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Impact of Adhesive Application and Moisture on the Mechanical Properties of the Adhesive Interface Determined by the Nano-indentation Technique

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Clinical Relevance

The vigorous rubbing action of acetone and ethanol/water-based adhesives into dry demineralized dentin resulted in high nanohardness and Young's modulus in the hybrid layer, and moisture increased the nanohardness and Young's modulus of Adper Single Bond Plus in the adhesive layer.

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SUMMARY

Objectives: This study assessed the nanohardness (NH) and Young's modulus (YM) of resin-dentin bonding components formed by an ethanol/water-based (Adper Single Bond Plus [SBP]) and an acetone-based system (One Step Plus [OSP]) under different moisture conditions and application methods.

Material and Methods: On 24 human molars, a flat, superficial dentin surface was exposed by wet abrasion. After acid-etching, two coats of SBP or OSP adhesive were applied on either a dry or rewetted dentin surface under vigorous rubbing action or inactive application. After polymerization of the adhesives (600 mW/cm²/20 seconds), composite buildups were constructed incrementally and the specimens were stored in water (37°C/24 hours). They were cross-sectioned perpendicular to the resin-dentin interface to obtain 1.5 mm-thick slices that were embedded and polished before the test. Nano-indentations

were made on the resin composite, adhesive system, hybrid layer and mineralized dentin. The results of NH and YM (GPa) of the adhesive system and hybrid layer were analyzed using three-way repeated measures ANOVA and Tukey's multiple comparison tests (α=0.05).

Results: When the dentin was kept wet, the mode of application did not affect the studied properties within the hybrid layer. On the other hand, the vigorous application mode increased the NH and YM of both adhesives applied in air-dried dentin. In the adhesive layer, the highest NH and YM were observed only for SBP, especially when applied in wet dentin under vigorous action.

Conclusion: It was concluded that: 1) the vigorous application of both adhesives in dry dentin resulted in high nanohardness and Young's modulus values in the hybrid layer and 2) in the adhesive layer, the moisture associated with the vigorous application mode increased the nanohardness and Young's modulus values of Adper Single Bond Plus.

INTRODUCTION

It is common knowledge that etch-and-rinse adhesive systems require previous dentin demineralization with phosphoric acid, and the resulting demineralized dentin must be kept moist in order to maintain interfibrillar porosity for resin monomer infiltration.[1-2] Studies have shown that bonding to demineralized dentin in an air-dried condition results in an improper adhesive infiltration, with as much as one-half of the zone of demineralized dentin[3-4] due to a reduction in the permeability to resin monomers.[5] This condition has been reflected by low early resin-dentin bond strength.[6-7]

A common and widespread way to reverse such an undesirable condition is by maintaining the demineralized dentin fully hydrated before adhesive application. This technique has been referred to as *wet bonding* and has been used for more than 15 years. Moist demineralized dentin provides a more porous collagen network, and thus greater infiltration of adhesive monomers[5-8] occurs. On the other hand, managing an adequate degree of moisture for the different solvent-based adhesives[9] is not easily accomplished, and residual water is not likely to be completely removed prior to polymerization.[10-11] Under ideal conditions, as the adhesive is applied, the water within the collagen fibrils should evaporate to provide space for the formation of a highly cross-linked polymer entangled with the collagen fibrils. However, HEMA, which is a primary component in many simplified etch-and-rinse commercial adhesives, can dramatically reduce water evaporation[10-11] by reducing its percentage in the solution. Consequently, water entrapped within the collagen network might cause phase separation of hydrophilic and hydrophobic monomers[12] and thus reduce the mechanical properties of the adhesive layer[13-14] that compromises the resin-dentin bonds.

Thus, any attempt to produce an increased rate of water and solvent evaporation, along with monomer penetration, might turn the adhesive interface stronger and more durable. Recent studies have shown that early and long-term resin-dentin bond strength was significantly improved by vigorously rubbing two-step etch-and-rinse adhesives into both wet and dry demineralized dentin.[15-16] The authors attributed this finding to improvement in the mechanical properties of the polymer formed within the demineralized dentin. However, this hypothesis has not currently been evaluated. Therefore, this study evaluated the effects of the degree of moisture and the application mode on the nanohardness and Young's modulus of components of the resin-dentin interface by the nano-indentation technique. The null hypothesis tested in this study was that no significant difference will be observed in the mechanical properties of the hybrid and adhesive layer under the different conditions of moisture and application mode.

METHODS AND MATERIALS

Twenty-four extracted, caries-free human third molars were used. The teeth were collected after obtaining the patient's informed consent. The University Estadual de Ponta Grossa Institutional Review Board approved this study under protocol #06257/06. The molars were disinfected in 1% thymol, stored in distilled water and used within six months of extraction.

A flat, superficial dentin surface was exposed on each tooth after wet grinding the occlusal enamel on #180-grit SiC paper. The enamel-free, exposed dentin surfaces were further polished on wet #600-grit silicon carbide paper for 60 seconds to standardize the smear layer. Two different solvent-based etch-and-rinse adhesive systems were tested: Adper Single Bond Plus (SBP, 3M ESPE, St Paul, MN, USA), an ethanol/water-based

Table 1: *Composition and Batch Number of the Adhesive Systems Employed in This Investigation*

Adhesive Systems	Composition	Batch #
Adper Single Bond Plus	BisGMA, HEMA, dimethacrylates, ethanol, water, photoinitiator and a methacrylate functional copolymer of polyacrylic and polyitaconic acids and 10% by weight of 5 nanometer-diameter spherical silica particles.	7KH
One Step Plus	Biphenyl, dimethacrylate, hydroxyethyl methacrylate acetone, dental glass.	0700004208

system, and One-Step Plus (OSP, BISCO Inc, Schaumburg, IL, USA), an acetone-based system (Table 1). The acid etching was performed with the respective acids of the different adhesives. Contrary to the manufacturer's instructions, the surfaces were rinsed with distilled water for 15 seconds and air-dried for 30 seconds using oil-free compressed air to collapse the collagen fibers. The adhesives were applied onto the surface, which was either kept dry or rewetted for 10 seconds, using different amounts of distilled water (approximately 1.5 or 3.5 µl, for SBP and OSP, respectively).[9] The differences in the amount of water used for rewetting the dentin was due to differences in the vapor pressure and Hansen's solubility parameters from solvents of each adhesive system.[9] The adhesives were applied onto the dentin as follows:

1) *No rubbing action (NRA)*: In this group, the adhesive was only spread over the entire surface for approximately three seconds and left undisturbed for seven seconds. Then, an air stream was applied for 10 seconds at a distance of 20 cm.

2) *Vigorous rubbing action (VRA)*: The adhesive was rigorously agitated with strong finger pressure on the entire dentin surface for approximately 10 seconds. An air stream was applied for 10 seconds at a distance of 20 cm. Before performing the adhesive application, with the aim of improving standardization of the equivalent manual pressure that would be placed on the surface of the demineralized dentin, the operator was trained in the surface of an analytical balance (Mettler, type H6; Columbus, OH, USA). In this group, the pressure was equivalent to approximately 37.5 ± 7.9 g. After the operator determined the load in the manual balance, this procedure was repeated seven times and a mean ± standard deviation was calculated. This procedure was repeated at the beginning of every laboratory setting in order to ensure the operator's calibration.

In both groups, a second coat of the adhesive layer was applied in the same manner as the first layer. The time lapse between the start of the adhesive application and the light-curing step (Optilux Demetron 401, Kerr, CA, USA at 600 mW/cm^2) was approximately 40 seconds. The light curing was performed for the respective recommended time (10 seconds). Resin composite buildups (Z250, 3M ESPE) were placed on the bonded surfaces (1 mm increments), which were individually light activated for 30 seconds. All bonding procedures were carried out by a single operator at 24°C room temperature.

After storing the bonded teeth in distilled water for 24 hours at 37°C, the teeth were longitudinally sectioned in a mesio-to-distal direction across the bonded interface using a diamond saw in a Labcut 1010 machine (Extec Corp, Enfield, CT, USA) under water cooling at 300 rpm to obtain 1.5 mm-thick bonded slices. The bonded slice from the center of the tooth was selected for the nano-indentation technique.

The resin-bonded dentin slices (n=3 for each experimental condition) were individually embedded in a self-cure polyester resin (Milflex, Milflex Indústrias Químicas, São Bernardo do Campo, SP, Brazil) and, after 24 hours, the molds were manually polished via waterproof silicon carbide papers of decreasing abrasiveness (600, 1000, 1200, 1500 and 2000). The samples were then polished using soft discs with diamond suspensions (1 and 0.25 µm) in an automatic polishing device (Aropol S; Arotec, Cotia, SP, Brazil) at 300 rpm. The polishing debris from each silicon carbide paper and diamond paste were ultrasonically removed for five minutes, then again upon completion of the procedure. All samples were kept in the ultrasonic device for 20 minutes.

For the nano-indentation measurements, the computer-controlled Nano Indenter XP (TPS Systems Corp, Oak Ridge, TN, USA) was employed, mounted with a triangular pyramidal diamond indenter—Berkovich. By means of the computer-controlled X-Y table, the dried specimen was transferred to the indenter. An accurate calibration of the distance between the microscope and the indenter was run before testing to ensure a precise transfer of the pre-programmed positions to the indenter.

Prior to starting the measurement, two groups of nine equally spaced indentation positions were programmed for each region by a remote video control (connected to the light microscope attached to the nano-indenter device). In order to obtain precise measurements, the interval of each indentation was twice the size of the indentation in each region, with the aim of avoiding corruption of the abutment.[17]

In the central area of the resin composite and adhesive layer, the surface approach rate of the nano-indenter was set at 10 nm/seconds and the duration of the loading and unloading indentation was set at five seconds each. The pre-programmed distance among indentations for the dentin, adhesive layer and resin composite were 10 µm and 20 µm, respectively, with a load of 5g. At the hybrid layer, the surface approach rate was the same; however, the load employed was 0.1 g and the indentations (n=9) were programmed for a distance of 3 µm. The reduction in load was required to reduce the indentation size so that the indentations could be positioned entirely within the area of the hybrid layer.

Epoxy resin replicas of all specimens were gold coated and analyzed under Scanning Electron Microscopy (Shimadzu, Kyoto, Japan) to verify the indentation geometry and the accurate positioning of the pre-programmed indentations. Those found to be outside the specified areas were excluded from the sample. The

nanohardness and Young's modulus of each area were computed following the method by Oliver and Pharr.[18]

The nanohardness and Young's modulus data obtained in the adhesive and hybrid layer were subjected to a three-way repeated measures analysis of variance (Adhesive system vs Moisture vs Mode of application) and Tukey's test for contrast of the means (α=0.05). A single mean and standard deviation taken from all specimens was calculated for the resin composite and mineralized dentin.

RESULTS

The mean values and standard deviations for nanohardness (GPa) in the resin composite and mineralized dentin were 1.02 ± 0.07 and 0.69 ± 0.11, respectively. For Young's modulus of elasticity, the mean values and respective standard deviations (GPa) were 14.94 ± 0.67 and 17.94 ± 1.84 for the resin composite and mineralized dentin, respectively. The total number of measurements for the resin composite and mineralized dentin was 27 nano-identations for each experimental condition.

Hybrid Layer

The overall nanohardness and Young's modulus values for the experimental groups are depicted in Table 2. Three-way repeated measures ANOVA showed a significant effect for the interaction Adhesive System vs Moisture vs Mode of application for the hardness (p=0.004) and Young's modulus (p=0.005).

Both the hardness and the Young's modulus were generally higher for the adhesive One Step Plus. The mode of application did not affect the studied properties within the hybrid layer when the dentin was kept moist before the adhesive application. On the other hand, the vigorous application mode increased the nanohardness and Young's modulus of both adhesives applied in air-dried demineralized dentin; however, this increase was only statistically significant for One Step Plus.

Adhesive System

The overall nanohardness and Young's modulus values for the experimental groups are depicted in Table 3. Three-way repeated measures ANOVA showed a significant effect for the interaction Adhesive System vs Moisture vs Mode of application for the hardness (p=0.004) and Young's modulus (p=0.006).

Table 2: *Means and Standard Deviations of Nanohardness (GPa) and Young's Modulus (GPa) From the Hybrid Layer for All Experimental Conditions*

Hardness				
Adhesive	**Vigorous Rubbing Action**		**No Rubbing Action**	
	Wet Dentin	**Dry Dentin**	**Wet Dentin**	**Dry Dentin**
Adper Single Bond Plus	0.27 ± 0.10 c	0.37 ± 0.14 b,c	0.29 ± 0.15 c	0.29 ± 0.22 c
One Step Plus	0.45 ± 0.12 b	0.64 ± 0.27 a	0.45 ± 0.16 b	0.39 ± 0.23 b,c
Young's Modulus				
Adhesive	**Vigorous Rubbing Action**		**No Rubbing Action**	
	Wet Dentin	**Dry Dentin**	**Wet Dentin**	**Dry Dentin**
Adper Single Bond Plus	6.87 ± 2.0 C,D	7.38 ± 3.1 C	5.69 ± 1.6 D	6.85 ± 0.2 C,D
One Step Plus	10.9 ± 3.5 B	14.2 ± 5.2 A	9.91 ± 2.7 B	8.97 ± 5.9 B

Similar lowercase letters indicate means statistically similar (p>0.05) for hardness. Similar uppercase letters indicate means statistically similar (p>0.05) for Young's modulus.

Table 3: *Means and Standard Deviations of Nanohardness (GPa) and Young's Modulus (GPa) from the Adhesive Layer for All Experimental Conditions*

Hardness				
Adhesive	**Vigorous Rubbing Action**		**No Rubbing Action**	
	Wet Dentin	**Dry Dentin**	**Wet Dentin**	**Dry Dentin**
Adper Single Bond Plus	0.39 ± 0.04 a	0.32 ± 0.05 b	0.37 ± 0.08 a	0.37 ± 0.06 a
One Step Plus	0.29 ± 0.04 b,c	0.31 ± 0.06 b,c	0.30 ± 0.07 b,c	0.28 ± 0.04 c
Young's Modulus				
Adhesive	**Vigorous Rubbing Action**		**No Rubbing Action**	
	Wet Dentin	**Dry Dentin**	**Wet Dentin**	**Dry Dentin**
Adper Single Bond Plus	7.13 ± 0.80 A	6.07 ± 1.00 C,D	6.76 ± 1.01 A,B	6.38 ± 1.01 B,C
One Step Plus	5.72 ± 0.82 D	6.09 ± 1.30 B,C,D	6.55 ± 1.61 A,B,C	6.38 ± 1.11 B,C

Similar lowercase letters indicate means statistically similar (p>0.05) for hardness. Similar uppercase letters indicate means statistically similar (p>0.05) for Young's modulus.

Interestingly, different findings were observed in the adhesive layer compared to the hybrid layer substrate. In the adhesive layer, the highest nanohardness and Young's modulus were observed for Adper Single Bond Plus, especially when applied in moist dentin under vigorous agitation. For One Step Plus, the nanohardness and Young's modulus of all conditions were similar, except for the Young's modulus under moist and vigorous agitation conditions.

DISCUSSION

Evidence from the literature shows that, when demineralized dentin is air-dried, the water within the collagen matrix is removed and the collagen fibrils are brought into close contact. The collagen fibrils form weak interpeptide bonds that render the matrix shrunk, stiff[19-20] and practically impermeable to resin adhesives, reducing the infiltration rate of the bonding resin within the hybrid layer to approximately 50% when applied to dry instead of wet dentin.[3-4,21]

Previous studies have reported that the adverse effect of over-drying could be reversed by vigorously rubbing the adhesive on the dentin substrate.[15-16] This approach enabled achievement of high early and long-term resin-dentin bond strength even to air-dried demineralized dentin. It also demonstrated that the values obtained under vigorous application were much higher than the slight or inactive application.[15-16] In fact, the current study partially corroborates with previous investigators, as the highest values of hardness and Young's modulus were obtained under vigorous rubbing action in a dry dentin.

Dal-Bianco and others[15] and Reis and others[16] speculated that two factors could have been responsible for such an increase in bond strength values under the vigorous application method. The first is that an improvement in the rate of water/solvent evaporation might occur. It seems obvious that, by rubbing the adhesive, solvent/water molecules entrapped between monomers from the inner layers of the hybrid and adhesive layer could be brought to the surface, likely resulting in their breaking away from the neighboring molecules and, therefore, increasing the rate of evaporation. In addition, the rubbing action can also cause a slight increase in local temperature, and the resulting alteration on the kinetics energy of the molecules could contribute to the high evaporation rate.

The second factor is that the rubbing action could have increased diffusion into the demineralized dentin, which is known to be limited under slight or inactive application.[21-23] The current investigation does not agree with the first hypothesis raised by Dal-Bianco and others[15] and Reis and others.[16,24] It only agrees with the second. If higher water/solvent evaporation had occurred, one would expect high nanohardness and Young's modulus values at the adhesive layer when the adhesive was vigorously applied in the dry environment. This was not the case for both adhesives and led the authors of the current study to reject the hypothesis that significantly higher solvent/water evaporation occurs with vigorous application under clinical application conditions.

Also, contrary to what was expected, the application of adhesive in the dry substrate reduced nanohardness and Young's modulus of the adhesive layer formed with Adper Single Bond Plus under vigorous rubbing action. Exactly the opposite response was expected, due to previous literature findings that demonstrated that the degree of polymerization is negatively correlated with the amount of solvent presented in the adhesive,[24-26] meaning that the higher the amount of water/solvent, the lower the degree of polymerization. The behavior of One Step Plus was indifferent, meaning that, regardless of the mode of application or degree of substrate moisture, the nanohardness and Young's modulus was not affected.

This difference between these two materials must rely on dissimilar viscosity of both systems. There is a known solvent concentration at which maximum conversion is reached, more or less solvent than this amount can decrease monomer conversion,[27] which seems related to the viscosity of the adhesive film.[24] Although not measured in this study, it was visually evident that OSP is far more fluid than SBP. Thus, one can assume that the remaining water from the wet bonding technique could have been beneficial to the SBP by increasing the flowability of the adhesive, enlarging the mobility of the reactive components during polymerization and resulting in increased nanohardness values.

Opposite results were measured in the hybrid layer when compared to the adhesive layer. Referencing Table 2, one can observe that vigorous rubbing improved the nanohardness and Young's modulus of both adhesives, primarily under dry conditions. The mechanical pressure applied to the demineralized dentin surface during the rubbing action might have compressed the collagen network like a sponge, after which release created a sucking pressure, pulling the adhesive solution into the collapsed collagen mesh,[28] causing per se an increase in the measured properties of these adhesives within the hybrid layer due to better resin infiltration. However, this hypothesis should be confirmed by imaging methods, such as Scanning or Transmission Electron Microscope.

Another important feature taken from the current study is that the properties of the OSP adhesive inside the hybrid layer were higher than those obtained with SBP. However, by looking at the same properties in the adhesive layer, the opposite can be seen. The One Step

system had already demonstrated possessing lower ultimate tensile strength than Single Bond[29-30] when tested as bar-like specimens following the microtensile method. This could be attributed to the high proportion of solvent/monomer concentration that prevents the monomers from contacting to form a high cross-linking polymer.

The same reason that was responsible for the lower mechanical properties of OSP at the adhesive layer could be used to explain why the properties of this material increased significantly within the hybrid layer. Since this material contains more solvents and is less viscous, its penetration within the hybrid layer was probably higher than that of SBP. In fact, this was already confirmed by a previous micro-Raman spectroscopic study.[31] According to the authors, the contribution of Single Bond adhesive is lower than 50% throughout nearly half of the demineralized dentin. In contrast, the penetration of One Step adhesive was superior to 50% throughout most of the demineralized dentin layer.[31]

The current study focused on the early mechanical properties of the adhesive interface. No attempt was made to evaluate the mechanical properties of this interface over time. However, it is known from the literature findings that, if the resin is poorly infiltrated or if the resin slowly hydrolyzes and leaches from the hybrid layer, the intrinsic collagenolytic and gelatinolytic activity of the dentin matrix can be expressed and attack the collagen, causing it to solubilize.[32] This weakens the hybrid layer and shifts more functional stress to the remaining fibrils, causing them to defibrillate and enlarging the porosities within the hybrid layer.[32] At the same time, water can cause softening of the polymer network[33] either inside the hybrid layer or at the adhesive interface, deteriorating the properties of this interface in a likely different manner, which still deserves further evaluation.

The nano-indentation measurements made on the resin composite and mineralized dentin were made in order to contrast with the literature findings. Although the values of hardness and Young's modulus of both substrates vary among studies, they are within the range published by other authors.[34-37]

For more than 15 years, the wet bonding technique has been recommended for dentin bonding. The rationale behind this is, as long as dentin is kept fully hydrated, the dentin matrix does not collapse and free space is available for resin infiltration; otherwise, monomers would not infiltrate and low bond strength would be achieved. However, it seems that bonding can also be accomplished in air-dried demineralized dentin. This collagen collapse can be reversed by altering the method by which adhesives are applied to demineralized dentin substrates. The vigorous application mode can provide better infiltration, yielding an increase in nanohardness and Young's modulus of the hybrid layer, as demonstrated in the current investigation. This explains why previous investigations found high early and six-month bond strength values for simplified etch-and-rinse adhesives under dry dentin substrate and should therefore be used in clinical scenarios.[15-16]

CONCLUSIONS

Based on the results of the current investigation, the authors can conclude that the vigorous rubbing action of both adhesives in dry demineralized dentin resulted in high nanohardness and Young's modulus in the hybrid layer, and moisture increased the nanohardness and Young's modulus of Adper Single Bond Plus in the adhesive layer.

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