The Influence of Tubule Density and Area of Solid Dentin on Bond Strength of Two Adhesive Systems to Dentin

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Purpose: To determine the correlation between the tubule density (TD) and the area occupied by solid dentin (ASD) with the bond strength of one conventional and one self-etching adhesive system to dentin.

Materials and Methods: The crown of extracted human third molars was transversally sectioned with a diamond saw to expose either superficial, middle, or deep dentin. The three groups of dentin surfaces were randomly divided and bonded with either Clearfil Liner Bond 2V (LB) or Prime & Bond 2.1 (PB) adhesive systems according to manufacturer's directions. Resin composite buildup crowns (10.0 mm high) were incrementally constructed on the bonded surfaces and the teeth stored in water at 37°C. After 24 h of storage, the teeth were vertically, serially sectioned in both x and y directions to obtain several bonded sticks of approximately 0.7 mm^2 cross-sectional area. Each stick was tested in tension in a EMIC DL-500 tester at 0.5 mm/min until failure. After testing, the dentin side of the fractured specimen was gently abraded with a 1000-grit SiC paper, etched with 37% phosphoric acid for 15 s and allowed to air dry. SEM micrographs at 1000X and 4000X magnification were taken to permit calculation of the TD (number of tubules/mm^2) and ASD (% of total area) at the site of fracture. Correlation between TD and ASD with the bond strength data was performed by linear regression. All statistical analysis was done with $\alpha = 0.05$.

Results: Overall bond strength (MPa) for LB was 26.0 ± 10.2, and 42.6 ± 15.2 for PB. There was a significant direct relationship between bond strength and ASD for both materials ($r^2 = 0.20$, $p < 0.05$ and $r^2 = 0.66$, $p < 0.01$, respectively for LB and PB). PB bond strength dropped significantly as the TD increased ($r^2 = 0.63$, $p < 0.05$), while LB was not sensitive to TD ($r^2 = 0.05$, $p > 0.05$). Mean bond strength of PB was significantly higher than LB for both superficial and middle dentin ($p < 0.05$), while there was no significant difference for deep dentin ($p > 0.05$).

Conclusion: Regional variations in TD and ASD may modify bond strength of both conventional and self-etching adhesive systems. Bonding sites with larger ASD seem to yield higher bond strengths regardless of the type of adhesive system used.

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High-quality hybrid layers require optimal infiltration of adhesive monomers into the demineralized dentin surface. It has been demonstrated that higher bond strength to dentin is achieved by a combination of micromechanical retention provided by resin tag formation into the dentinal tubules, hy-

brid layer formation into the intertubular dentin, and surface adhesion.[6] Due to the wide variation in the morphological characteristics of dentin as a function of depth, Pashley et al[11] proposed a mathematical model to predict bond strength values to dentin according to regional variances in the substrate. That model predicted that in superficial dentin, the larger surface area occupied by intertubular dentin favors the contribution of hybrid layer formation to the total bond strength. Conversely, deep dentin contains a much larger surface area occupied by dentinal tubules, therefore favoring the contribution of resin tags to the total adhesion. That model predicted that deep dentin would provide higher bond strengths than superficial dentin. In that study, however, the authors assumed that each bonding mechanism would be ideally achieved, such as fully infiltrated hybrid layers and resin tags that were properly hybridized with the lateral walls of the dentinal tubules, disregarding other factors that could interfere with the ideal bonding such as the high water content of deep dentin.

Dentin is a dynamic substrate,[14] and its morphological and functional characteristics are determinants of the quality of resin-dentin bonds achieved with adhesive agents.[10] Sclerotic, caries-affected and deep dentin have been considered unfavorable substrates for bonding.[8,22,23] Lower bond strengths have been reported for deep dentin using earlier, less hydrophilic bonding agents.[7] Increased wetness in deep dentin has been held responsible for diluting the resin monomers, thereby compromising adhesion.[16] More recent work continues to demonstrate lower bond strength in deep dentin using more hydrophilic adhesive agents.[15,22] Suzuki and Finger[17] demonstrated that the lower bond strengths usually observed in deep dentin were more related to the amount of solid dentin at the site of bonding than to the intrinsic wetness of dentin.

Among several factors that may interfere with the quality of bonding, the type of adhesive systems used is of great importance. Systems that employ a separate acid etching step are apparently more sensitive to the dentin characteristic depth than are self-etching systems.[15]

Most of the studies that investigated the effects of dentin depth on bond strength have simply identified dentin surfaces as having originated from superficial, middle, or deep dentin. Due to the anatomy of the pulp, it is likely that dentin previously classified as middle or superficial dentin may, in fact, be deep dentin or vice-versa. When using conventional shear or tensile testing, the problem may be even worse, because the large bonding area used with these tests may include dentin regions that are representative of different depths in one single specimen. The microtensile technique minimizes this problem by using much smaller bonding areas with less variance of the substrate within each specimen. Additionally, smaller bonding areas facilitate a more profound analysis of the dentin surface at the site of bonding.[12] The small surface area allows SEM observation of the bonded site to more fully characterize the dentin substrate.

The purpose of this study was to test the bond strength of two adhesive systems to different dentin depths and to correlate the bond strength values with the tubule density and the area occupied by solid dentin at the site of bonding. The null hypothesis tested here was that bond strength is not influenced by the characteristics of the substrate regardless of the type of adhesive system used.

MATERIALS AND METHODS

Nineteen extracted, caries-free human third molars that were stored for no longer than 3 months were used in this study. The crowns of the teeth were transversally sectioned with a diamond blade (PC 10, Imptech-Equilan, Diadema, SP, Brazil) under water irrigation just beneath the deepest occlusal fissure (n = 6), in the middle of the crown (n = 7), or next to the cemento-enamel junction (n = 6) to expose areas of superficial, middle, or deep dentin, respectively (Fig 1 A, B). The exposed dentin surfaces were wet-polished with 600-grit SiC paper to create a standard smear layer before being bonded with the adhesive systems.

Three superficial, 4 middle, and 3 deep dentin surfaces were bonded with Prime & Bond 2.1 adhesive system according to manufacturer's instructions, following etching with 36% phosphoric acid for 15 s and rinsing. Clearfil Liner Bond 2V adhesive system was applied to 3 superficial, 3 middle, and 3 deep dentin surfaces also according to manufacturer's instructions. The composition, application steps and manufacturers of the materials used are described in Table 1. After bonding, the entire dentin surfaces received several layers of Z-100 resin composite (Table 1) to build up a crown approximately 10.0 mm in height (Fig 1 C). Each layer

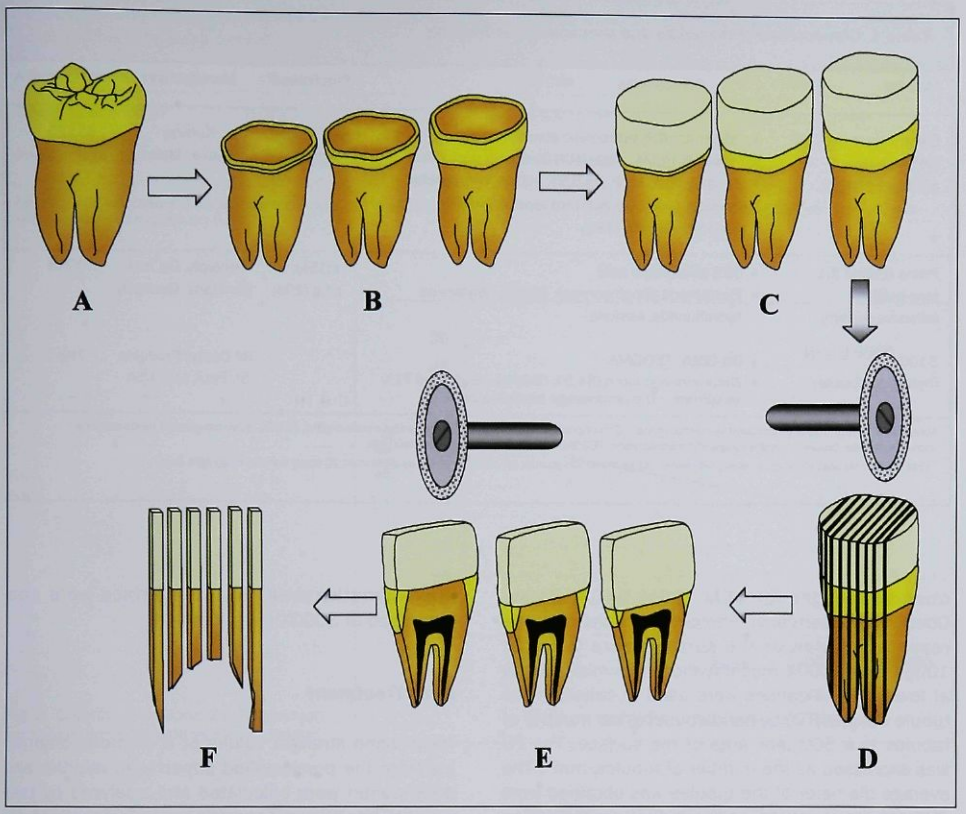

Fig 1 Schematic representation of specimen preparation.

was light cured for 40 s with a light-curing unit (Degulux, Degussa, Hanau, Germany) at 450 mW/cm^2. The bonded teeth were then stored in water at 37°C.

After 24 h of storage, the bonded teeth were vertically, serially sectioned into several 0.7-mm-thick slabs (Fig 1 D) with a diamond blade. Each slab was further sectioned to produce several bonded sticks of approximately 0.7 mm^2 (Fig 1 E,F). Each bonded stick was fixed to the grips of a Bencor testing device (Bencor Multi T, Danville, CA, USA) with cyanoacrylate glue (Zapit, DVA, Corona, CA, USA) and tested in tension in a testing machine (DL 500, Emic, SJ dos Pinhais, PR, Brazil) at 0.5 mm/min until failure. After testing, the specimens were carefully removed from the fixtures with a scalpel blade and the cross-sectional area at the site of fracture measured to the nearest 0.01 mm with a digital caliper (Starret 727-6/150, Starret, SP, Brazil) to calculate bond strength, expressed in MPa.

SEM Observations

The dentin side of failed specimens was lightly wet abraded with 1000-grit SiC paper to remove remnants of the adhesive agent, etched with 37% phosphoric acid for 15 s, washed and allowed to air dry. After drying, the surface was sputter-coated with gold (MED 010, Balzers, Balzers, Liechtenstein) and

Table 1 Composition of materials and procedures for bonding

Material	Components	Procedures*	Manufacturer	Lot number
Clearfil Liner Bond 2V (self-etching adhesive system)	• Primer A: MDP, hydrophilic dimethacrylate, CQ • Primer B: HEMA, water, N,N-Diethanol p-toluidine • Bond liquid A: MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, CQ, N,N-Diethanol p-toluidine, silanated colloidal silica	c;d;e(30s); f;g(20s)	Kuraray Osaka, Japan	61126
Prime & Bond 2.1 (one-bottle adhesive system)	• 36% phosphoric acid • Elastomeric dimethacrylate, PENTA, cetylamine hydrofluoride, acetone	a(15s); b; c;f;g (20s)	Dentsply, De Trey Konstanz, Germany	40293
Z-100 (hybrid composite)	• Bis-GMA, TEGDMA • Zirconium and silica (84.5% filled by weight and 71% by volume – 0.6 µm average particle size).		3M Dental Products St. Paul, MN, USA	7KE

Abbreviations: MDP: 10-methacryloyloxy methacrylate; CQ: camphorquinone; HEMA: 2-hydroxyethyl methacrylate; PENTA: dipentaerythritol penta-acrylate monophosphate; bis-GMA: bisphenol-glycidyl methacrylate; TEGDMA: triethylene glycol dimethacrylate.

* Procedures: (a) acid etching; (b) wash with water; (c) gently air dry dentin; (d) mix primer; (e) apply primer; (f) apply adhesive; (g) light cure.

observed under an SEM (DSM 940A, Zeiss, Oberkochen, Germany). Photomicrographs of a representative area of the surface were taken at 1000X and 4000X magnification. The micrographs at lower magnifications were used to calculate the tubule density (TD) by hand counting the number of tubules in a 500 μm^2 area of the surface. The TD was expressed as the number of tubules/mm^2. The average diameter of the tubules was obtained from direct measurements on the higher magnification micrographs using a digital caliper (Starret 727-6/150, Starret, SP, Brazil). The average was calculated by measuring the diameters of at least 4 dentinal tubules on the micrograph and the actual dimension calculated according to the scale bar. This value was then used to calculate the area occupied by the tubules and the percent area occupied by solid dentin (ASD). The latter was calculated as 100-TD.

Six more teeth (3 per material) were prepared according to the method described above until step E of Fig 1. The slabs were hand polished with 600-, 800-, 1000-, and 1200-grit SiC paper followed by diamond pastes (6 µm, 3 µm, 1 µm, and 0.25 µm), dehydrated in ascending acetone concentrations (30%, 50%, 70%, 90% and 100%), critical-point dried (CPD 030, Balzers, Balzers, Liechtenstein), sputter-coated with gold and examined under SEM. Representative areas of the interface were photographed at 3000X magnification.

Data Treatment

Mean bond strength values of specimens originating from the preclassified superficial, middle, and deep dentin were calculated and analyzed by two-way ANOVA (material x depth) and Duncan's Multiple Range test. Individual bond strength values were correlated with the respective TD and ASD and analyzed by linear regression. Statistical significance was established at $\alpha = 0.05$.

RESULTS

Mean bond strength values obtained with the two adhesive systems for the three preclassified dentin depths are showed in Table 2. There was no statistically significantly difference among bond strength values of LB for the three preclassified dentin depths ($p > 0.05$). This lack of sensitivity to dentin depth was confirmed by the absence of a significant relationship between bond strength and TD ($R^2 = 0.05$, $p > 0.05$, Fig 2) However, when individual bond strength values were correlated with their

Table 2 Average microtensile bond strength (MPa) of Clearfil Liner Bond 2V and Prime & Bond 2.1 to superficial, middle, and deep dentin

Adhesive System	Superficial	Middle	Deep
Clearfil Liner Bond 2V	29.9 ± 15.1 (n=9)[a]	24.3 ± 9.5 (n=9)[a]	23.9 ± 10.6 (n=9)[a]
	S	S	NS
Prime & Bond 2.1	61.7 ± 12.4 (n=9)[a]	41.1 ± 5.9 (n=12)[b]	25.6 ± 7.4 (n=9)[c]

Differences between materials are indicated by S = significant ($p < 0.05$) or NS = nonsignificant ($p > 0.05$). Same lower-case letters indicate no significant differences between dentin depths ($p > 0.05$).

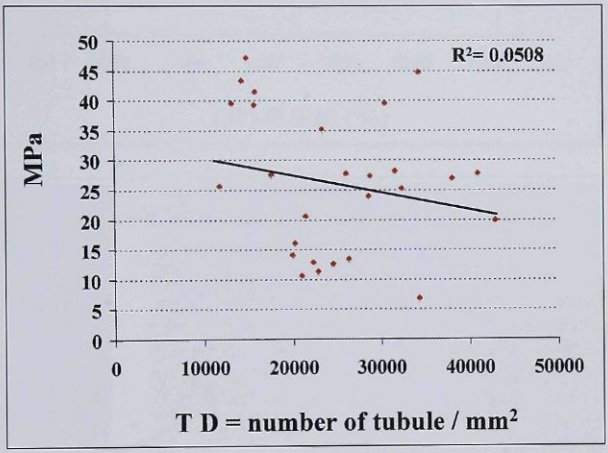

Fig 2 Clearfil Liner Bond 2V. Regression analysis of bond strength vs tubule density.

respective ASD, linear regression showed a weak, but significant relationship. There was a tendency for LB bond strength to increase as the area of solid dentin increased ($R^2 = 0.2$, $p < 0.05$, Fig 3).

There was a statistically significant difference among bond strength values of PB for the three preclassified dentin depths ($p < 0.05$). The bond strength of PB was significantly higher to superficial than to middle dentin, and this was also significantly higher than bonds made to deep dentin. Linear regression showed a strong inverse relationship between bond strength and TD for PB ($R^2 = 0.63$, $p < 0.05$, Fig 4). Conversely, bond strength of PB increased significantly with increasing ASD ($R^2 = 0.66$, $p < 0.05$, Fig 5).

Mean bond strength of PB was significantly higher than LB for both preclassified superficial and middle dentin ($p < 0.05$), but were not significantly different for deep dentin ($p > 0.05$).

Illustrative SEM micrographs of superficial, middle, and deep dentin are shown in Fig 6. Figures 7, 8, and 9 are representative micrographs of the bonded interfaces obtained with the two adhesive systems at superficial, middle, and deep dentin, respectively. Characteristic hybrid layer and resin tag formation was observed with the two bonding systems. The hybrid layer was always thicker with PB than with LB at all dentin depths evaluated.

DISCUSSION

Bond strengths to dentin have classically been reported as being lower to deep than to superficial dentin.[16-19] The main explanation for such findings relies on the fact that those studies employed earlier generations of bonding systems that were less hydrophilic and thus more sensitive to the higher in-

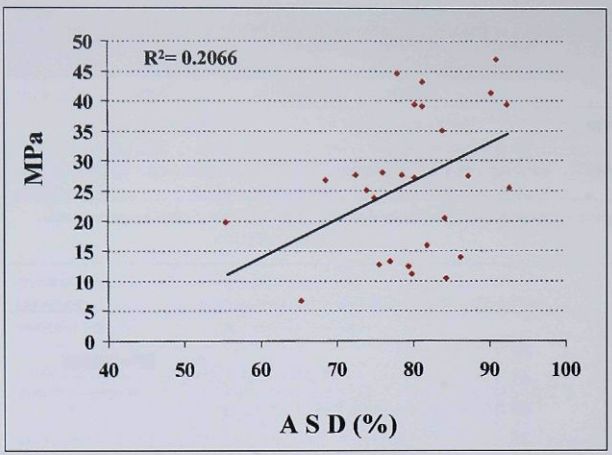

Fig 3 Clearfil Liner Bond 2V. Regression analysis of bond strength vs area of solid dentin.

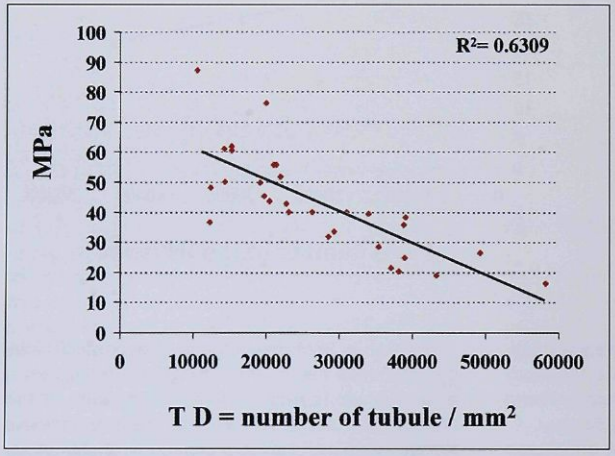

Fig 4 Prime & Bond 2.1. Regression analysis of bond strength vs tubule density.

trinsic wetness of deep dentin. However, more recent studies also reported lower bond strength to deep dentin using current, more hydrophilic adhesive systems.[15,22] In one of these studies,[15] the authors evaluated the bond strength of one acetone-based and one self-etching system to different regions of dentin (ie, periphery, center, or pulp horn) either with or without simulated pulpal pressure. The acetone-based system was very sensitive to both dentin depth and pulpal pressure, while the self-etching system bonded homogeneously in any situation. The authors explained their findings by the fact that enhanced permeability – resulting from the separate etching step with the acetone-based system – increased the surface wetness and may have compromised the bonding with that system because of the overwet phenomenon.[20] The same phenomenon did not occur with the self-etching system because its mild etching action permits smear plugs to remain within the dentinal tubules,

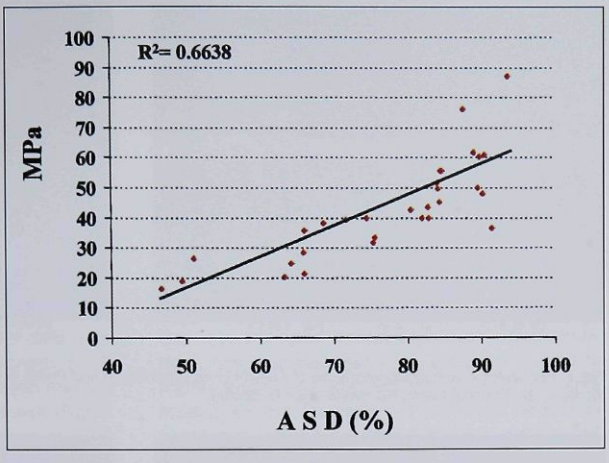

Fig 5 Prime & Bond 2.1. Regression analysis of bond strength vs area of solid dentin.

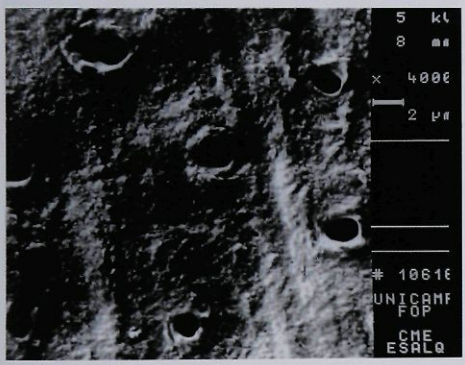

Fig 6a

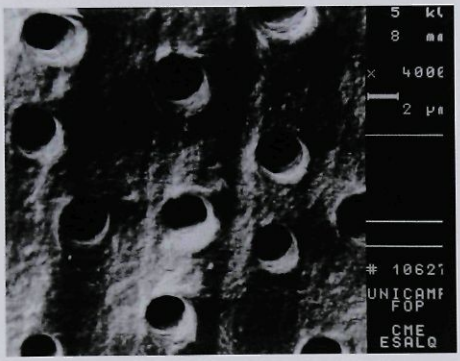

Fig 6b

Fig 6 Representative SEM micrographs of superficial (a), middle (b), and deep (c) dentin.

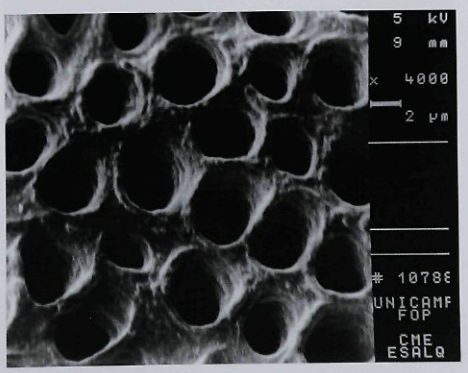

Fig 6c

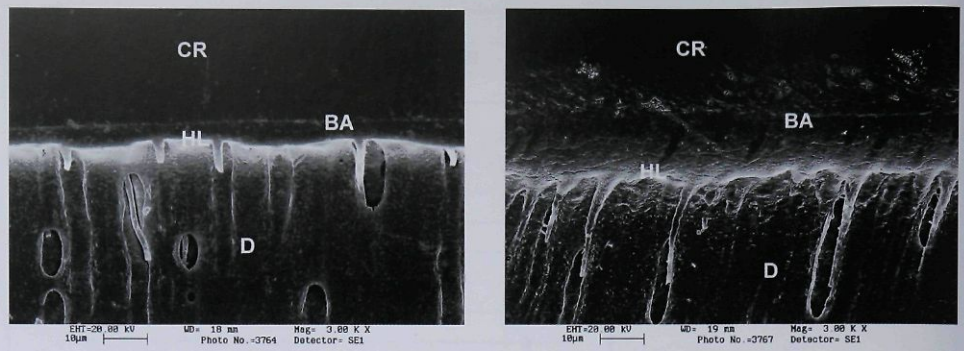

Fig 7 Representative bonded interfaces of Clearfil Liner Bond 2V (a) and Prime & Bond 2.1 (b) at superficial dentin (CR- composite resin, BA- bonding agent, HL- hybrid layer, D- dentin).

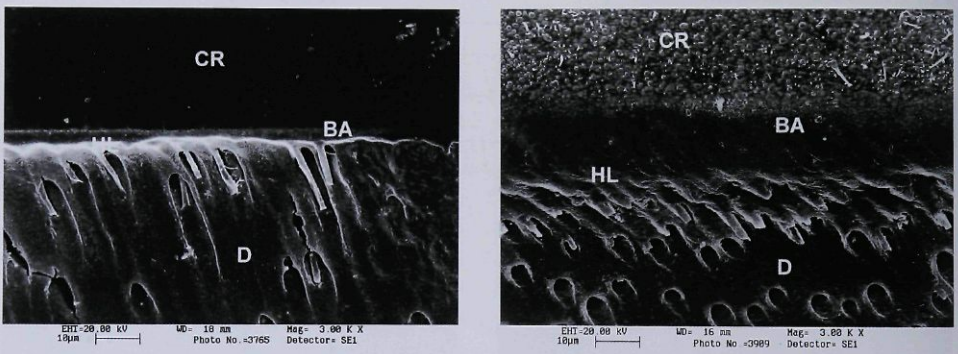

Fig 8 Representative bonded interfaces of Clearfil Liner Bond 2V (a) and Prime & Bond 2.1 (b) at middle dentin (CR- composite resin, BA- bonding agent, HL- hybrid layer, D- dentin).

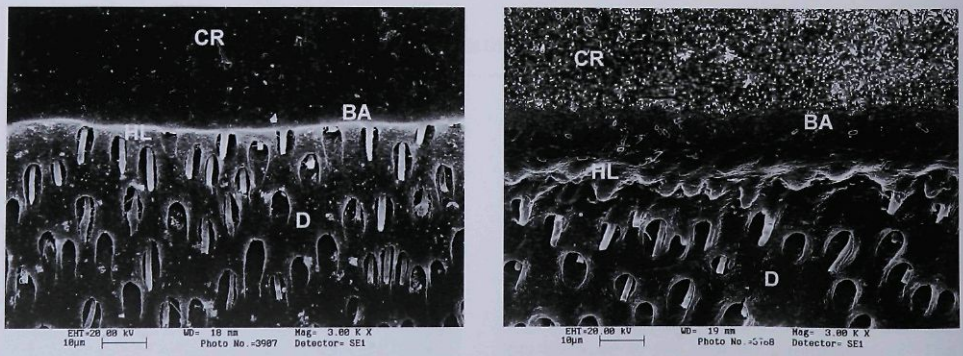

Fig 9 Representative bonded interfaces of Clearfil Liner Bond 2V (a) and Prime & Bond 2.1 (b) at deep dentin (CR- composite resin, BA- bonding agent, HL- hybrid layer, D- dentin).

thus reducing the permeability and surface wetness. The other study,[22] however, did not use simulated pulpal pressure and also demonstrated that bond strengths decreased with dentin depth for all the adhesive systems used in their study. In our study, simulated pulpal pressure was not used and the surface wetness was exclusively determined by the operator according to manufacturer's instructions.

Suzuki and Finger[17] pointed out that bond strengths to dentin are more related to the availability of solid dentin at the site of bonding than to other factors, such as surface wetness. This apparently was the case when we analyzed our data from the PB adhesive system. The bond strength of PB decreased significantly as the TD increased and the ASD available for bonding decreased as well (Figs 4 and 5). The higher bond strengths to more superficial dentin can be explained by the fact that more intertubular dentin is available for hybrid layer formation, this being the main bonding mechanism responsible for increased bond strength to dentin.[6] Theoretically, bond strengths should be higher to deep dentin whenever resin tags can be firmly bound (hybridized) to the lateral walls of the demineralized dentinal tubules.[11] However, we cannot rule out the fact that, even without simulated pulpal pressure, deep dentin is more porous and retains more water within its enlarged tubule openings, which may preclude adequate lateral bonding of the resin tags. Moreover, the wetter and more porous deep dentin is more likely to result in the overwet phenomenon,[20] which may entrap air within the blisters or within the dentinal tubules, also possibly compromising the polymerization of the resin bonding agent.[15] In that respect, while our study did not confirm the theoretical possibility of achieving higher bond strengths in deep dentin, others have shown that deep dentin produced bond strengths that were either not different than superficial dentin[1,9] or even higher[3] for some adhesive systems. It seems reasonable to admit that bond strengths to deep dentin can be higher than to superficial dentin. However, ideal bonding to deep dentin is largely dependent on the adequate bonding of resin tags within the wetter and larger dentinal tubules. This makes bonding to deep dentin more technique-sensitive and largely dependent on the ability of the operator to properly control the surface moisture and application technique for each adhesive system.

Our anticipated null hypothesis was only partially confirmed. It is evident from our findings that the self-etching system (LB) was less sensitive to dentin depth and TD than was PB (Table 2, Fig 2). The insensitivity of self-etching systems to surface variables such as dentin depth, intrinsic wetness, and presence, absence, or thickness of smear layer has been previously reported.[3,15,21] Apparently, because self-etching systems bond to the most superficial layer of dentin and do not completely remove smear plugs, the intrinsic wetness of dentin is less likely to interfere with bonding because the permeability of the tubules is reduced. Since the bonding mechanism of these systems relies on resin infiltration into the solid dentin underneath the smear layer, it is expected that bond strengths should be higher when more intertubular dentin is available. Indeed, we found a weak, but significant direct relationship between bond strength of LB and the ASD (Fig 3). For self-etching adhesive systems, resultant bond strengths are more largely dependent on hybrid layer formation than on resin tag retention. If we apply the modeling approach proposed by Pashley et al,[11] the contribution of hybrid layer to the total adhesion increases from the pulp to the periphery.

The stronger relationship between bond strength and both TD and ASD observed for PB can be explained by the wider range of values of both parameters. The bond strength of PB ranged from as low as 16.37 MPa (for a TD of 58,105 tubules/mm^2) to a maximum of 86.98 MPa (for a TD of 10,472 tubules/mm^2). The percentage of ASD ranged from approximately 45% for the deepest dentin up to approximately 94% for the most superficial. These same values for LB had a much smaller range. Bond strength values ranged from 6.64 MPa (for a TD of 34,290 tubules/mm^2) to 46.96 MPa (for a TD of 14,864 tubules/mm^2). The ASD varied from approximately 55% (one single specimen, Fig 6a) up to approximately 93% for deep and superficial dentin, respectively. The value range of both bond strengths and TD for LB were smaller than for PB; this may have reduced the power of regression analysis to identify a stronger interaction between bond strength and TD.

Our overall range of number of tubules per mm^2 is within the range of values usually reported in the literature.[2,4,5,13] For both PB and LB, most of the specimens were located within the range of 20,000 to 40,000 tubules per mm^2. These are more representative of middle than of very superficial or very deep dentin. Our flat dentin surfaces exposed for

bonding were obtained by transversally sectioning the crowns at three different distances from the cemento-enamel junction towards the cusps. The sections were preclassified as being deep, middle, and superficial dentin, respectively. Although our attempt to expose dentin at different depths was successful, the irregular anatomy of both dental pulp and peripheral enamel does not permit exposure of large areas of very deep or very superficial dentin. Therefore, care must be taken when interpreting data of bond strength of resins to different dentin depths, particularly when large bonding areas are used such as in the conventional shear or tensile tests. The microtensile technique offers the possibility of employing a much smaller bonding area, thus reducing the variability of the substrate on the site of bonding. This also allows for a more realistic SEM analysis of the susbtrate to which the bond was made.

CONCLUSIONS

The results of this work demonstrated that the bond strength of adhesive systems to dentin was dependent on the microstructure of the substrate at the site of bonding. This was more evident with the acetone-based system than with the self-etching system.

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