The effect of a peroxidase primer on bond strength of three luting systems to dentin

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Running title: Dentin bonding systems with a microperoxidase

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Eur J Oral Sci

Abstract

The purpose of the present study was to investigate the effect of an experimental primer containing a microperoxidase (MP-11) with 2-hydroxyethyl methacrylate on adhesive bonding of three different luting systems and dentin. The luting systems prepared were three etch-and-rinse systems (10-3/Super-Bond, 10-0/Super-Bond, 65PA/Super-Bond), a self-etching system (PanaviaF2.0), and a self-adhesive system (SA-Luting). These luting systems were used in conjunction with the MP-11 primer, and were designated as 10-3/MP-11/Super-Bond, 10-0/MP-11/Super-Bond, 65PA/MP-11/Super-Bond, MP-11/PanaviaF2.0, MP-11/SA-Luting, respectively. The dentin surfaces of human premolar teeth were treated, and then bonded with acrylic rods. Shear bond strengths were determined after 24 hours of storage in water. The maximum mean bond strength was obtained with 10-0/MP-11/Super-Bond (48.7±6.6 MPa), followed by 10-3/MP-11/Super-Bond (36.3±10.2 MPa), 65PA/MP-11/Super-Bond (32.9±9.2 MPa), 10-3/Super-Bond (26.6±6.7 MPa), MP-11/PanaviaF2.0 (21.4±5.6 MPa), MP-11/SA-Luting (17.2±3.5 MPa), PanaviaF2.0 (16.9±5.7 MPa), 65PA/Super-Bond (12.8±2.0 MPa), SA-Luting (11.2±5.4 MPa), and 10-0/Super-Bond (9.6±3.9 MPa). The additional use of MP-11 primer significantly improved the bond strengths in the etch-and-rinse systems with 4-META/MMA-TBB resin. It is suggested that the peroxidase has a potential to improve dentin adhesion in the etch-and rinse, self-etching, and self-adhesive systems.

Keywords: adhesion; enzyme; surface modification; dentin

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**Introduction**

Although various surface treatment agents and luting agents have been clinically used, the treatment outcome is not always maintained as problems such as detachment, marginal gap, and root fracture occur (1-3). In order to facilitate minimal intervention, the abutment tooth is minimally reduced, and a stronger bond between resin and dentin is desired.

In 1980’s, surface treatment of dentin with ferric chloride (4,5) or ferric oxalate (6) was reported, in which the ferric components were regarded as a mordant. An innovative bonding mechanism mediated by hybrid layer was proposed with the use of a dentin conditioner (10-3 liquid) containing 10wt% citric acid and 3wt% ferric chloride in conjunction with an auto-polymerizing resin containing 4-methacryloyloxyethyl trimellitate anhydride (4-META), methyl methacrylate (MMA), and tri-n-butylborane (TBB) (7). This luting system is commercially available as Super-Bond C&B (Sun Medical Co. Ltd., Moriyama, Japan) or C&B-Metabond Quick (Parkell Inc., Edgewood, NY, U.S.A.). When the adherend surface is limited to enamel, 65wt% phosphoric acid (65PA) is recommended for 4-META/MMA-TBB resin. However, without ferric chloride, neither phosphoric acid nor citric acid are effective as the 10-3 liquid in dentin adhesion (7-10).

Previous investigations have demonstrated that primers containing cytochrome c or a microperoxidase (MP-11) significantly enhance the bond strength of 4-META/MMA-TBB resin to bovine dentin etched with 10wt% phosphoric acid (11-13). MP-11 is a heme-undeca peptide complex derived from cytochrome c, in which the iron, porphyrin, and 11 amino acids are firmly coupled via thioether bridges (Fig. 1) (14). It was introduced as a nontoxic tracer that serves to define tissue or cellular compartments and their interconnecting channels in electron microscopy (15). Compared to cytochrome c, the smaller molecule is advantageous for tissue permeability (16).
MP-11 exhibited higher electrocatalytic efficiency (17), and the redox process includes transition of the heme-iron between Fe$^{2+}$ and Fe$^{3+}$. In general, peroxidase is a group of enzymes that catalyze the oxidation of a substance by a peroxide. The mechanism of polymerization of MMA initiated by alkylborones such as TBB was explained as follows. The alkylborones and the partly oxidized alkylborones were oxidized by O$_2$ to change some peroxides, and the created peroxides were decomposed to generate free radicals (18,19). It was revealed that MP-11 accelerated the polymerization of 4-META/MMA-TBB resin (13).

In contrast to the etch-and-rinse system using 4-META/MMA-TBB resin, the resin-base luting agents employing a self-etching or self-adhesive system are characterized by an etching procedure which is not time-consuming and their polymerization initiator consisted mainly of benzoyl peroxide and amine (20). Addition of the ferric component into a self-etching primer improved the bond strength of 4-META/MMA-TBB resin to dentin (21,22). However, with the exception of TBB-initiated resin, it is unknown whether MP-11 improves the adhesive bonding of the resin-based luting agent to dentin. Therefore, the present study was designed to investigate the efficacy of an experimental dentin primer containing MP-11 in conjunction with three different luting systems.

Materials and methods

The luting systems used are listed in Table 1. An experimental primer was prepared with 1.0 µmol/g MP-11, 35wt% 2-hydroxyethylmethacrylate (HEMA), and distilled water according to the previous study (13). The pH value of the primer was measured with a test strip (pH-Fix Papers, Macherey-Nagel GmbH & Co., Düren, Germany).
Sample preparation

Fifteen human premolar teeth were embedded in unfilled resin (Unifast II, GC Corp., Tokyo, Japan), and sectioned parallel to the long axis using a cutting apparatus (IsoMet Low Speed Saw, Buehler ltd., Lake Bluff, IL, USA). The exposed dentin surface was flattened by wet-grinding with #400 and #600 grit silicon carbide paper, and then the samples were cut perpendicular to the long axis to obtain 90 specimens of three areas (30 crown, 30 middle, and 30 root areas) (Fig. 2).

Adhesive masking tape with a 2-mm-diameter hole was affixed to the surface in order to define the bonding area and maintain a film thickness of 50 µm. The primer and luting agent was applied to the pretreated dentin surface (Table 2), and then the acrylic rod (5 mm in diameter, 5 mm long) was affixed under finger pressure. No light curing employed in the present study. All specimens were left in atmospheric environment at room temperature for 30 min, and stored in water at 37°C for 24 h.

Shear bond testing

The bonded specimens were embedded in an acrylic resin mold and placed in a shear testing apparatus (ISO/TR 11405 jig, Wago Industrial Ltd., Nagasaki, Japan). Shear load was applied to the bonded specimens by means of a universal testing machine (AGS-10kNG; Shimadzu Corp., Kyoto, Japan) at a crosshead speed of 0.5 mm/min.

The means and standard deviations of the six specimens were calculated for each group. Statistical analysis was performed using three-way factorial analysis of variance (ANOVA), in which luting system, dentin location, and MP-11 primer served as independent factors. The mean values were compared by post-hoc Tukey Compromise test (p=0.05) following one-way ANOVA.
After the shear test, the fractured surfaces were observed through an optical microscope (SMZ-10; Nikon, Tokyo, Japan) (20x) to identify the mode of bond failure. The failure modes were classified as being at the resin-dentin interface (adhesive failure, A), within resin (cohesive failure, C), and combinations of these failure modes (AC).

**Results**

The three-factor ANOVA showed that the bond strength was significantly influenced by the luting system (F-value=22.8, P-value=0.0001), and by MP-11 primer (F-value=143.2, P-value=0.0001) (Table 3). No significant effect was found with dentin location (F-value=3.0, P-value=0.06) and in the interactions (luting system/dentin location, dentin location/MP-11 primer, and luting system/dentin location/MP-11 primer). It means that the dentin location had no significant effect on bond strength statistically. Accordingly, all of the location results within a given luting system with or without MP-11 primer were combined for the following statistical analysis, as suggested in a previous study (23).

The mean bond strengths of nine specimens, the standard deviations, and the lowest bond strengths are listed in Table 4. Without MP-11 primer, the mean bond strength of 10-3/Super-Bond was greater than those of 10-0/Super-Bond, 65PA/Super-Bond, PanaviaF2.0, and SA-Luting. The difference between 10-0/Super-Bond, 65PA/Super-Bond, PanaviaF2.0, and SA-Luting was not significant. The mean bond strengths of 10-3/Super-Bond, 10-0/Super-Bond, and 65PA/Super-Bond were significantly enhanced with the use of MP-11 primer. No significant difference was found between PanaviaF2.0 and MP-11/PanaviaF2.0 or between SA-Luting and MP-11/SA-Luting. All of the lowest values of bond strength were increased with the use of MP-11 primer. The highest mean bond strength was obtained with 10-0/MP-11/Super-Bond (48.7±6.6 MPa), followed by 10-3/MP-11/Super-Bond (36.3±10.2
MPa) and 65PA/MP-11/Super-Bond (32.9±9.2 MPa). The difference of mean bond strength between 10-3/MP-11/Super-Bond and 65PA/MP-11/Super-Bond, and between MP-11/PanaviaF2.0 and MP-11/SA-Luting were not significant.

Forty specimens failed in combined adhesive and cohesive mode. No specimen failed within the dentin substrate or the interface between the luting agent and the acrylic rod. Pure adhesive failure was observed in groups 10-3/Super-Bond, 10-0/Super-Bond, 65PA/Super-Bond, SA-Luting, and MP-11/SA-Luting, while pure cohesive failure appeared in groups 10-3/MP-11/Super-Bond, 10-0/MP-11/Super-Bond, 65PA/MP-11/Super-Bond, and MP-11/PanaviaF2.0.

**Discussion**

The present study revealed that the use of MP-11 primer improved the bond strength of resin to dentin. The lowest bond strength values obtained in each group with the mean and standard deviation are presented (Table 4). When considering the safety factor, the lowest value is useful in evaluating bond strength (24). In the clinical situation, the safety factor should be taken into account in order to guarantee that even the lowest bond strength values are sufficiently strong (25). Although there were no clear statistically significant differences in mean bond strength between the groups PanaviaF2.0 and MP-11/PanaviaF2.0 and between the groups SA-Luting and MP-11/SA-Luting, the lowest values showed that the MP-11 primer also improved adhesive bonding of PanaviaF2.0 and SA-Luting to dentin.

The bonding mechanism can be explained in terms of diffusion of the monomer and polymerization of resin at the bonded interface. In the 65PA/Super-Bond and 10-0/Super-Bond systems, the peculiar polyelectrolytes of dentin should be removed by etching procedure (10,26) and air-drying collapsed the exposed dentin collagen (27-29). In the 10-
3/Super-Bond system, it is stressed that ferric ions promote diffusion of monomer into the
demineralized dentin, because the ferric ion stabilizes the dentin collagen against air-drying
(7). Ferric ion also works as a promoter or as an inhibitor for polymerization depending on its
concentration (30). These are the key points to explain why 10-3/Super-Bond showed higher
bond strength than 10-0/Super-Bond and 65PA/Super-Bond.

We speculated that the strong bonding is attributed to both diffusion of monomer into
dentin and acceleration of polymerization of the diffused monomer by the MP-11 primer.
With regard to the polymerization of resin, the luting agent generally polymerizes faster than
the area of the diffused monomer, because the polymerization proceeds with an increase in
the viscosity of the resin. When the polymerization is initiated at the dentin-resin interface,
polymerization shrinkage can be minimized (30). We previously reported that the primed
MP-11 shortened the polymerization time of 4-META/MMA-TBB resin, which contributed to
the bond strength (13). The results of 10-3/MP-11/Super-Bond (36.3 MPa), 10-0/MP-
11/Super-Bond (48.7 MPa), 10-3/Super-Bond (26.6 MPa), and 10-0/Super-Bond (9.6 MPa)
suggested that excessive ferric ions weakened polymerization in the 10-3/MP-11/Super-Bond
group, and also suggested that MP-11 is more active than ferric chloride.

Generally, peroxidase decomposes a peroxide to catalyze the oxidation of a substance by
the peroxide. TBB was oxidized by O₂ to change some peroxides, and the created peroxides
were decomposed to generate free radicals (18,19). In PanaviaF2.0 and SA-Luting, free
radicals are generated when benzoyl peroxide is decomposed. Accordingly, benzoyl peroxide
may be decomposed with MP-11.

The failure modes after shear testing tended to shift from adhesive to cohesive as the
bond strength increased. With 10-0/Super-Bond, the mean bond strength was the lowest, and
all of the specimens showed pure adhesive failure. At the highest mean bond strength (10-
3/MP-11/Super-Bond), five specimens showed pure cohesive failure within resin. The pure
cohesive failure suggests that the adhesive force at the resin-dentin interface area is greater than the cohesive strength of the resin.

Previous study showed that no strong bond was obtained in the absence of HEMA, even if MP-11 primer was used in conjunction with 4-META/MMA-TBB resin (13). The smear layer is a barrier to diffusion of the MP-11 primer into the underlying dentin. Once the smear layer is removed by etching, HEMA of the MP-11 primer should re-expand the collapsed collagen fibrils facilitating diffusion of the other monomers into the demineralized dentin (8,9). Taking the molecular size into consideration, deep diffusion of MP-11 into the demineralized dentin may be difficult. The difference between 10-0/MP-11/Super-Bond and 65PA/MP-11/Super-Bond suggests that acidity of etching agent also influence the bond strength.

Although bond strength test is not a definitive way to identify the weakest link in the bond, an ideal luting system would show a high mean bond strength, as well as a high lowest value, and have a narrow standard deviation. Among the luting systems evaluated in the present study, 10-3/MP-11/Super-Bond, 10-0/MP-11/Super-Bond, and 65PA/MP-11/Super-Bond appeared to better meet these requirements compared to commercial products (10-3/Super-Bond, PanaviaF2.0, and SA-Luting). Clinicians may fear marginal staining with some iron compounds. In order to predict clinical performance and reliability, more experiments on MP-11 are needed, such as microscopic analysis (31), microleakage test (32), and micro-tensile bond test (33).

Within the limitations of the present study of bond strength, it is suggested that the additional use of primer containing 1 μmol/g MP-11 with 35wt% HEMA serves to improve the adhesive bonding of the etch-and-rinse, self-etching, and self-adhesive systems to human dentin. The maximum mean bond strengths were obtained when the MP-11 primer was used in conjunction with 10wt% citric acid etching agent and 4-META/MMA-TBB resin.
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curing resins (4) bonding of dental self-curing resins to dentin surface by the use of


Figure legends

**Fig. 1.** Structural formula of microperoxidase MP-11. Val, valine; Glu, glutamic acid; Lys, lysine; Cys, cysteic acid; Ala, alanine; His, histidine; Thr, threonine\textsuperscript{14-16}.

\[
\text{Val - Glu(NH}_2\text{-Lys - Cys - Ala - Glu(NH}_2\text{-Cys - His - Thr - Val - Glu}}
\]

\[
\begin{array}{c}
\text{M:} \text{-CH}_3 \\
\text{P:} \text{-CH}_2\text{-CH}_2\text{-COOH}
\end{array}
\]

**Fig. 2.** Schematic illustration of the division of the tooth into crown, middle, and root segments.
Table 1

Luting systems used in the present study

<table>
<thead>
<tr>
<th>Material name</th>
<th>Components</th>
<th>Lot No.</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP-11 primer</td>
<td>1.0 µmol/g Microperoxidase MP-11 [pH=6.0] 35wt% 2-hydroxyethylmethacrylate</td>
<td>47H7000</td>
<td>Sigma Chemical Co., St. Louis, MO, U.S.A.</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>PAQ4710</td>
<td>Wako Pure Chemical Industries Ltd., Osaka, Japan</td>
</tr>
<tr>
<td>Super-Bond C&amp;B</td>
<td>Etching agent Red Activator (65PA): 65wt% phosphoric acid, Green Activator (10-3): 10wt% citric acid, 3wt% ferric chloride</td>
<td>RV1</td>
<td>Sun Medical Co. Ltd., Moriyama, Japan</td>
</tr>
<tr>
<td></td>
<td>Experimental (10-0): 10wt% citric acid</td>
<td>IFD02105</td>
<td>Hayashi Pure Chemical Industries Ltd.</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td></td>
<td>Osaka, Japan</td>
</tr>
<tr>
<td>Luting agent</td>
<td>Monomer liquid: 4-META, MMA</td>
<td>RR2</td>
<td></td>
</tr>
</tbody>
</table>
Clear powder: PMMA  
Initiator: Tri-$n$-butylborane derivative

PanaviaF2.0  
*Self-etching primer*  
Kuraray Medical Inc., Tokyo, Japan

ED Primer II, Liquid A: HEMA, MDP, water  
chemical initiator

Liquid B: Methacrylate monomer,  
water, chemical initiator

*Luting agent*  
Paste A: MDP, Methacrylate monomer,  
filler, photo initiator, chemical initiator

Paste B brown: Methacrylate monomer, sodium fluoride,  
filler, photo initiator, chemical initiator

Oxyguard II: Poly ethyleneglycol, accelerator  
00550A

Clearfil SA Luting  
*Luting agent*  
Kuraray Medical Inc.

(SA-Luting)  
A paste: Bis-GMA, TEGDMA, MDP,  
methacrylate monomer, filler,
photo initiator, initiator

B paste: Bis-GMA, methacrylate monomer, 00009a
sodium fluoride, filler, accelerator

4-META, 4-methacryloyloxyethyl trimellitate anhydride; MMA, methyl methacrylate; PMMA, poly(methyl methacrylate); HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; 5-NMSA, 5-(N-methacryloylamino)salicylic acid; Bis-GMA, 2,2-bis [4-(2-hydroxy3-methacryloyloxy-propoxy)phenyl]propane; TEGDMA, triethyleneglycol dimethacrylate
### Table 2

#### Bonding procedure

<table>
<thead>
<tr>
<th>Group name</th>
<th>Etching (seconds)</th>
<th>Rinse (seconds)</th>
<th>MP-11 primer (seconds)</th>
<th>ED primer II (seconds)</th>
<th>Luting agent</th>
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<tr>
<td>10-3/Super-Bond</td>
<td>10-3 (30)</td>
<td>(15)</td>
<td>–</td>
<td>–</td>
<td>Super-Bond</td>
</tr>
<tr>
<td>10-0/Super-Bond</td>
<td>10-0 (30)</td>
<td>(15)</td>
<td>–</td>
<td>–</td>
<td>Super-Bond</td>
</tr>
<tr>
<td>65PA/Super-Bond</td>
<td>65PA (30)</td>
<td>(15)</td>
<td>–</td>
<td>–</td>
<td>Super-Bond</td>
</tr>
<tr>
<td>PanaviaF2.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(30)</td>
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<tr>
<td>SA-Luting</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>SA-Luting</td>
</tr>
<tr>
<td>10-0/MP-11/Super-Bond</td>
<td>10-0 (30)</td>
<td>(15)</td>
<td>(30)</td>
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<td>Super-Bond</td>
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<tr>
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<td>(15)</td>
<td>(30)</td>
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<td>Super-Bond</td>
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<tr>
<td>MP-11/PanaviaF2.0</td>
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<td>–</td>
<td>(30)</td>
<td>(30)</td>
<td>PanaviaF2.0</td>
</tr>
<tr>
<td>MP-11/SA-Luting</td>
<td>–</td>
<td>–</td>
<td>(30)</td>
<td>–</td>
<td>SA-Luting</td>
</tr>
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Table 3  
Results of analysis of variance for shear bond strength

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<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F-value</th>
<th>P-value</th>
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<td><strong>(a) Three-way ANOVA</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Luting system</td>
<td>4</td>
<td>3611.5</td>
<td>902.9</td>
<td>22.8</td>
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<td>Dentin location</td>
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<td>235.9</td>
<td>118.0</td>
<td>3.0</td>
<td>0.06</td>
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<tr>
<td>MP-11 primer</td>
<td>1</td>
<td>5665.7</td>
<td>5665.7</td>
<td>143.2</td>
<td>0.0001</td>
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<tr>
<td>Luting system/Dentin location</td>
<td>8</td>
<td>396.7</td>
<td>49.6</td>
<td>1.3</td>
<td>0.3</td>
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<td>Luting system/MP-11 primer</td>
<td>4</td>
<td>3686.5</td>
<td>921.6</td>
<td>23.3</td>
<td>0.0001</td>
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<td>Dentin location/MP-11 primer</td>
<td>2</td>
<td>64.4</td>
<td>32.2</td>
<td>0.8</td>
<td>0.4</td>
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<td>Luting system/Dentin location/MP-11 primer</td>
<td>8</td>
<td>131.8</td>
<td>16.5</td>
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<tr>
<td>Residual</td>
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<td>2373.1</td>
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<td><strong>(b) One-way ANOVA</strong></td>
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<tr>
<td>Group</td>
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<td>Group name</td>
<td>Mean (SD) *</td>
<td>Lowest value</td>
<td>Type of bond failure**</td>
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<td>------------------------</td>
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<tr>
<td>10-3/Super-Bond</td>
<td>26.6 (6.7)</td>
<td>13.4</td>
<td>A(1) AC(8)</td>
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<tr>
<td>10-0/Super-Bond</td>
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<td>5.4</td>
<td>A(9)</td>
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<td>65PA/Super-Bond</td>
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<td>10.2</td>
<td>A(9)</td>
<td></td>
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<td>PanaviaF2.0</td>
<td>16.9 (5.7)</td>
<td>9.6</td>
<td>AC(9)</td>
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<tr>
<td>SA-Luting</td>
<td>11.2 (5.4)</td>
<td>4.1</td>
<td>A(9)</td>
<td></td>
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<td>10-3/MP-11/Super-Bond</td>
<td>36.3 (10.2)</td>
<td>20.4</td>
<td>AC(4) C(5)</td>
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<tr>
<td>10-0/MP-11/Super-Bond</td>
<td>48.7 (6.6)</td>
<td>40.1</td>
<td>AC(4) C(5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>65PA/MP-11/Super-Bond</td>
<td>32.9 (9.2)</td>
<td>16.2</td>
<td>AC(6) C(3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP-11/PanaviaF2.0</td>
<td>21.4 (5.6)</td>
<td>15.9</td>
<td>AC(7) C(2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP-11/SA-Luting</td>
<td>17.2 (3.5)</td>
<td>12.4</td>
<td>A(7) AC(2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Identical letters indicate that the values are not statistically different (n=9, p ≥0.05).

**A, adhesive failure at resin-dentin interface; C, cohesive failure within resin; AC, combined failure involving A and C modes.