Effects of different resin adhesives on the microleakage in a new model with simulated subgingival condition and pulpal pressure

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Resin adhesive restorations are susceptible to oral fluid contamination and greatly influenced by dentinal tubule fluid because of pulpal pressure, especially when the restorative cavities are near gingival tissues. This study designed a novel model to evaluate the microleakage of self-adhesive flowable composite and traditional resin adhesives under simulated subgingival cavity preparations and pulpal pressure. We applied three different adhesive systems, include Vertise Flow, Optibond all-in-one, and Optibond S, on premolars with V-shaped cavity. All samples exhibited good marginal sealing at resin-enamel interfaces. At resin-dentin interfaces, microleakage in control group was similar among different adhesive systems. Microleakage in group pulpal pressure was greater than that in control group for all adhesive systems except Vertise Flow. All adhesive systems in pulpal pressure and simulated subgingival group exhibited significantly greater microleakage. In total, Vertise Flow exhibited better marginal sealing under pulpal pressure than other traditional adhesives.

Keywords: Self-adhesive flowable composite, Pulpal pressure, Simulated gingiva, Microleakage

INTRODUCTION

Wedge-shaped lesions frequently observed at the cervical area of teeth are non-carious and create a V-shaped defect in the enamel of the tooth. Among various therapeutic strategies, conventional composite restorations have gained popularity because they involve a minimally invasive procedure and provide good aesthetic outcomes. Nevertheless, composite restorations are challenged with marginal microleakage, which can lead to dentin hypersensitivity, pulp irritation, pulpal necrosis, and recurrent caries1,2.

Currently, there are three main types of resin bonding agents, and each type has its own advantages. For instance, classic total-etch adhesive has reliable bond strength, especially on unprepared enamel surfaces3. Self-etch adhesive significantly reduces the operating time and technique sensitivity of bonding procedures. The recently developed self-adhesive flowable composite simplified the bonding procedures, though their clinical performance has not yet been fully evaluated.

Dentin of vital teeth is hydrated by an outward flow of dentinal fluid caused by positive pulpal pressure (about 15 cm H2O)4. The dentinal fluid extruded onto the surface of prepared cavities can remarkably affect the bonding between dentin and restorative materials5-7. Several studies have demonstrated a higher probability of microleakage when hydrostatic pulpal pressure existed8-10. However, the effect of pulpal pressure on bonding of self-adhesive flowable composite is still unknown.

Presence of moisture on the cavity surfaces is an extremely important factor in resin bonding. However, effective moisture control is a difficult issue for cavity preparations below the gingival margin. Although gingivectomy or crown lengthening might be possible strategies to control moisture, the cervical surfaces of those cavity preparations are still vulnerable to gingival fluid contamination. Previous studies have reported that the contamination by gingival fluid, saliva, or blood had a detrimental effect on marginal adaptation of resin restorations11-13. The major pitfalls of previous studies are that the bonding procedures were performed on artificially contaminated dentin surfaces that were uniformly brushed with some amount of gingival fluid, saliva, water, or other fluids. However, contamination of cavity surfaces is more likely to occur near gingival margins in vivo. The conditions of bonding procedures in previous studies differed greatly from the real oral environment.

In this study, we have successfully developed a novel model to partly imitate the cavity surface of vital teeth near gingival margin. This model was then used to examine the effects of hydrostatic pulpal pressure and presence of a subgingival cavity surface on marginal microleakage of restorations bonded by different resin adhesive systems.

MATERIALS AND METHODS

Sample selection
A total of 90 complete human premolars extracted for
orthodontic reasons within 3 months were used for this study. They were stored in 0.5% Chloramine T solution at 4°C. A V-shaped cavity was prepared on the buccal surface of each tooth using a cylindrical diamond bur (Strauss Industrial Diamonds, Palm Coast, FL, USA) mounted on a high-speed handpiece under water cooling condition. The cavity preparation was 3 mm in width and 1.5 mm in depth, with its center located at the cemento-enamel junction. These prepared teeth were randomly divided into three groups (n=30): Group C (control, C), no intervention; Group P (pulpal pressure, P), 15 cm H$_2$O hydrostatic pulpal pressure; Group PG (pulpal pressure and simulated gingiva, PG), 15 cm H$_2$O hydrostatic pulpal pressure with gingival margin of the cavity preparation located 0.5 mm below simulated gingival margin.

**Pulpal pressure model**
The pulp chambers of teeth in Groups P and PG were exposed and the apical section of each root was cut off 7 mm apical to the cervical margin of the cavity preparation using fissure burs (SS WHITE, Lakewood, NJ, USA) under water cooling condition. The pulp tissue was removed carefully using barbed broach (DENTSPLY, Tulsa, OK, USA) under dental microscope (OPMI PROergo, Carl Zeiss, Oberkochen, Germany). The buccal cervical surface of pulp chamber was unprepared to preserve pre-dentin surface. The pulp chambers were then irrigated with 2.5% sodium hypochlorite for 30 s followed by immersion in distilled water for 30 min. The root canal of each tooth was apically penetrated by a needle, fixed, and then sealed with flowable composite (revolution formula 2, Kerr, Orange, CA, USA) and resin adhesive (Optibond all-in-one). The chamber access hole was connected to a plastic tube (diameter: 2 mm) and the connection was fixed and sealed in the same way as mentioned above. High pressure water flow (40 cm H$_2$O) was applied for 1 min to eliminate air bubbles from pulp chamber followed by blocking the apical needle. The pulpal pressure device was activated for 24 h before the composite restoration was placed. The working pulpal pressure was 15 cm H$_2$O, which was within the normal pulpal pressure range in healthy vital teeth.

**Combined model of pulpal pressure and simulated subgingival condition**
A total of 30 specimens (Group PG) were randomly selected from the pulpal pressure models mentioned previously. Each tooth was fixed in a cylindrical container (height: 15 mm, diameter: 30 mm). To simulate a moist state of in vivo subgingival cavity surface, 1% agar solution was poured into the container until its liquid level exceeded 0.5 mm above cervical margin of cavity. All cavities were isolated by tin foil which was removed following coagulation of agar solution (Fig. 1). Composite restorations were placed as soon as the temperature of agar gel dropped to room temperature (20°C).

**Sample preparation**
Each group was divided into 3 subgroups (n=10) according to different resin adhesive systems including one self-adhesive flowable composite Vertise Flow (Kerr), one-step self-etch adhesive systems Optibond all-in-one (Kerr), and one total-etch adhesive system Optibond S (Kerr). Detailed information of these resin adhesive systems is listed in Table 1. Each adhesive system was applied according to manufacturer’s instructions. All specimens were restored using flowable composite (Revolution formula 2, Kerr) except the Vertise Flow.

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**Table 1** Chemical composition and application methods of tested materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
<th>Composition</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertise Flow</td>
<td>Kerr, Orange, CA, USA</td>
<td>GPDM; Prepolymerized filler, 1-micron barium glass filler, nano-sized colloidal silica, nano-sized Ytterbium fluoride</td>
<td>Air drying of the cavity, apply less than 0.5 mm, brush for 15–20 s, light-cure for 20 s.</td>
</tr>
<tr>
<td>Optibond All-in-one</td>
<td>Kerr</td>
<td>GPDM, GDM, HEMA, Bis-GMA, water, ethanol, acetone, silica filler, CQ, sodium hexafluorosilicate</td>
<td>Apply 20 s with scrubbing motion, apply second application for 20 s with scrubbing motion, gently air dry and light cure for 10 s.</td>
</tr>
<tr>
<td>Optibond S</td>
<td>Kerr</td>
<td>Ethanol, HEMA, Bis-GMA, GPDM, silica, barium glass, sodium hexafluorosilicate</td>
<td>Etch with 35% phosphoric acid for 15 s, apply to cavity surface using a light brushing action for 15 s, light-cure for 10 s.</td>
</tr>
</tbody>
</table>
subgroup which acted as both an adhesive system and a restorative material. After storage in distilled water at 37°C for 24 h, the simulation devices in Groups P and PG were dismantled. Then all 90 specimens were prepared for microleakage test.

**Microleakage test**

All surfaces of each tooth were coated with two layers of fast-setting nail varnish except the bonding interface area for 24 h. Then, all specimens were immersed in a 50% weight silver nitrate water solution for 24 h followed by a thorough rinse with distilled water. Next, they were placed into a photo-developing solution (Dental X-ray Developer, Kodak, Rochester, NY, USA) for 8 h, and rinsed with copious amounts of water. Lastly, teeth were cut into two halves in a buccal-lingual direction with a low speed saw (Minitom, Struers, Ballerup, Denmark) under continuous water cooling condition.

With a ruler placed beside teeth as measuring scale, each half of all the teeth were photographed under a dental microscope (OPMI PROergo, Carl Zeiss). The magnified images acquired were used to calculate the length ratio of silver nitrate impregnated bonding interfaces at both enamel margin (occlusal) and dentinal margin (cervical) to 1 mm in measuring scale ruler using the image analysis software Photoshop CS5 (Adobe, San Jose, CA, USA). The exact length of silver impregnated bonding interface was calculated according the following formula: the exact length of stained interface=(the length of stained interface in picture/the length of 1 mm in picture)×1 mm. Both halves of each tooth were measured and the greater length was adopted.

**Scanning electron microscopy (SEM)**

Two specimens were randomly selected from each subgroup and processed for SEM observation. The sections were polished with a series of silicon carbide paper with grits ranging from 300 to 1,200 under water cooling condition. They were then treated with 32% silica free phosphoric acid gel (Uni-Etch, Bisco, Schaumburg, IL, USA) followed by deproteinization in 2% sodium hypochlorite solution for 2 min. All specimens were subsequently dehydrated in an ascending series of aqueous ethanol solutions to absolute ethanol. Finally, they were dried using hexamethyldisilazane (HMDS, Best, Chengdu, China). Each section was observed under a scanning electron microscope (JSM6460, JEOL, Tokyo, Japan).

**Statistical analysis**

The data of microleakage in this study did not obey normal distribution through Kolmogorov-Smirnov test analysis. Therefore, the Kruskal-Wallis analysis of variance was used to assess the significance of differences in microleakage under various circumstances of bonding procedures. Mann-Whitney U tests was used for post hoc comparisons. The level of significance was set at p<0.05 in all tests, and the statistical analysis was performed using the SPSS 16.0 software (SPSS, Chicago, IL, USA).

**RESULTS**

The effects of pulpal pressure and simulated subgingival condition on microleakage

To detect the differences of three different resin adhesive system, we assessed 10 teeth for each group with our novel model. After measuring the depth of microleakage stained by silver nitrate, we found that the leakage depths of occlusal enamel-resin bonding interface were zero in almost all samples, indicating that all resin adhesive systems had a good marginal sealing capacity for enamel bonding.

On the contrary, there were obvious leakages on the dentin and resin interface. For the cervical microleakage, all three adhesive systems exhibited similar

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**Table 2** Descriptive statistics of microleakage at cervical bonding interface in Group C

<table>
<thead>
<tr>
<th>Material</th>
<th>n</th>
<th>Mean (mm)</th>
<th>SD</th>
<th>Median (mm)</th>
<th>25–75%</th>
<th>p&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertise Flow</td>
<td>10</td>
<td>0.26</td>
<td>0.12</td>
<td>0.21</td>
<td>0.16–0.36</td>
<td>A</td>
</tr>
<tr>
<td>Optibond All-in-one</td>
<td>10</td>
<td>0.28</td>
<td>0.09</td>
<td>0.27</td>
<td>0.22–0.33</td>
<td>A</td>
</tr>
<tr>
<td>Optibond S</td>
<td>10</td>
<td>0.25</td>
<td>0.13</td>
<td>0.28</td>
<td>0.21–0.33</td>
<td>A</td>
</tr>
</tbody>
</table>

Different capital letters label statistically significant differences.
In the pulpal pressure treated group, the microleakage depth of total-etch adhesive Optibond S was 0.79±0.19 mm, which was significant larger than that in Vertise Flow (0.28±0.10 mm) and Optibond All-in-one (0.44±0.21 mm) (Table 3, Fig. 2). Next, we evaluated the effects of pulpal pressure combined with simulated subgingival condition on microleakage in Group PG. Our results showed that the microleakage depth of Vertise Flow, Optibond All-in-one, and Optibond S were 2.42±0.46, 1.76±0.48 and 1.88±0.48 mm, respectively (Table 4, Fig. 2). These results suggest that on the enamel side, all three adhesive systems have an appreciated effect.
Fig. 5 Digital photographs and SEM photographs of specimens restored using Optibond S as adhesive. (A) Group C, (B) Group P, (C) Group PG. E=enamel, D=dentin, R=resin.

while only Vertise Flow showed an acceptable adhesive capacity under pulpal pressure on the dentin side.

**SEM**
To further classify the effects of all the adhesive systems on restorations placed under different conditions, we investigated the bonding interface by SEM. The leakage fissure can be clearly observed at silver-nitrate-impregnated bonding interface under SEM. Less leakage depth is positively associated with better adaptation between resin restorations and cavity surfaces. Consistent with the results of microleakage measurements, SEM showed that the teeth under pulpal pressure and simulated subgingival condition had massive microleakage at the cervical bonding interface and exhibited obvious wide gaps. No typical bonding layer near the cervical margin of V-shape cavity preparation can be found (Figs. 3–5). These results confirmed that Vertise Flow exhibited better marginal sealing under pulpal pressure than other traditional adhesives.

**DISCUSSION**

Hydrostatic pulpal pressure is a constant state in vital human teeth, and it can significantly influence the diffusion of monomers from composite and bonding resins. In addition, subgingival cavity surfaces near the gingival margin are susceptible to fluid contamination. Hydrostatic pulpal pressure and subgingival cavity surface are two important factors accounting for the failure of composite restorations of wedge-shaped defects. To the best of our knowledge, this is the first study to evaluate the effects of hydrostatic pulpal pressure and subgingival cavity surface on microleakage of resin restorations.

We successfully developed a new system to mimicking a part of the native conditions for evaluating the effects of hydrostatic pulpal pressure and subgingival cavity surface on microleakage of resin restorations. As the native conditions are much more complex, new models or modifications are needed to mimic the real condition during the whole process. There are still several improvements, such as gingival fluid, saliva, or blood in subgingival cavity, and pulpal pressure which is caused by blood rather than stable hydrostatic pressure. The agar gel which simulates gingiva in this study pretreated by soaking in gingival fluid and saliva could be a potential improvement in the future studies.

As no pulpal pressure and simulated subgingival condition was applied in the control group, it resembled the condition of non-vital teeth without gingival fluid contamination. Our results showed that all resin adhesive systems in the control group exhibited similar microleakage. The newly launched self-adhesive flowable composite Vertise Flow shows better marginal sealing capacity compared to traditional resin adhesives, which is consistent with several previous studies. The excellent marginal sealing of Vertise Flow may be partially attributed to its higher hygroscopic expansion, although its bond strength is lower than that of traditional bonding agents.

Dentin fluid flow driven by pulpal tissue pressure is closely correlated with successful dentin bonding in vital teeth. In our experiment, pulpal pressure had no impact on the microleakage of Vertise Flow. However, other two bonding agents were significantly affected by pulpal pressure. Alexandre et al. found that pulpal pressure could reduce the bond strength of adhesive bonding to dentin, with different adhesives being affected differently. Mahdan et al. confirmed that pulpal pressure could increase the microleakage of five all-in-one self-etch adhesives. Rosales-Leal et al. suggested that microleakage of total-etch adhesives would deteriorate more under simulated pulpal pressure, which was in agreement with our study. In our model with simulated pulpal pressure, we could assess the microleakage and bond strength of adhesives in vital teeth. If the model applied to alternated cooling and heating treatment under the continuous pulpal pressure, we may evaluate the long-term clinic effects of adhesives.

Dentin surfaces should be pre-etched with phosphoric acid when total-etch bonding agents are used. This etching procedure totally removes the smear layer that obstructs dentinal tubules. Thus, it may lead to the opening of dentinal tubules and subsequent dentinal fluid contamination of bonding surfaces, resulting in marginal microleakage. Although the use of self-etch
adhesives does not necessitate phosphoric acid etching, acidic agents in self-etch bonding agents can still dissolve and infiltrate into the smear layer\(^6\). This can reduce the blocking effect to the dentinal tubule, leading to dentinal fluid exudation. Even when the adhesive layer is light cured, dentinal fluid can still penetrate the bonding interface of several 7th generation adhesives\(^18\). Possible microleakage of self-etch adhesives is thus affected by pulpal pressure. However, due to higher viscosity and lower wettability of flowable composite compared to conventional resin adhesives, Vertise Flow may maximally protect the dentin smear layer. Hiraishi et al.\(^19\) found that the flow rate of dentinal tubule fluid was significantly lower on bonding surfaces pretreated with self-adhesive cement than on those pretreated with traditional self-etch resin bonding agents. This could explain why the microleakage of Vertise Flow was least affected by simulated pulpal pressure in our study, as the bonding mechanism of Vertise Flow is similar to that of self-adhesive cement\(^20\).

The material used to simulate gingiva in this study was 1% agar gel, which has water as the main component. The upper surface of agar gel was about 0.5 mm higher than cervical margin of the cavity preparation. Thus, the continuous moisture on the cervical margin of the cavity preparation produced by simulated gingiva mimicked the condition of in vivo subgingival cavity preparations. Our results revealed that microleakage in Group PG was larger than that in Groups C and P, suggesting that the simulated subgingival condition caused disastrous microleakage for all adhesive systems. Similarly, ineffective bonding near the cervical margin was observed for all three resin adhesive systems under SEM. However, effects of moisture on microleakage caused by simulated gingiva differed among different bonding agents. In our model with simulated subgingival condition and pulpal pressure, we could assess the microleakage of adhesives in vital teeth, but not the bond strength, as the moisture environment between the dentin and resin interface.

By analyzing phase separation of model dentin adhesives exposed to over-wet environments, resin adhesives are demonstrated to be susceptible to water contamination. Large amounts of water can separate components of adhesives into aqueous and hydrophobic resin phase\(^21\). Therefore, resin adhesives are not able to bond to dentin substrates in over-wet environments. In our study, the disastrous microleakage may have resulted from massive moisture produced by the simulated gingiva. The self-adhesive flowable composite Vertise Flow exhibited the largest microleakage under simulated gingiva. According to the manufacturer, the functional monomer glycerol phosphate dimethacrylate (GPDM), as well as other components in its compositions are not water-soluble\(^22\). Thus, formation of a liquid layer between resin restorations and the dentin substrate, even by a small amount of water contamination, can subsequently block its bonding to dentin and induce significant microleakage. Furthermore, air-drying the cavity preparation using an air-water syringe during bonding procedures can help evaporate water and organic solvent in adhesives, which may help reduce water contamination. The bonding procedures of Vertise Flow do not need air-drying, while other adhesive systems do, which might be partially responsible for the large amount of microleakage seen in Vertise Flow.

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