INTRODUCTION

Several factors are known to affect the performance of adhesive materials. One important factor is the dentin depth or remaining dentin thickness (RDT)\(^1\)-\(^4\). The RDT has been shown recently to have a significant effect on the microtensile bond strengths (\(\mu\)TBS)\(^1\). Furthermore, the bond strengths of one-step self-etch adhesive materials were shown to have a correlation with the RDT in short term water storage, whereas the two-step self-etch materials were not affected by RDT\(^1\).

The bonding performance of superficial dentin is generally better compared to that of deep dentin\(^2,3,5\)-\(^7\), especially when all-in-one systems are used\(^2,7\). Deep dentin is constituted mainly of larger funnel-shaped dentinal tubules with much less intertubular dentin\(^8\), and the contribution to resin retention is proportional to the intertubular dentin available for bonding\(^9\). In addition, the water content of dentin tissue is confined to dentinal tubules. The density of tubules changes with dentinal depth and their intrinsic wetness interferes with the bonding of the adhesive resin\(^9\).

Although the immediate resin-dentin bond strengths of contemporary adhesives are quite high, the values obtained in laboratory studies gradually decrease to 30% over 6 to 12 months\(^10,11\). One of the reasons for the adhesive degradation process over time is the activation of matrix metalloproteinases (MMPs) by weak acids, such as, lactic acid released by caries-producing bacteria, and acid-etchants used in adhesive bonding systems\(^11,12\). Another reason is incomplete resin infiltration into the dentin tubules\(^9\).

Therefore, the aim of this study was to evaluate the relationship between RDT and bond strength over time. The null hypothesis tested was that there is no difference in the bond strength or change in the interfacial morphology of resin-dentin bonds after 1 year of water storage.

MATERIALS AND METHODS

Specimen preparation and RDT measurement

Twenty-seven extracted non-carious human third molars were used in this study. They were stored in 0.5% chloramine-T solution at 4°C and used within 3 months after extraction. The teeth were collected under a protocol reviewed and approved by the university ethical committee (#2014-12). Twenty-seven human third molars were randomly divided into three groups: Clearfil Bond SE ONE (SE1, Kuraray Noritake Dental, Okayama, Japan), G-Bond plus (GB, GC, Tokyo, Japan) and Clearfil Mega Bond (MB, Kuraray Noritake Dental). Bonded specimens were stored in water at 37°C for 24 h. The teeth were then sectioned perpendicular to the adhesive interface to produce beams. RDT of each beam was measured digital calliper. Microtensile bond strength testing was carried out at a crosshead speed of 1 mm/min after 24 h and 1 year water storage. Thicker RDT produced higher bond strengths with one/two-step self-etch materials tested except for the group of 24 h MB. Nevertheless water storage time and RDT affected \(\mu\)TBS in all materials used.

Keywords: Remaining dentin thickness, Microtensile bond strength, Dentin adhesive, Long term bond stability
## Table 1  Chemical formulations and the individual manufacturer’s instructions

<table>
<thead>
<tr>
<th>Materials (Lot No.)</th>
<th>Abb.</th>
<th>Type of adhesive</th>
<th>Main composition</th>
<th>PH</th>
<th>Instruction for use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearfil Bond SE ONE (011111B)</td>
<td>SE1</td>
<td>One-step Self-etch</td>
<td>MDP, Bis-GMA, HEMA, Hydrophilic aliphatic dimethacrylate, Hydrophobic aliphatic methacrylate, Colloidal Silica, Sodium Fluoride, CQ, Accelerators Initiators, Water</td>
<td>2.3</td>
<td>1. apply bonding and leave 10 s 2. air-blowing gently for more than 5 s until the bond does not move 3. light-cure 10 s</td>
</tr>
<tr>
<td>G-Bond Plus (1009101)</td>
<td>GB</td>
<td>One-step Self-etch</td>
<td>4-MET, Phosphoric ester monomer, Dimethacrylate monomer, Silica Filler, Photo-Initiator, Acetone, Water</td>
<td>1.5</td>
<td>1. Shake bottle before use and immediately apply application 2. Leave 10 s after application 3. strong air-blowing 4. light-cure 10 s</td>
</tr>
<tr>
<td>Clearfil Mega Bond Clearfil SE Bond (LOT011528)</td>
<td>MB</td>
<td>Two-step Self-etch</td>
<td>PRIMER: MDP, HEMA, Hydrophilic aliphatic dimethacrylate, CQ, DEPT, Water BOND: MDP, Bis-GMA, HEMA, Hydrophobic aliphatic dimethacrylate, CQ, DEPT, Colloidal Silica</td>
<td>1.9</td>
<td>1. apply the primer and leave for 20 s 2. gentle air-blowing 3. apply the adhesive for 10 s 4. gentle air-blowing 5. light-cure for 10 s</td>
</tr>
</tbody>
</table>

Bis-GMA: bisphenol-A-diglycidyl methacrylate; CQ: camphorquinone DEPT: N,N-diethanol-p-toluidine DMAEMA: dimethylaminoethyl methacrylate; HDDDMA: 1,6-hexanediol dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate; MDPB: 12-methacryloyloxydodecylpyridinium bromide; TEGDMA: triethyleneglycol dimethacrylate; 4-MET: 4-methacryloxyethyl trimellitic acid

yielded 2–4 beams (cross-sectional area: 1×1 mm). The beams were randomly divided into 2 subgroups: a 24 h short-term test group and a 1 year long term group. The RDT was measured in all specimens. The dentin surfaces of the beams were observed by a glass magnifier (×20 magnification; Magnifier Light, Asone, Osaka, Japan). The average RDT of each beam was obtained by measuring the length of each dentin surface at their midpoints from the edge of the resin composite/dentin interface to the dentin/pulp interface with perpendicular tubule orientation (Absolute Digimatic, Mitutoyo, Kanagawa, Japan).

**Microtensile bond strength (μTBS) testing**
For the μTBS test, specimens in each group were attached to the jigs with a cyanoacrylate adhesive (Model Repair II Pink, Dentsply/Sankin, Tokyo, Japan), and the interface was stressed by testing at a crosshead speed of 1 mm/min (EZ Test, Shimadzu, Kyoto, Japan) until failure appeared. The μTBS was calculated to MPa.

**Statistical analysis**
The normality of all data was tested using the Kolmogorov-Smirnov test. The μTBS data were statistically analyzed by one-way ANOVA and Tukey’s HSD at 5% level of significance. The correlation between μTBS and RDT was evaluated with Pearson correlation test at 5% level of significance. All data were analyzed by SPSS 17.0 (SPSS statistics 17.0, SPSS, Chicago, IL, USA).

**Failure mode analysis**
After μTBS testing, the failure modes of the specimens were observed at ×20 magnification incident light microscope (Magnifier Light, Asone). The failure modes of the specimens were classified into the following three categories:

- Type 1: adhesive failure (fracture within the adhesive layer);
- Type 2: mixed failure (fracture within the adhesive layer and cohesive failure within dentin and/or resin);
- Type 3: cohesive failure (fracture within dentin or composite resin only).

**TEM observation**
After observation of the fractured specimens, they were observed by transmission electron microscopy (TEM) to analyze the resin-dentin interface. Specimen preparation for TEM followed the standard procedures for ultra-morphologic TEM examination of biological tissues. Specimens were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 1 min with the solution being changed three times. The specimens were then dehydrated in ascending grades of ethanol (50, 75, 95 and 100%) for 10 min each, with two changes of every new solution. This was followed by immersing the specimens in 1 by 1 absolute ethanol and epoxy resin (Poly/Bed 812 kit, Polyscience, Warrington, PA, USA) was stirred at a rate of 4 rpm over 8 h, before removing the specimen and placing them in 100% epoxy embedding resin in...
new bottles for rotation over 3 h. The resin-infiltrated specimens were embedded in a silicone rubber mold filled with 100% epoxy resin. The epoxy resin blocks in the mold were polymerized in an oven at 60°C for more than 48 h. TEM specimens were sliced parallel to the long axis of the teeth using a diamond knife (DiATOME, Bienne, Switzerland). They were cut to a thickness of about 70–100 nm using an ultramicrotome (Ultracut, Leica, Vienna, Austria) before examination by a TEM (JEM-1400, JEOL, Tokyo, Japan) at an accelerating voltage of 80 kV.

RESULTS

Relationship between \( \mu \text{TBS} \) and RDT

The means of bond strength results are shown in Table 2. The \( \mu \text{TBS} \) values of all long term groups were significantly lower than those of the short term groups using the same adhesives. The short term 24 h MB specimens showed the highest bond strength values (\( p<0.05 \)).

The \( \mu \text{TBS} \) values were generally lower in the 1 year groups, then in the short term groups. Figure 1 shows that the 1 year \( \mu \text{TBS} \) of SE1 is lower than that at 24 h. The trend lines show a similar tendency between the 1 year and 24 h groups indicating greater \( \mu \text{TBS} \) with increasing RDT values (\( p<0.05 \)) (Fig. 1). The GB groups showed similar results (Fig. 2). Interestingly, the 24 h group of the two-step self-etch adhesive, MB, did not indicate clear RDT dependency (Fig. 3). The trend line of the 1 year MB group presented a similar tendency as in SE1 and GB. Linear regression analysis carried out with SPSS software (SPSS statistics 17.0, SPSS) yielded the following data: short-term (24 h): SE1: \( R^2=0.735, p<0.05 \); GB: \( R^2=0.499, p<0.05 \); MB: \( R^2=0.192, p>0.05 \). Long-term (1 year): SE1: \( R^2=0.579, p<0.05 \); GB: \( R^2=0.656, p<0.05 \); MB: \( R^2=0.412, p<0.05 \).

Table 2 Summary fracture mode of bond systems

<table>
<thead>
<tr>
<th>Adhesive</th>
<th>( \mu \text{TBS} \pm \text{SD} ) (MPa)</th>
<th>Fracture mode (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Type1</td>
</tr>
<tr>
<td>SE1-24h</td>
<td>38.01±16.25e</td>
<td>10 (66.67)</td>
</tr>
<tr>
<td>SE1-1y</td>
<td>28.77±10.323^c</td>
<td>13 (86.67)</td>
</tr>
<tr>
<td>GB-24h</td>
<td>36.17±10.335bc</td>
<td>11 (73.33)</td>
</tr>
<tr>
<td>GB-1y</td>
<td>20.4±7.194</td>
<td>14 (93.33)</td>
</tr>
<tr>
<td>MB-24h</td>
<td>76.51±10.59τ</td>
<td>2 (13.33)</td>
</tr>
<tr>
<td>MB-1y</td>
<td>48.2±17.629</td>
<td>7 (46.67)</td>
</tr>
</tbody>
</table>

\( n=15 \) /group

Values having the same superscript are not significant: \( p>0.05 \), Tukey HSD test at \( \alpha=0.05 \)

Fracture mode categories:

Type 1: Adhesive failure, fractured with in adhesive layer
Type 2: Cohesive failure, only fractured with in dentin or resin
Type 3: Mixed failure, fractured with in adhesive and cohesive with in dentin or/and resin

Failure mode observation

Figures 1–3 show the failure mode for each specimen. The circles represent adhesive failure, triangles represent cohesive failure, and the squares represent mixed

Fig. 1 Relationship between the microtensile bond strength and remaining dentin thickness by 24 h and 1 year.

The material tested was SE1.

Fig. 2 Relationship between the microtensile bond strength and remaining dentin thickness 24 h and 1 year.

The material tested was GB.
Fig. 3  Relationship between the microtensile bond strength and remaining dentin thickness 24 h and 1 year. The material tested was MB.

Fig. 4  Figure showing the non-demineralized and stained TEM pictures of the resin/dentin interface of SE1. (A) It represents the superficial resin/dentin interface at 24 h. (B) represents the deep resin/dentin interface at 24 h. (C) shows superficial resin/dentin interface after 1 year. (D) shows deep resin/dentin interface after 1 year. After 1 year water storage, the adhesive layer showed de-bonding of filler particles. Bonded collagen fibrils along the dentin surface are partially unraveled (open arrow), the hydroxyapatite crystals are decreased and break away from hybrid layer. The thickness of hybrid layer is thinner than that of 24 h specimens and discontinuity (insert picture). A: adhesive resin, H: hybrid layer, D: unaffected dentin. Black arrow: silica filler particles.

Fig. 5  The non-demineralized and stained TEM images of the resin/dentin interface of GB. The adhesive layer showed de-bonding of filler particles. Bonded collagen fibrils along the dentin surface are partially unraveled and fracture apparent of deep dentin ((D): open arrow), the hydroxyapatite crystals are decreased and break away from hybrid layer. The thickness of hybrid layer is thinner than that of 24 h specimens and discontinuity (insert picture). A: adhesive resin, H: hybrid layer, D: unaffected dentin. Black arrow: silica filler particles.

Fracture modes. Overall, adhesive failure appeared to increase with time. MB showed more adhesive failures in deep dentin (lower RDT) with higher decreasing rate of bond strength after 1 year water storage compared to 24 h.

**TEM Observation**

Figure 4 shows the non-demineralized and stained TEM pictures of the resin/dentin interface of SE1. When focusing on the adhesive resin, the number of filler particles decreased in both long-term superficial and deep dentin specimens (Figs. 4(C) and (D)) compared to short-term ones (Figs. 4(A) and (B)). Low magnification of the specimens indicated the hybrid layers of interrupting non-continuous structures with different thicknesses less than 400 nm.

Figure 5 shows the non-demineralized and stained TEM pictures of the resin/dentin interface of GB. The filler particles within the adhesive resin layer after 24 h of water storage decreased over time (Figs. 5(A) and (B)) similarly to SE1 at 24 h. The hybrid layer within deep dentin in the 1 year specimens reveals loss of homogeneity.

Figure 6 shows the non-demineralised stained TEM images of the MB resin/dentin interface. There are two types of hybrid layer structures that can be seen in Figs. 6(B) and (D). The upper half of the hybrid layer contains
that bond strength was affected by RDT after 24 h of water storage. In contrast, studies by Pereira et al. and Sattabanasuk et al. proved that there is no effect of RDT on the bond strength for superficial and deep dentin. These findings are in line with previous studies, although no studies are available addressing this question in detail. Our study revealed that long term water storage affected bond strength, regardless of the material (adhesives) chosen. In any case, the RDT seems to have an important influence on the bond strength of dentin bonding systems.

One-step self-etch adhesives are vulnerable to water sorption and because they contain high concentrations of water and solvents, the adhesives behave like permeable membranes. This study indicated that the bond strength of SE1 and GB has a correlation with the RDT at 24 h. The microtensile test showed high bond strengths as the RDT increased on an occlusal flat dentin surface. Presumably, one-step adhesives are sensitive to the wetness of deep dentin. Presence of hydrophilic monomer and associated increased water sorption leads to plasticization of polymers, increases solubility, and decreases modulus of elasticity. After 1 year of water storage, the one-step adhesive materials, SE1 and GB, confronted more challenge to durability performance and the bond strength was also affected by RDT to show a lower result with deep dentin.

The two-step adhesive, MB, showed no significant difference between RDT and bond strength in immediate bond strength studies. Perhaps the separate demineralization and bonding steps can not only provide good bond performance, but also do not suboptimally cure the hydrophilic monomer moieties blended into the formulation.

In all adhesives (one-step and two-step), the long term $\mu$TBS bond strength decreased over time ($p<0.05$). One of reasons for this finding could be the area of intertubular dentin available for micro-mechanical retention through hybridization which decreases when the diameter and the number of dentinal tubules increase closer to the pulp. In addition, the latest iteration of most one-step self-etch adhesives are intricate mixes of hydrophobic and hydrophilic components that lead to compromise bond performance. In order to provide the acid monomer of self-etch adhesives to the treatment surface, water content in the adhesive is required for adequate ionization of the acidic monomer. On the other hand, improving the wetness of the dentin, thus increasing the water concentration reduces the monomer concentration at the same time and finally a loss of bond strength appears. The degradation pattern of filler reduction was typically observed in TEM pictures (Figs. 4–6) of all adhesives. Residual water contributed to bonding deterioration between the resin matrix and filler particles by hydrolysis over time. Compared to the 24 h specimen sectioned, all 1 year adhesive bond strength were decreased, and the incidence of adhesive fracture increased. Typically, the adhesive failure of resin composites were the weaknesses observed in specimens with low bond strength values. In this study,

**DISCUSSION**

Dentin tissue is a complex substrate. Dentinal tubule density is typically 30,000 tubules per square millimeter 2 mm from the pulp and the number of tubules per square millimeter increases from the dentino-enamel junction towards the pulp chamber. Due to this complex morphology, a lot of studies tend to focus on the distance from the bonding surface to the pulp tissue. The bonding performance at different dentin thicknesses is controversial. Some studies of immediate bond strength of self-etch adhesives to dentin show significant decrease when the pulp chamber is approached. In contrast, some studies have presented no significant correlation between dentin depth and the bond strength of two-step self-etch adhesives. Our study confirms the results of the study by Yoshikawa et al. on the 24 h bond test of a one-step bonding system that showed
many specimens showed low bond strengths with high percentages of adhesive failure of bonding resin in fractured surfaces (Table 2). These results indicate that the bond degradation of the adhesive contributes to reduction in bond strength over time (Figs. 4(C) and (D)) and GB (Figs. 5(C) and (D)). With regard to comparison of 1 year (Figs. 4(A) and (B)) and 24 h (Fig. 5(A) and (B)) TEM images of SE1, 1 year superficial and deep dentin images showed electron lucent and uncontinued hybrid layers. The quality of the hybrid layer of deep dentin is less compact. In addition, TEM images of MB at 1 year showed that the collagen fibrils and hydroxyapatite crystals of deep dentin appear to be decreased. Considering the images of the 24 h specimens, hydroxyapatite crystals were covered with resin. These may imply that degradation of resin occurred over time. In 1 year superficial specimens, the hybrid layer was more densely stained than the deep dentin counterpart. Therefore, the null hypothesis must be rejected, since there were differences in the bond strength or change in the interfacial morphology of resin-dentin bonds after 1 year of water storage.

In conclusion, this study confirmed that, in the laboratory environment, the effectiveness of the one-step adhesives is compromised with different RDT over time. In general, the adhesion of self-etch adhesives to dentin for 24 h water storage showed good bond strengths, but bond strengths decreased dramatically after 1 year of water storage.

REFERENCES


