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Adhesive Temperature: Effects on Adhesive Properties and Resin-Dentin Bond Strength

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Clinical Relevance

Preheating ethanol/water-based adhesive systems could be useful to improve the hybrid layer quality.

SUMMARY

Objectives: To evaluate the effect of adhesive temperature on the resin-dentin bond strength (µTBS), nanoleakage (NL), adhesive layer thickness (AL), and degree of conversion (DC) of ethanol/water- (SB) and acetone-based (PB) etch-and-rinse adhesive systems.

Methods: The bottles of the two adhesives were kept at each temperature (5°C, 20°C, 37°C, and 50°C) for 2 hours before application to demineralized dentin surfaces of 40 molars. Specimens were prepared for µTBS testing. Bonded sticks (0.8 mm^2) were tested under tension (0.5 mm/min). Three bonded sticks from each tooth were immersed in silver nitrate and analyzed by scanning electron microscopy. The DC of the adhesives was evaluated by Fourier transformed infrared spectroscopy.

Results: Lower µTBS was observed for PB at 50°C. For SB, the µTBS values were similar for all temperatures. DC was higher at 50°C for PB. Higher NL and thicker AL were observed for both adhesives in the 5°C and 20°C groups

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compared to the 37°C and 50°C groups. The higher temperatures (37°C or 50°C) reduced the number of pores within the adhesive layer of both adhesive systems.

Conclusions: **It could be useful to use an ethanol/water-based adhesive at 37°C or 50°C and an acetone-based adhesive at 37°C to improve adhesive performance.**

INTRODUCTION

The temperature of adhesive systems can alter some of the properties of monomer solutions, such as viscosity and degree of conversion, which are important bond effectiveness parameters.[1,2] The spreading velocity of etch-and-rinse adhesive systems,[3] as well as the vapor pressures of solvent,[4] can be influenced by temperature. Therefore, the adhesive temperature might play a role in the adhesive performance.

Although most manufacturers currently recommend storing adhesive materials at room temperature, many dentists still use the traditional practice of refrigerating materials to extend their shelf life.[5] It has been shown that when materials are taken from the refrigerator and used immediately, without allowing time for them to reach room temperature, their efficacy is reduced.[6,7] In this refrigerated condition, the overall conversion of monomers is lowered [8,9] and the adhesive viscosity increased,[2] which might reduce the penetration of adhesives into acid-etched dentin, resulting in a decrease in resin-dentin bond strength. Refrigeration might have further consequences, as it also affects solvent vapor pressure inhibiting the evaporation of the solvents from the adhesive layer.[10]

As opposed to this, temperatures around 40°C can be reached in places close to ovens, when exposed to direct sunlight or even in warm areas or seasons.[7] Several studies have demonstrated lower resin-dentin bond strength when ambient temperatures were increased to a level simulating intraoral conditions.[11,12] However, one cannot rule out the benefits of increased temperature on both radical and monomer mobility, resulting in higher overall monomer conversion[8,9] and lower viscosity.[13]

Several studies have observed that composite prewarming to around 54–60°C enhanced the maximal polymerization rate and overall monomer conversion of resin composites.[13-15] With increased temperature, free radicals and propagating polymer chains become more flexible as a result of decreased viscosity and react to a greater extent, resulting in a more complete polymerization reaction and greater crosslinking. This increase in polymerization may lead to improved mechanical properties.[14–16]

To the extent of our knowledge, the effects of temperature have been evaluated mainly for composites and self-etch adhesives.[6,7,17,18] Therefore, the aim of this *in vitro* study was to evaluate the effect of different temperatures on resin-dentin bond strength and nanoleakage of two-step etch-and-rinse adhesive systems. In addition, the degree of conversion and adhesive layer thickness were also evaluated.

MATERIALS AND METHODS

Forty extracted, caries-free human third molars were used. The teeth were collected after obtaining the patients' informed consent. The University Review Board approved this study. Teeth were disinfected in 1% thymol, stored in distilled water, and used within 6 months after extraction. A flat and superficial dentin surface was exposed on each tooth by wet grinding the occlusal enamel with number 180-grit silicon-carbide paper. The enamel-free, exposed dentin surfaces were further polished with wet number 600-grit silicon-carbide paper for 60 seconds to standardize the smear layer. Teeth were then rinsed with tap water before the bonding protocol.

Bonding and Restorative Procedure

Two etch-and-rinse adhesive systems having different solvents were tested: Adper Single Bond (SB; 3M ESPE, St. Paul, MN, USA), an ethanol/water-based system, and Prime&Bond 2.1 (PB; Dentsply De Trey, Konztanz, Germany), an acetone-based system (Table 1). Forty teeth were divided into eight groups according to the temperature to be tested. One bottle of each adhesive was stored in the refrigerator at 5°C, and the others were kept in the oven (incubator) at 20°C, 37°C, or 50°C for 2 hours before starting the adhesive procedure. One bottle of each adhesive system from the same batch number was used for each temperature. The temperature of the bottles of adhesive was checked with a thermometer before the restorative procedure. The surfaces were acid etched with the respective etchants of each adhesive system (Table 1) and rinsed off, and the dentin was left slightly moist before adhesive application. The adhesives Prime&Bond 2.1 (PB) and Adper Single Bond (SB) were removed from the oven or refrigerator and immediately applied according to the manufacturer's instructions. In all these groups, adhesives were light polymerized (VIP, Bisco,

Table 1: *Adhesive systems: Composition, groups, and application modes*

Adhesive Systems	Composition	Adhesive Temperature	Application Mode[a]
Prime&Bond 2.1 (Dentsply De Trey), batch no. 139207B	1. Caulk Tooth Conditioner Gel 34% phosphoric acid 2. Adhesive—UDMA, Bis-GMA, PENTA, butylated hydroxytoluene, 4-ethyl dimethyl aminobenzoate, cetilamine hydrofluoride, initiator, and acetone	5°C	a, b, c, d1, e, f, g
		20°C	
		37°C	
		50°C	
Adper Single Bond (3M ESPE), batch no. 2GM	1. Scotchbond Etchant: 37% phosphoric acid 2. Adhesive—Bis-GMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, initiators, water, and ethanol	5°C	a, b, c, d2, e, f, g
		20°C	
		37°C	
		50°C	

Abbreviations: UDMA, urethane dimethacrylate; Bis-GMA, bisphenol A diglycidyl methacrylate; PENTA, dipentaerythritol pentacrylate monophosphate; HEMA, 2-hydroxyethyl methacrylate.

[a] *a, acid-etch (15 s); b, rinse (15 s); c, air-dry (30 s); d1, dentin rewetted with water (3.5 μL for PB), 60 s; d2, dentin rewetted with water (1.5 μL for SB), 60 s; e, one coat of adhesive; f, air-dry for 10 s at 20 cm; g, light polymerization (10 s, 600 mW/cm^2).*

Schaumburg, IL, USA; 600 mW/cm^2) for the respective recommended time (10 seconds). Resin composite buildups (Opallis, shade A3.5; Batch number 090609; FGM, Joinville, SC, Brazil) were placed on the bonded surfaces (three increments of 1.5 mm each) and were individually light activated for 40 seconds each. All bonding procedures were carried out by a single operator at 24°C and 50% relative humidity. Five teeth were used for each experimental group. The adhesive bottles were removed from the oven or refrigerator only for as long as it took to remove two drops of the adhesive for application on the surface of each tooth. After this, the bottle was immediately put back into the oven/refrigerator.

Storage Time and Sticks Preparation

After storing the bonded teeth in distilled water at 37°C for 24 hours, they were longitudinally sectioned in both "x" and "y" directions across the bonded interface with a diamond saw in a Labcut 1010 machine (Extec Corp, Enfield, CT, USA), 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 the digital caliper to the nearest 0.01 mm and recorded for subsequent calculation of the microtensile bond strength (μTBS) (Absolute Digimatic, Mitutoyo, Tokyo, Japan). Approximately half the bonded sticks originating from the same teeth were randomly selected (Excel 2003, Microsoft Office software, Redmond, WA, USA) and used for microtensile testing. Another three sticks from each tooth were used for nanoleakage evaluation.

Microtensile Bond Strength Test (μTBS)

Each bonded stick was attached to a μTBS testing device with cyanoacrylate resin (Zapit, Dental Ventures of North America, Corona, CA, USA) and subjected to a tensile force in a universal test machine (Emic, São José dos Pinhais, PR, Brazil) at a speed of 0.5 mm/min. The failure modes were evaluated at 400× magnification (HMV-2, Shimadzu, Tokyo, Japan) and classified as cohesive (failure exclusive within dentin or composite, C), adhesive (failure at the resin/dentin interface, A), or adhesive/mixed (failure at the resin/dentin interface, which included cohesive failure of the neighboring substrates, A/M).

Nanoleakage Test (NL)

For each experimental condition, three bonded sticks per tooth from a total of five teeth were coated with two layers of nail varnish applied up to within 1 mm of the bonded interfaces. The specimens were rehydrated in distilled water for 10 minutes prior to immersion in the tracer solution for 24 hours. Ammoniacal silver nitrate was prepared according to the protocol previously described by Tay and others.[19] The sticks were placed in the ammoniacal silver nitrate in darkness for 24 hours, rinsed thoroughly in distilled water, and immersed in photo developing solution for 8 hours under a fluorescent light to reduce silver ions into metallic silver grains within voids along the bonded interface.

All sticks were wet polished with 600-grit SiC paper to remove the nail varnish. After this, the specimens were placed inside an acrylic ring that was attached to a double-sided adhesive tape 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. Specimens were polished with a 1000-grit SiC paper and 6, 3, 1, and 0.25 μm diamond paste (Buehler Ltd, Lake Bluff, IL, USA) using a polishing cloth. They were ultrasonically cleaned, air dried, mounted on stubs, and gold-carbon coated (MED 010, Balzers Union, Balzers, Liechtenstein). Resin-dentin interfaces were analyzed by means of a field-emission scanning electron microscope operated in the backscattered electron mode (LEO 435 VP, LEO Electron Microscopy Ltd, Cambridge, UK). The working distance was 10 mm and the accelerating voltage (ACCV) 15 Kv.

Three pictures were taken of each specimen. The first picture was taken in the center of the stick. The other two were taken 0.3 mm to the left and right of the first picture. As three sticks per tooth were evaluated and a total of five teeth were used for each experimental condition, 45 images were evaluated for each group. They were all taken by a technician who was blinded to the experimental conditions under evaluation. The relative percentage of SNU within the adhesive and hybrid layer areas was measured in all pictures using the UTHSCSA ImageTool 3.0 software (Department of Dental Diagnostic Science at the University of Texas Health Science Center, San Antonio, TX, USA) by an author blind to the test and control samples. First, the total area of the adhesive layer plus the hybrid layer was recorded. Then the area occupied by the silver nitrate deposits was delineated by a software tool and summed, and the relative ratio between the total area vs the impregnated areas was calculated to give the percentage of SNU within each specific bonding interface.

Adhesive Layer Thickness Measurement (AL)

Digital images were taken in three distinct regions of the same bonded stick used in SNU under the same 330× magnification. The adhesive layer thickness was measured using the image analysis software Corel Photo-Paint 8 (Corel Corp Ltd Dublin, Ireland) in three different regions of each picture, and the values obtained were averaged for statistical purposes.

Degree of Conversion (DC)

One drop (10 μL) of adhesive solution at each tested temperature was individually placed between acetate strips (8 mm × 8 mm) to achieve a thin film of approximately 8 mm in diameter. Before covering the drops of adhesive with the upper acetate strips, they were gently air-dried with a dry stream (10 seconds) to allow the solvent to evaporate. A Fourier transformed infrared (FTIR) spectrum of the unpolymerized material was recorded, and then the specimens were photoactivated for 10 seconds. Each specimen was carefully removed with a narrow surgical knife and stored for 24 hours in a dark, dry environment until the FTIR analysis of the DC (FTIR-8300, Shimadzu) was performed. The spectrum was obtained with 32 scans at 1 cm^{-1} resolution by a transmission method. The percentage of unreacted carbon–carbon double bonds (%C=C) was determined from the ratio of absorbance intensities of aliphatic C=C (peak height at 1640 cm^{-1}) against an internal standard before and after polymerizing the specimen. The aromatic carbon–carbon bond (peak height at 1610 cm^{-1}) absorbance was used as an internal standard. The DC was determined by subtracting the %C=C from 100%. Three specimens were tested for each group.

Statistical Analysis

The mean μTBS of all sticks from the same tooth was used for statistical purposes. The prematurely debonded specimens were included in the mean value for the tooth. The mean value attributed to specimens that failed prematurely during preparation was arbitrary and corresponded to approximately half the minimum bond strength value that could be measured in this study (~7.8 MPa).[20,21] The μTBS for every tested group was expressed as the mean of the five teeth used per group and expressed in MPa. For SNU (%) and AL (μm), the mean SNU and AL of all pictures taken of sticks that originated from the same tooth (three sticks per tooth, three pictures for stick) were averaged for statistical purposes. The SNU and AL of every test group were expressed as

Table 2: *Number and percentage of specimens (%) according to fracture pattern mode and the prematurely debonded specimens from each experimental condition*

Adhesive/ Temperature	**Prime&Bond 2.1**			**Adper Single Bond**		
	A/M	***C***	**PDS**	**A/M**	***C***	**PDS**
5°C	51 (85)	4 (6.7)	5 (8.3)	49 (80.3)	5 (8.2)	7 (11.5)
20°C	35 (67.3)	8 (15.4)	9 (17.3)	32 (51.6)	7 (11.3)	23 (37.1)
37°C	48 (82.8)	0 (0)	10 (17.2)	54 (84.4)	9 (14.1)	1 (1.5)
50°C	66 (89.2)	0 (0)	8 (10.8)	59 (83.1)	7 (9.9)	5 (7.0)

Abbreviations: A/M, adhesive/mixed fracture mode; C, cohesive fracture mode; PDS, prematurely debonded specimens.

the mean of the five teeth used per group and reported as a percentage.

Before submitting the data to analysis using the appropriate statistical test, the Kolmogorov–Smirnov test was performed to assess whether the data followed a normal distribution, and the Bartlett 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, all data were submitted to appropriate data analysis.

The μTBS (MPa), SNU (%), DC (%), and adhesive layer thickness (μm) data were subjected to a one-way analysis of variance for each adhesive, and a *post hoc* test (Tukey test at α=0.05) was used for pairwise comparisons.

RESULTS

The mean cross-sectional area ranged from 0.78 to 1.02 mm^2, and no difference was detected among the groups ($p>0.05$). The percentage of specimens with premature debonding specimens (PDS) and the frequency of each fracture pattern mode are shown in Table 2. The data showed equality of variances and normality (data not shown).

The overall μTBS and DC values for SB and PB are shown in Tables 3 and 4, respectively. No statistically significant difference was observed for SB in terms of μTBS or DC ($p>0.05$). For PB, the temperature was statistically significant for μTBS and DC ($p<0.05$). Lower μTBS and higher DC values were observed for PB at the higher temperature (50°C) ($p<0.05$). The overall SNU values for SB and

Table 3: *Overall microtensile resin-dentin bond strength values and the respective standard deviations (MPa) obtained under each experimental condition, as well as the statistical significance*[a]

Temperature	**Adhesive**	
	Prime&Bond 2.1	**Adper Single Bond**
5°C	38.5 ± 8.9 A	36.8 ± 6.2 a
20°C	39.6 ± 9.1 A	39.5 ± 3.8 a
37°C	43.5 ± 7.1 A	42.3 ± 4.7 a
50°C	25.8 ± 2.9 B	44.5 ± 3.8 a

[a] *Groups with same upper- or lowercase letter are not significantly different (Tukey test,* $p>0.05$*).*

Table 4: *Overall degree of conversion values and respective standard deviations (%) obtained under each experimental condition, as well as the statistical significance*[a]

Temperature	**Adhesive**	
	Prime&Bond 2.1	**Adper Single Bond**
5°C	37.9 ± 2.4 A,B	46.1 ± 3.2 a
20°C	30.8 ± 4.6 B	45.3 ± 2.9 a
37°C	37.9 ± 2.0 A,B	50.9 ± 1.5 a
50°C	38.3 ± 4.2 A	45.4 ± 3.7 a

[a] *Groups with same upper- or lowercase letter are not significantly different (Tukey test,* $p > 0.05$*).*

Table 5: *Overall silver nitrate uptake values and the respective standard deviations (%) obtained under each experimental condition, as well as the statistical significance*[a]

Temperature	Adhesive	
	Prime&Bond 2.1	Adper Single Bond
5°C	25.2 ± 5.1 B	29.5 ± 4.3 c
20°C	33.5 ± 5.4 C	34.2 ± 5.7 d
37°C	20.3 ± 3.2 A	18.3 ± 3.7 b
50°C	21.3 ± 2.2 A,B	13.5 ± 2.3 a

[a] *Groups with same upper- or lowercase letter are not significantly different (Tukey test,* p *> 0.05).*

PB are shown in Table 5. For both adhesive systems, a significant difference was observed ($p<0.05$). Lower SNU values were observed for SB at the higher temperature (50°C) ($p<0.05$) and for PB at 37°C and 50°C ($p<0.05$). For both adhesives systems, higher SNU values were found at 20°C ($p<0.05$).

Representative SEM images at the resin-dentin interfaces for the experimental conditions are shown in Figures 1 and 2. For both adhesive systems, one can observe that a higher amount of silver nitrate penetration occurred at the colder (5°C and 20°C) compared to the hotter (37°C and 50°C) temperatures. For the 5°C and 20°C groups, the silver nitrate uptake occurred mainly in the hybrid layer and in the adhesive layer. A lower amount of silver nitrate uptake can be seen in the 37 and 50°C groups.

The overall thickness of all values for SB and PB are shown in Table 6. Representative SEM images for PB are shown in Figure 3. A significant difference was detected for SB and PB ($p>0.05$). The colder solutions of SB and PB (5 and 20°C) formed adhesive layers with mean thickness values similar to each other and with higher thickness values than those formed by the hotter solutions (37°C and 50°C) ($p<0.05$). The lowest adhesive layer thickness was observed when the adhesive solutions were at the hottest temperature (50°C; $p<0.05$).

DISCUSSION

The results of present study showed that the number of PDS varied according to the experimental condition tested. Unfortunately, much of the literature has neglected the amount of PDS often recorded by μTBS testing, and this is a problem associated with this type of test.[22] The correct handling of samples that failed before they could be tested is still open to debate. However, it is generally accepted that PDS cannot just be omitted, as this will cause a bias toward a higher μTBS. Undoubtedly, when a high number of PDS are obtained and ignored, the material performance may be misleading.

Premature failures have been associated with lower quality of adhesion.[22] According to recent suggestions,[23] researchers may assign a bond-strength value of 0 MPa or a predetermined value to each PDS as, for example, the mean between lowest μTBS measured and zero.[20,21] In the present investigation, we opted to assign a predetermined bond strength value, as the actual bond strength values of the PDS are not known.

Another potential problem with the microtensile bond strength test is that each of these multiple specimens is often treated as an independent specimen statistically.[22,24] According to Neter and others,[25] an experimental unit is the smallest subunit of the experiment in which two different treatments can be applied. When the microtensile method is the test of choice, the several bonded sticks originating from one single tooth usually receive the same treatment; therefore, they cannot be considered separate experimental units. Nevertheless, assuming independence of sticks overstates the statistical significance levels for comparisons between materials.[26,27] This is the reason why the bond strength values of all sticks originating from the same tooth were averaged for statistical purposes.

The results of this study showed no significant difference in terms of microtensile bond strength, degree of conversion, and adhesive layer thickness between the refrigerated temperature (5°C) and the room temperature (20°C). In other words, this means that it does not matter if the refrigerated product is used soon after its removal from the refrigerator or after it reaches room temperature. However, it is worth pointing out that there is no consensus among authors on this matter. There are some studies demonstrating that cold temperatures can reduce the efficacy of adhesive systems,[6,7] while others show opposite findings.[5,28]

Differences in the adhesive system composition could explain this controversy. For instance, the studies that reported that refrigerated and at-room temperature adhesives can reach similar bonding efficacy evaluated etch-and-rinse systems.[5,28] The rationale behind etch-and-rinse materials being less

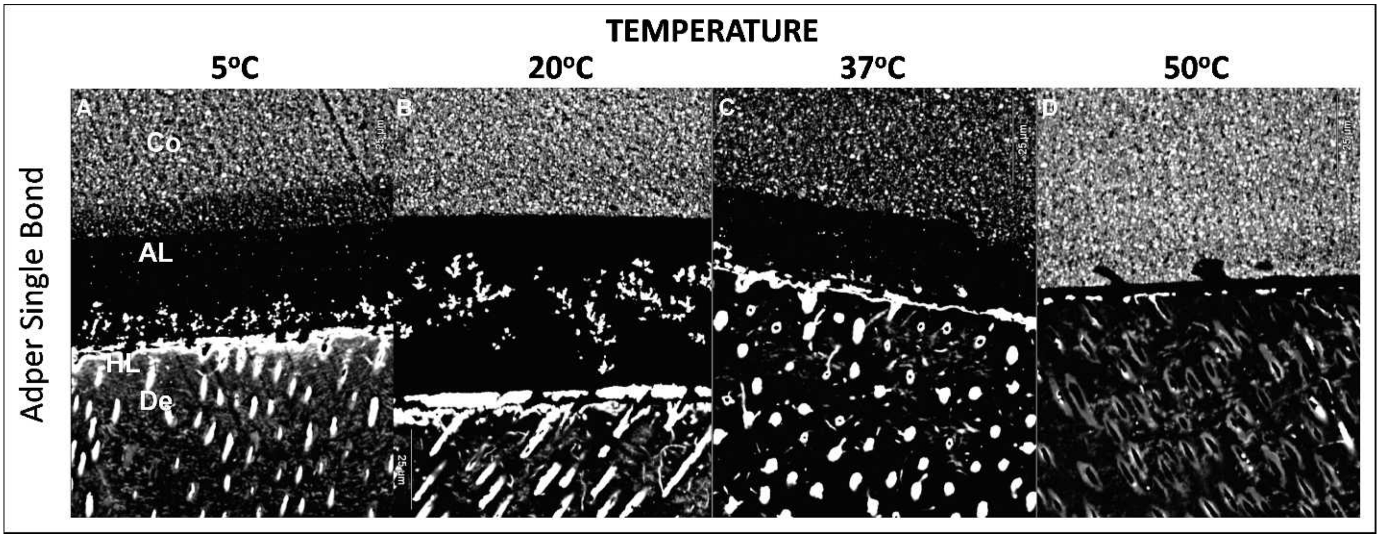

Figure 1. *Representative backscattered SEM images (200×) of the resin-dentin interfaces bonded with Adper Single Bond (A to D) applied at different temperatures. The amount of silver penetration in figures A and B was higher, and it occurred throughout the entire thickness of the HL and in several areas of the adhesive layer. In Figures C and D, lower silver nitrate uptake can be seen in the HL, mainly in the 50°C group. Observe that the AL formed at 5°C and 20°C is thicker than in the 37°C and 50°C groups. Co, composite; AL, adhesive layer; HL, hybrid layer; De, dentin.*

sensitive to temperature variations, mainly in the cold range, should be a matter of further investigation.

Under low temperature conditions (5°C-20°C), the viscosity of adhesive systems increases considerably.[1,2,5] It has been shown that the higher the viscosity of an adhesive, the more difficult is the substrate wetting[29] because the spreading velocity of the material is rather reduced.[3] This theoretically affects the diffusion of solvated comonomers into the acid-etched demineralized dentin matrix. Interestingly, this does not seem to be the case with the range of cold temperatures evaluated in the present study, as no significant difference in μTBS values and no difference in silver uptake were observed in the cold temperature groups (5°C and 20°C). Another consequence of using adhesives at low temperatures is that the adhesive layer formed tends to be thicker and more variable, as could be observed in the present investigation.

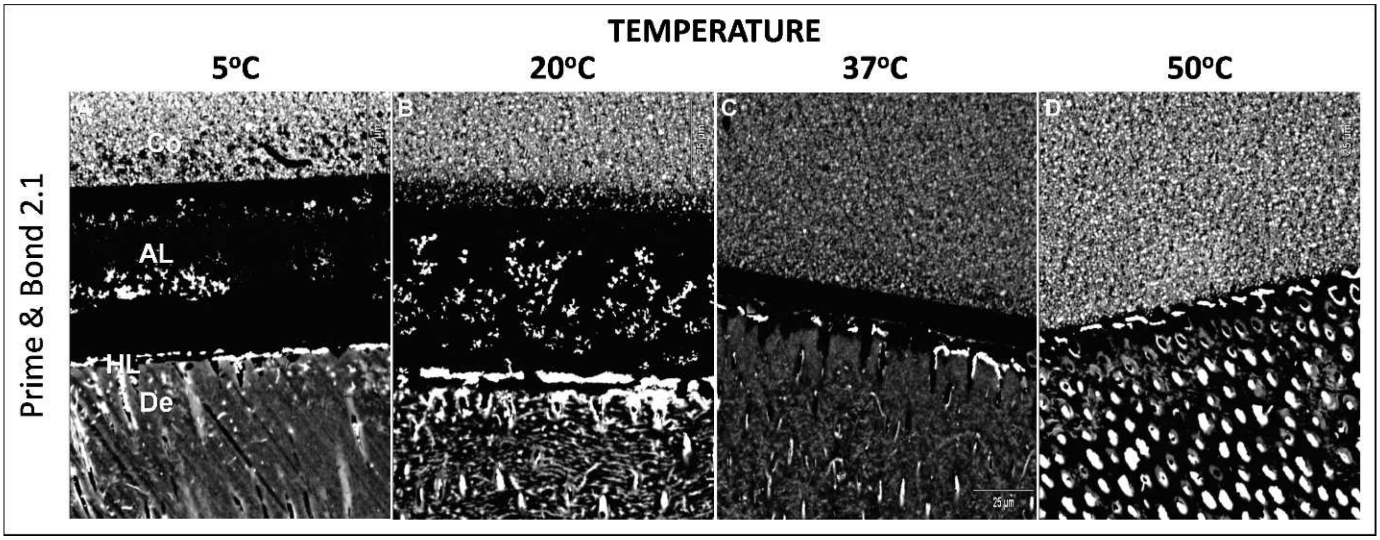

Figure 2. *Representative backscattered SEM images (200×) of the resin-dentin interfaces bonded with Prime&Bond 2.1 applied at different temperatures. It can be seen that at low temperatures (Figures A and B), the amount of silver nitrate penetration was higher and occurred in the HL and in the entire AL thickness. In Figures C and D, lower silver nitrate uptake can be seen in the HL. Observe that the AL formed at 5°C and 20°C is thicker than in the 37°C and 50°C groups. Co, composite; AL, adhesive layer; HL, hybrid layer; De, dentin.*

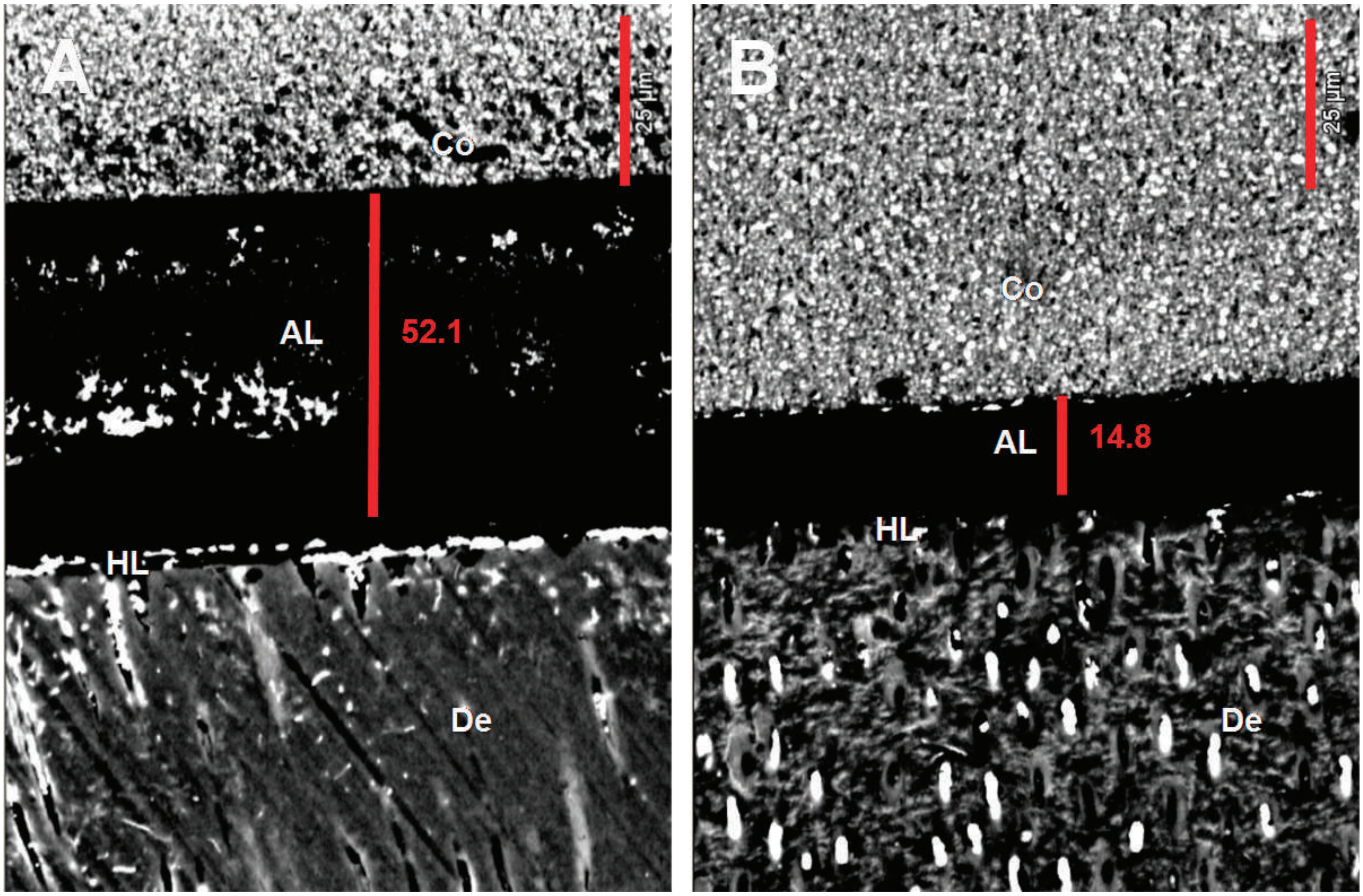

Figure 3. *Representative backscattered SEM images (200×) of the resin-dentin interfaces bonded with Prime&Bond 2.1 applied at 5°C (A) and 37°C (B). The thickness of AL is thicker in Figure A (52.1 µm) than in B (14.8). Co, composite; AL, adhesive layer; HL, hybrid layer; De, dentin.*

Another reported consequence of refrigerating polymer-based materials, such as adhesive systems, is that low temperatures may lower the vapor pressure of the solvents present in their composition, inhibiting their evaporation from the adhesive layer.[4,10] Moreover, the kinetic energy of the monomer molecules is reduced at low temperatures, reducing their chances of collision during the polymerization reaction. Although these two factors might theoretically affect the degree of conversion of the adhesive layer to a certain extent, the present study failed to prove this experimentally. No significant difference in the degree of conversion was observed when the temperatures of 5°C, 20°C, and 37°C of both adhesives were compared with one another.

On the other hand, by comparing the cold temperature groups with the hot groups, we could clearly note that the increase in temperature reduced the amount of silver deposits in both the hybrid layer (classic nanoleakage[30]) and the adhesive layer (water trees[31]) and lowered the adhesive layer thickness, with this being much more evident in the 50°C group. According to Tay and Pashley,[31] water trees in dentin adhesives, together with nanoleakage within the hybrid layers, represent water-rich interfacial regions from which the leaching of hydrophilic resin components may readily occur, indicating signs of potential degradation of the interfacial bond in the future. The regions of silver uptake probably represent areas of suboptimal conversion within the polymer matrix due to incomplete solvent removal.[32]

These regions of incomplete monomer penetration result in regions of incomplete polymerization and/or hydrogel formation. These regions may permit higher diffusional water fluxes within the hybrid layers that could accelerate water sorption and the extraction of unpolymerized or degraded monomers.[31,33]

By increasing the temperature, the flowability of the polymeric materials is also increased,[13] most likely causing an improvement in their penetration

Table 6: *Overall thickness of adhesive layer and respective standard deviations (%) obtained under each experimental condition, as well as the statistical significance*[a]

Temperature	**Adhesive**	
	Prime&Bond 2.1	**Adper Single Bond**
5°C	51.5 ± 6.4 A	54.3 ± 5.3 a
20°C	48.9 ± 7.4 A	51.2 ± 6.3 a
37°C	24.3 ± 4.6 B	34.5 ± 5.4 b
50°C	7.1 ± 3.3 C	19.2 ± 3.3 c

[a] *Groups with same upper- or lowercase letter are not significantly different (Tukey test,* $p > 0.05$*).*

into the acid-etched dentin and increasing their spreading velocity.[3] Furthermore, when a substance is heated, energy comes in and can be used to increase the kinetic energy of the molecules, causing an increase in temperature, or the heat can be used to increase the potential energy of the molecules, causing a change in the state.[34] One could hypothesize that under the conditions of the study, the intrinsic adhesive heat could have altered the manner in which molecules bond to one another. Consequently, this increased the evaporation rate of solvents from bonding interface, allowing the achievement of better hybrid layer formation with thin adhesive layer formation, as observed in the present investigation. This was also enhanced when a warm airstream was used for solvent evaporation of the adhesive systems.[35]

However, the adhesives did not respond homogeneously to the temperature increase. Although a numerical increase in the resin-dentin bond strengths was observed for SB, the acetone-based adhesive system presented a significant reduction in the bond strength values when the adhesive solution was at 50°C. Furthermore, the thickness of the adhesive layer of PB at this temperature was approximately one-third of that measured for SB. This may be explained by the faster evaporation rate of acetone at high temperatures. Acetone has a lower boiling temperature (56.5°C) and a higher vapor pressure (200 mm Hg) when compared with ethanol (78.3°C/43.9 mm Hg) and water (100°C/17.5 mm Hg).[4] This means that when adhesive systems are heated to 50°C, faster solvent evaporation might occur with acetone-based systems because this temperature is very close to the boiling temperature of acetone (56.5°C). Some authors have suggested that PB contains a higher volume of acetone (52–81 wt%[4,36]), and this explains the thinnest adhesive layer formed with this group. Under the conditions in the other groups, acetone could not evaporate completely, making room for the formation of a thicker adhesive layer.[37]

The thinner the adhesive layer, the more susceptible it is to polymerization inhibition by oxygen. Some authors suggested that 20 μm was the minimum adhesive thickness to avoid polymerization inhibition by the oxygen.[38] In the present study, PB at the hottest temperature (50°C) presented an adhesive layer of only 7 μm, which, according to the previous authors, could have been partially inhibited by oxygen.

Unfortunately, there is no consensus about the ideal minimum thickness for the adhesive layer. Some authors have observed that the average adhesive thickness was lower than the suggested "ideal minimum"; however, this did not prevent the adhesives from reaching high bond strength results.[39-42,41] The real benefit of thin or thick adhesive layers for bonding layers is still a controversial issue and deserves future investigations.

Clearly, the ability of an adhesive to spread without becoming overly thin, such as with acetone at the highest temperature, or overly viscous, such as at cold temperatures for both adhesive systems, is very important during resin bonding.[43] The results of this study indicated that the increase in temperature (37°C-50°C) of the ethanol/water two-step etch-and-rinse adhesive system can be a useful tool to improve the quality of the resin-dentin bonds, as a thin adhesive layer was obtained and lower silver nitrate uptake occurred. For the acetone two-step etch-and-rinse adhesive system bottle, the heating can be done only with temperatures up to 37°C. Further studies are still required to evaluate the effects of the adhesive temperature on the long-term resin-dentin bonds.

CONCLUSIONS

The use of an ethanol/water-based adhesive at 37°C or 50°C and an acetone-based adhesive at 37°C could be useful to improve the quality of the hybrid layer since it might reduce the number of pores within the adhesive layer. The increase of temperature did not improve the bond strength results for an ethanol/water-based adhesive; however, the hottest temperature (50°C) of one acetone-based adhesive decreases the bond strength values. Thicker adhesive layers were observed for both adhesives in cold temperatures (in the 5°C and 20°C groups). Degree of conversion was significantly better for acetone-based adhesive only at hot temperature (50°C).

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