Effect of different remaining dentin thickness and long term water storage on dentin bond strength

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Key words: Remaining dentin thickness, Microtensile bond strength, Dentin adhesive, Long term bond stability

Number of reprints: 50

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ABSTRACT

The purpose of this study was to investigate the effect of remaining dentin thickness (RDT) and long term water storage on dentin bond strength in-vitro. Twenty-seven third molars were randomly divided into 3 groups: Clearfil Bond SE ONE (SE1, Kuraray Noritake Dental Incorporated, Tokyo, Japan), G-Bond plus (GB, GC Corporation, Tokyo, Japan) and Clearfil Mega Bond (MB, Kuraray Noritake Dental Incorporated, Tokyo, Japan). 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 1mm/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 μTBS in all materials used.
INTRODUCTION

Several factors are known to affect the performance of adhesive materials. One important factor is the dentin depth or remaining dentin thickness (RDT)\textsuperscript{1-4}. The RDT has been shown recently to have a significant effect on the microtensile bond strength (μTBS)\textsuperscript{1}). Furthermore, the bond strength 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\textsuperscript{1}).

The bonding performance of superficial dentin is generally better compared to that of deep dentin\textsuperscript{2,3,5-7}, especially when all-in-one systems are used\textsuperscript{2-7}). Deep dentin is constituted mainly of larger funnel-shaped dentinal tubules with much less intertubular dentin\textsuperscript{8}), and the contribution to resin retention is proportional to the intertubular dentin available for bonding\textsuperscript{7}). 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\textsuperscript{9}).

The adhesive bond is challenged by hydrolysis through water uptake and subsequent swelling or shrinking of the resin matrix over time. Although the immediate resin-dentin bond strengths of contemporary adhesives are quite high, these values gradually decrease to 30% over 6 to 12 months\textsuperscript{10,11}). One of the reasons for the adhesive degradation process 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\textsuperscript{11, 12}). Another reason is incomplete resin infiltration into the dentin tubules\textsuperscript{13}).

Therefore, the aim of this study was to evaluate the relationship between remaining dentin thickness 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 human third molars were randomly divided into three groups for use with three adhesive materials: Clearfil Bond SE ONE (SE1, Kuraray Noritake Dental Incorporated, Okayama, Japan), G-Bond Plus (GB, GC Corporation, Tokyo, Japan) and Clearfil Mega Bond (MB, Kuraray Noritake Dental Incorporated, Okayama, Japan) as listed in Table 1. Each adhesive group was then subdivided into two further groups: a short term group and a long term group. After removal of the crown segment of each tooth, 600-grit silicon carbon paper was applied to polish the dentin surface under water for 60 seconds. The application procedures for the adhesives were performed following the individual manufacturer’s instructions. Subsequently, CLEARFIL AP-X composite resin (Kuraray Noritake Dental Incorporated, Okayama, Japan) was applied onto the bonded dentin surface. After storage in 37°C water for 24 hours, the teeth in each group were sectioned perpendicularly to the adhesive interface...
to produce beams, using an Isomet diamond saw under water lubrication as previously
reported\(^{11}\). Each tooth yielded 2-4 beams (cross-sectional area: 1×1mm). 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.

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 challenged by testing at a crosshead speed of 1 mm/min (EZ Test,
Shimadzu Co., Kyoto, Japan) until failure appeared. The μTBS was quantified by
MPa, and all data were statistically analyzed by the Tukey test and regression line test
in the SPSS software (\(p<0.05\)).

Failure mode analysis

After μTBS testing, the failure modes of the specimens were observed at ×20
magnification (Magnifier Light, Astone, Osaka, Japan). 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 minutes 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 Inc., PA, USA) for 8 hours with rotation at 4 rpm (Taab Rotator type N, Taab Laboratories Eqt., Aldermaston, UK), before removing the specimen and placing them in 100% epoxy embedding resin in new bottles for rotation over three hours. 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 hours. 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 μTBS and RDT

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

The μTBS values were generally lower in the 1 year groups, then in the short term
groups. Figure 1 shows that the 1 year μTBS of SE1 is lower than that at 24 hours.
The trend lines show a similar tendency between the 1 year and 24 hour groups
indicating greater μTBS with increasing RDT values (p<0.05) (Figure 1). The GB
groups showed similar results (Figure 2). Interestingly, the 24 hour group of the
two-step self-etch adhesive, MB, did not indicate clear RDT dependency (Figure 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
Inc., Chicago, USA.) yielded the following data: short-term (24 hours): SE1:
R²=0.735, p<0.05; GB: R²=0.499, p<0.05; MB: R²=0.192, p>0.05. Long-term (1 year):
SE1: R²=0.579, p<0.05; GB: R²=0.656, p<0.05; MB: R²=0.412, p<0.05.

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 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 hours.

**TEM Observation**

When focusing on the adhesive resin, the number of filler particles decreased in both long-term superficial and deep dentin specimens (Figures 4C, 4D) compared to short-term ones (Figures 4A, 4B). Low magnification of the specimens indicated the hybrid layers of interrupting non-continuous structures with different thickness less than 400nm.

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 hours of water storage decreased over time (Figure 5A and 5B) similarly to SEI at 24 hours. 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 Figures 6B and 6D. The upper half of the hybrid layer contains collagen fibrils as shown in Figure 6D. The bottom of the hybrid layer contains mostly hydroxyapatite crystals that appear dark. Figures 6C and 6D showed greater loss of filler in both superficial
and deep dentin comparing 24 hours with 1 year. The electron densities stained within
the hybrid layers are significantly decreased.

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 a study by Yoshikawa et
al on the 24 hour bond test of a one-step bonding system that showed that bond
strength was affected by RDT after 24 hours of water storage. In contrast, studies by
Pereira et al and others 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\textsuperscript{19).} In this study, the bond strength to dentin surface of SE1 and GB indicates that there is a high correlation with intertubular dentin dimensions. The microtensile test showed high bond strengths as the RDT increased on an occlusal flat dentin surface. Perhaps one-step adhesives are sensitive to the wetness of deep dentin tissue and hydrophilic monomer efficacy is not completely by regulated by water absorbability, then lead to plasticizes polymers, increases solubility, and decreases modulus of elasticity at the same time\textsuperscript{2,20).} 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 of 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\textsuperscript{1,21).}

In all adhesives (one-step and two-step), the long term μTBS 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\textsuperscript{3,22).} In addition, the latest generation of most one-step self-etch adhesives are intricate mixes of hydrophobic and hydrophilic components that lead to
compromise bond performance\textsuperscript{23). 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\textsuperscript{24). There is also the possibility of activated endogenous dentin matrix metalloproteinases (MMPs)\textsuperscript{11). Once MMPs are activated during the bonding procedure they can degrade the collagen fibrils, causing failure of the adhesive-dentin bonds\textsuperscript{25).}

The degradation pattern of filler reduction was typically observed in TEM pictures (Figures 4-6) of all adhesives. Residual water contributed to bonding deterioration between the resin matrix and filler particles by hydrolysis over time\textsuperscript{19, 26). Compared to the 24 hour specimen sectioned, all adhesives bond strength of 1 year 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, 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 (Figures 4c, d) and GB (Figures 5c, d). TEM images of SE1 at 1 year compared to 24 hours (Figures 4a, b) (Figure 5a, b). 1 year superficial and deep dentin images showed electron dense zone lucent and uncontinued hybrid layers. The quality of the hybrid layer of deep dentin appear more incompact. 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 hour
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 the effectiveness of the one-step adhesives
is compromised with different RDT over time. In general, the adhesion of self-etch
adhesives to dentin remained stable, but bond strengths decreased dramatically after 1
year of water storage.

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2) Yoshikawa T, Wattanawongpitak N, Cho E, Tagami J. Effect of remaining dentin
thickness on bond strength of various adhesive systems to dentin. Dent Mater J 2012;
31: 1033-1038.


<table>
<thead>
<tr>
<th>Materials</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</td>
<td>SE1</td>
<td>One-step</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 10s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Self-etch</td>
<td></td>
<td></td>
<td>2. air-blowing gently for more than 5 s until the bond does not move</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3. light-cure 10 s</td>
</tr>
<tr>
<td>G-Bond Plus</td>
<td>GB</td>
<td>One-step</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</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Self-etch</td>
<td></td>
<td></td>
<td>2. Leave 10 s after application</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3. strong air-blowing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4. light-cure 10 s</td>
</tr>
<tr>
<td>Clearfil Mega Bond</td>
<td></td>
<td>Two-step</td>
<td>PRIMER: MDP, HEMA, Hydrophilic aliphatic dimethacrylate, CQ, DEPT, Water</td>
<td>1.9</td>
<td>1. apply the primer and leave for 20 s</td>
</tr>
<tr>
<td>Clearfil SE Bond</td>
<td>MB</td>
<td>Self-etch</td>
<td>BOND: MDP, Bis-GMA, HEMA, Hydrophobic aliphatic dimethacrylate, CQ, DEPT,</td>
<td></td>
<td>2. gentle air-blowing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Colloidal Silica</td>
<td></td>
<td>3. apply the adhesive for 10 s</td>
</tr>
<tr>
<td></td>
<td>(LOT011528)</td>
<td></td>
<td></td>
<td></td>
<td>4. gentle air-blowing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>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; HDDMMA: 1,6-hexanediol
dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate; MDPB: 12-methacryloyloxydodecylpyridinium bromide; TEGDMA: Triethylene glycol dimethacrylate; 4-MET: 4-methacryloyloxyethyltrimellitate; 4-META: 4-methacryloyethyl trimellitic acid;
Table 2 Summary fracture mode of bond systems (n=15/group)

<table>
<thead>
<tr>
<th>Adhesive</th>
<th>μTBS±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>38.0±16.2abc</td>
<td>10(66.67)</td>
</tr>
<tr>
<td>SE1·1y</td>
<td>28.7±10.32a</td>
<td>13(86.67)</td>
</tr>
<tr>
<td>GB·24h</td>
<td>36.1±10.33bc</td>
<td>11(73.33)</td>
</tr>
<tr>
<td>GB·1y</td>
<td>20.4±7.19d</td>
<td>14(93.33)</td>
</tr>
<tr>
<td>MB·24h</td>
<td>76.5±10.59a</td>
<td>2(13.33)</td>
</tr>
<tr>
<td>MB·1y</td>
<td>48.2±17.62c</td>
<td>7(46.67)</td>
</tr>
</tbody>
</table>

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
Figure 1 -SE1-Ting et al.
Figure 2 - GB-Ting et al.
Figure 3 -MB-Ting et al.
Figure 4

TEM: NON DE-MINERALIZED, STAINED (SE1)

A. 24h-superficial
B. 24h-deep
C. 1Y-superficial
D. 1Y-deep
TEM: NON DE-MINERALIZED, STAINED (GB)