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\textsuperscript{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\textsuperscript{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.

*J Adhesive Dent 2001;3:315-324.* Submitted for publication: 16.05.01; accepted for publication: 14.09.01.

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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-
braid layer formation into the intertubular dentin, and surface adhesion. Due to the wide variation in the morphological characteristics of dentin as a function of depth, Pashley et al. 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, and its morphological and functional characteristics are determinants of the quality of resin-dentin bonds achieved with adhesive agents. Sclerotic, caries-affected and deep dentin have been considered unfavorable substrates for bonding. Lower bond strengths have been reported for deep dentin using earlier, less hydrophilic bonding agents. Increased wetness in deep dentin has been held responsible for diluting the resin monomers, thereby compromising adhesion. More recent work continues to demonstrate lower bond strength in deep dentin using more hydrophilic adhesive agents. Suzuki and Finger 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.

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. 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.
was light cured for 40 s with a light-curing unit (Degulux, Degussa, Hanau, Germany) at 450 mW/cm². 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 mm² (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

<table>
<thead>
<tr>
<th>Material</th>
<th>Components</th>
<th>Procedures*</th>
<th>Manufacturer</th>
<th>Lot number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearfil Liner Bond 2V</td>
<td>• Primer A: MDP, hydrophilic dimethacrylate, CQ</td>
<td>cc(x30s);</td>
<td>Kuraray</td>
<td>61126</td>
</tr>
<tr>
<td>(self-etching</td>
<td>• Primer B: HEMA, water, N,N-Diethanol p-toluidine</td>
<td>fg(20s)</td>
<td>Tokyo, Japan</td>
<td></td>
</tr>
<tr>
<td>adhesive system)</td>
<td>• Bond liquid A: MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, CQ, N,N-Diethanol p-toluidine, silanated colloidal silica</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prime &amp; Bond 2.1</td>
<td>• 36% phosphoric acid</td>
<td>a(15s); b:</td>
<td>Dentsply, De Trey</td>
<td>40293</td>
</tr>
<tr>
<td>(one-bottle</td>
<td>• Elastomeric dimethacrylate, PENTA, cetylamine</td>
<td>c; f(20s)</td>
<td>Konstanz, Germany</td>
<td></td>
</tr>
<tr>
<td>adhesive system)</td>
<td>hydrofluoride, acetone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z-100</td>
<td>• Bis-GMA, TEOSDMA</td>
<td></td>
<td>3M Dental Products</td>
<td>7KE</td>
</tr>
<tr>
<td>(hybrid composite)</td>
<td>• Zirconium and silica (84.5% filled by weight and 71% by volume - 0.6 μm average particle size)</td>
<td></td>
<td>St. Paul, MN, USA</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MDP: 10-methacryloyoxy methacrylate; CQ: camphorquinone; HEMA: 2-hydroxyethyl methacrylate; PENTA: dipentaerythritol penta acrylate monophosphate; Bis-GMA: bisphenolglycidyl 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 μm² area of the surface. The TD was expressed as the number of tubules/mm². 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 α = 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 (R² = 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

<table>
<thead>
<tr>
<th>Adhesive System</th>
<th>Superficial</th>
<th>Middle</th>
<th>Deep</th>
</tr>
</thead>
</table>
| Clearfil Liner Bond 2V | 29.9 ± 15.1 (n=9)
   | S           | 24.3 ± 9.5 (n=9)
   | 23.9 ± 10.6 (n=9) |
| Prime & Bond 2.1     | 61.7 ± 12.4 (n=9)
   | S           | 41.1 ± 5.9 (n=12)
   | NS          |
|                      |             |         | 25.6 ± 7.4 (n=9) |

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. 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-
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 6c  

Fig 6 Representative SEM micrographs of superficial (a), middle (b), and deep (c) dentin.
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 wet-
ness. The other study, however, did not use simu-
lated 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 instruc-
tions.

Suzuki and Finger pointed out that bond
strengths to dentin are more related to the availabili-
ty of solid dentin at the site of bonding than to
other factors, such as surface wetness. This appar-
ently 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 superfi-
cial dentin can be explained by the fact that more
intertubular dentin is available for hybrid layer for-
mation, this being the main bonding mechanism re-
ponsible for increased bond strength to dentin.

Theoretically, bond strengths should be higher to
deep dentin whenever resin tags can be firmly
bound (hybridized) to the lateral walls of the de-
mineralized dentinal tubules. 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, which may entrap air
within the blisters or within the dentinal tubules,
also possibly compromising the polymerization of
the resin bonding agent.11 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 superfi-
cial dentin or even higher for some adhesive
systems. It seems reasonable to admit that bond
strengths to deep dentin can be higher than to su-
perficial dentin. However, ideal bonding to deep
dentin is largely dependent on the adequate bond-
ing of resin tags within the wetter and larger denti-
nal 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 in-
sensitivity of self-etching systems to surface vari-
ables such as dentin depth, intrinsic wetness, and
presence, absence, or thickness of smear layer has
been previously reported. Apparently, because self-etching systems bond to the most superfi-
cial layer of dentin and do not completely remove
smear plugs, the intrinsic wetness of dentin is less
likely to interfere with bonding because the perme-
ability of the tubules is reduced. Since the bonding
mechanism of these systems relies on resin infiltra-
tion 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 rela-
tionship between bond strength of LB and the ASD
(Fig 3). For self-etching adhesive systems, resultant
bond strengths are more largely dependent on hy-
brid layer formation than on resin tag retention.
If we apply the modeling approach proposed by Pash-
ley et al, the contribution of hybrid layer to the
total adhesion increases from the pulp to the per-
iphery.

The stronger relationship between bond strength
and both TD and ASD observed for PB can be ex-
plained by the wider range of values of both para-
eters. The bond strength of PB ranged from as
low as 16.37 MPa (for a TD of 58,105 tubules/
mm²) to a maximum of 88.98 MPa (for a TD of
10,472 tubules/mm²). The percentage of ASD
ranged from approximately 45% for the deepest
dentin up to approximately 94% for the most super-
fiicial. 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/mm²) to
46.96 MPa (for a TD of 14,864 tubules/mm²). 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 mm²
is within the range of values usually reported in the
literature. For both PB and LB, most of the
specimens were located within the range of 20,000
to 40,000 tubules per mm². These are more repre-
sentative 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 reclassified 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 substrate 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.

ACKNOWLEDGMENTS

The adhesive systems used in this study were generously supplied by Kuraray Company, Osaka, Japan, and Dentsply Ind. e Com. Ltda., Petrópolis, RJ, Brazil. The authors are indebted to Dr. E.W. Kitajima (NAP-MEPA/ESALQ-USP) for technical electron microscopy support. This study was supported, in part, by grants DE 06427 from (the NIDCR, USA, and 300481/95-0 from CNPq, Brazil.

REFERENCES
