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Multiple Adhesive Layering Influence on Dentin Bonding and Permeability

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ABSTRACT

The aim was to evaluate the effect of the number of layers on the microtensile bond strength (μTBS), permeability and nanoleakage of an etch-and-rinse adhesive (Adper Single Bond 2). Different numbers of layers (1, 2 and 4) were applied on dentin substrate. Specimens were obtained and tested for μTBS. Stick-shaped samples were analysed by scanning electron microscope, to observe silver nitrate penetration. One-way ANOVA and Tukey tests were applied (α=0.05) for μTBS values. The fracture mode was evaluated under a stereomicroscope and a scanning electron microscope (SEM). Groups with two and four layers of bond showed higher μTBS to dentin and they also showed lower adhesive permeability than ONE layer. The silver nitrate uptake occurred in the hybrid layer in practically all groups. Fracture occurred predominantly at the mixed interface. The authors recommend the application of more than one layer of Adper Single Bond 2, because a single layer showed lower μTBS and higher permeability values.

INTRODUCTION

Clinical progress in adhesive restorations has often been marked by simplifying procedures. Classical multi-step adhesives have been replaced by simplified “single-step” systems that are apparently not only simpler, but faster to use. However, contemporary dental adhesives have been simplified at the expense of increasing incorporation of hydrophilic monomers.[1]

These simplified adhesives contain both hydrophilic and hydrophobic monomers to bond to an intrinsically wet surface, and this has increased the adhesive layer potential to absorb water.[2] The absorbed water may degrade both polymers and the bonded interface.[3] As certain resin components are leached into water, minute water-filled voids are produced within the polymer matrix,[4] forming a permeable interface along the restoration margin.

Resin/dentin bond instability has been attributed to the porous nature of the hybrid layer,[5] that behaves as a permeable membrane after polymerization.[2,6] Obtaining an adherent and impermeable hybrid layer seems to be a prerequisite for achieving satisfactory immediate performance and preventing early chemical and mechanical degradation of bonded interfaces.[7]

Studies have also proposed changes in the application methods of adhesive systems to increase the bond strength to dental substrate, such as increasing the duration, or vigorous (active) application of the adhesive to demineralized dentin; methods that have shown higher bond strength values.[8,9] This could be attributed to the higher degree of monomer penetration into the decalcified dentin, and higher level of solvent/water evaporation before light polymerization, thereby improving the long-term resin–dentin bond strength.[10]

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Another way to improve the adhesive infiltration into the collagen network is by increasing the number of layers, leading to a considerable spread in the bond strength to dentin, and a decrease in nanoleakage values of etch-and-rinse adhesive systems.[11-14] The superior composition of the hybrid layer is capable of significantly improving bond properties.[15]

However, there is no consensus about how many layers are required to achieve good results. While some authors have indicated that only two layers are enough,[11,13,15] others have indicated that several layers lead to better results.[12,14] Apart from the above reports, no study has so far evaluated the *in vitro* effect of the number of layers of adhesive on the water-fluid flow within resin–dentin bonds after bonding procedures with etch-and-rinse adhesive systems. Multiple layerings may result in higher saturation of monomers and less residual solvent, which facilitates water evaporation[16] and subsequently, protection of the demineralized collagen.[17] Thus, spaces previously occupied by water after conditioning, can be replaced by the bonding agent upon application of multiple layers.

Based on the observations above, the purpose of this study was three-fold: 1) to evaluate the immediate resin/dentin bond strength after the application of several layers of an etch-and-rinse adhesive system; 2) to examine the nanoleakage pattern after application of several layers of an etch-and-rinse adhesive system; and 3) evaluate the adhesive permeability under the above-mentioned application modes. This study tested three null hypotheses: 1) the number of layers would not affect the bonding effectiveness of a two-step etch-and-rinse adhesive to dentin; 2) the number of layers would not affect the nanoleakage pattern of a two-step etch-and-rinse adhesive to dentin; and 3) the number of layers would not affect the permeability of a two-step etch-and-rinse adhesive to dentin.

MATERIAL AND METHODS

TOOTH SELECTION AND EXPERIMENTAL DESIGN

Thirty freshly extracted, non-carious human third molars were selected for the study. All teeth were cleaned and stored at 4ºC in a saturated solution of thymol in distilled water for up to 6 months before preparation and testing procedures. Several layers (1, 2 and 4) of an etch-and-rinse adhesive system (Adper Single Bond 2) were applied.

PREPARATION FOR MICROTENSILE BOND STRENGTH TESTING AND SILVER NITRATE UPTAKE

Fifteen (15) teeth were used for evaluating μTBS (n=5). A flat, superficial dentin surface was exposed on each tooth after wet grinding the occlusal enamel on #180-grit silicon-carbide (SiC) paper. The enamel-free, exposed dentin surfaces were further polished on wet #600-grit SiC paper for 30 seconds.[18]

The adhesive systems were applied according to the description in Table 1. After adhesive application, resin composite build-ups (Opallis) were placed on the bonded surfaces (3 increments of 1.5 mm each), that were individually light activated with a halogen light set at 600 mW/cm^2 (VIP) for 40 s each. All bonding procedures were carried out by a single operator in a room with controlled temperature and humidity (24 °C and 50% relative humidity).

After 24 h storage, they were longitudinally sectioned in both "x" and "y" directions across the bonded interface with a diamond saw in a precision cutter (Isomet 1000) under water cooling, at 300 rpm, to obtain bonded sticks with a cross-sectional area of approximately 0.8 mm^2. The number of prematurely debonded sticks (D) per tooth during specimen preparation was recorded. The cross-sectional area of each stick was measured with a digital caliper to the nearest 0.01 mm and recorded, to subsequently calculate the microtensile bond strength (Absolute Digimatic).

Table 1. Composition and application mode of adhesive systems used in this study.

Adhesive	Composition	Application mode		
		Acid etching	Coat applications	Air-dry and light-polymerization
Adper Single Bond 2 (3M/ESPE)	1 - 35% phosphoric acid 2 - Adhesive: Bis-GMA, HEMA, dimethacrylates, polyalknoic acid copolymer, initiators, water and ethanol	1 – Acid etching (15 s), rinsing (15 s) and air-drying (10 s) leaving dentin moist	2 – One Layer: Apply one layer with strong agitation; application for 10s; 2 - Two Layers: Apply two layers applied with strong agitation, each layer for 10s; 2 - Four Layers: Apply four layers applied with strong agitation; each layer for 10s;	3 – Air-dry (10 s at 20 cm); 4 - Light-activation (10 s - 600 mW/cm^2)

Abbreviations: BisGMA= 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy)]- phenyl propane; HEMA = 2-hydroxyethylmethacrylate.

At each storage time interval, each bonded stick was attached to a modified device for microtensile bond strength testing, with cyanoacrylate resin (Super-Bonder Gel), and subjected to a tensile force in a universal testing machine (Emic) at 0.5 mm/min. The failure modes were evaluated by stereomicroscope at 40× magnification (HMV-2) and classified as cohesive resin (failure exclusively within composite, CR), cohesive dentin (failure exclusively within dentin, CD), adhesive/mixed (failure at resin/dentin interface, A/M). Two specimens of each group were randomly selected for evaluation of the interface by scanning electron microscopy (TM 3030).

SILVER NITRATE UPTAKE: SCANNING ELECTRON MICROSCOPE

Two bonded sticks from each tooth - prepared for the μTBS - were used, so that a total of 10 bonded sticks were evaluated per group. The specimens were layered with two layers of nail varnish applied up to 1 mm from the bonded interfaces. The specimens were rehydrated in distilled water for 10 min before immersion in the tracer solution for 24 h. The sticks were placed in the ammoniacal silver nitrate in darkness for 24 h, rinsed thoroughly in distilled water and immersed in photo developing solution under a fluorescent light for 8 h, to reduce silver ions into silver metal grains within voids along the bonded interface.

All the sticks were wet-polished with 600 SiC paper to remove the nail varnish. The specimens were then placed inside an acrylic ring and embedded in epoxy resin. After the epoxy resin had set, the thickness of the embedded specimens was reduced to approximately half by grinding with silicon carbide papers under running water. The specimens were polished with 1000-grit SiC paper and 6, 3, 1 and 0.25 μm diamond paste (MetaDi) using a polishing cloth. They were ultrasonically cleaned, air dried, mounted on stubs and gold layered (MED 010). Resin-dentin interfaces were analysed by scanning electron microscope (SSX-500) operated in back-scattering electron mode, to identify silver nitrate penetration. The working distance was 10 mm and accelerating voltage (ACCV) was 15 kV with a small spot medium.

ADHESIVE PERMEABILITY MEASUREMENTS

Fifteen teeth were used in this part (n=5) of the study. Dentin disks were cut from crown segments, parallel to the occlusal surface at the top of the pulp chamber with a low-speed saw (Isomet 1000). Occlusal enamel was removed by making a second cut parallel to the dentin section, resulting in specimens approximately 1.3 mm thick. The specimens were reduced to a thickness of 1 mm with abrasive paper (# 600) to create a smooth, uniform surface on both sides. All polishing procedures were performed by the same operator who measured the thickness with an electronic digital caliper (Absolute Digimatic) accurate to within 0.03 mm.

The method used for measuring hydraulic conductance was derived from Gregóire *et al.*[18] Each crown-segment was connected to the Permeability machine (TTHD03) via polyethylene tubing under a constant physiological hydrostatic pressure (16.87 cm H_2O). A pressure gradient between the water reservoir and the specimen induced fluid movement through the specimen. The fluid movement rate was measured by following the displacement of a tiny air bubble that was introduced into a glass capillary located between the water reservoir and the specimen. Displacement of the air bubble was detected by digital caliper (Absolute Digimatic). The linear displacement was manually converted to fluid flow (μL min^{-1}) by using the Excel program. The fluid flow rate across dentin was measured twice, sequentially, as follows: 1) after the dentin surface was acid-etched with phosphoric acid gel for 15 s to determine the maximum baseline conductance; 2) After this, dentin disks were hybridized according to one of the bonding procedures described *(Table 1)* and the fluid flow rate across dentin was measured again (second measurement) three times, sequentially. For each specimen, the fluid flow (μL. min^{-1}) across the adhesive bonded dentin was expressed as a percentage of the maximum permeability derived from the acid-etched dentin (assigned as 100%). To compensate for the great variations in penetrating capacity of the various dentin specimens, all subsequent permeability measurements made on the same specimen were expressed as a percentage increase or decrease of the initial hydraulic permeability.[19] This allowed each specimen to serve as its own control, since the same surface area was used in all three measurements.

STATISTICAL ANALYSIS

The experimental unit in this study was the tooth. The mean microtensile bond strength (MPa) of all sticks from the same tooth was averaged for statistical purposes. The microtensile bond strength means for every test group was expressed as the average per group of the five teeth used. Before submitting the data to the appropriate statistical analysis, the Kolmogorov–Smirnov test was performed to assess whether they followed a normal distribution. The Barlett test for equality of variances was performed to determine if the assumption of equal variances was valid. After observing the normality of the data distribution and the equality of the variances, the microtensile bond strength (MPa) and adhesive permeability (%) data were subjected to a one-way analysis of variance (layers) and post-hoc test (Tukey test at α=0.05). The silver nitrate uptake was only evaluated qualitatively.

RESULTS

The percentage (%) of specimens with premature debonding and the frequency of each fracture pattern mode are shown in Table 2. Adhesive/Mixed failure was predominant as we observe in these results *(Figure 1)*.

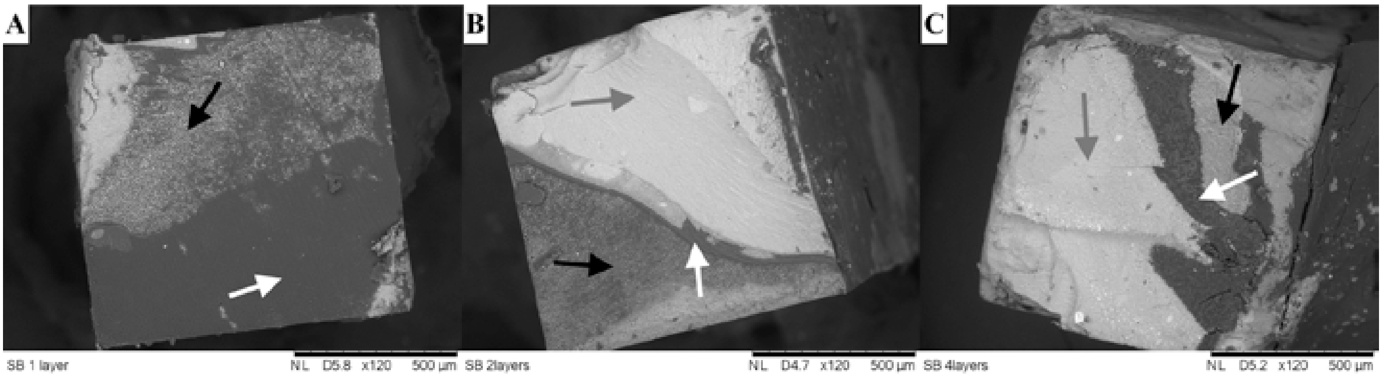

Figure 1: Representative SEM images of an adhesive/mixed failure of dentin side of fractured specimen. (A) One layer: Fractured specimen with a visualization of dentinal tubules (black arrow) and a thin layer of the adhesive system (white arrow). (B) Two layers: Dentinal tubules exposed (black arrow), a thicker layer of adhesive system (white arrow) and the presence of composite resin (gray arrow). (C) Four layers: (D) Adequate demineralization in the dentin, with visualization of some open dentinal tubules (black arrow), specimen shows a large amount of the adhesive layer (white arrow) and composite resin (gray arrow).

Table 2. Percentage of specimens (%) according fracture mode pattern

Adhesive	Number of layer								
	ONE			TWO			FOUR		
	A/M	CR	CD	A/M	CR	CD	A/M	CR	CD
Adper Single Bond 2	76%	16%	8 %	71,5 %	14,25%	14,25%	71%	16%	13%

Table 3 - Overall microtensile bond strength values and the respective standard deviations (MPa) obtained in each experimental condition, and statistical significance (*)

Adhesive	Number of layers		
	ONE	TWO	FOUR
Adper Single Bond 2	40.8 ± 3.0 B	63.2 ± 7.1 A	60.2 ± 3.8 A

(*) Groups with the same letter do not differ significantly (Tukey test, $p > 0.05$).

Table 4 - Overall dentin permeability values and the respective standard deviations (%) obtained in each experimental condition, and statistical significance (*)

Adhesive	Number of layers		
	ONE	TWO	FOUR
Adper Single Bond 2	79.4 ± 23.4 B	48.9 ± 24.2 A	44.9 ± 9.6 A

(*) Groups with the same letter do not differ significantly (Tukey test, $p > 0.05$).

MICROTENSILE BOND STRENGTH TEST

The results of μTBS values of all experimental conditions are shown in Table 3. One-way ANOVA showed different means, and the Tukey test revealed that groups TWO and FOUR layers showed higher μTBS than ONE layer ($p = 0.001$).

NANOLEAKAGE TEST

Representative SEM images at the resin-dentin interfaces for Adper Single Bond 2 are depicted in Figure 2. All experimental conditions showed a similar amount of silver nitrate uptake. There was silver nitrate uptake at the hybrid layer and in few dentin tubules in practically all specimens *(Figure 2)*. It was possible to see extended uniform adhesive layer thickness when more layers were applied, and more resin tags in dentin channels.

ADHESIVE PERMEABILITY MEASUREMENTS

Fluid conductance was expressed as percentages of the maximum permeability that occurred at baseline in the acid-etched dentin. The overall adhesive permeability (%) values of all experimental conditions are shown in Table 4. One-way ANOVA revealed statistically significant difference ($p < 0.05$). For the adhesive system, two or four layers showed lower adhesive permeability through dentin than one layer.

DISCUSSION

The bond to dentin depends on adequate resin monomer infiltration into demineralized dentin by the acid, providing high quality hybrid formation.[20] The number of layers of adhesive system appears to be a fundamental factor for this to occur, since the bond strength, nanoleakage and dentin permeability results vary with the increase in the number of layers of adhesive. Therefore, all the hypotheses were rejected.

The results of the study showed that several adhesive layers significantly affected bond strength to dentin. In agreement with these results, other studies have found that by using two different etch-and-rinse adhesive systems, resin-dentin bonds of additional quality may be obtained by following a multilayering technique.[15,20]

On the other hand, the lowest μTBS values were obtained when only one layer of the adhesive system tested was applied. A possible explanation for these results would be that the smaller thickness of the adhesive *(Figure 1)* has a more pronounced effect on inhibiting the adhesive polymerization by oxygen when a single layer is applied.[21] This hypothesis seems to have been confirmed by SEM of the samples with one layer, in which we observed silver nitrate within the adhesive system; and also by the high dentin permeability values in the condition of one layer *(Figure 2 and Table 4)*. Hashimoto *et al.*[12,13] observed a low bond strength and high nanoleakage at the bond interface when a single layer of adhesive instead of several layers was used. Even with the observation of a very thin layer on the one layer group *(Figure 1A)* and a thicker layer to the other groups *(Figure 1B and 1C)*, the fractographic analysis indicated a higher incidence of adhesive/mixed failure *(Table 2)*.

The increase in bond strength with the application of several layers of adhesive, (2 and 4) when compared with a single layer, is in agreement with the literature. Loguercio *et al.*[22] demonstrated that the use of several layers of an acetone-based adhesive increased the survival of restorations in teeth with non-carious cervical lesions.

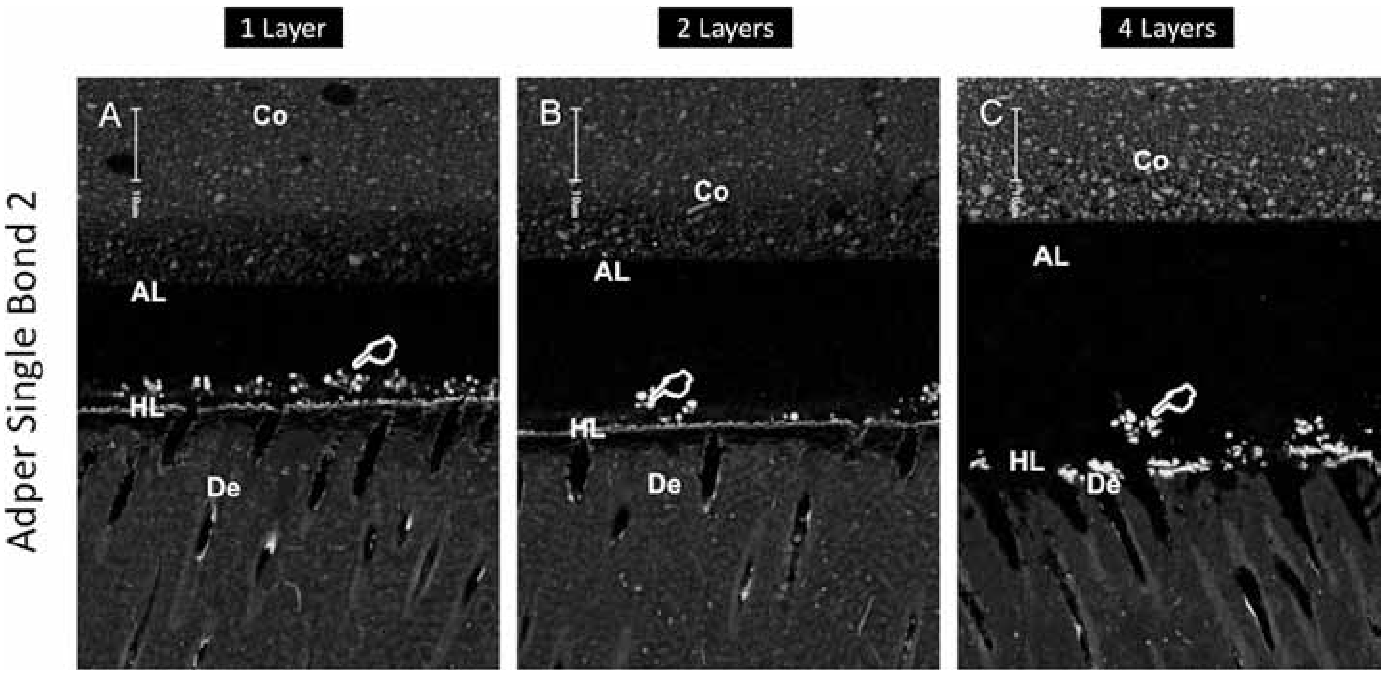

Figure 2: Representative SEM images of the resin-dentin interfaces bonded with Adper Single Bond 2 with one (A), two (B) and four (C) layers. The amount of silver penetration was similar between groups and occurred practically only in the hybrid layer (white hand). Observe that the adhesive layer was improved by increasing the number of layers (Co = composite; AL = adhesive layer; HL = hybrid layer and De = dentin)

There are two techniques for the application of several layers of etch-and-rinse adhesive systems that are as yet, not clear: 1) when they are polymerized separately, the goal is to increase the adhesive layer thickness, believing that this increase may protect the hybrid layer from the stress generated during composite polymerization;[23] 2) when polymerizing several layers in one operation, the goal is to potentiate infiltration of the adhesive into the collagen network and evaporation of the solvents, thereby increasing the degree of conversion of the adhesive system.[20]

The layers were applied consecutively and polymerized at the end, by using a methodology similar to that of Hashimoto *et al*.[20] who evaluated the application of 1 to 8 layers of a simplified conventional adhesive, without light polymerization between them. They obtained an increase in bond strength up to the number of 4 layers, but a decline when 6 or 8 layers were used. In our study, we found no statistically significant difference in the bond strength values between the applications of 2 to 4 layers.

Most manufactures suggest 2 layers for single bottle adhesives, because the first application allows monomer diffusion through demineralized dentin and the second removes resin bubbles and replaces monomer in the residual water spaces.[20] This is a favourable protocol, because conventional adhesives are more sensitive in monomer diffusion, to protect demineralized collagen.

Although the dentin permeability test showed the lowest values, when we applied up to 2 layers of adhesive, in no group was able to block the permeability of initially perfused deep human dentin in any of the groups. Due to their intrinsic hydrophilic characteristics,[2] these simplified etch-and-rinse adhesives behave as semi-permeable membranes, allowing fluid-flow movement across the adhesive interface.[24]

Adper Single Bond 2 is a HEMA-based, etch-and-rinse adhesive, whose preliminary etching step inevitably increases the permeability of dentin owing to removal of the smear layer and smear plugs.[13] In addition to this inherent disadvantage, the adhesive contains 13% polyalkenoic acid polymer in an ethanol-water solvent. Polyalkenoic acid copolymers have multiple pendant carboxylic acids along a linear backbone, which tend not only to bind water in the adhesive but also to preclude its penetration into interfibrillar spaces due to its high molecular weigh.[25]

The nanoleakage test demonstrated impregnation of silver nitrate in all the groups, and almost all occurred in the hybrid layer. However, the smallest quantity of silver nitrate infiltration occurred in the group in which we applied 2 layers of adhesive system *(Figure 2*).

These results suggested that 2 layers of these adhesives should be applied, but further researches should be encouraged to evaluate the bond durability of this technique in the long-term.

CONCLUSION

Our results in dentin, means that applying only one layer of the adhesive Adper Single Bond 2 should be avoided, because it showed lower μTBS and higher permeability values. Two or four layers showed favourable μTBS results: two layers demonstrated lower silver nitrate penetration and fewer clinical steps when compared with four layers.

MANUFACTURERS' DETAILS

- Adper Single Bond 2, 3M/ESPE, St. Paul, MN, USA
- Opallis, FGM, Joinville, SC, Brazil
- VIP, Bisco, Schaumburg, IL, USA
- Isomet 1000, Buehler, IL, USA
- Absolute Digimatic, Mitutoyo, Tokyo, Japan
- Super-Bonder Gel, Loctite, São Paulo, SP, Brazil
- Emic, São José dos Pinhais, PR, Brazil
- HMV-2, Shimadzu, Tokyo, Japan
- Hitachi TM 3030, Tokyo, Japan
- MetaDi, Buehler Ltd., Lake Bluff, IL, USA
- MED 010, Balzers Union, Balzers, Liechtenstein
- SSX-500, Shimadzu, Tokyo, Japan
- TTHD03, Odeme Prod. Odont. Ltda, Joaçaba, SC, Brazil

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