Effect of double-application of all-in-one adhesives on dentin bonding

Yasuko Nakaoki, Wataru Sasakawa, Seiko Horiuchi, Futami Nagano, Takatsumi Ikeda, Toru Tanaka, Satoshi Inoue, Shigeru Uno, Hidehiko Sano, Sharanbir K. Sidhu

Cariology, Operative Dentistry and Endodontology, Department of Oral Health Science, Graduate School of Dental Medicine, Hokkaido University, North 13 West 7, Kita-ku, Sapporo 060-8586, Japan
School of Dental Medicine, Hokkaido University, Sapporo, Japan
Division for General Dentistry, Hokkaido University, Dental Hospital, Sapporo, Japan
Department of Dentistry, Toranomon General Hospital, Tokyo, Japan
Restorative Dentistry, School of Dental Sciences, University of Newcastle, Newcastle, UK

Received 26 October 2004; received in revised form 19 January 2005; accepted 7 February 2005

KEYWORDS
All-in-one adhesives; Dentin; Double-application; Micro-shear bond strength; SEM

Summary
Objectives: To evaluate the effect of double-application of all-in-one adhesives using the micro-shear bond test.

Methods: The occlusal surfaces of extracted human third molars were ground perpendicular to the long axis of each tooth to expose a flat dentin surface. Three commercially available and one experimental bonding system were used in this study: Adper Prompt L-Pop (APL, 3M ESPE), REACTMER BOND (RB, Shofu), XENO III (Xeno, Dentsply-Sankin) and newly developed OBF-2 (OB2, Tokuyama Dental). These adhesives were applied on the dentin surfaces by either the manufacturers’ instructions or by an experimental method (single-application or double-application). Resin composite was then placed and light-cured for 40 s. After 24 h immersion in water, a micro-shear bond test was carried out and the fractured dentin surfaces were observed microscopically. The data were analyzed by one-way ANOVA and Tukey’s HSD tests (p < 0.05).

Results: The mean bond strengths of APL, RB, Xeno and OB2 with single-application were 22.7, 28.3, 30.3 and 34.6 MPa, respectively. The mean bond strengths of APL, RB, Xeno and OB2 with double-application were 29.5, 27.2, 29.6 and 32.5 MPa, respectively. There were no statistically significant differences in micro-shear bond strengths between the single- and double-application methods for each adhesive system (p > 0.05). The morphological observation of the fractured dentin surfaces revealed differences between the single- and double-application groups especially for APL and OB2.
Conclusions: Micro-shear bond strengths of all-in-one adhesives in this study showed no statistically significant differences between the single-application and the double-application method.

© 2005 Elsevier Ltd. All rights reserved.

Introduction

In the last decade, the two-step self-etch adhesive technology led to the development of all-in-one bonding systems. The advantage of all-in-one adhesives is the relatively simple procedure involved, which minimizes the steps of the bonding process and reduces the technique sensitivity. All-in-one bonding systems do not require water rinsing nor drying. Consequently, technique sensitivity on blotting process to obtain maximum performance and postoperative sensitivity should be reduced compared to the adhesives that involve phosphoric acid etching. Another advantage of some current dentin adhesives is the incorporation of a cariostatic component, e.g. a fluoride-complex. Fluoride-releasing materials are considered to prevent secondary caries formation and to enhance the remineralization of tooth structure.

On the other hand, the bond strength of all-in-one adhesives to dentin is reported not to exceed that of two-step self-etch systems. An in vitro study by Bouillaguet et al. indicated that the bond strength of a two-step self-etch adhesive was higher than that of an all-in-one adhesive when bonded to ground dentin. Inoue et al. suggested that one-step self-etch adhesives tend to have lower micro-tensile bond strength than two-step self-etch and two-step total-etch adhesives. De Munck et al. evaluated the bond strength of one- and two-step self-etch adhesives in comparison with a total-etch approach. They found that two-step self-etch adhesives showed statistically the same or higher bond strengths than one-step self-etch adhesives.

Recently, it was reported that the application of two coats (double-application) of an all-in-one bonding system (Adper Prompt L-Pop) was successful to increase the tensile bond strength to sound dentin. Morphological observation of the resin-dentin interface using TEM revealed that bonding of Prompt L-Pop to dentin was improved by the application of a second adhesive layer after light-curing the first layer. Multiple consecutive coatings of another bonding system, the one-bottle total-etch system, also improved its bond strength and reduced the nanoleakage. The current trends in bonding appear to favor single-application of adhesives. However, it is speculated that the double-application method is an effective technique to improve the bond to dentin. The purpose of this study was to evaluate the effect of double-application of all-in-one adhesives to human dentin by means of the micro-shear bond test. The null hypothesis was that the bond strengths of all-in-one adhesives are not significantly different between the single-application method and the double-application method.

Materials and methods

Three commercially available systems, Adper Prompt L-Pop (APL, 3M ESPE, Seefeld, Germany), REACTMER BOND (RB, Shofu, Kyoto, Japan), XENO III (Xeno, Dentsply-Sankin, Tochigi, Japan) and one experimental bonding system OBF-2 (OB2, Tokuyama Dental, Tokyo, Japan) were used in this study (Table 1).

Twenty-four caries-free extracted human third molars stored in an aqueous solution of 0.5% chloramine-T were used in this study. They were obtained according to protocols approved by the relevant institutional review board of the Graduate School of Dental Medicine, Hokkaido University. A dentin slice, approximately 1.0 mm thick, was cut perpendicular to the long axis of each tooth from the upper middle coronal region using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL) under water coolant. The occlusal surfaces of slices were ground with silicon carbide paper up to # 600 grit to expose a flat dentin surface. They were randomly assigned to four equal groups. Then, the adhesives were applied on the dentin surfaces by using one of

<table>
<thead>
<tr>
<th>Table 1 All-in-one bonding systems used in this study.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesive</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Adper Prompt L-Pop</td>
</tr>
<tr>
<td>REACTMER BOND</td>
</tr>
<tr>
<td>XENO III</td>
</tr>
<tr>
<td>OBF2</td>
</tr>
</tbody>
</table>
two methods: single-application or double-application. The manufacturers’ instructions stated single-application for all adhesives except for APL, which requires double-application. In the double-application method, the application of the adhesive and mild air drying were repeated for each group. After application of the adhesive to each dentin surface and prior to irradiation, three or four cylinder (internal diameter: 0.79 mm, height: 1.0 mm) of micro bore tygon tubing (R-3603, Norton Performance Plastic Co, Cleveland, OH) were placed on the flat dentin at different locations. After irradiation, each tubing was filled with restorative resin composite (Clearfil AP-X, Kuraray Co., Okayama, Japan) and then light-cured for 40 s. The specimens were stored under the moist conditions at room temperature at 23°C for 1 h prior to removing the tygon tubing. They were then immersed in water at 37°C for 24 h.

The specimens were examined with an optical microscope (SZ-PT, Olympus, Tokyo, Japan) (30×) with particular reference to the integrity of the resin-dentin interface. The cylinders that showed apparent interfacial gap formation, bubble inclusion, or any other relevant defects were excluded from the study. The cylinders were then subjected to the micro-shear bond test (MSBT). The micro-shear bond testing apparatus is shown in Fig. 1. Each dentin slice with the resin cylinders was adhered to the testing device (modified Ciucchi’s jig), with a cyanoacrylate adhesive (Model repair II Blue, Dentsply-Sankin, Otahara, Japan). The jig was placed in a universal testing machine (EZ-test-500N, Shimadzu Co., Kyoto, Japan) for shear bond testing. A thin wire (diameter 0.20 mm) was looped around each resin cylinder, making contact through half of the cylinder base, and was placed as close as possible to the resin-dentin interface. A shear force was applied to each specimen at a crosshead speed of 1.0 mm/min until failure occurred. The resin-dentin interface of the specimens and the wire loop were aligned as straight as possible to ensure that the same orientation in shear was maintained. The loads at failure were recorded and the data were analyzed by one-way ANOVA and Tukey’s HSD test (p<0.05). All the debonded dentin surfaces after the MSBT were examined under an optical microscope at 30× magnification. Representative specimens for each group were sputter-coated with gold, and examined using a field-emission scanning electron microscope (FE-SEM) (S-4000, Hitachi, Tokyo, Japan).

Results

The mean micro-shear bond strengths of the tested adhesives are shown in Table 3 and Fig. 2.

![Diagram of the micro-shear bond test apparatus](image-url)
There were no significant differences between the single-application and the double-application for each adhesive ($p > 0.05$). The optical observation of the fractured dentin surfaces revealed mixed failure of adhesive resin and resin-dentin interface for all groups.

Figs. 3-6 indicate the fractured dentin surfaces of representative specimens for each group. For APL, morphological differences between single- and double-application was noted (Fig. 3). Collagen fibrils were clearly identified on the single-application surface, showing a porous appearance and partially dissolved peritubular dentin (Fig. 3(a)). In contrast, the intertubular dentin of the double-application group appeared dense compared with the single-application group and most of the peritubular dentin was dissolved (Fig. 3(b)).

RB showed similar fractured surfaces for single-application and double-application. For both groups, the peritubular dentin appeared intact while the intertubular dentin showed a slightly porous image (Fig. 4). The only difference was resin tag formation, which was identified in every tubule only for the double-application group (Fig. 4(b)).

For Xeno, porous intertubular dentin was found in both groups (Fig. 5). The size of the resin tags was greater in the double-application group than that of single-application. It is difficult to distinguish all the resin tags from the peritubular dentin in the double-application group (Fig. 5(b)), whereas peritubular dentin appears to be preserved for the single-application group (Fig. 5(a)).

For OB2, there was also a different morphological appearance between single- and double-application (Fig. 6). Double-application revealed porous intertubular dentin and dissolved peritubular dentin (Fig. 6(a)) compared to single-application (Fig. 6(b)). Resin tags remained within the dentinal tubules on both specimens.

**Discussion**

All-in-one adhesives have the acidic monomer ingredient, which demineralize the subsurface of the dentin, remove or modify the smear layer and improve the infiltration of the adhesive resin through the residual smear layer into the underlying dentin. These adhesives may be categorized as mild or strong self-etch adhesives, depending on their pH and therefore their etching potential. If the adhesive's capacity to dissolve the smear layer is limited, the bond strengths to the dentin with a thick smear layer may be reduced. On the other hand, smear-removing adhesives with strong acidity exhibited the formation of a demineralized collagen layer under the resin-hybridized dentin. Insufficient infiltration and low polymerization rate of adhesive resins are reported to result in the formation of exposed collagen fibrils over time, which possibly degrade both in vitro and in vivo.

Multiple application of adhesives is considered to be effective to prevent the above-mentioned defects of resin bonding and improve the bond performance to dentin. Therefore, the effect of double-application of current all-in-one bonding systems on the bond strength and the quality of the resin-dentin interface is of crucial interest.

In APL, there was no significant difference in bond strength between the single-application and the double-application ($p > 0.05$). However, the double-application group tended to show greater bond strength than that of single-application. Nara et al. reported that two-coats
Double-application of all-in-one adhesives to dentin

(double-application) of APL could increase the tensile bond strength to sound dentin. On the other hand, when observing the fractured surfaces, the morphology of the intertubular dentin greatly varied between the two application methods. The single-application produced a porous and fibrous appearance (Fig. 3(a)), which is supposed to be overetched. In double-application, the intertubular dentin appeared dense compared with the single-application group. Most of the peritubular dentin was dissolved.

Figure 3  (a) SEM image of the fractured dentin surface of APL specimen using the single-application method. Collagen fibrils were clearly identified on the surface, showing a porous appearance. Peritubular dentin was partially dissolved. (b) SEM image of the fractured dentin surface of APL specimen using the double-application method. The intertubular dentin appeared dense compared with the single-application group. Most of the peritubular dentin was dissolved.

The bond strength of RB showed equivalent values between the two application methods ($p > 0.05$). The morphology of the fractured surface was similar between the two groups and was not so porous as shown in other adhesive groups (Fig. 4(a) and (b)). This fractured surface may represent adequate infiltration of adhesive resin into demineralized dentin. RB is categorized as a mild self-etch adhesive among the all-in-one systems. 16 Regardless of single- or double-application of the adhesive, high pH of RB (Table 2) would moderate the demineralization of the dentin surface, resulting in similar surface morphology. For RB, single-application appears to be enough to obtain good bond strength.

Figure 4  (a) SEM image of the fractured dentin surface of RB specimen using the single-application method. The peritubular dentin appeared intact while the intertubular dentin showed a slightly porous image. (b) SEM image of the fractured dentin surface of RB specimen using the double-application method. The intertubular and peritubular dentin showed similar image to that of the single-application group. The resin tag formation was identified in every tubule for this double-application specimen.
The Xeno group presented no significant difference between single- and double-application in this study \((p > 0.05)\). However, it is reported by Ito et al.\(^{24}\) that the bond strength of Xeno increased with multi coating before light curing compared with that of a single coat. The reason for this variation is not clear. On Xeno fractured specimens, exposure of collagen fibrils-like structures was observed for both groups of single- and double-application (Fig. 5(a) and (b)). Xeno is categorized as the smear-removing self-etch system because of low pH (Table 2). However, in contrast to APL, the second application of the adhesive did not promote the infiltration of resin monomers. The thicker resin tag formation implies that demineralization of peritubular dentin may be accelerated, though it is not clear if this large tag formation contributes to the bond strength.

In OB2, significant difference in bond strength was not identified between the two application methods \((p > 0.05)\). It is of interest that the appearance of intertubular dentin of OB2 for double-application (Fig. 6(b)) is more porous than that of the single-application group (Fig. 6(a)). The OB2 protocol recommends active application by rubbing the dentin surface with an adhesive-containing applicator before light curing. The etching effect of this first application is strong enough to remove the dentin smear layer completely (FE-SEM observation.)
of the adhesive-conditioned dentin surface, not shown). It is speculated that both low pH (Table 2) and rubbing technique will greatly influence to the demineralization degree of tooth substrate. In this study, the second active application might accelerate further demineralization of the dentin surface, which is recognized as overetch. In OB2, double-application does not increase the bond strength nor contribute to the resin-rich interface between the resin and dentin.

In this study, double-application of APL had an effect on the bond strength and the interfacial morphology. However, for OB2 with rubbing technique, double-application had an adverse effect and led to overetched dentin. The other adhesives used in this study, RB and Xeno, did not show large differences in bond strength and fractured surface morphology between the application methods. From these results, when adopting new methodology for all-in-one bonding to dentin, practitioners must consider both the characteristics of the adhesives and the original protocols to handle these materials properly.

In this research, the effect of double-application of all-in-one adhesives was detected and the null hypothesis was rejected. Micro-shear bond strengths of all-in-one adhesives used in this study showed no statistically significant differences between the single-application method and the double-application method, although the morphology of the fractured dentin surface indicated differences in the resin infiltration (Table 3).

### Table 2: The chemical formulations, procedures and pH of all-in-one adhesives investigated.

<table>
<thead>
<tr>
<th>Adhesive</th>
<th>Component</th>
<th>Ingredients</th>
<th>Bonding procedures</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>APL</td>
<td>Liquid in two reservoirs</td>
<td>Methacrylated phosphoric acid ester, phosphine oxide, fluoride complex, water, photoinitiator</td>
<td>a, b-1 (keep rubbing for 15 s), c-2, d (for 10 s)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>RB</td>
<td>Bond A, bond B</td>
<td>4-MET, 4-META, HEMA, UDMA, water, photoinitiator, PRG filler, fluoro-aluminosilicate glass</td>
<td>a, b-2 (leave it for 20 s), c-1, d (for 20 s); the manufacturer’s instruction</td>
<td>2.8</td>
</tr>
<tr>
<td>Xeno</td>
<td>Universal, catalyst</td>
<td>METP, phosphasen monomer, UDMA, HEMA, ethanol, water, photoinitiator, fluoride complex, submicron silicate filler</td>
<td>a, b-2 (leave it for 20 s), c-2, d (for 10 s); the manufacturer’s instruction</td>
<td>&lt;1</td>
</tr>
<tr>
<td>OB2</td>
<td>Bonding agent A, bonding agent B</td>
<td>MAC-10, phosphoric ester monomer, polyfunctional monomer, HEMA, water, photoinitiator, submicron fluoro-alumino silicate glass</td>
<td>a, b-1 (keep rubbing for 10 s), c-2, d (for 10 s); the manufacturer’s instruction</td>
<td>1.1</td>
</tr>
</tbody>
</table>

4-MET, 4-methacryloyloxyethyl trimellitic acid; 4-META, 4-methacryloyloxyethyl trimellitate anhydride; HEMA, 2-hydroxyethyl methacrylate; UDMA, urethane dimethacrylate; PRG filler, pre-reacted glass-ionomer filler; METP, Methacrylate Pyrophosphate; MAC-10, 10-methacryloyloxydecamethylene malonic acid. Procedures: (a) mix liquids; (b-1) apply adhesive with rubbing; (b-2) apply adhesive without rubbing; (c-1) air-dry; (c-2) mildly air-dry; (d) light-cure. The bonding procedure without ‘the manufacturer’s instruction’ means the experimental one.

### Table 3: Micro-shear bond strength of all-in-one adhesives to dentin using single- and double-application (MPa).

<table>
<thead>
<tr>
<th>Adhesive</th>
<th>APL</th>
<th>RB</th>
<th>Xeno</th>
<th>OB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-application</td>
<td>22.7±8.7a (11)</td>
<td>28.3±6.7a,b (11)</td>
<td>30.3±7.1a,b (12)</td>
<td>34.6±4.9b (12)</td>
</tr>
<tr>
<td>Double-application</td>
<td>29.5±9.2a,b (11)</td>
<td>27.2±6.1a,b (11)</td>
<td>29.6±6.4a,b (11)</td>
<td>32.5±6.4a (11)</td>
</tr>
</tbody>
</table>

Mean±SD values with the same superscript letter within each group are not statistically different (p>0.05). The numbers in parentheses refer to the number of specimens.
Acknowledgements

The authors would like to thank Dr Yasushi Shimada and Prof. Junji Tagami in Tokyo Medical and Dental University for their contribution to this study. OB2 is now commercially available as One-Up Bond F Plus. This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for Promotion of Science #15390573.

References