Comparative study of the dentin bond strength of a new universal adhesive

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This study compared the dentin bond strength of a new universal adhesive with that of contemporary multi-step dentin adhesives. Six experimental groups were prepared according to the adhesives used and their application modes: Optibond FL (OB), Adper Single Bond Plus (SB), One-Step Plus (OS), Clearfil SE Bond (CS), All-Bond Universal using etch-and-rinse mode (ABE), and All-Bond Universal using self-etch mode (ABS). Micro-tensile bond strength (µTBS) and failure mode were evaluated for each group. The bonded interface was analyzed using transmission electron microscopy (TEM). As a result, µTBS of 6 experimental groups was followed as: OB=ABE=SE=ABS>SB>OS group. TEM micrographs of ABE and ABS groups revealed a homogenous adhesive layer formation. In conclusion, a new universal adhesive can make reliable bond to dentin, regardless of the application mode.

Keywords: Universal adhesive, Micro-tensile bond strength, Failure mode, Transmission electron microscope

INTRODUCTION

Dentin adhesives have been developed continuously to minimize the application step and to reduce technique sensitivity1). As a result, single-step self-etch adhesives (commonly called “all-in-one” adhesives) were introduced in the early 21st century. They contained all the necessary components for bonding in a single solution such as various solvents (acetone, ethanol, buthanol, or water), resin components, photo-initiators, inhibitors2). They are so-called as 7th generation dentin adhesive and undoubtedly the most convenient. However, various studies about their bonding effectiveness revealed some disadvantages for them3-7). For these reasons, the most commonly used dentin adhesives are multi-step dentin adhesives.

Prior to “all-in-one” dentin adhesive, dentin adhesives are generally classified into 3-step etch-and-rinse, 2-step etch-and-rinse and 2-step self-etch type. They have their respective pros and cons. For the etch-and-rinse dentin adhesives, the smear layer can be completely removed by phosphoric acid etching to form a predictable hybrid layer. However, it may cause post-operative hypersensitivity if the demineralized dentin is not infiltrated properly with dentin adhesive8). The use of self-etch dentin adhesive enables to minimize post-operative hypersensitivity, but its disadvantage is an insufficient enamel etching ability resultant from their less acidity2). Thus, it is very important to use these dentin adhesives properly in various clinical situations.

Recently, so-called “universal” dentin adhesives have been introduced into the market. They can be applied using either etch-and-rinse or self-etch mode, enabling the clinician to choose the appropriate application mode. Some studies have been reported regarding the bonding performance of these universal adhesives9-10), and yet it remains controversial whether they are capable of bonding properly or not.

In this study, the dentin bond strength of a new universal adhesive was investigated by comparing micro-tensile bond strength (µTBS) and fracture mode with 4 contemporary multi-step dentin adhesives. The bonded interface was analyzed using transmission electron microscopy (TEM). The null hypothesis was that the dentin bond strength of a new universal adhesive differs significantly from that of other multi-step dentin adhesives.

MATERIALS AND METHODS

Specimen preparation

The protocol of this study was approved by the Institutional Review Board (IRB) of Kyung Hee Medical Center (KHD IRB 1204-3). Twenty four caries-free mandibular 3rd molars were selected. The superficial enamel was removed, and flat dentin surface was exposed under water irrigation using a low-speed diamond saw (ISOMET™, Buehler, USA). Each dentin surface was ground with 320-grit silicon carbide abrasive paper to produce a standard smear layer. The teeth were randomly assigned to 6 experimental groups according to the adhesive used as well as its mode of application: Optibond FL (Kerr, Orange County, CA, USA; OB group); Single Bond Plus (3M ESPE, St. Paul, MN, USA; SB group); One-Step Plus (Bisco, Schaumburg, IL, USA; OS group); Clearfil SE Bond (Kuraray, Osaka, Japan; CS group); All-Bond Universal using the etch-and-rinse mode (Bisco; ABE group); and All-Bond Universal using the self-etch mode (ABS group). The chemical composition and instruction for use of all the adhesives are summarized in Table 1.
<table>
<thead>
<tr>
<th>Material</th>
<th>LOT No.</th>
<th>Chemical composition</th>
<th>Instruction for use</th>
</tr>
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<tbody>
<tr>
<td>OptiBond FL (Kerr, Orange, CA, USA)</td>
<td>Primer 5143466</td>
<td>Etchant: 35% H₃PO₄, Primer: hydroxyl-ethyl methacrylate (HEMA), glycerol phosphate dimethacrylate (GPDM), mono-2-methacryloyloxyethyl phthalate, water, ethanol, photoinitiator</td>
<td>1. Apply etchant for 15 s, rinse and blot dry. 2. Apply primer with scrubbing for 15 s. 3. Gentle air stream. 4. Apply adhesive with brushing motion for 15 s. 5. Light-cure for 20 s.</td>
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<tr>
<td></td>
<td>Adhesive 5143467</td>
<td>Adhesive: bisphenol A diglycidyl methacrylate (Bis-GMA), HEMA, GPDM, barium-aluminum borosilicate glass, disodium hexafluorosilicate, silica</td>
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<tr>
<td>Single Bond Plus (3M ESPE, St. Paul, MN, USA)</td>
<td></td>
<td>Etchant: 35% H₃PO₄, Adhesive: ethanol, silane treated silica, Bis-GMA, HEMA, copolymer of acrylic and itaconic acids, glycerol, 1,3-dimethacrylate, diurethane methacrylate, water</td>
<td>1. Apply etchant for 15 s, rinse and blot dry. 2. Apply 2 separate coats with scrubbing for 10–15 s. 3. Gentle air stream. 4. Light-cure for 10 s.</td>
</tr>
<tr>
<td>One-Step Plus (Bisco, Schaumburg, IL, USA)</td>
<td></td>
<td>Etchant: 35% H₃PO₄, Adhesive: biphenyl dimethacrylate (BPDM), Bis-GMA, HEMA, acetone, fluoroaluminosilicate glass fillers, photoinitiator</td>
<td>1. Apply etchant for 15 s, rinse and blot dry. 2. Apply 2 separate coats with scrubbing for 10–15 s. 3. Gentle air stream. 4. Light-cure for 10 s.</td>
</tr>
<tr>
<td>Clearfil SE Bond (Kuraray, Osaka, Japan)</td>
<td>Primer 01241A</td>
<td>Primer: 10-methacryloyloxycetyl dihydrogenphosphate (10-MDP), HEMA, photoinitiator, water hydrophilic dimethacrylate, Bond: 10-MDP, HEMA, Bis-GMA, hydrophobic dimethacrylate, colloidal silica, photoinitiators</td>
<td>1. Dry surface. 2. Apply primer for 20 s. 3. Gentle air stream. 4. Apply adhesive. 5. Light-cure for 10 s.</td>
</tr>
<tr>
<td></td>
<td>Bond 3J0223</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All-Bond Universal (Bisco)</td>
<td></td>
<td>10-MDP, BPDM, Bis-GMA, HEMA, water, ethanol, photoinitiator</td>
<td>Etch-and-rinse mode 1. Apply phosphoric acid etchant for 15 s, rinse and blot dry. 2. Apply two separate coats with scrubbing for 10–15 s per coat. 3. Gentle air stream. 4. Light-cure for 10 s. Self-etch mode 1. Dry surface. 2. Apply two separate coats with scrubbing for 10–15 s per coat. 3. Gentle air stream for 10 s. 4. Light-cure for 10 s.</td>
</tr>
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</table>

Each adhesive was applied to a prepared dentin surface according to the manufacturer's instructions and light-cured using a Quartz-Tungsten-Halogen (QTH) light curing unit (VIP Junitor™, Bisco) with a light intensity of 600 mW/cm². Next, 4 mm of composite resin (Z250™, 3M ESPE) was incrementally built-up, each layer measuring 2 mm. Each composite layer was light-cured for 40 s. The specimens were stored in distilled water at 37°C for 24 h.

Micro-tensile bond strength (µTBS) test and fracture mode analysis
After 24 h of storage, the specimens were sectioned using a low-speed diamond saw to yield 1 mm thickness slabs. These slabs were further sectioned into 1x1 mm² composite-dentin beams, using the non-trimming version of the µTBS test. Twenty composite-dentin beams were selected and assigned to each experimental group. Each composite-dentin beam was then mounted onto a µTBS testing jig with cyanoacrylate adhesive
Table 2  Micro-tensile bond strength (µTBS) and failure mode of all experimental groups (n=20)

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Bond strength in MPa (S.D)</th>
<th>Failure mode (A/M/CC/CD)</th>
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<tbody>
<tr>
<td>Optibond FL (OB)</td>
<td>38.44 (7.57)*</td>
<td>(14/5/0/1)</td>
</tr>
<tr>
<td>Single Bond Plus (SB)</td>
<td>27.06 (9.05)b</td>
<td>(14/2/2/2)</td>
</tr>
<tr>
<td>One-Step Plus (OS)</td>
<td>26.05 (4.42)bc</td>
<td>(12/2/4/2)</td>
</tr>
<tr>
<td>All-Bond Universal using etch-and-rinse mode (ABE)</td>
<td>38.81 (7.75)*</td>
<td>(13/2/3/2)</td>
</tr>
<tr>
<td>Clearfil SE-Bond (CS)</td>
<td>39.33 (6.95)*</td>
<td>(15/2/1/2)</td>
</tr>
<tr>
<td>All-Bond Universal using self-etch mode (ABS)</td>
<td>39.02 (10.81)*</td>
<td>(15/3/0/2)</td>
</tr>
</tbody>
</table>

*Different superscript means a significant statistical difference (p<0.05). A: adhesive failure, M: mixed failure, CC: cohesive failure in composite, CD: cohesive failure in dentin.

Transmission electron microscopy (TEM) analysis

TEM specimen was prepared according to Tay et al.16). In each experimental group, 2 additional mandibular 3rd molars were selected. The specimens were prepared concurrently with the µTBS test. Two central slabs were sectioned from each tooth, yielding 4 slabs in total. Two coats of nail varnish were applied to the slabs, leaving 1 mm between the varnish and the adhesive interface. The specimens were immersed in 50 wt.% ammoniacal silver nitrate solution (pH 9.5) for 24 h in a dark room; they were then irrigated using distilled water and stored in a developing solution for 8 h under fluorescent light, during which time they were once again irrigated using distilled water. The specimens were fixed in Karnovsky’s solution (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium phosphate buffer [pH 7.4]) for 8 h, and then washed for 3×10 min using 0.1 M sodium phosphate buffer. Post-fixation was performed using a 1% osmium tetroxide solution for 2 h. Subsequently, each specimen was washed for 3×10 min each. The post-fixed specimens were dehydrated in ascending grades of ethanol (50, 70, 80, 90, 95, and 100%) for 10 min each and embedded in epoxy resin. After screening a 200 nm semi-thin section of each specimen using an ultra-microtome equipped with a diamond knife, an approximately 70 nm ultra-thin section was sliced and collected on a 75 mesh formvar-coated copper grid. After drying, the samples were examined using TEM (JEM-1010™, JEOL, Tokyo, Japan) at 100 kV.

RESULTS

Micro-tensile bond strength (µTBS) test and fracture mode analysis

The µTBS and failure mode in all experimental groups are shown in Table 2. For etch-and-rinse mode, the µTBS of OB and ABE groups were significantly higher than that of SB and OS groups (p<0.05). OS group showed the lowest µTBS among 4 etch-and-rinse dentin adhesive (p<0.05). For self-etch mode, µTBS of SE group was not different statistically from that of ABS group (p>0.05). In failure mode distribution, the predominant fracture pattern was an adhesive failure for all the experimental groups.

Transmission electron microscopy (TEM) analysis

Figures 1–6 show TEM micrographs of all experimental groups. In OB group, well-infiltrated resin tags were formed in the adhesive layer (Fig. 1A). No silver nitrate infiltration was observed in the hybrid layer, and a completely demineralized dentin surface could be seen (Fig. 1B). The adhesive interfaces of SB and OS groups were similar to that of OB group (Figs. 2 and 3). The adhesives infiltrated well into the exposed dentinal tubule, and no silver nitrate infiltration was found in the hybrid layer (Figs. 2A and 3A). In addition, the morphological distinction between the hybrid and adhesive layers was obvious (Figs. 2B and 3B). TEM micrographs of ABE group revealed a well-infiltrated adhesive layer (Fig. 4A); nonetheless, slight silver nitrate infiltration was observed in the hybrid layer (Fig. 4B). In SE group, TEM micrographs revealed that no resin tags had formed in the adhesive layer, and slight silver nitrate infiltration was observed in the hybrid layer (Fig. 5A). Partially demineralized dentin was observed on the dentin surface (Fig. 5B). TEM micrographs of ABS group was similar to that in the SE group; however, the thickness of adhesive layer was narrower than that of SE group (Fig. 6).
Fig. 1  TEM micrographs of Optibond FL.
(A) Well-infiltrated resin tags can be seen (black arrows). The adhesive layer is about 20 µm thick. (B) The distinction between dentin and adhesive is obvious (white arrows). No silver nitrate was deposited in the adhesive layer.
D: Dentin, A: Adhesive, C: Composite

Fig. 2  TEM micrographs of Single Bond Plus.
(A) Well-infiltrated resin tags are observed. Polyalkenoic copolymer clusters are visible in the adhesive layer (black arrows). The thickness of adhesive layer is about 25 µm.
(B) There is no silver nitrate deposit in the adhesive layer. The distinction between the adhesive and hybrid layers is clear (black triangles).
D: Dentin, H: Hybrid layer, A: Adhesive layer, C: Composite

Fig. 3  TEM micrographs of One-Step Plus.
(A) Representative resin tags can be observed. Adhesive fillers are distributed throughout the adhesive layer (black arrows). The thickness of the adhesive layer is approximately 20 µm.
(B) The hybrid layer is clearly distinct from the adhesive layer (black triangles). Silver nitrate has not been deposited in the adhesive layer.
D: Dentin, H: Hybrid layer, A: Adhesive layer, C: Composite
Fig. 4  TEM micrographs of All-Bond Universal using etch-and-rinse mode.
(A) A homogenous adhesive layer is formed. The thickness of adhesive layer is about 15 µm. (B) The hybrid layer is clearly distinct from the adhesive layer (black triangles). Slight silver nitrate infiltration is observed in the hybrid layer (black arrow).
D: Dentin, H: Hybrid layer, A: Adhesive layer, C: Composite

Fig. 5  TEM micrographs of SE-Bond.
(A) The thickness of adhesive layer is about 40 µm. A slight silver nitrate deposit can be observed throughout the hybrid layer (black arrows). (B) Partially remineralized dentin was observed on the top of dentin surface (white arrows).
D: Dentin, H: Hybrid layer, A: Adhesive layer, C: Composite

Fig. 6  TEM micrographs of All-Bond Universal using self-etch mode.
(A) The adhesive layer is homogenous and it is approximately 15 µm thick. (B) Partially demineralized dentin is visible in the hybrid layer (black arrows). Silver nitrate is not present in the adhesive layer.
D: Dentin, H: Hybrid layer, A: Adhesive layer, C: Composite
**DISCUSSION**

So-called “universal” dentin adhesives were recently introduced to the market. Although their development was an innovation in adhesive dentistry, it is still questionable whether they are appropriate for all adhesive procedures or not. In this study, All-Bond Universal showed similar or higher μTBS regardless of application modes compared to other dentin adhesives, thus the null hypothesis was rejected.

To compare the dentin bond strength in the etch-and-rinse mode, Optibond FL, Single Bond Plus, and One-Step Plus were used in this study. Optibond FL is a conventional 3-step dentin adhesive, and considered as “gold-standard” product. On the other hand, Single Bond Plus and One-Step Plus are 2-step dentin adhesives, and their bonding performances are known as reliable. In this study, there is no statistical difference between the μTBS of ABE and that of OB groups (p>0.05). In addition, μTBS of ABE group was significantly higher than that of SB and OS groups (p<0.05). Single Bond Plus includes polyalkenoic copolymer in its composition that can improve moisture tolerance of the adhesive. It was shown in the TEM micrographs and its morphology was similar to that of other studies, however there has been no evidences that it could enhance the bond strength so far. One-Step Plus uses an acetone as its solvent. Acetone has a so-called “water-chasing” effect, thus it can infiltrate rapidly into the exposed dentinal tubules. However, its vapor pressure is much higher than that of other solvents like ethanol or water, and the adhesive may not infiltrate sufficiently in some situations. Considering the TEM micrographs of OS group, it was hardly possible that the high vapor pressure of acetone adversely affected μTBS of OS group.

For self-etch mode, 2-step dentin adhesive, Clearfil SE-Bond was only used in this study. μTBS of ABS group was not different significantly from that of SE group (p>0.05). Clearfil SE-Bond is considered as the “gold standard” among all the self-etch adhesives, and it is the first dentin adhesive that includes 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) as its functional monomer. According to recent researches, it can chemically adhere to hydroxyapatite crystals, thus forming a stable calcium-phosphate salt. In addition, it can create so-called “nano-layering” with hydroxyapatite, forming a stable adhesive layer and enhancing the durability of adhesion.

In the failure mode analysis, adhesive and mixed failures were predominant except OS and ABE groups. The percentage of cohesive failures (both in composite and dentin) are 30% (6/20) for OS group and 25% (5/20) for ABE group. It might partly affect μTBS of these groups because the fracture usually begins at the weakest point of the specimen.

All-Bond Universal is the first commercial, single-bottle universal dentin adhesive. It is similar to single-step dentin adhesives except that it can be applied with etch-and-rinse mode. Most single-step dentin adhesives are very hydrophilic so that they can interact with underlying dentin. However, it may form water-permeable adhesive layer, thus compromising bonding performance. To overcome this problem, All-Bond Universal contains minimum amount of ethanol and water as their solvent. The exact amount of solvent was not figured out so far, the manufacturer stated that the pH of All-Bond Universal is about 3.2. In addition, it contains biphenyl dimethacrylate (BPDM) and 10-MDP as its functional monomers. The partition coefficient (log P) of BPDM and 10-MDP are 4.0 and 4.1 each. It means that they are very hydrophobic, thus can enhance its bonding performance. These changes in its composition might be attributed to reliable μTBS regardless of application mode in this study.

TEM analysis of the adhesive interfaces showed various micro-morphological aspects. All the etch-and-rinse dentin adhesives showed representative resin tags that were well-infiltrated into the dentinal tubules. In addition, the top of the dentin surface was completely demineralized by the phosphoric acid etching. In the highly magnified view, the adhesive layer was clearly distinct from the hybrid layer in groups except the Optibond FL (Figs. 1B, 2B, 3B, and 4B). When All-Bond Universal is used, there was slight silver nitrate deposit in the hybrid layer (Fig. 4B). It is generally regarded as a reticular pattern of nanoleakage, and caused by incomplete water removal of the adhesive. It must be evaporated completely because it can reduce the durability of adhesion.

Two self-etch dentin adhesives showed interfacial micro-morphologies that were rather different from those of etch-and-rinse dentin adhesives. There were no resin tags in the adhesive layer, and partially demineralized dentin was observed. They are less acidic than 35–37% phosphoric acid used in the etch-and-rinse approach. When dentin surface is treated with these adhesives, undemineralized hydroxyapatite crystals remains on the treated dentin surface. Due to the difference of electron transmittance in the TEM analysis, the area of partially demineralized dentin was shown as brighter than underlying undemineralized dentin, and the distinction between adhesive layer and dentin was unclear (Figs. 5B and 6B). TEM micrographs of SE group revealed slight silver nitrate infiltration in the adhesive layer (Fig. 5). Its pattern was similar to that of All-Bond Universal with etch-and-rinse mode, and it was also caused by the incomplete removal of water. The major difference between the 2 self-etch dentin adhesives was the thickness of adhesive layer. Whereas the adhesive thickness of the SE-Bond was about 40 μm, that of All-Bond Universal was about 10 μm. Although the thickness of adhesive layer does not adversely affect the bonding performance, it may cause ill-fitting of indirect restorations in some clinical situations.

The development of universal dentin adhesive is a challenge to adhesive dentistry. Within the limitations of this study, universal dentin adhesive showed reliable immediate bond strength and interfacial morphology regardless of application mode. However, more studies regarding the durability of adhesion and compatibility
with resin cement should be performed.

ACKNOWLEDGMENTS

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