength of different solvent-based adhesive systems gradually decreases over time regardless of the variable moisture pattern used for the bonding procedure.[5, 6, 7]

The evolution of aesthetic restorative materials has become increasingly able to correct the discrepancies in shape, texture, placement, color and function of the teeth. Nevertheless, the union still finds the tooth problems as hydrolytic degradation, polymerization shrinkage, microleakage, permeability and nanoleakage[8]. The durability of bonds between adhesive resin systems and dentin is important for the longevity of bonded restorations. Self-etching adhesives are widely employed, mainly because of their ease of use and low sensitivity technique. However, the longevity of adhesive bonds is still an area of interest in adhesive dentistry.[9]

Faced with the need for adhesives system each day more durable, the purpose of this study, was to analyze and compare the micro-shear bond strength of adhesive systems stored in water for a 1-year period.

MATERIAL AND METHODS

Four adhesive systems were used: Adper Sigle Bond 2 (3M / ESPE); Adper Scotchbond Multi-Purpose Plus (3M/ESPE); Adper Easy One (3M/ESPE), Clearfil SE Bond (Kuraray) were applied on the surface of dentin according to the manufacturer's recommendations (Table 1).

Table 1. Chemical composition of tested materials.

MATERIAL/ MANUFACTURER	GENERAL COMPOSITION	MANUFACTURERS' INSTRUCTIONS OF USE
Scotchbond Multi-Purpose- 3M/ ESPE	Primer: HEMA, polyalkenoic acid polymer, water. Bond: Bis-GMA, HEMA, tertiary amines (both for light-cure and self-cure initiators),PI photo-initiator	• Acid etched for 15 s with 35% phosphoric acid. • The surface was rinsed with water for 10 s. • Excess water was removed with absorbing paper • Apply primer with a brush and leave for 20 seconds. • Air-blow for 10 seconds. • Apply bond with a brush and air-thin. • Light cure for 20 seconds.
Adper Single Bond 2-3M/ ESPE	BisGMA, HEMA, DMA, ethanol,water, PI and a methacrylate functional copolymer of polyacrylic and polyitaconic acids	• Acid etched for 15 s with 35% phosphoric acid. • The surface was rinsed with water for 10 s. • Excess water was removed with absorbing paper • Two layers of adhesive were applied with gentle air drying for 10 s after each layer application • Light polymerization for 20 s
Clearfil SE Bond - Kuraray	Primer: Water, MDP, HEMA, CQ, DET and hydrophilic : DMA. Bond: MDP, Bis-GMA, HEMA, DMA, CQ, DET, silanated colloidal silica. .	• Dispense primer in the well. • Apply primer with a brush and leave for 20 seconds. • Air-blow for 10 seconds. • Dispense bond in the well. • Apply bond with a brush and air-thin. • Light cure for 20 seconds.
Adper Easy One- 3M/ ESPE	HEMA, Bis-GMA Methacrylated phosphoric esters, DMA, Methacrylate functionalized Polyalkenoic acid (Vitrebond™ Copolymer), silica filler, Ethanol, Water, CQ, Stabilizers.	• Apply primer with a brush and leave for 20 seconds. • Air-blow for 10 seconds. • Light cure for 20 seconds

Abbreviations: Bis-GMA: bis-phenol A diglycidylmethacrylate; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate; DMA: dimethacrylate CQ: camphorquinone; MMA: methylmethacylate; Det: N,N- diethanol p-toluidine; PI:photoinitiators.

To evaluate the effect of water storage on the microshear bond strength, 48 bovine incisors were used, after extraction were subjected to a scraping with a scalpel blade # 12 and polishing with rubber cup for removing organic debris. The roots were removed by micromotor with double face diamond disc (KG Sorensen). After that the crowns were kept in stored at 4°C in phosphate buffered saline (PBS), containing 0.002% sodium azide.

The teeth were mounted horizontally, so that the buccal surface was exposed in cold curing acrylic resin (Acropars 200, Iran) using plastic circular molds. Superficial coronal dentin was exposed with 180 and 320-grit sandpaper under water. After trimming, the resulting surfaces were finished using 600-grit sandpaper was then used for 50 seconds to form a standardized smear layer. The prepared teeth were assigned to five groups of sixteen each.

Teeth were randomly divided into 4 groups: each group was divided into two subgroups. One subgroup was subjected to the micro shear test after 24 hours storage in distilled water at 37°, the second subgroup was tested after 1 year (T1) the preparation of specimens.

For production of these specimens a matrix of silicone tubing with 1 mm high with an internal diameter of 0.7 mm thickness (Tygon tubing TYG-030, Saint-Gobain Performance Plastic, Maime Lakes, FL, EUA) were used to obtain the resin cylinders with the same dimension. These arrays were placed on the dentin after application of the adhesive system were applied according to manufacturers' recommendations. The composite resin (Z250 3M ESPE) was placed in these Tygon tubing and light cured for 40s using a tested LED Radii-Cal (SDI Dental Equipment Products, Australia). Five resin cylinders were made for each tooth, totaling 15 cylinders per group (n = 15), each cylinder representing a specimen. Then the specimens were stored in distilled water, at 37°C , some groups for 24 hours and the other for 1 year (distilled water was changed every week).

For analysis of bond strength tests were performed microshear in a universal testing machine (Shimadzu, AGS-X Universal Testing Machine). A thin steel wire (3 mm D) was looped flush between the load cell projection and the resin cylinder, making contact with the lower half-circle of the cylinder and touching the tooth surface. The force was applied at a crosshead speed of 0.5 mm/minute until failure. Care was taken to keep the composite cylinder in line with the center of the load cell and the wire loop, parallel to the load cell movement direction and the bonded surface, in order to maintain a shear stress orientation at the bonding interface. The maximum load at the time of failure was recorded for each adhesive. After microshear testing, the fractured surface of the specimen was inspected by a stereomicroscope at 40x magnification (Olympus, Tokyo, Japan) to evaluate the mode of failure. The mode of the failures was classified into cohesive in dentin or cohesive in resin, adhesive at the dentin/resin interface and mixed.

Data were analyzed using SPSS software. Two-way ANOVA (α= 0.05) was used to compare differences in mean micro shear bond strength values of the groups, the Tukey's test was used to compare the differences between paired groups.

RESULTS

MICROSHEAR BOND STRENGTH TEST

After 24h, Scothbond Multi-Purpose showed higher bond strength 39.68 (± 11.55)^{A}; followed by Adper Single Bond 2 22.71 (± 4.07)^{B}, SE Bond Clearfill 18.94 (± 7.29)^{BC} and Adper Easy One 13.30 (± 3.94)^{C} respectively. After 1 year only Scothbond Multi-Purpose 33.95 (± 6.35)^{A} and Clearfill SE Bond 13.59 (± 2.63)^{BC} kept their bonding resistance values ($p \geq 0.05$), Single Bond 2 10.62 (± 4.32)^{CD} and Adper Easy One 4.49 (± 2.49)^{D} showed a decrease in values due time (Table 2).

FRACTURE SURFACE OBSERVATION

There were no pretest failures in any group. Overall, failures were predominately adhesive or mixed. The incidence of cohesive failures in composite was more common with increasing bond strength. The complete results are presented in Table 3.

After 24 hours of water storage, the fracture patterns of the bonded specimens were adhesive (83,33%) at the resin-dentin interface. Also, mixed types of failures were shown in 16,67% of the specimens, while 0% showed cohesive failure either in dentin or composite.

After one year of wear storage, failures were also mainly adhesive (91,67%). The mixed type of failure was seen in 8, 23% of the specimens. Cohesive failures were not observed.

DISCUSSION

Acid etching of dentin results in an increased surface moisture of dentin, so the use of a hydrophilic monomer is necessary to prepare the substrate for the adhesive (hydrophobic monomer) application.[10] The possibility that the resin monomers do not diffuse in depth through the demineralized dentin remains, fact that can compromise the adhesion of the conventional adhesive systems. Several factors can influence in the formation of the hybrid layer: depth of demineralization; thickness of the smear layer; type, concentration and duration of acid etch application; the dentinal fluid flow towards the demineralized surface, which can act as an physical barrier to the acid etch, as well as contribute to their dilution; the degree of mineralization of the substrate; depth of the cavity; washing and drying procedures of demineralized tissue; the composition and way of use of the adhesive system.[11]

Table 2. Microshear bond strength of the tested adhesives to dentin (MPa ± SD).

MATERIALS	n	24-HOURS WATER STORAGE (SD)	1-YEAR WATER STORAGE (SD)
Adper Easy One	15	13.30 (± 3.94)^{C}	4.49(±2.49)^{D}
Adper Single Bond 2	15	22.71 (±4.07)^{B}	10.62(±4.32)^{CD}
Scothbond Multi- Purpose	15	39.68 (±11.55)^{A}	33.95 (±6.35)^{A}
Clearfill SE Bond	15	18.94 (±7.29)^{BC}	13.59 (±2.63) ^{BC}

Identical capital letters denote no significant differences among groups ($p < 0.05$).

Table 3. Failure patterns of the tested adhesives to dentin after 24-hours and 1-year water storage.

MATERIAL	STORAGE	FAILURE		
		ADHESIVE	MIXED	COHESIVE
Adper Easy One	24-hours	12	3	0
	1-year	15	0	0
Adper Single Bond 2	24-hours	12	3	0
	1-year	13	2	0
Scothbond Multi- Purpose	24-hours	13	2	0
	1-year	13	2	0
Clearfill SE Bond	24-hours	13	2	0
	1-year	14	1	0

To obtain an increased longevity of restorative treatments, the resin-dentin bond should remain stable. Several methods are used to simulate restorative clinical situations, such as thermal cycling or water storage. Water is one of several substances used in research protocols due to its capacity of promote the degradation of unprotected collagen fibers, in samples stored for certain periods.[12-15] Most of these studies report significant decreases in bond strengths, even after relatively short storage periods. The decrease in bonding effectiveness after water storage was supposed to be caused by degradation of interface components by hydrolysis (mainly resin and\or collagen). Also water can infiltrate and decrease the mechanical properties of the polymer matrix by swelling and reducing the frictional forces between the polymer chains. This causes plasticization of the resin, which makes it weaker.[8, 9, 15-17]

The self-etching primer can simultaneously cause both acid-conditioning and priming in one step. These adhesive systems eliminate the separate steps of acid etching and water rinsing, thus simplifying bonding procedures. Such simplification may reduce the technical errors that often follow the use of total-etch adhesives, such as over-etching, over wetting or over dehydration of the prepared tooth surface, contributing to a reduction in post-operative sensitivity and improved sealing[9]. Such systems are the result of an constant search for operational efficiency and reduction of working time, which came not tied to a genuine technological breakthrough, this simplification also decreases sensitivity since they are all dry adhesion.[18]

After the removal of smear layer by acid etching, there is a possibility that the collagen fibers are not completely covered by the adhesive. These fibers become more susceptible to the action of proteolytic enzymes, leading to the dissolution of hybrid layer. Such enzymes are known as endogenous metalloproteinases (MMP) and are present in the dentin.[8, 19]

Several studies[2, 6, 12, 14, 20] claim that the durability of adhesion is altered in all adhesive systems . Long-term studies have shown that single step self-etching adhesives have lower bond strength[13] and higher solubility[6] when compared to conventional or two steps systems. Although some studies[21] have shown that the two-step self-etching adhesives exhibit a more stable bond to dentin, other studies have shown that bond remained stable for longer periods. [22] Conventional adhesives have shown, in short-term evaluation, good formation of hybrid layer, but after1 year of storage voids can be observed in the adhesive interface, indicating an increased dissolution of the hybrid layer and the appearance of pores[23], results corroborated by this research.

Self-etching one-step adhesive systems like Adper Easy one, due to its higher concentrations of hydrophilic and ionic resin monomers and the lack of subsequent application of hydrophobic resin coverage, behave as a permeable membrane after polymerization.[24] This allows water diffusion from dentin through the cured dentin adhesive, remaining along the adhesive-composite interface. Increased adhesive permeability then allows the dissolution of adhesive components, which can happen if there is an insufficient polymerization within the hybrid layer due to excessive dentin moisture during application of the adhesive[14], resulting in decreased bond strength with longer water exposure , with may explain the results obtained in this research.

Okunda et al[5], studying whether the nano leakage of adhesive systems was directly related to an decrease in bond strength, evaluated two conventional adhesive system, Single Bond (3M ESPE) and One Step (Bisco). Teeth were divided into 4 groups, according to the time of adhesive strength evaluation (1 day, 3 months, 6 months and 9 months). Results agreed with the present study, and a decrease in the bon strength of both adhesives over time was noted. This can be explained by the fact that conventional two-step adhesives have both primer and adhesive in the same flask, losing its effectiveness due to organic solvents and hydrophilic monomers mixture with the adhesive, resulting in an increased permeability and hydrolytic degradation over time.[25]

Hashimoto[13] evaluated the durability of adhesive systems stored for 10 years in water, and concluded that the bond degradation results from water flow through the interface, with a subsequent degradation of the resin-dentin interface, although they point that more studies, with the use of transmission electron microscope are needed to clarify this question.

The results of the present study corroborated with Hogan and Burrow[26] which evaluated the effect of water on the bond strength of three adhesive systems (All Bond 2, Scotchbond Multi -Purpose Adhesive, Clearfil LB Bond) after 1 , 7 , 28 , 84 and 168 days of storage and compared with the results of the specimens that were kept dry. Results showed that materials kept dry showed significantly higher bond strength between the first and seventh day. Materials stored in waters behaved the opposite way, with samples immersed for 1 day presenting the highest bond strength evaluated. Scotchbond presented on day 1 higher bond strength than all other periods ($p < 0.05$).The tensile strength of the specimens decreased significantly between 28 and 84 days. Authors concluded that the effect of water was the reduction of the bond strength of all materials tested for 168 days.

To demonstrate that self-etching adhesives are permeable membranes when bonded to dentin, they are applied on moist dentin, light-cured, and composite resins is applied over the interface. The light-cure of resin is performed after the sample is stored for 10-20 minutes in the dark. Water will flow through the adhesive layer and be trapped as bubbles inside the adhesive- resin interface, and, since the resin is hydrophobic, it will show the negative impression of these bubbles on its inner surface, a phenomenon known as osmotic blistering. When the adhesive interface is broken, a surface with the appearance of honeycomb is visualized.[24] Since Scothbond Multi Purpose and Clearfill SE Bond are presented with primer and adhesive in separate flasks, it is observed that after the use of a hydrophilic primer, a hydrophobic adhesive layer that prevents passage of water is applied. This may explain why both systems didn't show a decrease in bond strength over a year of water immersion in the present study.

The sealing interface proportioned by contemporary adhesives should be better than the smear layer alone, in which the smear plugs represent 86 % of the resistance to the fluids movement.[24] It is believed that a perfect seal would be established if the dentinal tubules were infiltrated with resin, with the assumption that the polymerized resins are impermeable to dentinal fluid.[18]

CONCLUSION

Of the adhesives tested through microshear test, Adper Easy One and Adper Single Bond 2 had their bond strength reduced after one year of water storage. ScotchBond Multi-Purpose and Clearfill SE Bond showed statistically the same resistance after one year. We can conclude that the adhesive systems that are applied in separate steps (with hydrophilic and hydrophobic components in different flasks) have shown greater bond strength to dentin after long-term water immersion, and hydrophobic adhesives should be preferred when used on dentin.

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