Influence of Differently Oriented Dentin Surfaces and the Regional Variation of Specimens on Adhesive Layer Thickness and Bond Strength

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ABSTRACT

Statement of the Problem: Adhesive systems can spread differently onto a substrate and, consequently, influence bonding.

Purpose: The purpose of this study was to evaluate the effect of differently oriented dentin surfaces and the regional variation of specimens on adhesive layer thickness and microtensile bond strength (MTBS).

Materials and Methods: Twenty-four molars were sectioned mesiodistally to expose flat buccal and lingual halves. Standardized drop volumes of adhesive systems (Single Bond [SB] and Prime & Bond 2.1 [PB2.1]) were applied to dentin according to the manufacturer's instructions. Teeth halves were randomly divided into groups: 1A-SB/parallel to gravity; 1B-SB/perpendicular to gravity; 2A-PB2.1/parallel to gravity; and 2B-PB2.1/perpendicular to gravity. The bonded assemblies were stored in 37°C distilled water for 24 hours and then sectioned to obtain dentin sticks (0.8 mm^2). The adhesive layer thickness was determined in a light microscope (×200), and after 48 hours the specimens were subjected to MTBS test. Data were analyzed by one-way and two-way analysis of variance and Student–Newman–Keuls tests.

Results: Mean values (MPa ± SD) of MTBS were: 39.1 ± 12.9 (1A); 32.9 ± 12.4 (1B); 52.9 ± 15.2 (2A); and 52.3 ± 16.5 (2B). The adhesive systems' thicknesses (µm ± SD) were: 11.2 ± 2.9 (1A); 18.1 ± 7.3 (1B); 4.2 ± 1.8 (2A); and 3.9 ± 1.3 (2B). No correlation between bond strength and adhesive layer thickness for both SB and PB2.1 ($r = -0.224$, $p = 0.112$ and $r = 0.099$, $p = 0.491$, respectively) was observed.

Conclusions: The differently oriented dentin surfaces and the regional variation of specimens on the adhesive layer thickness are material-dependent. These variables do not influence the adhesive systems' bond strength to dentin.

CLINICAL SIGNIFICANCE

Adhesive systems have different viscosities and spread differently onto a substrate, influencing the bond strength and also the adhesive layer thickness. Adhesive thickness does not influence dentin bond strength, but it may impair adequate solvent evaporation, polymer conversion, and may also determine water sorption and adhesive degradation over time. In the literature, many studies have shown that the adhesive layer is a permeable membrane and can fail over time

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because ofits continuous plasticizing and degradation when in contact with water. Therefore, avoiding thick adhesive layers may minimize these problems and provide long-term success for adhesive restorations.

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INTRODUCTION

The success of adhesion procedures depends on the adequate infiltration of monomers into the demineralized collagen network, providing a hybrid layer formation that avoids restoration dislodgement and sealing the tooth structure.[1,2] However, because of the structural complexity of dentin,[3] such as variations on permeability[4] and tubule orientation,[5–8] adhesion to this substrate is still a limiting factor on the long-term stability of adhesive restorations.[3,9,10] Notwithstanding, other factors such as etching agent[2,11–13] and proper etching time[12,14] may also influence adequate hybrid layer formation and bond strength.

Most dentin adhesive studies are performed on coronal dentin sectioned perpendicularly to the teeth long axis, where tubules are exposed perpendicularly to the surface. However, few studies use buccal and lingual dentin halves as substrate, where tubular orientation varies from parallel to oblique, with respect to the long axis of the tubules. Moreover, there is no consensus about the correlation between tubule orientation and hybrid layer formation[6–8] or bond strength.[7] On coronal dentin sectioned perpendicularly to the tooth long axis, there is more intertubular than peritubular dentin to form the hybrid layer.[1,4,6,9] On the other hand, on buccal and lingual dentin surfaces or on dentin sectioned mesiodistally to the tooth long axis, more peritubular dentin is exposed, proportionally to that found on perpendicularly sectioned dentin. Throughout a cavity wall, adhesion to dentin may be provided according to its morphology and chemical/mineral composition,[15] as adhesion depends on these parameters.[16] Thus, once peritubular and intertubular dentins are structurally and chemically different, it seems that etching and monomer infiltration would be also different between them,[3,17] thus perhaps influencing adhesion.

On the brief adhesive clinical application time, the gravitational effect may also influence adhesion on the cavity walls according to the tooth position,[12] if perpendicular or parallel to the gravitational force. Some authors[18] studied theoretically the effect of gravity on the rough solid-liquid and have shown that the apparent contact angle formed can be raised by gravity. Once the contact angle[16] and the adhesive viscosity[19] are relevant parameters for wettability and adhesion,[20] one can argue how adhesion would be if a less viscous adhesive were applied on a surface submitted to the effect of gravity.

Adhesive viscosity depends on the chemical composition of adhesives and greatly influences the wettability of the adhesive system on the substrate.[21,22] It has been shown that the higher the viscosity of an adhesive, the more difficult it is to wet a substrate.[19] If an adhesive is spread rapidly over a cavity wall, it may form an adhesive layer with a variable thickness and may accumulate on the internal angles of the cavity before polymerization. Consequently, the formation of a variable adhesive layer thickness may decrease the resin composite polymerization stress relief[23,24] and also the adhesive solvent evaporation. This provides monomers' phase separation and creation of porosities on the adhesive.[25]

There is no consensus about the correlation between bond strength and adhesive layer thickness. Some authors[26] reported that there is no correlation when using an acetone-based adhesive, but others[25] noted that there is an inverse correlation when using an ethanol/water-based adhesive system.

The aim of this study was to evaluate the effect of differently oriented dentin surfaces and the regional variation of specimens on adhesive layer thickness and bond strength. The null hypotheses were: (1) there is no difference in bond strength despite material and dentin orientation, (2) for each material there is no difference in bond strength despite the regional variation of specimens, (3) there is no difference in adhesive layer thickness despite the material and dentin orientation, (4) for each material there is no difference in adhesive layer thickness despite the regional variation of specimens, and (5) there is no correlation between bond strength and adhesive layer thickness despite the material used.

MATERIALS AND METHODS

This study was approved by the Ethical Committee of Bauru School of Dentistry—University of São Paulo (Bauru, São Paulo, Brazil). Twenty-four extracted caries-free human molars were selected and stored in 0.1% thymol solution. The teeth were sectioned in a mesiodistal direction with a slow-speed water-cooled diamond saw in a section machine (Labcut 1010, Extec Corp., Enfield, CT, USA) to expose flat buccal and lingual dentin halves. The dentin surfaces were polished with #600 silicon carbide abrasive paper to create a standard smear layer. Each buccal (or lingual) half of each tooth was divided into one of the four groups: according to the restorative treatment and to the different dentin orientation (parallel or perpendicular to gravity), simulating a possible inclination of the teeth during dental treatment. Although this study did not test groups without the earth's gravitational pull, the authors studied the effect of gravitational force on the spreading of adhesive systems applied on supported (perpendicular) and unsupported (parallel) dentin surfaces. So the authors adopted the terms "perpendicular" and "parallel" to refer to this phenomenon in the text.

In group 1A, buccal (or lingual) halves were mounted parallel to the direction of gravity. This was performed using a protractor and a base with wax to make teeth stable. A 35% phosphoric acid gel was applied for 15 seconds to the dentin surfaces, washed away, and dried with absorbent paper. Subsequently, two consecutive layers (3.5 µL each) of Single Bond (SB) (3M-ESPE Dental Products, St. Paul, MN, USA) were applied to the substrate using a microbrush. Solvent evaporation was facilitated using gentle air-drying for 5 seconds and, after 30 seconds, light-cured for 10 seconds, according to the manufacturer's instructions.

In group 1B, buccal (or lingual) halves were mounted perpendicular to the direction of gravity. This was performed using a protractor and a base with wax to make teeth stable. Restorative procedures were the same as for group 1A.

In group 2A, teeth halves were mounted as described for group 1A. After 15 seconds of 37% phosphoric acid gel etching, the dentin was washed and gently dried. Prime & Bond 2.1 (PB2.1) (Dentsply Ind. e Com. Ltda, Petrópolis, RJ, Brazil) was applied to the substrate (3.5 µL) using a microbrush and, after 30 seconds, solvent evaporation was promoted by gentle air-drying for 5 seconds and light-cured for 10 seconds. Another coat (3.5 µL) of PB2.1 was applied, dried, and light-cured according to the manufacturer's instructions.

In group 2B, teeth halves were mounted as described for group 1B, and the restorative procedures were the same as for group 2A.

Adhesive systems volumes were standardized for all groups by means of a micropipette

(Pipetman, Gilson Medical Electronics S.A., Villiers-le-Bel, France). Resin composite Z250 (3M-ESPE Dental Products) was built up incrementally to a height of 5 mm to ensure sufficient bulk for the microtensile bond strength (MTBS) test. Each increment of resin composite was light-cured for 20 seconds. A curing unit (3M Curing Light XL 1500, 3M-ESPE Dental model 5518AA, Toronto, ON, Canada) with 500 mW/cm^2 power density was used for light-curing, being periodically monitored by a radiometer (Curing radiometer, Model 100P/N-150503, Demetron Research Corp., Danbury, CT, USA).

Teeth halves were stored in 37°C distilled water for 24 hours. After that, the resin-bonded halves were serially sectioned in both "x" and "y" directions across the bonded interface with a diamond saw in a section machine (Labcut 1010) to obtain sticks with a cross-sectional area of approximately 0.8 mm.[2,27] The adhesive layer thickness of the sticks was determined in transmission mode by a digitized image analyzer attached to a microscope (Olympus BX50F4, Olympus Optical Co. Ltd., Tokyo, Japan) at 200× magnification. For each experimental group, MTBS and adhesive thickness data were recorded according to the regional variation of specimens (occlusal or cervical thirds). For each stick, 16 images were taken along the adhesive interface (four images for each one of the four specimens' surfaces). Forty-eight hours after adhesion procedures, the sticks were attached to a Bencor Multi-T apparatus (Danville Engineering, Danville, CA, USA), with a cyanoacrylate adhesive (Zapit, Dental Ventures of America, Corona, CA, USA), and the MTBS was measured in a testing machine (EMIC, Equipamentos e Sistemas de Ensaio Ltda., São José dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm/min. After testing, the fracture mode of each specimen was determined by examination under a dissecting microscope at 40× magnification (DF Vasconcellos S.A., São Paulo, SP, Brazil).

Statistical Analysis

Specimens were analyzed according to their regional variation (cervical or occlusal thirds) in teeth halves for both bond strength and adhesive layer thickness. One- and two-way ANOVA and Student–Newman–Keuls tests were performed to determine the differences in MTBS and adhesive layer thickness among groups. For each group, separately, the data (MTBS and adhesive thickness) were statistically analyzed (one-way ANOVA) according to the regional variation of specimens (occlusal or cervical thirds). Then, the data for each study group (dentin orientation and material) were analyzed together (two-way ANOVA), despite the regional variation of specimens.

The correlation between the variables (bond strength and adhesive layer thickness) was compared with Pearson product moment correlation. Statistical significance was defined as $p < 0.05$. Statistical calculations were done using SigmaStat (version 2.03, Jandel Scientif Software, Chicago, IL, USA).

RESULTS

One-way ANOVA results showed that there were differences in MTBS among different subgroups ($p < 0.05$). However, results of the multiple comparison test indicated that there was no difference in the bond strengths of occlusal versus cervical dentin in each of the orientation–dentin combinations ($p > 0.05$) (Table 1).

The regional variation of specimens significantly influenced the adhesive thickness only for group 1B, being greater for the cervical third, followed by the occlusal one (Table 1). Group 1B showed the higher adhesive layer thickness, followed by group 1A ($p < 0.05$). Although having the greater bond strength values, both groups 2A and 2B showed the lowest adhesive layer thickness ($p < 0.05$). No differences were observed in adhesive layer measurements between the PB2.1 groups ($p > 0.05$).

TABLE 1. MEAN VALUES OF BOND STRENGTH AND ADHESIVE LAYER THICKNESS ACCORDING TO THE REGIONAL VARIATION OF SPECIMENS FOR EACH SEPARATE GROUP.

Groups		Microtensile Bond Strength (MPa) Mean ± SD	Adhesive Thickness (µm) Mean ± SD
(1A) SB parallel	Cervical	39.4 ± 13.6[a]	11.8 ± 2.4[a]
	Occlusal	39.1 ± 13.1[a]	11.1 ± 3.1[a]
(1B) SB perpendicular	Cervical	29.4 ± 11.9[b]	21.2 ± 7.1[b]
	Occlusal	35.6 ± 12.5[b]	16.2 ± 6.7[c]
(2A) PB2.1 parallel	Cervical	56.2 ± 12.6[c]	3.5 ± 2.1[d]
	Occlusal	50.9 ± 16.7[c]	4.6 ± 1.5[d]
(2B) PB2.1 perpendicular	Cervical	56.8 ± 11.7[d]	4.1 ± 1.2[e]
	Occlusal	50.4 ± 18.2[d]	3.8 ± 1.4[e]

SB = Single Bond; PB2.1 = Prime & Bond 2.1.
Same superscript letters indicate no statistically significant difference ($p > 0.05$).

TABLE 2. MEAN VALUES OF MTBS AND ADHESIVE LAYER THICKNESS MEASUREMENTS.

Groups	MTBS (MPa) Mean ± SD (*N*)	Adhesive Thickness (µm) Mean ± SD (*N*)
(1A) SB parallel	39.1 ± 12.9 (23)[a]	11.2 ± 2.9 (23)[a]
(1B) SB perpendicular	32.9 ± 12.4 (28)[a]	18.1 ± 7.3 (28)[b]
(2A) PB2.1 parallel	52.9 ± 15.2 (26)[b]	4.2 ± 1.8 (26)[c]
(2B) PB2.1 perpendicular	52.3 ± 16.5 (24)[b]	3.8 ± 1.3 (24)[c]

SB = Single Bond; PB2.1 = Prime & Bond 2.1; *N* = number of specimens.
Same superscript letters indicate no statistically significant difference ($p > 0.05$).

TABLE 3. NUMBER OF SPECIMENS (*N*) FOR EACH GROUP AND FRACTURE MODES.

Groups	Fracture Mode: Adhesive/Mixed	Cohesive Resin	Cohesive Dentin
(1A) SB parallel	23	5	1
(1B) SB perpendicular	28	4	1
(2A) PB2.1 parallel	26	7	0
(2B) PB2.1 perpendicular	24	7	0

SB = Single Bond; PB2.1 = Prime & Bond 2.1.

Data from occlusal and cervical thirds of each group were mixed, and the results of adhesive layer thickness measurements and MTBS are shown in Table 2. The highest bond strengths were found in groups 2A and 2B. These groups showed statistically higher values than groups 1A and 1B ($p < 0.05$). Groups with the same adhesive systems presented no statistically significant differences on the bond strength between them.

The fracture mode of tested groups is shown in Table 3. The adhesive/mixed fracture mode was observed in most specimens. Specimens with a resin or dentin cohesive mode were not used for statistical analyses.

There was no correlation between bond strength and adhesive layer thickness for both SB and PB2.1 (Pearson product moment correlation; $r = -0.224$, $p = 0.112$ and $r = 0.099$, $p = 0.491$, respectively) (Figures 1 and 2).

DISCUSSION

Some factors such as dentin permeability,[4] tubule orientation,[5–8] etching agent,[2,9,12,13] and proper etching time[12,14] are mentioned as relevant to influence the adequate hybrid layer formation and bond strength. However, the chemical composition of the adhesive systems and their

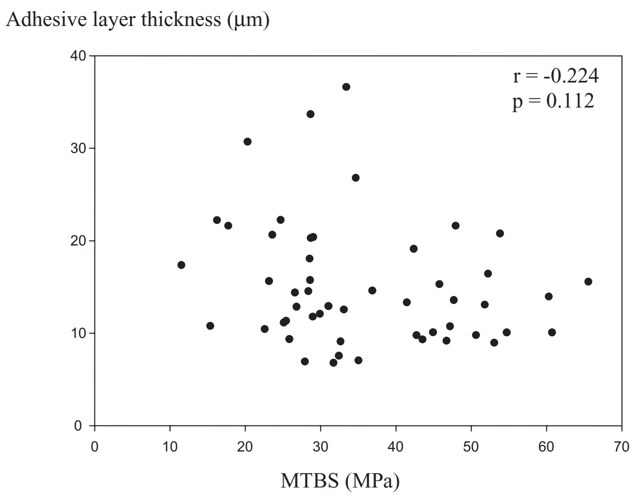

Figure 1. Scatter plot of all individual values of the correlation between microtensile bond strength (MTBS) and adhesive layer thickness of Single Bond.

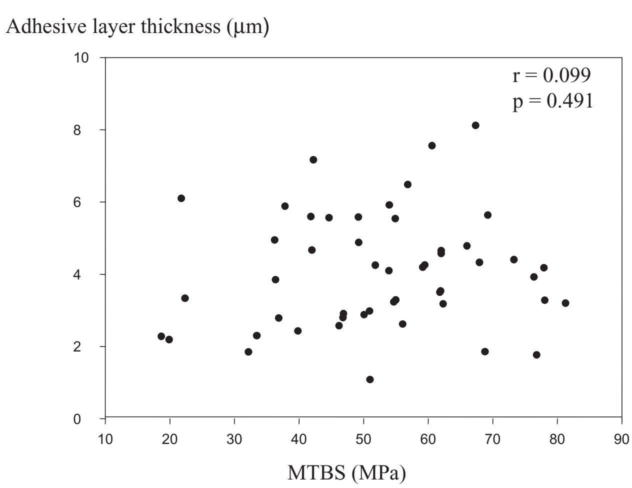

Figure 2. Scatter plot of all individual values of the correlation between microtensile bond strength (MTBS) and adhesive layer thickness of Prime & Bond 2.1.

wettability, viscosity,[19–22,28] and elastic modulus[23,24,29] can also influence adhesion.

In the present study, the area for adhesion on buccal and lingual halves showed mostly parallel and oblique cut dentin tubules, as reported by other authors.[30] Although these tubule orientations provide adhesion on both peritubular and intertubular dentin, which have different etching characteristics,[11,17] they did not influence the bond strength values compared with other studies that were performed on perpendicular dentin tubules using SB,[7,25,27,31] or PB2.1.[32,33] Notwithstanding, there is no literature consensus about the correlation between bond strength and dentin tubule orientations.[6–8,12]

The regional variation of specimens (cervical or occlusal thirds) did not influence dentin bond strength (Table 1), as also mentioned by others authors,[34] when using both conventional and self-etching adhesive systems.

The different chemical composition of adhesive systems can greatly influence their ability to wet and infiltrate demineralized dentin. This is because of the heterogeneous chemical composition altering the viscosity, surface tension, and contact angle of adhesive systems,[21,22,28] which are relevant parameters to provide adequate adhesion.[20]

Some authors[12] suggested that a low-viscosity adhesive can spread out of a cervical cavity because of the gravitational effect, thus providing less contact between adhesive and dentin. As adequate contact angle[16] and proper contact time[14] between the adherent and adhesive is necessary to provide adhesion, we hypothesized that adhesive systems with different characteristics (e.g., viscosity) could yield different bond strengths when applied on dentin surfaces parallel or perpendicular to gravity. This was observed in the present study, but it was influenced by the different adhesive systems and not by the different dentin orientations (parallel or perpendicular). SB is a viscous adhesive, probably because of the presence of the viscous monomer BISGMA[21] and the low percentage of solvent in its composition (31% of ethanol/water), compared with PB2.1 (81% of acetone).[31]

According to an interesting study,[19] the viscosity of adhesives impairs their ability to wet a substrate. Additionally, the vapor pressures of ethanol and water are lower (43.9 and 17.5 mmHg, respectively)[35] than the vapor pressure of acetone (200 mmHg).[27] The presence of water in SB and the 30 seconds of infiltration time indicated by the manufacturer may not provide enough time to evaporate the entire solvent and residual water from the demineralized dentin, thus affecting the adhesion procedure and yielding lower bond strength results compared with PB2.1. Acetone is known as having a tendency to wet properly the demineralized dentin and to greatly evaporate residual water.[36] Despite these properties, the results of SB bond strength in the present study corroborate the data reported in other studies.[7,13,25,27,31]

The great percentage of acetone solvent in PB2.1 provides a thin adhesive layer and can create cracks because of solvent evaporation.[26] One could speculate that the 81% of acetone in this adhesive system may have quickly evaporated from the substrate, and yielded the irregular superficial appearance under light microscopy. However, the possible presence of cracks on the adhesive layer did not seem to occur in the present study as high bond strength values for both acetone-based groups were found (Table 2).

Even though the same total volume (7 μL) of adhesive system was dispensed in the microbrush, significant differences between SB and PB2.1 adhesive layer thicknesses were observed, despite the differently oriented dentin surfaces. The PB2.1 adhesive layer thickness was significantly thinner than in the SB groups (Table 2). This may be explained by the different vapor pressures of the solvents (ethanol/water—SB and acetone—PB2.1).[37] Probably, almost all the acetone evaporated in the 30 seconds prior to light-curing, resulting in the thinning of the adhesive layer, thus contributing to reaching higher bond strength results. This does not apply to the SB adhesive layer, according to its lower solvents vapor pressures and its greater viscosity.

There was no influence of dentin orientation on the adhesive layer thickness of groups 2A and 2B. Actually, it was expected that PB2.1 could spread differently over an inclined surface. Probably, the rapid acetone evaporation and its tendency to properly wet the dentin surface[36] provided similar adhesive layer formation for groups 2A and 2B. On the other hand, as expected, higher thickness of SB layers was observed when it was applied to the dentin perpendicularly to gravity (group 1B) than to dentin parallel to gravity (group 1A). A recent study showed lower spreading velocity for SB than for PB2.1,[38] suggesting that these materials spread differently.

Interestingly, different adhesive layer thickness was found on occlusal and cervical thirds only for group 1B (Table 1). The greater results were noted for the cervical third, but could not be clearly justified as the SB was applied to the dentin perpendicularly to gravity, giving no additional chance for the adhesive to spread over.

Some authors suggested 20 µm as the minimum ideal thickness for the adhesive layer to avoid polymerization inhibition by the oxygen.[39] In the present study, the average adhesive thicknesses for both PB2.1 and SB were lower than that suggested "minimum ideal" thickness, and the lower adhesive layer thickness found in the acetone-based groups (2A and 2B) provided the higher bond strength results. According to some authors,[40] some adhesives may have their inhibited adhesive layer "removed" when a resin composite is applied over them. This might occur because the resin composite may dislodge or absorb the residual monomers from the adhesive layer.[40] Also, it was suggested that the heat generated from the light-curing device during the resin composite photopolymerization might convert some monomers in polymers.[39]

Other studies[25,29] also showed thin adhesive layer thickness. SB in a thin layer (<7.5 µm) provided similar bond strengths as higher thickness (between 7.5 and 25 µm and between 25 and 50 µm).[25] When applied in one or two coats, SB can reach 5- and 10-µm thicknesses, respectively.[29]

The Pearson product moment correlation test did not show a correlation between adhesive layer thickness and bond strength to dentin, despite the adhesive system used. An interesting study[25] showed no correlation between ethanol/water-based adhesive layer thickness and bond strength when the adhesive layer is 7.5- to 50-µm thick. However, when the authors applied a thicker adhesive layer (50–430 µm), an inverse correlation with bond strength was observed. On the other hand, authors[26] have found no correlation between experimental acetone-based adhesive thickness and bond strength to dentin. Notwithstanding, those authors noted that lower acetone percentage yielded a thinner adhesive layer. These observations indicate that, despite adhesive layer thickness, adhesive systems may properly wet demineralized dentin and yield high bond strengths.

In the literature, adhesive layer thickness is correlated to resin composite stress relief.[24] Thick adhesive layer of a high-elastic-modulus adhesive system yields good resin composite stress relief,[23,24] as does a thin adhesive layer of a low-elastic-modulus adhesive system.[24] Even though it was mentioned that an ideal adhesive layer thickness should be between 50 and 150 µm[24] to provide adequate stress relief, the present study has found thin adhesive layers and high dentin bond strength values. This could be explained because we used flat dentin surfaces for adhesion, and those recommendations were based on cavity designs by means of finite element analyses. Probably, thin adhesive layers might have different findings when correlated to the influence of the C-factor.[4]

As there was no correlation between adhesive layer thickness and bond strength, the authors suggest the application of a minimum adhesive layer to avoid inadequate solvent evaporation,[25] polymers conversion,[41] and chemical degradation after water sorption by the hydrophilic monomers.[42,43] Additional studies should be carried out to evaluate hybrid layer quality under the same variables of the present study.

In accordance with the hypotheses tested in this study, and based on the results found here, we accept the null hypotheses 2 and 5 and reject the null hypotheses 1, 3, and 4.

CONCLUSIONS

SB yielded thicker adhesive layers than PB2.1, despite the dentin orientation (parallel or perpendicular) to the gravitational force. However, the groups with thinner adhesive layers (2A and 2B) showed higher bond strengths than the others (1A and 1B). The regional variation of specimens did not influence bond strength results, but significantly influenced the adhesive thickness for group 1B.

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