The effectiveness of newly developed commercial dentin bonding systems (SB, MB II and KB) was evaluated by measuring the contraction gap width of a resin composite restored into a cylindrical dentin cavity prepared in an extracted human molar and by measuring the tensile bond strength to the flat dentin surface. In addition, calcium loss during dentin conditioning was analyzed using electron microanalyses. An experimental dentin bonding system composed of EDTA conditioning, GM solution priming and a bonding agent containing 10-MDP was employed as a control in which it was presumed that contraction gap formation was prevented completely. However, gap formation was observed using the three commercial simplified dentin bonding systems. SEM observation showed that the gap was formed between the resin composite and the top surface of the dentin cavity wall indicating that the fracture occurred at the adhesive interface, but never inside the dentin nor inside the resin composite.

Key words: Dentin bonding, Contraction gap, Remaining calcium content

INTRODUCTION

The procedures of resin composite restoration are theoretically composed of the steps of enamel etching, dentin conditioning, dentin priming, and dentin bonding agent application prior to resin composite filling, although most of the commercial systems have been simplified by combining some of these five steps. In these procedures, the smear layer on the cavity wall is removed and the surface characteristics of the dentin surface are alternated to be suitable for bonding with resin materials. The detailed mechanism of dentin priming and the possible chemical or physical interaction between the adhesive monomer in the dentin bonding agent and the component in the substrate dentin have not been clarified completely. In addition, the bonding mechanism between the resin and the dentin has been explained by the impregnation of a monomer into the microspace of the interfibrous collagen network which is exposed by acid etching of the dentin conditioner and then expanded by the dentin primer. This speculation suggests that the resin materials bond not to the inorganic component but physically to the organic component of the dentin.

However, Wu et al. and Manabe et al. clarified that both the calcium in the dentin cavity wall and the functional monomer such as 10-methacryloxyethyl dihydrogen phosphate (10-MDP) or 4-methacryloxyethyl trimellitate anhydride (4-...
META) were mandatory to obtain marginal adaptation of the resin composite in the dentin cavity. Furthermore, they reported that the contraction gap width of a light-cured resin composite filled into an experimental dentin cavity increased in relation both to the reduction of calcium content in the dentin cavity wall and to the absence of a functional monomer in the dentin bonding agent. These findings strongly suggested that the chemical interaction between the calcium in the dentin and the phosphate or the carboxylate groups of the functional monomer was essential for marginal integrity of the resin composite restoration.

In spite of the above described speculation regarding the dentin bonding mechanism, the restorative procedures of the commercial dentin bonding systems have been simplified by the introduction of the total-etching technique or the self-etching dentin primer, although no consistent evaluation of their bonding effectiveness has been reported.

The purpose of the present study was to examine the effectiveness of three commercial dentin bonding systems.

**MATERIALS AND METHODS**

Three commercial dentin bonding systems were tested and are listed in Table 1.

**Measurement of the contraction gap width**

The proximal enamel of an extracted human molar, which was stored in a refrigerator for a maximum of four weeks after extraction, was flatly eliminated on a wet

<table>
<thead>
<tr>
<th>Code</th>
<th>System</th>
<th>Manufacturer</th>
<th>Lot number</th>
</tr>
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<tbody>
<tr>
<td>KB</td>
<td>10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) 2-HEMA, polyfunctional methacrylate, water photoinitiator</td>
<td>Kuraray</td>
<td>KPA-7, KPB-6</td>
</tr>
<tr>
<td></td>
<td>10-MDP, 2-HEMA, polyfunctional methacrylate photoinitiator</td>
<td></td>
<td>KBA-6</td>
</tr>
<tr>
<td>MB</td>
<td>methacryloxydecyl propanedioic acid (MAC-10) methacryloxyethyl dihydrogen phosphate, ethanol, water,</td>
<td>Tokuyama</td>
<td>4303757</td>
</tr>
<tr>
<td></td>
<td>MAC-10, 2-HEMA, Bis-GMA, TEGDMA, photoinitiator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SB</td>
<td>35% phosphoric acid methylacrylates pendant polyalkenoic acid copolymer, 2-HEMA, Bis-GMA, dimethacrylate, ethanol, water, photoinitiator</td>
<td>3M</td>
<td>7EE, 7AD</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>neutralized 0.5 mol/L EDTA (pH 7.4)</td>
<td>Experimental</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35 vol% of glycerol mono-methacrylate</td>
<td>Experimental</td>
<td></td>
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<tr>
<td></td>
<td>Clearfil Photo Bond (10-MDP, 2-HEMA, Bis-GMA, TEGDMA, ethanol, photoinitiator, Benzoil Peroxide)</td>
<td>Kuraray</td>
<td>262, 365</td>
</tr>
</tbody>
</table>
Table 2 Bonding procedures of KB-1300

1. Conditioning and priming enamel and dentin with DC primer A+B for 30 sec.
2. Apply DC bond followed by 20 sec irradiation.
3. Resin composite filling.

Table 3 Bonding procedures of Single Bond

1. Etching enamel and dentin with 35% phosphoric acid gel for 15 sec.
2. Single Bond Adhesive application with 2 coats on enamel and dentin followed by 10 sec irradiation.
3. Resin composite filling.

Table 4 Bonding procedures of Mac Bond II

1. Conditioning and priming with primer A+B on enamel and dentin for 20 sec.
2. Bonding agent application on enamel and dentin followed by 10 sec irradiation.
3. Resin composite filling.

carborundum paper grit number of 220, and a cylindrical cavity approximately 3 mm in diameter and 1.5 mm in depth was prepared in the exposed dentin. The cavity wall was then treated according to the manufacturer's instructions and a commercial light-cured resin composite (Silux Plus; SP, 3M, MN, USA) was slightly overfilled. The composite surface was covered with a plastic matrix, flattened with a glass plate, and irradiated for 40 sec (Wite Lite, Takara Belmont, Co., Osaka, Japan). After storing the specimens in water at a room temperature of 24±1°C for 10 min, the overfilled resin composite was eliminated and the exposed dentin and composite surface were polished with a linen cloth mediated with an alumina slurry grain size of 0.03μm. The marginal adaptation of the resin composite was inspected under a light microscope (Orthoplan, Leiz, Wetzlar, Germany) and the width of any contraction gap was measured at eight points every 45 degrees along the cavity margin. The gap value was calculated as the sum of the diametrically opposing gap width in percentage to the cavity diameter, and the maximum of four gap values was given as the contraction gap of the specimen. In the positive control group, the cavity wall was conditioned with 0.5 mol/L ethylenediamine tetraacetic acid (EDTA) neutralized to pH 7.4 by sodium hydroxide for 60 sec followed by rinsing and drying. The cavity wall was primed using a 35 vol% glycerol mono-methacrylate solution (Blemmer GLM, Nihon Oil & Fat Co., Tokyo, Japan; GM) for 60 sec and the cavity was dried completely. A commercial dual-cured dentin bonding agent containing a functional monomer of 10-MDP (Clearfil Photo Bond, Kuraray, Osaka, Japan) was applied to the cavity and irradiated for 10 sec after removing any excess material with a blast of compressed dry air. The resin composite filling and measurement of the gap width
was performed using the same method as that in the experimental groups. Ten specimens for each group, 40 in total, were prepared.

**Tensile bond strength measurement**

A flat dentin surface extracted from human teeth and embedded in an epoxy resin as a substrate was prepared using a wet carborundum paper, grit number 1000. Prior to embedding in an epoxy resin, the root apexes of the human teeth were capped with zinc phosphate cement. Then a split Teflon mold with an inner diameter of 3.6 mm, an outer diameter of 20.0 mm and a height of 5.0 mm was clamped on the dentin. The dentin surface was treated using the above-described three commercial dentin bonding agents or the positive control from the top window of the center hole of the mold, and the light activated resin composite (SP) was placed in a mold with a thickness of not more than 2 mm and irradiated for 40 sec. The upper part of the center hole of the mold was filled with a chemically cured resin composite (P-10, 3M, MN, USA) and a round bar was inserted in the unpolymerized resin composite to provide a grip for the bond strength measurement. After storing the specimens in water at a room temperature of 24±1 °C for 24 hours, the tensile bond strength was measured using a universal testing machine (Instron 1123, Boston, Mass, USA) with a crosshead speed of 5 mm/min. Ten specimens for each group, 40 in total, were prepared.

**Analysis of the calcium loss in the conditioned dentin**

Dentin disk of extracted human molars was prepared by grinding the occlusal enamel of extracted human molars. Half of the dentin was covered with an adhesive tape and the other half was conditioned with the conditioner from the commercial dentin bonding system. Then the dentin surface was coated with carbon and the calcium content of the conditioned and not-conditioned dentin was analyzed at ten points on both surfaces using an energy dispersion electron microanalyser (EDS) (Delta-IV EDX, Kevex, CA, USA) mounted on a scanning electron microscope. The remaining calcium content was calculated as the calcium content of the conditioned dentin as a percentage of the non-conditioned dentin. Five specimens for each dentin bonding system, 20 in total, were prepared.

**SEM observation**

The cervical margin of the cylindrical dentin cavity described in the contraction gap measurement was dehydrated in gradual ethanol and vacuum evaporated with carbon and platinum. The morphology of the marginal adaptation was observed using a scanning electron microscope (Hitachi S-700, Tokyo, Japan) with an acceleration voltage of 15 kV.

**Statistical analyses**

The values of tensile bond strength and calcium loss due to dentin conditioning were analyzed statistically by Student’s t-test.
RESULTS

The contraction gap, tensile bond strength and remaining Ca–content after dentin conditioning are shown in Table 5. Complete marginal adaptation was obtained in the positive control group. In the specimens of the three commercial simplified dentin-bonding systems, gaps were observed in seven, nine and nine out of ten speci-

Table 5 Measurements of contraction gap, tensile bond strength and remaining Ca–content measured

<table>
<thead>
<tr>
<th></th>
<th>Contraction gap (%)</th>
<th>Tensile Bond Strength (MPa)</th>
<th>Remaining Ca–content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KB</td>
<td>0.08±0.074 (3)</td>
<td>12.1±2.8</td>
<td>93.87±0.30</td>
</tr>
<tr>
<td>MB II</td>
<td>0.16±0.133 (1)</td>
<td>17.3±6.6</td>
<td>85.14±5.25</td>
</tr>
<tr>
<td>SB</td>
<td>0.22±0.170 (1)</td>
<td>14.0±4.4</td>
<td>36.35±7.71</td>
</tr>
<tr>
<td>Experimental</td>
<td>0 (10)</td>
<td>18.5±4.0</td>
<td>94.94±1.82</td>
</tr>
</tbody>
</table>

N=10, **N=5

*: mean±SD of the gap values and the number of gap-free specimens are in ( ).

In the experimental group, the dentin surface was conditioned with 0.5 mol/L EDTA for 60 sec, primed with 35 vol% of glycerol mono-methacrylate solution for 60 sec and Clearfil Photo Bond was applied prior to the resin composite (Silux Plus) filling. Groups joined by the same line were insignificantly different (t-test, p<0.05).

Fig. 1 SEM photograph of complete marginal adaptation between Silux Plus and dentin cavity margin treated with EDTA conditioning and GM priming (×2000). The bar represents 5 μm.

Fig. 2 Complete marginal adaptation between Silux Plus and dentin cavity margin treated with an experimental dentin bonding system composed of EDTA conditioning, GM priming and Clearfil Photo Bond (×5000).
mens for KB, MB II and SB, respectively. The mean tensile bond strength of the tested bonding system varied from 12.1 to 18.5 MPa, and the positive control and the MB II specimen exhibited significantly higher bond strengths than KB or SB (t-test, p<0.05). However, fractures occurred inside the resin composite cylinder in nine specimens of KB and MB II, and seven specimens of SB. Furthermore, all specimens of the positive control exhibited cohesive fractures inside the resin composite. Adhesive fractures in the substrate dentin were not observed. Calcium loss due to dentin conditioning was significant in the SB specimen though decalcification by EDTA conditioning and self-etching dentin priming of KB was significantly mild; over 90% of the calcium was residual after conditioning (t-test, p<0.05). With the SEM study, marginal integrity was observed in the positive control specimens (Figs. 1 and 2). In addition, hybrid layer formation was not distinct when the dentin cavity wall was conditioned with EDTA solution. When the resin composite was filled after KB treatment, the hybrid layer was not definite and a gap was observed at the adhesive interface (Figs. 3 and 4). In the specimens of SB and MB II, the hybrid layer was observed to be approximately 3 μm in thickness, and a contraction gap was formed between the resin composite and the top surface of the hybrid layer (Figs. 5-8).

Fig. 3 Marginal discrepancy between Silux Plus and dentin cavity margin treated with KB-1300 (×2000).

Fig. 4 High magnification observation of Fig. 3 (×5000). Hybrid layer formation is not definite and a contraction gap is observed at the adhesive interface.
Fig. 5 Marginal discrepancy between Silux Plus and dentin cavity margin treated with Single Bond ($\times 2000$).

Fig. 6 Hybrid layer approximately 3 $\mu$m in thickness is formed and wide contraction gap is formed in spite of the hybrid layer formation ($\times 5000$).

Fig. 7 Marginal discrepancy between Silux Plus and dentin cavity margin treated with Mac Bond II ($\times 2000$).

Fig. 8 Cavity margin treated with Mac Bond II ($\times 5000$). Hybrid layer is observed to be approximately 3 $\mu$m in thickness.
DISCUSSION

The detailed mechanism of dentin bonding has been explained as the impregnation of an adhesive monomer into the microspace between the dentin collagen which has collapsed and re-expanded due to the etching of the dentin conditioner and the dentin primer, respectively. Such a speculation suggests that the dentin adhesive physically bonded to the organic component in the dentin collagen. However, Chiba et al. found that the reduction of dentin hardness after dentin conditioning correlated well with the width of the contraction gap of the light activated resin composite in an experimental dentin cavity. Furthermore, as mentioned above, both a calcium-rich dentin cavity wall and the functional monomer in the dentin bonding agent are essential for dentin bonding. Therefore, it was speculated that the bonding between the resin composite and the dentin cavity wall was established by the possible chemical interaction between the adhesive monomer and the inorganic component in the dentin. The adhesive monomers tested in this study, 10-MDP, MAC-10 and polyalkenoate and 2-HEMA copolymer, were designed to bond to the inorganic component and not to the organic component in the dentin, although this effect was not complete. The effect of the dentin primer has been explained as being due to the wetting and expanding of the dentin collagen consequently enlarging the interfibrous microspace between the collagen fibers. However, Chigira et al. claimed that the glycerol mono-methacrylate solution exhibited a complete priming effect because it possibly disturbed the penetration of the adhesive monomer and kept the monomer concentration high at the adhesive interface which was observed with a TEM as a high density zone. Furthermore, the 2-HEMA primer promoted monomer diffusion into the dentin, resulting in a low monomer concentration at the adhesive interface. Thus, complete marginal adaptation was not obtained by 2-HEMA priming. Such a discrepancy between the above described two speculations about the bonding mechanism, one based on monomer diffusion into the organic components and the other based on the possible chemical interaction between the functional monomer and the inorganic component in the substrate dentin, is due to the difference in the methods used to estimate the effectiveness of the dentin bonding system. The effectiveness of a dentin bonding system has been widely evaluated by the bond strength measurement to the flat tooth surface. However, it is recognized that the specimens frequently exhibit fractures in the resin composite cylinder or the substrate dentin, although these two fractures are never experienced in the clinical cavity of the resin composite restoration. Furthermore, it has been reported that bond strength was easily influenced not only by the dentin bonding system employed but also by various factors including mechanical strength of the resin composite bonded, depth or the kind of dentin, string condition of the extracted teeth and site of the dentin. In addition, no consistent maximum limit of bond strength has been reported that was sufficient to obtain marginal integrity of the resin composite in the dentin cavity.

Yanagawa et al. reported that clinical cavity adaptation of the resin composite
correlated well with that observed in the cylindrical dentin cavity for contraction gap width measurement. This finding strongly suggests that the effectiveness of the dentin bonding system should be evaluated consistently by the contraction gap measurement. In order to obtain bonding of the dentin and resin composite, it is important to maintain the attachment between the unpolymerized resin composite paste and the dentin cavity wall until the completion of polymerization contraction of the resin composite in a three dimensional dentin cavity. Therefore, it is mandatory for the establishment of complete marginal adaptation to keep the calcium and monomer concentration at the adhesive interface high and to eliminate polymerization inhibitors, such as oxygen in the air and water from the dentin. Thus, it is possible to prevent interfacial separation between the resin composite and the dentin cavity wall. Discussion about the stress destroying the two-dimensional bonding between the resin composite and the flat dentin (i.e. bond strength measured) has little significance for the marginal adaptation of the resin composite. In this examination, significantly high bond strength was obtained by using MB II although the effectiveness of this system was judged to be poor because complete marginal adaptation was observed in only one out of the ten prepared specimens.

Of the three commercial dentin bonding systems tested in this study, the degree of decalcification by phosphoric acid with SB was remarkable. Such a significant loss of calcium in the dentin cavity wall caused poor marginal adaptation of the resin composite because the functional monomer lost the bonding target in the cavity wall although the hybrid layer formation was most definite. Therefore, hybrid layer formation is not always mandatory for dentin bonding; and the undesirable side effect of the demineralization by the dentin conditioner showed more serious damage for dentin bonding.

The process of the 10-MDP and methacryloyxethyl dihydrogen phosphate in the self-etching dentin primer in the KB and MB II was not complete. This finding strongly supported the report by Watanabe et al.20) in which the methacryloyxethyl hydrogen phenyl phosphate was a more effective acidic monomer than these two examples. Although this conclusion was only based on the bond strength measurement. As demonstrated in this study, marginal integrity was not observed with the three newly developed commercial dentin bonding systems which instructed two steps prior to the resin composite filling. The use of contraction gap-free dentin bonding systems in clinic should be recommended, even though the restorative procedures are still complex.

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