Evaluation of Microtensile Bond Strength and Microleakage of a Self-adhering Flowable Composite

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\textbf{Purpose:} To evaluate the microtensile bond strength (μTBS) and marginal sealing ability of a self-adhering flowable composite between dentin and composite interfaces, as well as the microleakage of Class V restorations.

\textbf{Materials and Methods:} The occlusal thirds of 40 third molars were removed and randomly divided into 4 groups according to the applied adhesive: Adper Easy One (AEO, 3M ESPE), Clearfil SE Bond (CSEB, Kuraray), Prime & Bond NT (PBNT, Dentsply) and a self-adhering flowable composite (Dyad Flow, DF, Kerr). Filtek Flowable (3M ESPE) resin composite crowns were then built up in the first three groups; in group DF, composite crowns were built up without the application of an adhesive. Thirty stick-shaped microspecimens were prepared per group, 10 of which were used for morphological observation of bonded interfaces by scanning electron microscopy (SEM) after decalcification. The remaining microspecimens underwent microtensile bond strength testing and the failure mode was analyzed. Microleakage evaluation was performed on 10 premolars per group in which standardized box-shaped Class V cavities were prepared. After 500 thermocycles, the premolars were immersed in 1% methylene blue for 24 h, and three slices from each tooth were observed under a stereomicroscope and scored. Statistical analysis was performed using one-way ANOVA, Student-Newman-Keuls and chi-square tests.

\textbf{Results:} The PBNT group presented the highest μTBS values, followed by the CSEB and AEO groups, which did not differ significantly from each other. The DF group showed the lowest μTBS values. No significant differences in microleakage were observed among these four groups.

\textbf{Conclusion:} Although individual usage of the self-adhering flowable composite showed the lowest bond strength, the same marginal sealing ability was observed as that of combining self-etching and etch-and-rinse adhesives with flowable composite.

\textbf{Keywords:} microtensile bond strength, microleakage, self-adhering, flowable composite, adhesive.

\textit{J Adhes Dent} 2015; 17: 535–543. Submitted for publication: 08.08.14; accepted for publication: 21.10.15
doi: 10.3290/j.jad.a35253

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Various flowable composites have been continuously developed since 1996, when the first generation was introduced to restore Class V lesions. These flowable composites have desirable handling properties which allow the material to be injected through special syringes, thus simplifying the placement procedure and extending the range of applications suggested by manufacturers. They are less viscous because they contain less fillers (weight: 60%-70%; volume: 46%-65%) compared with their hybrid analogues (weight: 70%-80%; volume: 60%-75%). Currently, flowable composites are ideal for a variety of indications, such as base/liner, pit-and-fissure sealant, small Class III and V restorations, amalgam margin repairs, enamel defects, and incisal edge repairs in anterior sites.

Flowable composites generally showed higher shrinkage and lower elastic modulus than traditional nonflowable composites because of the lower filler content. The interfacial shrinkage stresses between dental tissue...
and composites indicate a potential to increase the possibility of microleakage. In addition, adhesive technique sensitivity could limit the clinical success of bonded restorations. Therefore, self-adhering flowable composites which incorporate an adhesive resin in the flowable composite seem clinically promising.

By incorporating glycercophosphate dimethacrylate (GPDM), a functional monomer, self-adhering flowable composites (Dyad Flow or Vertise Flow, Kerr) can greatly simplify direct restorative procedures. A GPDM adhesive monomer acts like a coupling agent. It has an acidic phosphate group for etching the tooth structure and chemically bonding to the calcium ions within the tooth structure; it also possesses two methacrylate functional groups for copolymerization with other methacrylate monomers to provide increased crosslinking density and enhanced mechanical strength for the polymerized adhesive (manufacturer’s information, Kerr). This new composite eliminates the need for a separate bonding application step. Therefore, this composite could lessen clinical application time and significantly reduce technique sensitivity or the risk of making errors during application and manipulation.

One study showed that self-adhering flowable composite presented the highest modulus and hardness, as well as remarkably strong elastic properties, compared with other flowable composites. It exhibited the highest hygroscopic dimensional expansion and water sorption after 150 days of water immersion, compared with conventional flowable and composite resins. A higher hygroscopic dimensional expansion manifests as better cavity sealing. The self-adhering flowable composite also showed the highest reliability, degree of conversion, and micromechanical properties, as well as suitable mechanical properties, compared with other flowable resin-based composites. However, very few studies have been conducted to test the bonding abilities of self-adhering flowable composite.

With shear bond strength tests on dental substrate, one study showed that the self-adhering flowable composite resulted in lower bond strengths but better marginal sealing ability compared with all-in-one adhesive systems. When the self-adhering flowable composite was used to bond brackets to enamel, it initially achieved suitable shear bond strength, but this decreased significantly after thermal cycling. In addition, self-adhering flowable composite combined with an adhesive resin provided greater bond strength and better marginal sealing than the composite alone. Therefore, more studies should be carried out to evaluate the adhesive performance of this composite and provide useful information on its clinical applications.

The purpose of this study was to investigate the bond strength between dentin and the self-adhering flowable composite interfaces, as well as assess the marginal sealing ability of Class V cavities restored by the self-adhering flowable composite compared with that of frequently used dentin bonding systems. The null hypotheses were that (1) the bond strength of the self-adhering flowable composite was the same as that of combination of adhesives and flowable composites, and that (2) no differences in marginal sealing ability were present between the self-adhering flowable composite and other dentin bonding systems.

**MATERIALS AND METHODS**

The materials used in this study are outlined in Table 1. A total of 100 permanent human premolars and third molars were obtained from West China Hospital of Stomatology, Sichuan University. After extraction, the teeth were immediately cleaned and ultrasonicated to remove residual soft tissue, plaque, and other pit-and-fissure debris, then stored in 1% chloramine solution at 4°C for less than four weeks before use. Out of the 100 teeth, 20 that exhibited stains, demineralization, decay, fluorosis, enamel defects, or restorations were excluded, resulting in 40 premolars for microleakage testing and 40 third molars for μTBS testing. Ethical and protocol approval was obtained from the Medical Ethics Committee at West China School of Stomatology, Sichuan University.

**Microtensile Bond Strength Testing and Ultrastructural Morphology**

**Microspecimen preparation for μTBS testing**

Figure 1 shows the μTBS microspecimen production and testing method.

A total of 40 third molars without stains, demineralization, decay, fluorosis, enamel defects, or restorations were selected. The occlusal third of the molar crowns was removed using a water-cooled slow-speed diamond saw (Miniotm, Struers; Ballerup, Denmark) to expose the dentin surfaces. Afterward, the surfaces were polished with water-cooled silicon carbide paper (320, 500 and 600 grit) on a rotating polishing machine (Struers). To make sure no enamel remained, the surfaces were examined using a stereoscopic microscope (SMZ100, Nikon; Tokyo, Japan).

The teeth were randomly divided into 4 groups (n = 10) according to the type of adhesive restorative system used. All treatment interventions were performed by a trained dental investigator. For groups AEO (Adper Easy One, 3M ESPE; St Paul, MN, USA), CSEB (Clearfil SE Bond, Kuraray Noritake; Tokyo Japan) and PBNT (Prime & Bond NT, Dentsply; York, PA, USA), three dentin adhesive systems were applied to the dentin surfaces, and resin composite crowns of Filtek Z350 flowable restorative were then built up incrementally in 1.5-mm layers to a height of approximately 4 mm, following the manufacturers’ instructions (Table 1). For group DF (Dyad Flow, Kerr), the resin composite crowns were incrementally built up on the dentin surfaces to a height of approximately 4 mm using the self-adhering flowable composite without the application of adhesives, following the manufacturers’ instructions (Table 1). After immersion in water at 37°C for 24 h, the teeth were sectioned perpendicular to the bonding surface both mesiodistally and buccolingually along their long axis into about 1 mm × 1 mm × 8 mm stick-shaped specimens.
with a slow-speed diamond saw. For each tooth, only 4 to 6 central sticks were used and carefully examined in a stereoscopic microscope, resulting in 30 sticks per group, 10 of which were used for SEM examination of the bonding interfaces and the rest for μTBS evaluation.

SEM examination of bonding interfaces
The surfaces of the specimens were polished with 600-grit silicon carbide paper, decalcified for 30 s in 37% phosphoric acid, deproteinized for 2 min in 10% NaOCl, and then rinsed. The bonding interfaces between adhesive systems and dentin were air dried, sputter coated with gold, and observed using a scanning electronic microscope (Inspect F50; FEI; Hillsboro, OR, USA).

μTBS testing
The microspecimens were individually attached to a custom-made apparatus (Fig 1) for tensile testing in a computer-controlled Universal Testing Machine (MTS; Eden Prairie, MN, USA) and stressed to failure at a crosshead speed of 0.5 mm/min. The cross-sectional area at the site of failure of each stick was measured with an electronic digital-display micrometer (Shenzhen XinHeCheng Technology; Guangdong, China) and re-
corded. The μTBS of each specimen was calculated in MPa, by dividing the imposed force (in N) at the time of fracture by its bonded area (in mm²). All specimens were maintained moist throughout the whole preparation and test procedure. Premature failures were excluded from further analysis and were not included in the calculation of the mean μTBS, but the number of premature failures was recorded.

**Failure mode analysis and SEM examination**

After the μTBS test, the test surfaces of the dentin and resin were examined using a stereoscopic microscope under 100X magnification. The failure modes were recorded as follows: adhesive failure, if 100% of the bonded interface failed between the dentin and the bonding resin; cohesive failure, if 100% of the failure was in the flowable restorative; or mixed failure, if the failures were partially adhesive and partially cohesive.42

Representative specimens were examined using SEM to observe the ultrastructural micromorphology of the failures.

**Statistical analysis**

Statistical analysis was performed using SPSS 17.0 for Windows (SPSS; Chicago, IL, USA). After normal distribution and homogeneous group variances of the μTBS data were confirmed, one-way ANOVA was applied, followed by the Student-Newman-Keuls multiple comparisons test for post-hoc comparisons. A chi-square test was used to compare the incidence of different failure modes among the four groups. The significance level was set at p < 0.05.

**Microleakage Evaluation**

**Sample preparation**

A total of 40 premolars, free of demineralisation, decay, enamel defects, or restorations were selected. Standardized box-shaped (4 mm mesiodistally, 2 mm occlusogingivally, and 2 mm deep) Class V cavities were prepared on the buccal surfaces of each tooth with a diamond bur in a water-cooled high-speed handpiece by a trained dentist. After each cavity preparation, the electronic digital-display micrometer and a periodontal probe were used to check the accuracy of the dimensions. The cavities were located at the cementoenamel junction level, with the occlusal margin in enamel, the gingival margin in cementum-dentin, and a short bevel at the occlusal enamel margin. All samples were restored according to the groups AEO, CSEB, PBNT, and DF (n = 10). Bonding and filling procedures were done following manufacturers’ instructions (Table 1). Final finishing and polishing procedures were performed immediately after filling. The teeth were then stored in water at 37°C for 24 h.

**Microleakage testing**

The samples were thermally cycled 500 times between 5°C and 55°C water baths, with a dwell time of 30 s at each temperature and a transfer time of 5 s between baths. After cycling, the samples were dried. For microleakage testing by dye penetration, the apices of the teeth were sealed with autopolymerizing acrylic resin and the entire surface was coated with nail vanish, except for the restoration and 1 mm of tooth surface adjacent to the restoration. They were then immersed in 1% methylene blue at 20°C to 25°C for 24 h. After removing them from the dye solution, the samples were washed under tap water, dried, and mounted in the autopolymerizing acrylic resin to facilitate handling during sectioning.

**Microscopic observation**

Each tooth was sectioned longitudinally in the occlusogingival direction with a water-cooled slow-speed diamond saw. The position of the first cut was 0.5 mm distal to mesial edge of the restoration. The following cuts were successively moved 1 mm distally to yield three 1-mm-thick slices. Both sides of each slice were examined at the occlusal and gingival margins with a stereomicroscope at 50X magnification by two investigators without knowledge of the assigned groups. If the two investigators disagreed on the score given to a certain slice, they re-evaluated and reached a consensus. The extent of microleakage in both occlusal and gingival margins was evaluated using the following scoring system (Fig 2):10 0: no dye penetration; 1: dye penetration
up to one-third of cavity depth; 2: dye penetration up to two-thirds of cavity depth; 3: dye penetration towards the cavity floor.

Only the side with the highest score in occlusal or gingival margins of each tooth was considered in the statistical analysis.

**Statistical analysis**
The Kruskal-Wallis H non-parametric test was used to independently compare the four groups for occlusal and gingival margins. Occlusal microleakage was compared to gingival microleakage within each group using the Wilcoxon signed-rank test. The significance level was set at $p < 0.05$.

**RESULTS**

**SEM Observation of the Bonding Interfaces**
Representative micrographs of the bonding interfaces for the different adhesive systems are shown in Fig 3. The bonding region of groups AEO (Fig 3a) and CSEB (Fig 3b) exhibited similar morphological aspects, characterized by varying lengths of resin tags and a relatively thin hybrid layer. Group PBNT (Fig 3c) showed particularly thick hybrid layers, as well as long, robust resin tags. Figure 3d illustrates the thinnest hybrid layer and short resin tags that were not able to penetrate into dentinal tubes.

**μTBS**
No significant differences in μTBS were observed between groups AEO and CSEB. The bond strength values of group PBNT were the highest ($p < 0.05$), whereas group DF exhibited the lowest μTBS ($p < 0.05$) (Table 2).

**Failure Mode Analysis**
The distribution of failure modes (adhesive, cohesive and mixed) for the different adhesive systems is depicted in Fig 4. SEM images of the failures of representative specimens are shown in Fig 5. A predominance of adhesive failure mode was observed for all groups tested, but statistical analysis (chi-square test), showed that no significant differences in failure modes were
observed among the groups (p > 0.05). No failures were detected through dentin. Only group PBNT showed 5% cohesive failure mode.

Microleakage
The Kruskal-Wallis H non-parametric test indicated no significant differences among the four groups for occlusal and gingival microleakage independently. The Wilcoxon signed-rank test revealed that the microleakage was significantly higher in the gingival than in the occlusal margin for group PBNT (p < 0.05). The occlusal and gingival microleakage scores among the other groups showed no significant differences (p > 0.05) (Table 3).

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* Total number (n = 10) of teeth evaluated. Same uppercase letters indicate no significant differences among the four groups for occlusal and gingival microleakage independently (p > 0.05). Different lowercase letters indicate that the microleakage was significantly higher in the gingival margin than in the occlusal margin of group PBNT (p < 0.05). For group abbreviations, see Table 2.

DISCUSSION
The μTBS test is presently considered the most valuable test for evaluating adhesion bond strength.7,33 Thanks to an axial tensile load on a small, square-shaped interface, the μTBS test provides more favorable stress distribution better representing the true ultimate strengths, thus eliminating most of the cohesive failures in composite or dentin.3,23 Furthermore, when the cross section was less than 2 mm², the μTBS test reflected the highest tensile force (ie, bond strength), because the cohesive strength of dentin and composite was the least under this small interface.34 This study provided evidence of predominance of the adhesive failure mode, with no failure in dentin for any of the groups tested.

In the present study, the highest μTBS was observed in the group treated with PBNT and flowable compos-
ite (37.96 ± 7.15 MPa). As evident in the SEM image (Fig 3c), a thick, uniform, and compact hybrid layer, as well as resin tags and their lateral branches are apparent on the bonding interface. The etch-and-rinse technique was used in this group, which removes the smear layer completely, with dentinal tubules opening via acid etching with 34% phosphoric acid. The use of phosphoric acid on dentin may result in complete dissolution of the peritubular dentin, allowing more resin infiltration into the dentinal tubules. The adhesive strength mediated by PBNT principally comes from the combined effect of micromechanical interlocking of resin molecules into an exposed dentin collagen fiber network and resin tags into the opened dentinal tubules. Thus, the highest bond strengths were observed when PBNT systems were applied, and the μTBS value showed significant differences from the other two adhesive systems and the self-adhering flowable composite. However, a previous study found acid etching to induce moderate to severe pulp inflammatory reactions. This is due to the fact that acid etching of dentin, regardless of the concentration of phosphoric acid, removes the smear layer and exposes the apertures of dentinal tubules, consequently providing a passageway for bacteria and their products into the pulp chamber. Therefore, the etch-and-rinse technique is often applied in enamel bonding rather than dentin bonding, to decrease the possibility of postoperative sensitivity.

Unlike the etch-and-rinse technique, the self-etching technique did not completely dissolve or remove the smear layer formed by the organic and inorganic debris. Such remnants will partly integrate into the hybrid layer. The hydrophilic and acidic monomers in self-etching adhesives can simultaneously demineralize and penetrate dentin. However, the acidity of the self-etching primers is lower than that of phosphoric acid. Thus, the presence of the smear layer will prevent infiltration of resin into dentin, so that the bond strength of the self-etching systems was lower than that of etch-and-rinse systems, as observed in this study. Groups AEO and CSEB showed a characteristic interfacial morphology with a thin hybrid layer and resin tags of various lengths (Figs 3a and 3b). The μTBS values of both groups (AEO: 34.90 ± 8.33 MPa; CSEB: 35.63 ± 5.23 MPa) were lower than that of group PBNT, and no significant difference was found between these two groups.

The self-adhering flowable composite exhibited the lowest μTBS (32.66 ± 8.20 MPa), which is supported by other studies which found that the μTBS to dentin of the self-adhering flowable composites was significantly lower than that of other adhesive systems combined with composites. The functional monomer GPDM most likely acted as an etching agent rather than as an adhesive to hydroxyapatite because of its weak penetration ability, although OptiBond FL (Kerr), which contains the same functional monomer, has proven to be among the top-performing adhesives in both laboratory and clinical studies. The poor wettability of this composite might be another reason of such a result. Dyad Flow was more viscous than the other adhesive systems tested, because it contained no solvent. One drawback of this material was its insufficient wettability when it was bonded to self-etch collagen fibrils. The initial layer of this material was dispensed on a dry surface, brushed for 15 to 20 s with moderate pressure, and then light cured for 20 s following the manufacturer's recommendation. However, the present study did not find the μTBS of Dyad flow to be enhanced, which supports the rejection of the first null hypothesis. The clinical performance of another self-adhesive flowable composite, Fusio Liquid Dentin, did not show an acceptable success rate in restoration of noncarious cervical lesions after 6 months. Although Dyad Flow showed higher μTBS than Fusio Liquid Dentin, the highest μTBS was recorded when Dyad Flow was applied after the acid etching of dentin. The sole use of Dyad Flow on dentin does not provide adequate μTBS. The SEM image of the interface between Dyad Flow and dentin showed thin tags and lack of a uniform hybrid layer (Fig 3d). The result was in accordance with a previous study, which concluded that the bond strength values of the self-adhering flowable composite to dentin and enamel were the lowest when compared with five combinations of self-adhesive systems and the proprietary flowable composites.

The clinically important minimum dentin bond strength required for successful bonding is still unknown. A systematic review showed significant differences in the pooled mean bond strength, as the weighted bond-strength means per adhesive class range from 31 MPa for three-step etch-and-rinse adhesives, 29 MPa for two-step self-etching adhesives, 26 MPa for two-step etch-and-rinse adhesives, 20 MPa for one-step self-etching adhesives. In the present study, all adhesive systems, including Dyad Flow, showed higher bond strength compared to these weighted bond-strength means. This finding means that the self-adhering flowable composite may be used alone to eliminate the complexity of bonding technique and reduce the application time for dentists. Further studies should focus on the clinical outcome of applying the self-adhering flowable composite to different classes of cavities.

Microleakage occurred when gaps were present between the composite restoration and the walls of the prepared cavity caused by the polymerization shrinkage of all composites. The evaluation of microleakage was imperative to test the bonding systems and restorative materials. Class V cavities were prepared in this study because they have high C-factors, with a high ratio of bonded flow-inactive to free flow-active surfaces. Thermocycling was used to imitate the intraoral environment by exposing the restored teeth to temperature extremes. As reported, small numbers of thermocycles are sufficient to induce microleakage in restorations, and thermocycling increased the microleakage of Class V composite restorations. Thus, the results were more reliable because of the use of these two standard procedures.

According to the microleakage evaluation results, no significant difference was observed in the marginal sealing ability between the tested adhesive systems both in the occlusal and gingival margins, meaning the second null hypothesis was accepted. This result was in accord-
ance with Owens et al,25 who observed no significant differences among the self-etching and etch-and-rinse adhesives when the dentin margin was evaluated. When the self-adhering flowable composite was used in Class I cavities in vitro, it presented better marginal sealing ability compared with all-in-one adhesive systems.38 This result was different from ours. The authors speculated that it was the hygroscopic expansion and the reduction of the competition between bonding and curing stress that favored marginal adaptation of this material.38 Self-adhering flowable composite could also be applied as a pit-and-fissure sealant because of the satisfactory sealing ability compared with the marketed sealants.21 The self-adhering flowable composite alone exhibited better sealing ability at the dentin interface of Class V cavities than did preliminary phosphoric acid etching of dentin combined with the self-adhering flowable composite.29 However, a different study6 reported that the self-adhering flowable composite presented higher microleakage than did a three-step etch-and-rinse adhesive combined with flowable composite in enamel and dentin. The microleakage of the self-adhering flowable composite could be significantly reduced when enamel was pretreated.9 Whether self-adhering flowable composite maintains its favorable marginal sealing ability when studied in vitro is debatable.9 Gingival microleakage was significantly higher than occlusal microleakage in group PBNT. This finding may be due to the use of a separate phosphoric acid etchant when bonding. This step can improve enamel penetration and the subsequent attachment of adhesive monomers. Conversely, the use of phosphoric acid caused hyper-decalcification of the dentin surface and subsequent collapse of the collagen fibrillar network.

CONCLUSIONS

The results of the present research showed that individual usage of the self-adhering flowable composite yielded the lowest bond strength and similar marginal sealing ability compared with self-etching and etch-and-rinse adhesives combined with flowable composite. Further studies in vitro are expected to clarify whether the bond strength and sealing ability of Dyad Flow self-adhering flowable composite are clinically adequate.

ACKNOWLEDGMENTS

This study was supported by grants from the National Natural Science Foundation of China (Grant Nos. 81170958 and 51073102), the Specialized Research Fund for the Doctoral Program of Higher Education (Grant No. 20100181110056), and Special Fund for Scientific Research in the Public Interest (Grant No. 201000207).

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Clinical relevance: The findings suggest that clinical application of the self-adhering flowable composite alone should be carefully considered. The simplified procedure and proper marginal sealing ability encourages further clinical investigations on restoring Class V cavities.