Effect of Pulp Pressure on the Micropermeability and Sealing Ability of Etch & Rinse and Self-etching Adhesives

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Clinical Relevance
Etch & rinse adhesives are much more micropermeable and affected by pulp fluid compared with self-etching adhesives. Pulp pressure reduces dentin sealing with etch & rinse adhesives but not with self-etching adhesives. Pulp pressure has no effect on enamel sealing, which is lower when self-etching adhesive is used.

SUMMARY
This research evaluated the effect of pulp pressure on the micropermeability and sealing ability of etch & rinse and self-etching adhesives. Two etch & rinse adhesives (Prime&Bond NT and Admira Bond) and one self-etching adhesive (Xeno III) were used. Adhesive layer micropermeability was evaluated by using confocal laser scanning microscopy (CLSM). Eighteen molars were connected to a pulp pressure device and divided into two groups. One group was restored with pulp pressure and the other group without. Each group was divided into three subgroups according to the adhesive used. The adhesives were rhodamine-labeled and Class V cavities were restored. After restoration, all specimens were kept under pulp pressure conditions for 24 hours with fluorescein-labeled pulp fluid. The specimens were sectioned and the axial wall was observed under CLSM. A microleakage test was performed to evaluate the sealing. Thirty molars were divided into two groups. One group was prepared with a pulp pressure device and the other group without. Each group was divided into three subgroups as a function of the adhesive used. Class V cavities were restored and the specimens were immersed in fuchsin and sectioned. Microleakage and dentin penetration were recorded in the occlusal and gingival walls. A CLSM study showed that the etch & rinse adhesives had higher micropermeability compared to the self-etching adhesives and pulp pressure made all the adhesives more permeable. In the occlusal wall, the best sealing (hermetic) was...
obtained when etch & rinse adhesives were used. Xeno obtained the lowest occlusal sealing values. In the gingival wall, Xeno obtained the best sealing, followed by Admira and Prime&Bond. Pulp fluid decreased gingival wall sealing when etch & rinse adhesives were used but not when self-etching adhesive was used.

INTRODUCTION

The ability of adhesives to seal dentin is one of the most important requirements for the durability of a composite restoration.1 Bonding to dentin and complete sealing of the exposed dentinal surfaces remain problematic, because of the highly hydrated and complex nature of the tissue, which is formed by intertubular dentin, peritubular dentin and dentin tubules. Dentin tubules constitute 20% to 39% of dentin, and the fluid within them represents 22% of dentin volume.2 The natural tissue pressure of the pulp promotes outward fluid movement into the dentin tubules and, when a cavity is prepared, the dentin surface is uniformly wetted.3

The adhesive must prevent water fluid movement from pulp, while sealing the dentin tubules and exposed collagen layer. Water from pulp can damage the resin adhesive during application (overwet phenomenon).4 If the adhesive cannot properly seal the dentin layer and tubules and, therefore, becomes damaged by water, the cavity sealing will be compromised and microleakage is more likely to occur.4

It is well documented that the adhesive layer acts as a micropermeable layer and can absorb water from the oral medium.5 However, there is inadequate information about the movement of pulp fluid into the adhesive layer6 and the impact on sealing of pulp pressure-induced variations in the hybrid layer and adhesive micropermeability.

Two different adhesive systems are currently used: etch & rinse adhesives and self-etching adhesives. Etch & rinse adhesives require an initial acid-etching step, which promotes a demineralization front with tubule opening. After etching, the adhesive is applied and a hybrid layer is formed.1 Self-etching adhesives perform substrate etching and infiltration simultaneously; they have a weaker acid effect and produce a smaller etching front and a smaller tubule opening versus etch & rinse adhesives.7 Therefore, the effects of pulp pressure may also differ between these adhesive systems.

This study evaluated the effect of pulp fluid on the micropermeability of the adhesive layer and on the sealing ability of two etch & rinse adhesives and one self-etching adhesive. The null hypothesis was that pulp pressure has no influence on dentin micropermeability or sealing of the adhesives, regardless of the type of adhesive used.

METHODS AND MATERIALS

Study Design

This study evaluated the effect of fluid movement from pulp to the bonding layer of two etch & rinse adhesive systems (Prime&Bond NT, Dentsply, Kostanz, Germany and Admira Bond, Voco, Cuxhaven, Germany) and a self-etching adhesive system (Xeno III, Dentsply) (Table 1). Two in vitro testing procedures were used. First, a novel confocal laser scanning microscope (CLSM) procedure was developed to evaluate the micropermeability of the dentin-bonding layer to the pulp fluid, testing morphological variations in the bonding layers as a consequence of fluid movement from the pulp. Second, a microleakage test was used to study the effect of pulp pressure on sealing ability.

Pulp Pressure Device (Figure 1)

Human third molars were used within three months of extraction and were stored in an aqueous 1% chloramine T solution at 4°C until prepared. The apical third of each tooth was removed. The permeability of two root canals to the pulp chamber was verified by means of endodontic K-files (#20). Each tooth was then apically penetrated from these root canals to the pulp chamber by two needles fixed to the root with Vitrebond (3M, St Paul, MN, USA). The needles were connected to a simulated pulp circuit of incoming and outgoing water under pressure. High pressure (40 mm Hg) was applied for five minutes to eject air bubbles from the pulp chamber via the outgoing circuit, which was then closed. The

Figure 1: Preparation of tooth and pulp pressure device.
pulp device was activated in the specimens for 24 hours before completing the restoration to ensure correct fluid movement through the dentin tubules. The working pulp pressure was 15 cm H2O (11.14 mm Hg), which is within the 7.5-22 cm H2O range of normal pulpal pressures in non-inflamed human teeth. To avoid interference with reagents used in the CLSM experiment, the fluid in the pulp pressure circuit was saline (0.9% sodium chloride).

Confocal Laser Scanning Microscopy

Eighteen third molars were prepared using the pulp pressure device. In each tooth, two standardized Class V cavities (3 mm x 2 mm x 2 mm [depth] with a 1 mm and 45° enamel bevel) were prepared with diamond coated #330 burs at high speed under water-cooling. The specimens were divided into two groups, a “non-pulp pressure group,” in which the adhesives were applied without activating the pulp device and a “pulp pressure group,” in which the adhesives were applied under pulp pressure conditions. Each group was divided into three subgroups as a function of the adhesive used (three specimens [six cavities] per adhesive and pulp condition). The cavities were filled according to manufacturers recommendations (Table 1). Prior to restoring, the bonding systems were labeled with rhodamine B isothiocyanate (Merck, Darmstadt, Germany) at a concentration of approximately 0.1%.

The filling material was placed in two increments, with each increment being light-cured for 40 seconds. The restoration was finished with polishing discs (3M). The restored teeth were immersed in water at 37°C for 24 hours. Pulp pressure was applied during the 24-hour water-immersion period only in the “pulp pressure group.”

After the 24-hour water immersion, all specimens in both groups (“no pulp pressure group” and the “pulp pressure group”) remained connected to the pulp pressure device for an additional 24 hours, using water labeled with fluorescein (Kraeber, Ellerbek, Germany) at a concentration of 5%. The apical needles were then removed and the specimens embedded in acrylic resin. Three 1-mm thick bucco-lingual sections were obtained from each resin-embedded specimen by means of a cutting machine (a total of nine sections [18 cavities] per adhesive and pulp condition). The sections were polished with fine emery cloth (P 4000 grade) and mounted on glass slides. The dentin/adhesive interface of the axial cavity wall was examined using a Leica TCS-SP2-AOBS CLSM (Leica, Wetzlar, Germany) equipped with two lasers (Ar laser operated at 488 nm and He-Ne laser operated at 543 nm). The images were obtained using a 100x oil immersion objective with 10x ocular and phototube. The reflection and fluorescent images were recorded, digitized and processed using the Leica Confocal Software (Leica). Observation of the rhodamine distribution (Ar laser) revealed the morphology of the adhesive dentin infiltration. Observation of the fluorescein distribution (He-Ne laser) showed pulp fluid penetration into the hybrid and adhesive layer (micropermeability of the bonding layer). The following

<table>
<thead>
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<th>Table 1: Materials Tested</th>
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<td><strong>Adhesive</strong></td>
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data were obtained from the images: thicknesses of the hybrid and adhesive layers (in microns); percentage of cases with infiltration of the hybrid layer by pulp fluid and percentage of infiltration; percentage of cases with sealed tubules and the length of seal in tubules (sealing length); and the percentage of cases with infiltration of the adhesive layer by pulp fluid and percentage of infiltration.

**Microleakage Test**

Thirty third-molars were divided into two groups, a “no pulp pressure group” of specimens that were prepared (see above) and tested without pulp pressure, and a “pulp pressure group” of specimens that were prepared and tested with pulp pressure. In each specimen, Class V cavities were prepared as described above. Specimens in each group were divided into three subgroups as a function of the adhesive used (5 specimens [10 cavities] per adhesive and pulp condition). The cavities were filled according to manufacturers recommendations (Table 1). In the “pulp pressure group,” the restoration was done under pulp pressure conditions.

The restored teeth were kept in water at 37° for 24 hours, then the apical needles were removed. After sealing the roots with IRM (Dentsply), the teeth were covered with two coats of nail varnish, leaving a 1-mm varnish-free margin around the restoration. The specimens were then immersed in a 0.5% water solution of basic fuchsin for 24 hours and rinsed for five minutes with distilled water. Next, the specimens were embedded in acrylic resin, and three bucco-lingual slices 1 mm thick were obtained for each specimen (15 slices [30 cavities] per adhesive and pulp condition). The slices were coded and randomly examined under the microscope by an experienced examiner in a blinded fashion. The grade of microleakage at the occlusal and gingival walls was categorized as follows: 0: hermetic seal, no leakage; 1: mild microleakage, dye on no more than half of the wall; 2: moderate microleakage, dye on more than half of the wall but not including the axial wall; 3: massive microleakage, dye on the entire wall, including the axial wall. For each group and wall, the microleakage was scored by multiplying the percentage of specimens with each grade of microleakage by the grade number (0-3) and adding these results together. Dentin penetration was evaluated as negative (absence of dye solution in dentin tissue) or positive (presence of dye solution in dentin tissue).

**Statistical Analysis**

Parametric micropermeability data were analyzed by means of a two-way ANOVA test (pulp pressure and material as independent variables), and multiple post-hoc comparisons between pairs of means were performed by using the Newman Keuls test. A Student’s t-test was used to compare variables between the “no pulp pressure” and “pulp pressure” groups. Non-parametric micropermeability data were compared by using the exact Fisher test. Microleakage analysis was performed with the non-parametric Kruskal-Wallis H-test and Mann-Whitney U-test. The exact Fisher test was used to evaluate dentin penetration. Given the large number of tests and comparisons, significance was established using Bonferroni’s correction. Statistical significance was considered at a confidence level of 95% ($p<0.05$)

### RESULTS

Table 2 lists the micropermeability data obtained, and Figures 2-7 depict representative CLSM images. In each Figure, image A shows the two fluorochromes together and the co-localization between rhodamine and fluorescein, while image B shows the pathway of the fluorescein-labeled pulp fluid. Rhodamine is visualized in red, fluorescein in green and co-localization in yellow or orange.

The hybrid layer was thickest with Prime&Bond (Figures 2-3), followed by Admira (Figures 4-5), then Xeno (Figures 6-7). The bonding layer was thicker with Xeno (Figure 6) than with Admira and Prime&Bond, which showed no differences between them (Figures 2-4). Pulp pressure had no influence on the thickness of
the hybrid or bond layers except with the use of Xeno, when a thicker hybrid layer was produced.

Pulp fluid infiltrated 100% of the hybrid layer when etch & rinse adhesives were used, both with and without pulp pressure (Figures 2-5). When self-etch adhesives were used, the hybrid layer was not always infiltrated (Figure 6); however, this infiltration was more frequent under pulp pressure conditions (Figure 7). When infiltrated, 100% of the self-etch hybrid layer was penetrated by fluorescein (Figure 7). Tubule sealing was only observed when self-etching adhesive was used, and it was more frequent with longer sealing length when applied without pulp pressure (Figure 6). The dentin tubules were not sealed when etch & rinse adhesives were used (Figures 2-5).

Infiltration of the adhesive layer differed according to the adhesive used and pulp pressure conditions. When etch & rinse adhesive was used without pulp pressure, a lower percentage of cases with infiltration of the adhesive layer and a lower percentage of infiltration were observed with Prime&Bond compared to Admira. However, under pulp pressure conditions, the adhesive layer of all specimens was infiltrated, with Admira showing the highest percentage of infiltration and Xeno the lowest. With the use of Xeno, there was a lower percentage of cases with infiltration when pulp pressure was applied.

Table 3 lists the microleakage and dentin penetration data obtained. Cavity sealing differed according to the cavity wall, adhesive type and pulp pressure. In the occlusal wall, Prime&Bond and Admira obtained the same hermetic sealing, while Xeno showed leakage but no positive dentin penetration. In the gingival wall, Xeno obtained the lowest leakage and dentin penetration, followed by Admira, while Prime&Bond obtained the highest leakage and dentin penetration. Sealing was higher in the occlusal wall than in the gingival wall when etch & rinse adhesives were used and, similarly, between the occlusal and gingival walls when self-etching adhesive was used.

Pulp pressure only affected sealing of the gingival wall. In the occlusal wall, the same leakage and dentin penetration values were obtained with and without pulp pressure. Etch & rinse adhesives produced more leakage and dentin penetration under pulp pressure conditions. However, when self-etching adhesive was used, similar leakage values were obtained with and without pulp pressure, and there was only a slight increase in dentin penetration under pulp pressure conditions.

Table 3: Microleakage Test Results

<table>
<thead>
<tr>
<th>Adhesive System</th>
<th>Intrapulpar Pressure</th>
<th>Cavity Wall</th>
<th>Microleakage (grades)</th>
<th>Positive Dentin Penetration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prime&amp;Bond NT</td>
<td>No pulp pressure</td>
<td>Occlusal</td>
<td>100 0 0 0 0</td>
<td>a 0 A</td>
</tr>
<tr>
<td>Prime&amp;Bond NT</td>
<td>No pulp pressure</td>
<td>Gingival</td>
<td>33 49 8 10 95</td>
<td>b 10 B</td>
</tr>
<tr>
<td>Prime&amp;Bond NT</td>
<td>Pulp pressure</td>
<td>Occlusal</td>
<td>100 0 0 0 0</td>
<td>a 0 A</td>
</tr>
<tr>
<td>Admira Bond</td>
<td>No pulp pressure</td>
<td>Occlusal</td>
<td>100 0 0 0 0</td>
<td>a 0 A</td>
</tr>
<tr>
<td>Admira Bond</td>
<td>No pulp pressure</td>
<td>Gingival</td>
<td>81 9 10 0 29</td>
<td>d 3 A</td>
</tr>
<tr>
<td>Admira Bond</td>
<td>Pulp pressure</td>
<td>Occlusal</td>
<td>100 0 0 0 0</td>
<td>a 0 D</td>
</tr>
<tr>
<td>Admira Bond</td>
<td>Pulp pressure</td>
<td>Gingival</td>
<td>66 17 15 2 53</td>
<td>e 20 E</td>
</tr>
<tr>
<td>Xeno III</td>
<td>No pulp pressure</td>
<td>Occlusal</td>
<td>75 22 3 0 28</td>
<td>d 0 A</td>
</tr>
<tr>
<td>Xeno III</td>
<td>No pulp pressure</td>
<td>Gingival</td>
<td>80 15 5 0 25</td>
<td>d 0 A</td>
</tr>
<tr>
<td>Xeno III</td>
<td>Pulp pressure</td>
<td>Occlusal</td>
<td>70 28 2 0 32</td>
<td>d 0 A</td>
</tr>
<tr>
<td>Xeno III</td>
<td>Pulp pressure</td>
<td>Gingival</td>
<td>77 16 7 0 30</td>
<td>d 2 D</td>
</tr>
</tbody>
</table>

Values with the same letter were statistically similar (p<0.05). (Sig: significance).
DISCUSSION

CLSM is a useful technique for exploring the sealing of bonding materials.\textsuperscript{6,10-11} A double labeling technique was applied in this study, using two fluorochromes with different wavelength excitation ranges (rhodamine and fluorescein) and exciting them with different lasers to minimize possible artifacts.\textsuperscript{12} Using this method, the location of each fluorochrome could be separately visualized. Rhodamine dissolves in acetone or ethanol-based liquids, similar to the adhesives under study, but it does not dissolve in water. Therefore, unlike pulp fluid, rhodamine does not move from resins into the water medium.\textsuperscript{10} In contrast, fluorescein dissolves in water and reaches the same places as water. The combination of these fluorochromes allows micropermeability of the adhesive layer to be evaluated by examining the movement of the resin material (rhodamine) and pulp water (fluorescein).

Current adhesives contain hydrophilic and hydrophobic components, increasing their potential to absorb water.\textsuperscript{5,13-14} The presence of numerous nanochannels in the hybrid layer was recently demonstrated, and the hybrid layer can be considered a micropermeable membrane.\textsuperscript{5,14-15} The current CLSM study demonstrated that pulp fluid can be absorbed by the hybrid and adhesive layers.

Micropermeability was higher with the etch & rinse adhesives tested than with the self-etching adhesive. The main difference between phosphoric acid and self-etching primer is the dentin permeability promoted by the former.\textsuperscript{16} Phosphoric acid removes the peritubular dentin and fully opens the dentin tubules. Phosphoric etching was reported to produce an increase of 200-300\% in hydraulic conductance,\textsuperscript{17} and ground dentin showed a fluid flow rate of 0.1 \(\mu\)l/minute compared to 0.9 \(\mu\)l/minute for phosphoric etched dentin.\textsuperscript{18} The adhesive must stop the flow of fluid and seal the entire exposed surface. However, etch & rinse adhesives have been reported to maintain a percentage of increased hydraulic conductance after composite placement.\textsuperscript{15} In contrast, when self-etching adhesive is applied, the dentin surface is smear layer sealed, reducing dentin permeability.\textsuperscript{18} and there is a lesser tubule opening\textsuperscript{16} and a reduced fluid flow. A 68%
increase in hydraulic conductance was reported after application of a self-etching acid, followed by a return to baseline conductance levels after composite placement. The above explains why the hybrid layer was always 100% infiltrated by pulp fluid when etch & rinse adhesive was applied (Figures 2-5). In contrast, self-etching adhesive was not always infiltrated, because tubule sealing was achieved in some cases (Figure 6). This finding was previously reported.

Although the adhesive layer is micropermeable, the hybrid layer must first be infiltrated. Fluid flow to the adhesive layer after hybrid infiltration differs according to the adhesive used. For the reasons described above, etch & rinse adhesives show a higher adhesive permeability compared with self-etching systems. In addition, the hybrid layer demonstrated less water absorption in self-etching versus etch & rinse adhesives, with a consequent reduction in adhesive layer micropermeability.

There were some differences between the etch & rinse adhesives studied. Higher micropermeability was observed with Admira than with Prime&Bond. Admira, an ormocer-based polymer, is more hydrophilic than Prime&Bond and can absorb more water. Therefore, under the same sealing conditions, there will be more fluid movement into the adhesive with Admira than with Prime&Bond.

Pulp pressure increased the micropermeability of all three adhesives, since it produces extra water on the surface and creates more microchannels for water movement after polymerization. This was more evident when etch & rinse adhesives were used, because of the more permeable surface created by phosphoric acid. It is more difficult to fully seal open tubules that exude water (etch & rinse adhesives) than partially sealed smeared tubules (self-etching adhesive).

Interestingly, when Xeno was used, the thickness of the hybrid layer was greater under pulp pressure conditions. A previous study reported that a higher water concentration improved acidic monomer ionization, with a consequent increase in thickness of the hybrid layer. Extra water from the pulp would increase the water concentration and acidic monomer ionization and, consequently, the etching depth.

Micropermeability has potentially detrimental effects. Collagen fibers are structurally unstable when in contact with water and become damaged over time. In the hybrid layer, adhesive resin has a protective function around infiltrated collagen fibrils, and this function may eventually be compromised by water sorption and hydrolytic degradation of the hydrophilic resin components in bonding systems. Water in the nanochannels may contribute to the direct degradation of resins by extracting unpolymerized monomers or small oligomers over time. The plasticizing effects of water on polymers have been documented. When water sorption occurs, intermolecular interactions among polymer chains are broken, with a detrimental effect on the union over time.

The effect of pulp fluid on the interface bonding microstructure is reflected in the sealing efficacy of the adhesive, as shown by the microleakage test results of this study. In the occlusal wall, which is bordered by enamel, pulp pressure had no effect on any adhesive. Etch & rinse adhesives obtained hermetic sealing. Enamel is mainly composed of mineral, and the etch-...
ing depth produced by phosphoric acid is adequate for the resin to produce a good seal, which is always higher in the occlusal versus the gingival wall.\textsuperscript{26-27} On the other hand, the self-etching adhesive showed slight leakage, because the weaker acidity of the primer achieves inadequate etching depth. In the gingival wall, pulp pressure decreased the sealing with etch & rinse but not with self-etching adhesives. As explained above, the higher permeability and hydraulic conductance produced by phosphoric acid compromises the sealing of the etched dentin. In contrast, when a self-etching adhesive is applied, there are no open tubules, and the simultaneous etching and infiltration facilitates tubule sealing.\textsuperscript{28-29} These differences explain the lower penetration of dentin by dye when self-etching was used. Although self-etching adhesives have lower etching power, it has been demonstrated that the bonding result is not influenced by etching depth or hybrid layer thickness.\textsuperscript{30} 

The results of this study have clinical repercussions. From the perspective of the authors of this study, self-etching adhesives are more appropriate for dentin bonding, because they use a dry technique that produces an improved bonding union. It has been proposed that a dry union is preferable in order to avoid the deleterious effects of water over time.\textsuperscript{25}

**CONCLUSIONS**

The null hypothesis is rejected and the conclusions of this study can be summarized as follows:

1. The most micropermeable union was obtained with Admira, followed by Prime&Bond, then Xeno.
2. Micropermeability was increased by pulp pressure when etch & rinse adhesives were used, but this increase was only small when self-etching adhesive was used.
3. The occlusal wall was hermetically sealed when etch & rinse adhesives were used, but not when self-etching adhesive was used.
4. Self-etching adhesive produced better gingival wall sealing versus etch & rinse adhesives. Between the etch & rinse adhesives, Admira obtained better gingival sealing than Prime&Bond.
5. Pulp pressure reduced gingival sealing with etch & rinse adhesives, but not with self-etching adhesive. Pulp pressure had no effect on occlusal sealing.

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**References**


