The influence of light intensities irradiated directly and indirectly through resin composite to self-etch adhesives on dentin bonding

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This study was designed to evaluate effects of light-irradiated intensities directly and indirectly through resin composites to one- and two-step self-etch adhesives on dentin bonding. One-step (Clearfil S Bond; TS, Bond Force; BF) or two-step (Clearfil SE Bond; SE) self-etch adhesives was applied to dentin surface. The adhesive agent was light-cured with light-intensity of 350 or 600 mW/cm², and then resin composite with different colors (translucent or opaque shade) was filled and light-cured with the same light-intensity as the bonding procedure. After 24 h water storage, bond strengths to dentin were determined using µTBS test. For the 600 and 350 mW/cm² groups, translucent shade resin obtained higher µTBS than opaque shade resin. Using SE and BF, the 350 mW/cm² group in translucent shade resin was higher µTBS than the 600 mW/cm² group in opaque shade resin, while TS showed no different µTBS between them.

Keywords: One-step self-etch adhesives, Two-step self-etch adhesives, Light irradiation intensity, Light transmission intensity, Micro-tensile bond strength

INTRODUCTION

Bonding to dentin has significantly evolved through the continuous development of adhesive systems since their first appearance. Self-etch adhesives have become widely used in the clinic because of their reduced technique-sensitivity3 and good bonding ability to tooth substrates.

Self-etch adhesives can be classified into two-step and one-step adhesives. A two-step self-etch adhesive consists of a self-etching primer and bonding agent. The self-etching primer with functional acidic monomers and water, can partially demineralize and simultaneously penetrate monomers into dentin subsurface, and is followed by the application of a solvent-free bonding agent, which contains mainly hydrophobic cross-linking monomers, which provides high mechanical properties1,2. On the other hand, a one-step self-etch adhesive contains a mixture of acidic monomers, hydrophilic and hydrophobic monomers, water and organic solvents in one bottle1,2. In the presence of water, the acidic monomer is ionized, acidifying the adhesive, and an organic solvent is added to combine the hydrophobic and hydrophilic ingredients. After applying a one-step self-etch adhesive to tooth substrates, air-blowing is an essential step in the bonding procedure in order to remove the excess water/solvent from the tooth surface. However, some researchers4-6 have indicated that even after air-blowing, residual water/solvent is present in the bonding layers, which imparts some disadvantages such as a reduction in the degree of conversion, and droplet formation due to phase separation. Moreover, a one-step self-etch adhesive exhibits higher hydrophilicity even after polymerization. Therefore, the bonding resins can act as permeable membranes6, resulting in nanoleakage in the bonding layer14-19. These phenomena might be dependent upon the degree of conversion of the bonding resin, which is strongly influenced by the intensity of light irradiation to the bonding resin.

When restoring a tooth with resin composite using a one-step or two-step self-etch adhesive system, the bonding layer will receive light irradiation twice. That is, the bonding layer receives light irradiation directly during the bonding procedure and indirectly through the filled resin composite. Direct light irradiation intensity to the bonding layer is dependent upon clinical scenarios such as cavity preparation, tooth position or the existence of adjacent teeth10-12. When the distance between the light source tip and irradiated surface increases, the intensity of the light irradiation reaching the bonded surface decreases. On the other hand, indirect light irradiation intensity of bonding layer is concerned with the type and shade of filled resin composites13 and their thickness, which affect the light transmission characteristics14-19 of the resin composite. Some studies have demonstrated that a reduction in light irradiation intensity causes lower bond strengths to dentin10,13. However, few studies have focused on the influence of indirect light irradiation through resin composite on bond strengths to dentin.

In addition, indirect light irradiation through the resin composite may affect the join between the bonding layer and the filled resin composite, which is another
important factor for the clinical success of adhesive restorations, as well as bonding to tooth substrates. This join is completed by co-polymerization between the bonding resin and resin composite. After direct light irradiation to the bonding resin, the top surface of the bonding resin is covered with an uncured resin layer due to oxygen inhibition of the polymerization reaction. This uncured bonding resin layer becomes a site for co-polymerization with the uncured matrix of the resin monomers of the resin composite\textsuperscript{20). In the case of a one-step self-etch adhesive, residual water/solvents may be present within the uncured bonding resin layer, which might affect co-polymerization between the bonding resin and resin composite, because the presence of residual water/solvents causes a reduction in the degree of conversion\textsuperscript{21–23). Therefore, the bonding of one-step self-etch adhesives might be influenced by the intensity of the light irradiation directly and indirectly through the resin composite more than two-step self-etch adhesives with a solvent-free bonding agent. However, there is no data, no published research on the influence of different intensities of direct and indirect light irradiation on the strength of the bonding layer to dentin and nanoleakage in the adhesive layer, using one-step and two-step self-etch adhesives.

Therefore, the purpose of this study was to evaluate effects of light-irradiated intensities directly and indirectly through resin composites to one-step and two-step self-etch adhesives on dentin bonding and nanoleakage expression of the adhesive interfaces. The null hypothesis tested was that there were no differences in dentin bond strength and nanoleakage between one-step and two-step self-etch adhesives under different direct and indirect light irradiation intensities.

**MATERIALS AND METHODS**

Two commercially available one-step self-etch adhesives [Clearfil S\textsuperscript{3} Bond (TS; Kuraray Medical Inc., Tokyo, Japan) and Bond Force (BF; Tokuyama Dental Corp., Tokyo, Japan)], and a two-step self-etch adhesive [Clearfil SE bond (SE; Kuraray Medical Inc., Tokyo, Japan)] were used in this study (Table 1).

**Tooth preparation**

Thirty-six non-caries extracted human third molars were used in this study, according to the protocol approved by the Human Research Ethics Committee, Tokyo Medical and Dental University, Japan. After removing the occlusal enamel using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA), the dentin surface was ground using 600-grit SiC papers to expose a flat dentin surface. The specimens were then randomly divided into 12 groups, based on the combination of light intensities of the curing light, adhesive systems and the different coloured resin composites. The bonding area was demarcated on the prepared dentin surface of each tooth using a black plastic ring, 2 mm in height and 8 mm in bore diameter. The adhesive systems were applied to the dentin surfaces according to the manufacturers' instructions, and then light-irradiated using an experimental LED light-curing hand-piece based on DENTAPORT (J. Morita Mfg. Corp., Kyoto, Japan) with an intensity of 350 or 600 mW/cm\textsuperscript{2}, which was equipped with an additional function of changing its light intensities in 30 levels, ranging from 200 mW/cm\textsuperscript{2} to 775 mW/cm\textsuperscript{2} (Fig. 1). Before the light irradiation, the light intensity was measured three times, using a spectroradiometer (Model 100 Optilux Radiometer,

**Table 1 Chemical composition and application mode of the adhesive materials tested in this study**

<table>
<thead>
<tr>
<th>Materials</th>
<th>Composition</th>
<th>Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearfil SE Bond</td>
<td>Primer: 10-MDP, HEMA, hydrophilic dimethacrylate, photoinitiator, water</td>
<td>Apply for 20 s</td>
</tr>
<tr>
<td>(SE; Kuraray Medical)</td>
<td>Bond: 10-MDP, HEMA, Bis-GMA, hydrophobic dimethacrylate, photoinitiator,</td>
<td>Air blow gently</td>
</tr>
<tr>
<td>pH=2.0*</td>
<td>silanated colloidal silica</td>
<td>Light cure for 10 s</td>
</tr>
<tr>
<td>Clearfil S\textsuperscript{3} Bond</td>
<td>10-MDP, HEMA, Bis-GMA, water, ethanol, silanated colloidal silica,</td>
<td>Apply for 20 s</td>
</tr>
<tr>
<td>(TS; Kuraray Medical)</td>
<td>photoinitiator</td>
<td>Dry with strong air pressure for 5 s</td>
</tr>
<tr>
<td>pH=2.7</td>
<td></td>
<td>Light cure for 10 s</td>
</tr>
<tr>
<td>Bond Force</td>
<td>Methacryloyloxyalkyl acid phosphate, HEMA, Bis-GMA, TEGDMA, water, isotro</td>
<td>Apply for 20 s</td>
</tr>
<tr>
<td>(BF; Tokuyama)</td>
<td>propyl alcohol, Glass Filler, photoinitiator</td>
<td>Dry with moderate, strong air pressure for 5 s</td>
</tr>
<tr>
<td>pH=2.3</td>
<td></td>
<td>Light cure for 10 s</td>
</tr>
</tbody>
</table>

Abbreviations: HEMA: 2-hydroxyethyl methacrylate; Bis-GMA: bisphenol-A-diglycidyl methacrylate; TEGDMA: triethylene glycol dimethacrylate; 10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate

*pH of the primer.
Sybron-Kerr, West Collins Orange, CA, USA) to check the output light power.

After the bonding procedure, two kinds of resin composites with different translucencies; translucent color [Clearfil Photo Core (PC; Kuraray Medical Inc.)] or opaque shade [Clearfil Majesty OA2 shade (MJ; Kuraray Medical Inc.)] (Table 2) were packed into the plastic ring by means of a bulk filling technique. The top of the ring was flattened using a plastic matrix strip and a glass slide then light-cured for 20 seconds with the same light intensities as for the bonding procedures.

**Micro-tensile bond strength test**

The bonded assemblies were stored in water at 37°C for 24 hours, then the stored teeth were vertically cross-sectioned perpendicular to the bonded surface to obtain beams with bonded area dimensions of approximately 0.7×0.7 mm using the diamond saw, under water cooling. 18 beams were tested for each adhesive/resin composite/intensity combination. The beams were fixed by their ends to the micro-tensile testing jig of a universal testing machine (EZ test, Shimadzu Co., Kyoto, Japan) with a cyanoacrylate adhesive (Model Repair II Blue, Sankin Industry Co., Tokyo, Japan) and tested at a crosshead speed of 1 mm/min (Fig. 1).

**Failure mode analysis**

After the bond test, the fractured surfaces were observed

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**Table 2** Chemical composition of the resin composites used in this study

<table>
<thead>
<tr>
<th>Resin composite</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearfil Photo Core (PC; Kuraray Medical)</td>
<td>Bis-GMA, TEGDMA, fillers, dl-Camphorquinone</td>
</tr>
<tr>
<td>Clearfil Majesty OA2 shade (MJ; Kuraray Medical)</td>
<td>Bis-GMA, Hydrophobic aromatic dimethacrylate, fillers, dl-Camphorquinone</td>
</tr>
</tbody>
</table>

Abbreviations: Bis-GMA: bisphenol-A-diglycidyl methacrylate; TEGDMA: triethylene glycol dimethacrylate
using an optical microscope (Nikon SMZ1000-1, Nikon Corp., Kanagawa, Japan) at 100× magnification. Fracture modes were classified into four categories as follow: Cohesive failure in resin composite (CR), Adhesive failure (A), Cohesive failure in dentin (CD) and Mixed failure (M).

Statistical analysis
The bond strength data were analyzed by two-way analysis of variance (ANOVA). Afterwards, multiple comparisons were performed using Bonferroni tests between groups (significance level p<0.05).

Nanoleakage observation
A further 36 teeth were used for nanoleakage evaluation of adhesive interface using a scanning electron microscope (SEM; JSM-5310LV, JEOL, Tokyo, Japan). The bonded specimens prepared as previously mentioned, were sectioned occluso-gingivally into 1.0 mm thick and placed in a 50 wt% ammoniacal silver nitrate solution for 24 hours in the dark. Ammoniacal silver nitrate was prepared by the dissolution of 25 g of silver nitrate crystals (Wako, Osaka, Japan) in 25 mL of distilled water. Concentrated (28%) ammonium hydroxide (Sigma, Tokyo, Japan) was used to titrate the black water. After gold sputter coating, the adhesive interface and cohesive failure in resin and/or dentin. However, only the BF/MJ/350 mW/cm² group showed nanoleakage expressions along adhesive-dentin interfaces. However, only the BF/MJ/350 mW/cm² group showed nanoleakage expressions along adhesive-composite interface as well as adhesive-dentin interface (Fig. 3(f)).

Fracture mode
The dominant fracture modes observed in all the experimental groups were mixed failure with adhesive interface and cohesive failure in resin and/or dentin. Only three of the specimens of the BF/MJ/350 mW/cm² group cohesively failed in resin.

RESULTS
Table 3 summarizes the means and standard deviations of the micro-tensile bond strengths (µTBS) to dentin. Two-way ANOVA revealed that there were significant differences between the materials (p<0.0001) and light irradiation condition (p<0.0001). There was a significant interaction among materials and light irradiation condition (p<0.0001). SE demonstrated significantly higher µTBS than the tested one-step self-etch adhesives for each light irradiation condition (p<0.05). The PC/600 mW/cm² group for all the tested adhesives had higher µTBS than the MJ/600 mW/cm² group. For SE and BF, the MJ/600 mW/cm² group showed significantly lower µTBS than the PC/350 mW/cm² group (SE; p=0.001, BF; p<0.0001), while for TS, there were no significant differences between the MJ/600 mW/cm² and PC/350 mW/cm² groups (p>0.05). The MJ/350 mW/cm² group for all the tested adhesives had the lowest µTBS among all the light irradiation conditions.

DISCUSSION
In this study, the micro-tensile bond strength test was performed to evaluate the effects of different light intensities irradiated directly and indirectly through resin composite on dentin bonding. The results of this study showed that for both one-step and two-step self-etch adhesives, light irradiation with 600 mW/cm² resulted in higher µTBS to dentin than that of 350 mW/cm², regardless of the type of filled resin composite. These results are in agreement with previous reports, in which the bonding ability of self-etch adhesives is dependent upon light irradiation intensity. It has been reported that attenuation of light reduced the degree of conversion of carbon double bonds (C=C) and lowered the mechanical properties, leaving uncured resin monomers. Therefore, the lower bond strength of the 350 mW/cm² groups would have been the result of poor mechanical properties of the bonding resin.

Translucent color (Clearfil Photo Core (PC)) and opaque shade resins (Clearfil Majesty OA2 shade (MJ)),

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Micro-tensile bond strength to dentin in MPa</th>
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<tbody>
<tr>
<td></td>
<td>PC</td>
</tr>
<tr>
<td></td>
<td>600 (mW/cm²)</td>
</tr>
<tr>
<td>SE</td>
<td>66.4 (5.5)</td>
</tr>
<tr>
<td>TS</td>
<td>46.4 (5.7)</td>
</tr>
<tr>
<td>BF</td>
<td>50.1 (13.0)</td>
</tr>
</tbody>
</table>

Values are means (standard deviations).
- Within the same row, means with the different capital superscript letter are statistically different (p<0.05).
- Within the same column, means with the different lowercase superscript letter are statistically different (p<0.05).
Fig. 2  Representative SEM images of adhesive interface with silver uptake in the PC group.
(a) SE/600 mW/cm², (b) TS/600 mW/cm², (c) BF/600 mW/cm², (d) SE/350 mW/cm², (e) TS/350 mW/cm², (f) BF/350 mW/cm². For all groups, nanoleakage expression is identified along the adhesive-dentin interface (white arrow).

Fig. 3  Representative SEM images of adhesive interface with silver uptake in the MJ group.
(a) SE/600 mW/cm², (b) TS/600 mW/cm², (d) SE/350 mW/cm², (e) TS/350 mW/cm². For the SE and TS groups, nanoleakage expression is identified along the adhesive-dentin interface (white arrow). (c) BF/600 mW/cm²; Nanoleakage expression is identified along the adhesive-dentin interface (white arrow). (f) BF/350 mW/cm²; Nanoleakage expression is identified along the adhesive-composite interface (white arrowheads) as well as adhesive-dentin interface (white arrow).
2 mm in thickness, were used in this study. Light transmission characteristics are dependent upon shade, translucency, and thickness of resin composite. In our pilot study, light transmission intensities through each resin composite were measured. Resin composite disks (8 mm in diameter) were made and covered with celluloid strips on glass plates, which were separated by spacers of 2 mm thick. Three specimens were prepared for each material. After curing with the light-curing hand-piece for 60 seconds each from the top and bottom sides, the strips and glass plates were removed. The light transmission intensity was measured using a spectroradiometer (Model 100 Optilux Radiometer, Sybron-Kerr, West Collins Orange, CA, USA), placing the disk between the sensor and the light tip. The light intensity through each resin composite was measured three times at each resin composite/light intensity combination. In PC, a light intensity irradiation of 600 mW/cm² decreased to 150 mW/cm² after going through 2 mm thickness of resin composite, and 350 mW/cm² to 120 mW/cm². In MJ, a 600 mW/cm² intensity irradiation decreased to 50 mW/cm², and 350 mW/cm² to 20 mW/cm². The light transmission intensity of MJ for 600 mW/cm² irradiation was 1/3 compared with that of PC, and with 350 mW/cm² had a lower transmission intensity than PC with 350 mW/cm².

Regarding the two-step self-etch adhesive, SE, with 600 mW/cm² irradiation, the PC group demonstrated significantly higher µTBS than the MJ group. In addition, with 350 mW/cm² irradiation, there were also significant differences in µTBS to dentin between the PC and MJ groups. For the MJ group, although the direct light irradiation intensity was of the same power as the case of the PC group, less indirect light irradiation reached the bonding resin through the resin composite. These results indicate that less indirect light irradiation to the bonding resin through the resin composite reduced the µTBS to dentin even when sufficient light intensity was irradiated directly on bonding resin. On the other hand, the PC/350 mW/cm² group, in which the direct light intensity was low but the indirect light intensity was relatively high, showed significantly higher bond strength than the MJ/600 mW/cm² group, in which the direct light intensity was high but the indirect intensity was low. These results would indicate that light transmission intensity through resin composite played an important role in SE achieving higher bond strengths to dentin.

For the one-step self-etch adhesive, BF, the µTBS results showed the same tendency as observed in SE; the indirect light intensity through resin composite was a significant factor in achieving higher bond strengths to dentin. However, when the PC/600 mW/cm² and MJ/350 mW/cm² groups were compared, BF showed a larger reduction in µTBS to dentin than SE. When both the direct and indirect light irradiation intensities were low, the µTBS of BF reduced to 16.6 MPa from 50.1 MPa, while for SE, it reduced to 35.7 MPa from 66.4 MPa. Generally, insufficient direct and indirect light irradiation intensities to the bonding layer would produce large amounts of uncured resin within the bonding layer, leading to lower mechanical properties for the bonding resin. The mechanical strength of the bonding resin is mainly provided by polymerization of the network of hydrophobic cross-linking monomers. In the case of one-step self-etch adhesives, the solvents and functional monomers usually make up almost 50% of the adhesive. Therefore, the concentration of hydrophobic monomers in one-step self-etch adhesives is drastically reduced compared with the bonding agent of a two-step self-etch adhesive, resulting in lower mechanical properties. Moreover, the presence of residual water within the bonding resin would lead to domains of incomplete polymerization of bonding resin. For the one-step self-etch adhesive, BF, lower direct and indirect light irradiation intensities to the bonding resin might strongly impair optimal formation of the cross-linking network of hydrophobic monomers because of the lower concentration of hydrophobic cross-linking monomers and incomplete polymerization of the bonding resin due to residual water/solvents, leading to a drastic reduction in the bond strength to dentin compared with SE.

Moreover, a reduction in the indirect light irradiation intensity through filled resin composite would aggravate the co-polymerization between bonding resin and filled resin composite. When light-cured resin composite is irradiated, the hydrophobic resin matrix of the filled resin composite can co-polymerize with the uncured bonding resin produced on cured bonding layer as well as polymerizing itself. Solvents in one-step self-etch adhesives retard the polymerization process, leading to the creation of thicker oxygen inhibited uncured layer on the cured bonding resin. Presumably, there would be remnants of water/solvent in the uncured bonding layer of a one-step self-etch adhesive. These residual water/solvents might affect co-polymerization between the hydrophilic bonding resin and hydrophobic resin composite, in which light intensities irradiated directly and indirectly through resin composite would affect the join between resin composite and the bonding resin of the one-step self-etch adhesive. For BF, the MJ/350 mW/cm² group showed nanoleakage expressions along both the adhesive-composite and the adhesive-dentin interfaces (Fig. 3(f)), however in the other groups, nanoleakage expression was not observed at adhesive-composite interface. These results would indicate that the join between the bonding layer of BF and resin composite was impaired. Residual water within the uncured bonding layer of BF might inhibit co-polymerization between the hydrophobic resin composite and bonding resin when less indirect light irradiation reaches the join between resin composite and bonding resin.

On the other hand, the one-step self-etch adhesive, TS, maintained relatively stable bond strengths to dentin within the experimental groups except for the MJ/350 mW/cm² group. TS also showed the lowest µTBS in the MJ/350 mW/cm² group, but the difference between the MJ/350 mW/cm² and PC/600 mW/cm² groups was
less than that for BF. In addition, nanoleakage was not observed along the adhesive-composite interface of TS, only along the adhesive-dentin interface. Recently, Itoh et al. revealed that TS showed significantly lower water sorption value and solubility than BF\(^{30}\). Both TS and BF contain the hydrophilic monomer HEMA, but utilize different organic solvents (TS: ethanol, BF: isopropyl alcohol). The vapor pressure (at 25°C) is 44 mmHg for isopropyl alcohol and 54.1 mmHg for ethanol. These values indicate that it is more difficult to evaporate isopropyl alcohol than ethanol by air-drying. Therefore, in the case of TS, the solvent (ethanol) may have been easily removed by air-drying. As a result, polymerization of the bonding resin would be less affected since the amount of remaining solvent would be lower.

Moreover, for TS, there was no significant difference between the PC/350 mW/cm\(^2\) and MJ/600 mW/cm\(^2\) groups. This result is in disagreement with those of SE and BF, in which the PC/350 mW/cm\(^2\) group had significantly higher µTBS than the MJ/600 mW/cm\(^2\) group. These results necessitate partial rejection of the null hypothesis. In this study, the influence of direct and indirect light irradiation intensities on the bonding layer on dentin bonding ability was dependent upon the tested materials, in which the classification of the adhesive was not a significant factor. The polymerization process of the bonding resin is influenced by the type and/or concentration of photo-initiators and catalysts utilized in the adhesive system as well as light irradiation intensity. Therefore, the photo-initiation system and light irradiation method would have a complex relationship with regards to their influence on the mechanical properties of the bonding resin. Presumably, the photo-initiation system would be different between the tested adhesive materials. This might be a reason why TS exhibited relatively stable bond strengths to dentin under different intensities of direct and indirect light irradiation.

Within the limitations of this study, it was concluded that indirect light irradiation intensity of the bonding layer through filled resin composite, as well as direct light irradiation was important for achieving higher bond strengths to dentin. In the case of SE and BF, higher indirect light irradiation intensities improved their bonding performance to flat dentin surfaces even when the intensity of the direct light irradiation was relatively low, while for TS, direct and indirect light irradiation intensities had a complementary activity on bonding performance. However, these influences of direct and indirect light irradiation intensities on bonding performance might be different at the cavity walls, because contraction stress induced by light-curing the resin composite causes reduction of bond strength to restricted cavity floor dentin, while for flat surfaces, it is released by free surface area, resulting in less influence on bond strength\(^{11}\). Further research is required to pursue the effects of direct and indirect light irradiation on the bonding layer on dentin bonding performance within the cavity and co-polymerization with filled resin composite.

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