

Effect of hydration variability on hybrid layer properties of a self-etching versus an acid-etching system

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Abstract

The hypothesis tested in this study was that the self-etching system (Clearfil SE Bond, CSE) is less sensitive to surface moisture variability than the system that uses a separate acid-etching step (Single Bond, SB). Eighteen dentin specimens were bonded to composite using CSE or SB. Three different surface moisture conditions per bonding type (overwet, w; dry, d; and visibly moist, n [normal]) were applied prior to bonding dentin to composite. One cross section of each sample was analyzed with lines of nanoindentations crossing perpendicular to the bonding interface. An additional set of bonded samples was fixed and cross sectioned before the hybrid layer thickness was measured in scanning electron microscopy. The nanoindentations revealed significant differences in indentation modulus (E_i) and hardness (H) for the hybrid layer comparing SBn, $E_i = 2.7(\pm 1.6)$; $H = 0.24(\pm 0.1)$ GPa with SBd, $E_i = 0.9(\pm 0.7)$; $H = 0.07(\pm 0.05)$ GPa, respectively, while CSE showed no differences among the groups. A significantly greater demineralized zone below the hybrid layer was found for SBd. The hybrid layer was wider for both CSEd and SBd. In conclusion the hypothesis was verified; CSE exhibited no significant changes of hybrid layer properties (E_i , H) at different hydration conditions, while SB had significant differences, especially after air-drying.

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1. Introduction

The resin–substrate interface in dental restorations is a key factor in their durability. The surface condition (e.g. moisture content) of dentin has a major impact on the strength of this interface as well as the type of solvent in the primer [1–3]. Nakabayashi et al. [4] introduced the concept of the hybrid layer to explain dentin bonding. Since that time, the hybrid layer has been considered a biologic composite composed of polymerized resin that encapsulates partially demineralized collagen fibrils of the dentin matrix. Dentin

surface conditions have been shown to be important in bonding [5,6]. The “wet-bonding” technique is commonly used for most three-step bonding systems (etch, prime, bond). Maintaining a wet surface to prevent the collapse of the acid-etched matrix by air-drying and using a solvent to improve penetration of adhesive monomers are important aspects of this technique [5,6].

Micromorphological studies have shown that the wet-bonding technique [6] is sensitive to moisture content [7–9]. After acid etching and rinsing, some water is necessary to maintain the demineralized dentin structure, but excess water introduces phase changes and water blisters that decrease the strength of the bonded interface. Tay et al. [7–9] showed that the bonding to overwet dentin created blister like structures, representing regions previously occupied by water along the hybrid layer–primer interface. Another study claimed

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that excess water would compete with a hydrophilic resin monomer (HEMA) for space in the demineralized zone, decreasing the monomer density within the collagen network, and probably interfering with its polymerization [3]. Thus, excess water compromises the resin–dentin bonding, as does desiccation prior to bonding [10]. There is an obvious need for less technique sensitive bonding systems.

This study compared the technique sensitivity of two currently used bonding systems with respect to dentin surface hydration: one acid-etching system and another self-etching system were studied. Self-etching systems have become increasingly popular in the last decade [11–13]. The combination of etchant and primer into a two-step self-etching primer system is advantageous in that it reduces the application time. Since there is no separate etching process involved, the hydration of the surface after etching should not be a problem for clinicians. This study tested the hypothesis that the self-etching system Clearfil SE Bond (CSE) is less sensitive to surface moisture variability than a system that uses a separate acid-etching step, Single Bond (SB). The hybrid layer thickness and indentation modulus E_i were compared for both systems under three different hydration conditions.

2. Materials and methods

2.1. Sample preparation

Dentin samples were obtained from 18 randomly selected human non-carious third molars according to a protocol approved by the UCSF Committee on Human Research. All teeth were recently extracted (less than 3

months) from patients needing extractions as part of their dental treatment. They were sterilized using gamma radiation in a cesium 137 radiation chamber as described by White et al. [14]. Each tooth was sectioned horizontally to prepare a 2.5 mm thick midcoronal dentin slice. The upper surface of each specimen was finished with 320 grit SiO₂ paper, followed by surface moisture application, resin bonding and application of resin to composite. Three different surface moisture conditions were created prior to bonding: overwet (w), dry (d) and visibly moist (normal, n).

The overwet specimens were prepared by depositing 10 µl of deionized water on the 3.2 mm diameter hole in the mylar strip covering the dentin surface that defined the area for bonding. This resulted in a pool of water about 1 mm thick on the surface. For the d group specimens, the exposed dentin surface in the mylar hole was air-dried using medium air pressure with a dental air syringe for 30 s at a distance of 5 cm with a total air volume of about 5 m³. The air-drying resulted in a dull desiccated surface. Specimens with a moist exposed dentin surface, which were created by removing excess water using paper tissue as recommended by the manufacturer, served as normal controls.

The dentin specimens in the SB group were etched before they received the different surface moisture treatments: w, d or n. The self-etching material CSE was applied directly to the different surface moisture conditions on dentin. The materials used in this study are displayed in Table 1. Three specimens for each group CSEw, CSEd, CSEn, SBw, SBd and SBn were prepared by bonding the resin composite to the occlusal dentin slab using CSE or SB as bonding agent, under different moisture conditions as described above. All materials were polymerized with a curing light (Model

Table 1
Restorative and adhesive materials

Material	Code	Composition	Batch #	Function
Single Bond adhesive, 3M ESPE, St. Paul, MN	SB	35% phosphoric acid, Bisphenol A diglycidyl ether dimethacrylate, HEMA, dimethacrylate, solvent, water	7AT	Adhesive system
Clearfil SE Bond, Kuraray America, USA	CSE	Primer-10-MDP, HEMA, hydrophilic dimethacrylate, DL-camphorquinone, <i>N,N</i> -diethanol- <i>p</i> -toluidine, water	Primer 00113A	Adhesive system
		Resin-10-MDP, bis-GMA, HEMA, hydrophilic dimethacrylate, DL-camphorquinone, <i>N,N</i> -diethanol- <i>p</i> -toluidine, silanated colloidal silica	Adhesive 00051A	Adhesive system
Z-100, 3M ESPE, St. Paul, MN	Z 100	Bisphenol A diglycidyl ether dimethacrylate; silanated zirconium silica synthetic mineral	IEA	Restorative material
35% phosphoric acid, 3M ESPE, St. Paul, MN	PA	35% phosphoric acid	9LX	Acid conditioner

100, Demetron Research Corporation, Danbury, CT, USA). The light's intensity was monitored periodically with a curing radiometer (Model 100, Demetron Research Corporation, Danbury, CT, USA) (acceptable range of 500–600 mW/cm²). After bonding, the specimens were cut using a diamond saw (Buehler Ltd., Lake Bluff, IL, USA) perpendicular to the bonding zone to obtain a cross section of the specimen. Each surface was polished through a series of SiO₂ abrasive papers down to 2000 grit (Carbimet Buehler-met, Buehler Ltd., Lake Bluff, IL) followed by polishing with diamond pastes through 0.25 μm on a hard cloth (Buehler Micropolish, Buehler Ltd., Lake Bluff, IL). All specimens were ultrasonically cleaned in deionized water at the end of each polishing step for 1 min to remove polishing agents and remnant smear layer. During the specimen preparation and until testing, all samples were kept in Hank's balanced salt solution (HBSS) to minimize surface changes [15] and were tested within 48 h of final polishing.

2.2. Properties of the dentin–adhesive interface

2.2.1. Rationale for the measurement of indentation modulus and hardness of the hybrid layer

The different moisture contents can alter the physical properties of the hybrid layer (hl). Decreased indentation modulus and hardness of the hybrid layer may indicate poorly infiltrated demineralized dentin (Table 2). The nanoindentations were performed on an atomic force microscope (AFM) (Nanoscope III Digital Instruments, Santa Barbara, CA), where the standard head was replaced by a Triboscope indenter system (Hysitron Inc., Minneapolis, MN) as previously described by Balooch et al. [16]. A diamond cube corner indenter with a tip radius of about 20 nm was used for imaging and indentation. All images and indentations for indentation modulus and hardness across the adhesive–dentin interface were made with the specimen submerged in water. Indentations at intervals of 1–2 μm were made in a straight line, starting from the adhesive to the dentin

with a load of approximately 300 μN, as seen in Fig. 1. A large indent (1000–3000 μN) in the adhesive served as a fiduciary mark. When indenting different zones (adhesive layer, hybrid layer or dentin), the load was adjusted accordingly prior to each indentation to keep the size of the indentation constant. After the indentations were performed, an image was captured with the AFM to analyze the exact distance between the indentations. To determine which indentations were made within the hybrid layer, the location and width of the hybrid layer had to be determined. Since this was difficult in the AFM, specimens were studied by scanning electron microscopy (SEM). The specimens were treated to reveal the hybrid layer, using the Schneider et al. [17] method (1 min 0.1 mol HCl, 2 min rinsing, 1 min 10% NaOCl, 2 min rinsing), followed by dehydration using a graded series of ethanol, with final drying in HMDS [18]. After sputter coating with 200 nm gold/palladium (Hummer VII Sputtering System, Anatech LTD., Alexandria, VA), the SEM images (ISI ABT SX-40A SEM, Topcon Instruments, Pleasanton, CA) were used to identify which indents were present in the hybrid layer based on the number of indents from the fiduciary marker. These measurements were performed using both backscattered and secondary electron modes. In addition, these locations were verified by measuring

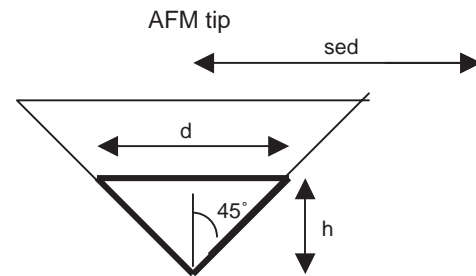


Fig. 1. Schematic of an AFM tip, where “sed” is the safe distance to the adjacent indent, “d” is the diameter of the tip at plastic deformation and “h” is the depth of plastic deformation. To calculate the safe distance to the adjacent indentation the equations: $sed = 1.5 \times d$ and $d = 2(h \times \sin 45^\circ)$ have been used.

Table 2

Indentation modulus and hardness of hybrid layers (hl), demineralized zone below hybrid layer and width of hybrid layer^a

	Clearfil SE Bond (Kuraray)			Single Bond (3M)			Interaction <i>p</i> -Value
	Visibly moist	Wet	Dry	Visibly moist	Wet	Dry	
E_i of hl (GPa)	5.5 (1.1)	6.9 (1.3)	7.0 (1.3)	2.7 (1.6)	3.1 (2.9)	0.9 (0.7) ^b	0.014
H of hl (GPa)	0.36 (0.07)	0.39 (0.1)	0.41 (0.08)	0.24 (0.11)	0.24 (0.2)	0.07 (0.05) ^b	<0.001
DZ (μm)	2.3 (0.84)	1.9 (0.7)	1.5 (0.7)	3.8 (0.7)	3.0 (0.7)	7.4 (0.7) ^b	<0.001
width of hl (μm)	2.0 (0.4)	1.5 (0.2)	2.8 (0.4) ^b	7.1 (0.4)	6.2 (0.6)	2.8 (0.4) ^b	<0.001

^a Assessment of E_i , H and demineralized zone determined with nanoindentations (mean (stdev)). The width of the hybrid layer at the side of the indentations was evaluated using SEM analysis. The number of specimens was $n = 6$ for each group. Interaction values demonstrated differences in adhesive response to the hydration conditions.

^b Significantly different from visibly moist.

the thickness of the hybrid layer in the SEM. We recognized that this layer may have undergone shrinkage, as demineralized dentin may undergo 10–30% shrinkage, during SEM processing [19]. Both methods gave consistent results and allowed us to determine which indents were below the hybrid layer in the AFM images. Therefore, it was possible to plot mechanical properties versus location based on the AFM information without regard to possible shrinkage of either the hybrid layer or the partially demineralized dentin below it. The hybrid layer thickness was measured along the three individual indentation lines for each specimen using image analysis software (Ultrascan 2.1.1, Soft Imaging Software, KeveX Sigma, Noran Instruments Inc., Madison, WI). The indentation modulus of elasticity (E_i) and the hardness (H) from the indents in the hybrid layer were computed using the TriboScope software (version 3.5.6.1, Hysitron, Minneapolis, MN), as previously described [15].

2.2.2. Demineralized zone determination

Moisture differences prior to bonding can affect not only the hybrid layer properties but also the dentin below the hybrid layer. If a partially demineralized zone exists, it can be detected by analyzing the E_i data across the interface. The width of any such zone was determined as the distance from the bottom of the hybrid layer (defined from the SEM images) to the location where the E_i values reached those of normal dentin. To identify the end of the partially demineralized zone, a statistical mixed model was applied. The mixed model analyzed when the gradient in mechanical properties ended at a plateau, indicating unaltered dentin substrate. Using this statistical mixed model analysis, means and standard deviations of the partially demineralized gradient zones for the CSE and SB groups were established. This method of estimation began with the two right-most data points (indents in dentin) to construct an initial standard deviation and mean (Fig. 2). The standard deviation was multiplied by a constant factor, whose value is described below. The third right-most data point was compared to the result of (above mean) – ((factor)*(above standard deviation)).

(1)

If the third right-most data point was within this result, then this point was considered to be within the plateau of dentin. The program then moved forward one point and recomputed the mean and standard deviation using all three points. The fourth point was compared to Eq. (1). This process continued until either a point fell below the result from Eq. (1), that meant a gradient zone, or all data points were exhausted, that meant no gradient zone. Once a point was assigned to be in the gradient zone, the program backed up one point and used the previous point as the start of the dentin plateau.

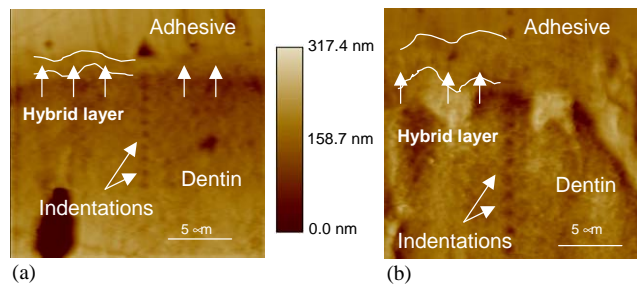


Fig. 2. (a) Topographic AFM image of the cross section of a visibly moist treated CSE sample containing nanoindentations from the adhesive layer toward dentin. (b) Topographic AFM image of an SB visibly moist treated sample; the hybrid layer is remarkably wider than in the CSE sample. No indents are evident in the hybrid layer (hl), revealing a material with low E_i modulus and visco-elastic deformation.

The factor multiplying the standard deviation in Eq. (1) was selected by examining values between 1 and 2 in increments of 0.1. For each factor value, the program estimated the width of the demineralized zone for all lines of indentations. Both E_i and H estimates of the width provided a correlation coefficient for each value of the factor. The chosen factor of 1.4 maximized the correlation between the two estimated widths, while minimizing the standard deviation of each.

For all indentations it is important to have sufficient space between the indents. The cube corner causes a plastic deformation in the substrate that can affect the validity of the measurement from the adjacent indentation if it is placed too close, e.g. less than some safe spacing. The safe spacing to the adjacent indent was calculated as 1.5 times the diameter of the tip at the depth when plastic deformation occurs (Fig. 1). The depth is determined using the Hysitron software when indenting the substrate. This method calculated a specific safe spacing for each indent. If the radius of the safe spacing overlapped with the adjacent zone, the indents were too close and were considered invalid and were not used in the analysis. Indents with sufficient space were selected for the E_i and H analysis of the hybrid layer and the E_i data were used for the demineralized zone evaluation.

2.2.3. Evaluation of the width of the hybrid layer

To determine if the moisture conditions and bonding agents resulted in variations in hybrid layer thicknesses, bonded specimens were prepared for SEM examination. This part of the study was performed to capture the most realistic morphology of the dentin adhesive interface. An additional set of 18 freshly extracted human third molars was prepared as described in the specimen preparation section. An important difference in specimen preparation for this set of teeth was the storage of the bonded samples in a fixing solution before they were cut. In order to stabilize the morphological structure of

the entire specimen, they were stored in 3 vol% glutaraldehyde with 0.2 mol/l sodium cacodylate buffer solution for 24 h at 4°C in darkness [18]. Specimens were then cut in cross section, treated with HCl and NaOCl [17] and dehydrated [18] as described above. The hybrid layer thickness was measured in the SEM following sputter coating with 200 nm of gold/palladium. Three randomly selected locations per specimen were analyzed for hybrid layer thickness using backscattered and secondary electron modes. Six specimens per group were analyzed. Eighteen measurements per group resulted in a total of 108 hybrid layer thickness measurements.

2.3. Statistical analysis

The width of the hybrid layer, the E_i and H data of the hybrid layer, as well as the demineralized zone, were analyzed for significant differences using one-way ANOVA with Dunnett's test at a 95% level of confidence (Table 1). The visibly moist groups CSEn and SBn served as controls. To assess the interaction values for differences in adhesive response to the hydration conditions, two-way ANOVAs were applied for the data of the hybrid layer, E_i , H and the demineralized zone. For both tests, StatView 4.0 statistical software package (SAS Institute Inc., Cary, NC, USA) was used.

3. Results

Fig. 2 shows representative AFM images for CSEn and SBn specimens. In both images, the indents in the

hybrid layer almost disappeared, while indents in adhesive and dentin remained visible, reflecting differences in the mechanical properties. The insert of Fig. 3 shows continuous data across the resin–dentin interface. In that sample, the value for E_i dropped slightly in the middle of the hybrid layer. The mechanical properties of the hybrid layer represented by E_i and H revealed no statistically significant differences for the CSE material group (Table 1), but the SB materials showed a significant difference between the dry group and the visibly moist group for both E_i and H in one-way ANOVA and Dunnett's test. Dunnett's test reveals statistical differences between n and w as well as between n and d for SB bonded specimens.

The extremely low E_i and H values for SB materials also had high standard deviations. Since the width of the hybrid layer in SBw was much greater than in the SBd, about two times more data points could be generated. No significant difference could be found between the SBw group and the SBn group. The modulus in the CSE groups was approximately double that in the SB groups. The two-way ANOVA analysis with the factors adhesive type and moisture variability revealed highly significant interaction p -values of 0.014 and <0.001 for E_i and H , respectively. The interaction was caused by the SB group with significant differences of mean E_i and H values of the hybrid layer at different moisture conditions. Drying the sample, before the bonding agent was applied, significantly reduced the mechanical properties of this zone. The width of the partially demineralized zone was estimated based on E_i values and revealed gradient zones of 2.3 (0.8), 1.9 (0.7) and 1.5 (0.7) μm for CSEn, CSEw and CSEd, respectively, which were not significantly different. SB materials exhibited a

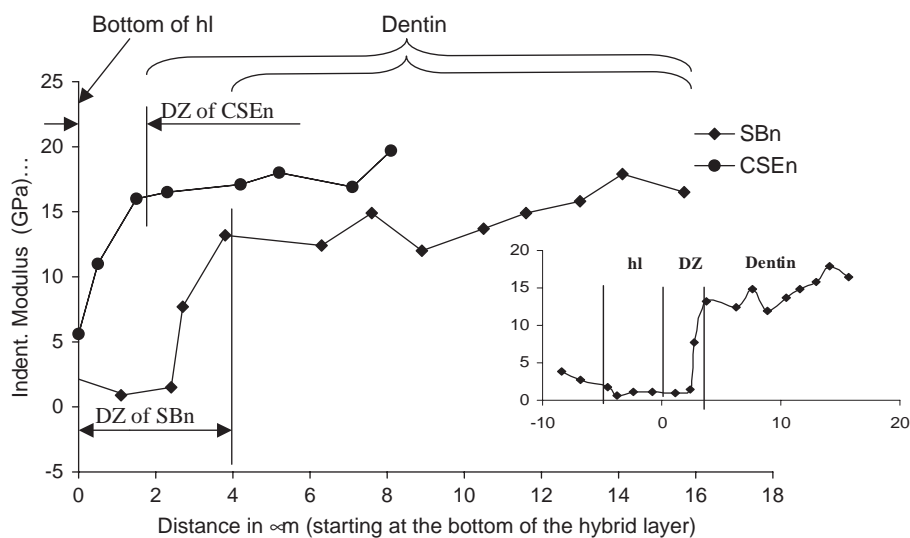


Fig. 3. Graphs show modulus versus distance for the two very different bonding zones. The SBn data exhibit a low E_i below the hybrid layer, corresponding to a partially demineralized zone (DZ) about 3.8 μm wide. In contrast, the CSEn sample reached the dentin plateau after about 2 μm . The range of E_i of all data for intertubular dentin was 12.0–22.3 GPa. The inset shows continuous data of SBn from the main graph across the hybrid layer (hl) to display the variation within the hybrid layer. CSE data are not displayed because the hybrid layer showed no variation with this method.

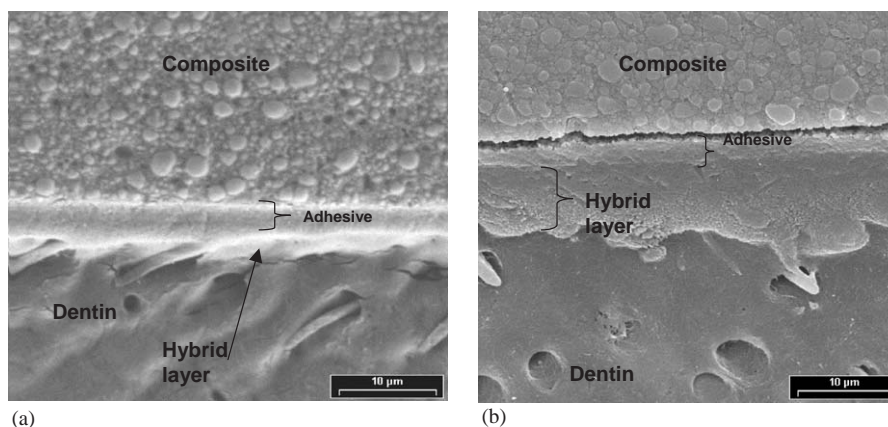


Fig. 4. (a) SEM image in backscattered mode of a cross section of a CSEn sample showing a small hybrid layer and (b) SEM in secondary mode of an SBn sample in cross section presenting a wider hybrid layer and a crack between the adhesive and the composite. The crack is an artifact of the SEM vacuum.

significant difference between SBd (width = 7.4 (0.7) μm) and SBn (width = 3.8 (0.7) μm). The interaction factor for the width of the demineralized zone for adhesive type and moisture variability was highly significant, even though the values for SBn and SBw groups did not differ much from those for the CSE groups. Drying the demineralized dentin after etching resulted in a wide demineralized zone and thin hybrid layer, suggesting that the dentin was only partially impregnated with adhesive. The significantly wider zone of the SBd group compared to the SBn group caused the high interaction factor. Fig. 3 shows a CSEn sample with a steep increase in E_i below the hybrid layer, suggesting only a small gradient zone of 1.5 μm . The SBn sample had a 3.8 μm partially demineralized zone below the hybrid layer. Fig. 4 shows two representative SEM images with clearly revealed hybrid layers in backscattered mode (Fig. 4a) and in secondary mode (Fig. 4b). Hybrid layer width was between 1.5 and 2.8 μm in the CSE groups, while it ranged between 2.8 and 7.1 μm in the SB groups. The width for the demineralized zones for the d condition in both groups were significantly different than the n condition. The reason for the significant interaction ($p < 0.001$) was again due to the difference in behavior of the two adhesive types with moisture content. Drying the dentin for CSEd increased the width of the hybrid layer significantly, as compared to CSEn, while in the SBd group the width of hybrid layer was significantly lower than SBn.

4. Discussion

This study examined the effects of surface hydration on mechanical properties of the hybrid layer and the subjacent dentin of two different bonding approaches. The comparison of self-etching and acid-etching bond-

ing systems is difficult, because the self-etching material includes etching and priming in one step. Thus, the critical time for defining the hydration state is different for the two systems. For CSE, the hydration stages were established before the self-etching primer was applied. This was in contrast to the acid-etched material, SB, where the hydration conditions were established after the acid-etching process. Fig. 2 shows the AFM images of indentation lines from self-etching and acid-etching systems. In both cases, no indentations were visible in the hybrid layer area. This is due to the visco-elastic properties of the hybrid layer resulting in no visible permanent deformation.

Previous studies have shown that the hydration conditions of the dentin are very important and can influence substantially the hybrid layer formation. Tay et al. [7–10] and Carvalho et al. [20] showed that moist bonded specimens had significantly higher bond strength than those bonded under dry conditions. Hashimoto et al. [21] confirmed these findings while another study has shown that dry bonding does not necessarily lead to an insufficient hybrid layer formation [22]. In the latter study, it was assumed that the amount of water present in adhesive systems with hydrophilic water based primers, suffices to re-hydrate and re-expand the air-dried and collapsed collagen network. The present study showed that the self-etching system was relatively insensitive to moisture content in comparison to the acid-etch system. The bond strength of the acid-etching system significantly decreased with either excess moisture or drying, as shown by others [5,6,21,23,24]. In contrast, the self-etching system showed no differences in bond strength with hydration state. Different qualities of the hybrid layer were created with the two different systems with the result that the lowest E_i for the SB material was found within the hybrid layer (Fig. 3) where in contrast for the CSE

system the lowest E_i was found within the adhesive layer (data not shown). The results were also reflected in variations in hybrid layer thickness and subjacent dentin demineralization. The etching process in a self-etching system produced a much smaller hybrid layer than in the acid-etching system (Table 1), due to the fact that phosphoric acid alone demineralizes apatite faster than the acidic monomer, which has a higher pH. Even though CSEd materials exhibited slightly, but significantly wider hybrid layers compared to CSEn, this did not result in significant differences in mechanical properties of the hybrid layers. Statistical analysis showed that E_i and H of the hybrid layer of the CSE material exhibited no difference for different hydration groups, which suggests that, in a clinical situation, there are no quality differences in hybrid layer formation whether the dentist air-dries the dentin surface or leaves excess water. Self-etching materials may yield consistent results regardless of the moisture condition. This would permit practitioners to visualize a dry field preparation. Another major concern is whether or not the demineralized dentin can be penetrated completely by the monomer, or if a layer of demineralized dentin remains. The self-etching materials reduce the discrepancy between the depth of dentin affected by demineralization and the depth of resin infiltration, since both processes occur together, but they do not eliminate the uninfiltreated layer [25,26]. The clinical consequences of such a layer are of concern. The ideal adhesive system would create three-dimensional diffusion channels that would surround dentin collagen fibrils and be filled by resin [1]. Although both adhesive systems had a layer of reduced mechanical properties below the hybrid layer, which suggested the presence of a less-impregnated microporous zone (Table 1), the SB system had a much thicker demineralized zone than the CSE system. This was related to the differences in pH of the two systems. In addition, the partially demineralized zone beneath the hybrid layer is topographically the least likely polymerized zone. Even though primer might be present there, it can remain unpolymerized and result in reduced mechanical properties. This zone could not be identified with SEM after fixation but was apparent from the mechanical properties. Future studies need to validate this zone using different techniques, e.g. digital stiffness imaging with high resolution [27].

In conclusion, these data suggest that the self-etching material exhibited no significant changes in hybrid layer properties (E_i and H) at different hydration conditions, while the conventional acid-etching system revealed significant changes, especially after air-drying. Therefore, the hypothesis was verified, the self-etching material was less technique sensitive compared to the material with the separate etching step.

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