Microtensile bond strength of a newly developed resin cement to dentin

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Keywords: Adhesive resin cement, Microtensile bond strength, Fracture modes, SEM, TEM

The purpose of this study was to evaluate the microtensile bond strength (µTBS) of a newly developed resin cement, ECD-89 (ECD, Tokuyama Dental) to dentin and to observe the interfacial micromorphology by comparing with two commercial resin cements, Multilink Automix (MA, Ivoclar Vivadent AG, Schaan, Liechtenstein) and Panavia F2.0 (PF, Kuraray Noritake Dental). Flat dentin surfaces of human third molars were exposed using #600 SiC. After application of primer and cement to the dentin surface, each cement was applied and cured with light (light condition) or without light (dark condition). The teeth were sectioned to obtain beams (1 mm×1 mm) after 24 h of water storage. The mean bond strengths and SDs (MPa) were: ECD: 68.6±14.9, MA: 39.2±18.9, PF: 39.4±18.5 and ECD: 54.5±22.4, MA: 36.7±15.6, PF: 13.4±4.46 when cured in light and dark condition, respectively. In both conditions, ECD-89 showed statistically higher µTBS than the others.

INTRODUCTION

In clinical situations, adhesive resin cements are very convenient for bonding indirect restorations. Many factors could influence the performance of luting materials, including the clinical scenario1, polymerization methods and degree of conversion2, physical/chemical properties3 and biologic aspects regarding pulp response4. It has also been claimed that the adhesive systems used in association with the cementing agents are of paramount importance in preventing an early dislodgement and providing long-term bonding stability2,5-7. Hence, conventional adhesive resin cements require special luting procedures using the adhesive steps5-7. The majority of the adhesive systems used with resin cements are simplified systems because of clinical trends for reduced steps during adhesive procedures9. To ease the pretreatment procedures of the tooth tissue, self-adhesive resin cements were recently developed5,9.

As self-adhesive resin cements bond to the substrate, more specifically to dentin, by polymerization no pre-treatment with adhesive systems is necessary. Though the application time and the technique sensitivity might be reduced; however, recent studies showed that the self-adhesive resin cements cannot alternate conventional resin cements8-13 because of their inability to demineralize/dissolve the smear layer completely. In addition, limited decalcification/ infiltration of dentin morphology was also observed which is also a drawback14. Consequently, in terms of two very important characteristic of adhesive resin cements, such as bond strength and long term durability, those of conventional resin cements were better than the self-adhesive ones13,14. Therefore, the pre-treatment with adhesive systems could be a significant factor to achieve a better bond between luting materials and tooth structure.

Recently, a new adhesive resin cement, ECD-89 (ECD, Tokuyama Dental, Tokyo, Japan), classified as a conventional adhesive resin cement, was developed. The nobility of this resin cement was to contain a newly developed functional monomer, 3D SR monomer15,16. This 3D SR monomer can not only form strong three-dimensional cross-linking polymers after polymerization17 but also, it bonds chemically with the tooth structure18. Yoshida et al. revealed that this monomer formed a hydrolysis-resistant Ca-salt on the dentin. This result suggested that the 3D SR monomer could contribute to better bond durability18. Fu et al. also reported about this functional monomer, which was a component of a new self-bonding system for direct restoration19. In their study, 3D SR monomer could produce good bonding with dentin structure.

In comparison with adhesives for direct restoration, the resin cements for indirect restoration should show good bonding performance under dark conditions because less curing light could reach through thick indirect restorations. That is why, almost all of the adhesive systems contained in the conventional resin cement, employed the “dual-curing systems”. These materials used chemical initiators in order to obtain the maximum polymerization under dark conditions. But the bonding performance of the adhesive systems in the conventional resin cements under both conditions is yet to be evaluated.

Therefore, the purpose of this study was to evaluate the bonding performance between dentin and 3D SR monomer containing ECD-89 under both “light” and “dark” conditions by µTBS testing, fracture mode
The null-hypothesis to be tested was that there were no differences in bonding efficiency between the new adhesive cement and two commercial resin cements under “light” and “dark” conditions.

### MATERIALS AND METHODS

**Teeth used**
Thirty extracted human molars were used in this study to evaluate three different adhesive resin cement systems. The teeth were collected under a protocol reviewed and approved by the ethical committee of Hokkaido University. Each system consisted of ten teeth which were further divided into two groups for “light” and “dark” curing conditions. The teeth were stored at 4°C in an aqueous solution of 0.5% Chloramine-T, and used within four months after extraction. Flat dentin surfaces were obtained by removing the coronal enamel of each tooth in a gypsum model trimmer with the water coolant, leaving the surrounding enamel. After that, the dentin surfaces were ground with #600 SiC paper for 60 s under continuous water-cooling to produce standardized smear layers prior to bonding.

**Adhesive resin cements**
Three adhesive resin cements were used. A newly developed experimental adhesive resin cement (ECD-89: ECD, Tokuyama Dental, Tokyo, Japan) was evaluated. In addition, two commercially available ones, Multilink Automix (MA, Ivoclar Vivadent AG, Schaan, Liechtenstein) and Panavia F2.0 (PF, Kuraray Noritake Dental, Tokyo, Japan) were employed for this experiment as control materials.

The chemical formulations and the respective manufacturer's instruction for usage of three adhesive resin cements were indicated in Table 1.

**Bonding procedure**
Cementation and polymerization procedures are described in Table 2. Each adhesive cement was bonded

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<table>
<thead>
<tr>
<th>Adhesive Cements</th>
<th>Materials</th>
<th>Lot no.</th>
<th>Compositions</th>
<th>pH of Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECD-89 (Tokuyama Dental, Tokyo, Japan)</td>
<td>CP-89 (Paste)</td>
<td>PEC1U2505</td>
<td>BPO, The data of other compositions could not avail.</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>DBC600 (Primer A)</td>
<td>101207</td>
<td>3D SR-monomer, phosphoric acid monomer, Bis-GMA, TEGDMA, HEMA, acetone, alcohol, water</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>DBC600 (Primer B)</td>
<td>100729</td>
<td>Borate Catalyst, acetone, peroxide</td>
<td>2.1</td>
</tr>
<tr>
<td>Multilink Automix (Ivoclar Vivadent AG, Schaan, Liechtenstein)</td>
<td>Paste</td>
<td>N51455</td>
<td>Dimethacrylates, HEMA, BPO, Inorganic fillers, Ytterbium trifluoride, Initiators, Stabilizers, Pigments, Barium glass filler, Silica filler</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Primer A</td>
<td>N47994</td>
<td>Sulfonate Amine</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Primer B</td>
<td>N78969</td>
<td>Methacrylate modified polyacrylic acid, Phosphoric acid acrylate, HEMA, Stabilizers</td>
<td>2.1</td>
</tr>
<tr>
<td>Panavia F2.0 (Kuraray Noritake Dental, Tokyo, Japan)</td>
<td>A paste</td>
<td>00486A</td>
<td>10-MDP, Hydrophobic aromatic dimethacrylate, Hydrophobic and hydrophilic aliphatic demethacrylate, dl-Camphorquinone, Silanated barium glass filler, Surface treated sodium fluoride, Catalysts, Initiators</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>B paste</td>
<td>00247A</td>
<td>Hydrophobic aromatic dimethacrylate, Hydrophobic and hydrophilic aliphatic demethacrylate, Silanated barium glass filler, Surface treated sodium fluoride, Catalysts, Accelerators, Pigments</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>ED primer2 Liquid A</td>
<td>00298A</td>
<td>10-MDP, HEMA, N-Methacryloyl-5-amino-salicylic acid, Water, Accelerators</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>ED primer2 Liquid B</td>
<td>00172A</td>
<td>N-Methacryloyl-5-amino-salicylic acid, Water, Catalysts, Accelerators</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Bis-GMA: bisphenol A diglycidyl methacrylate; BPO: Benzoyl Peroxide; HEMA: 2-hydroxyethyl methacrylate; TEGDMA: triethylene glycol dimethacrylate; 10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate
to dentin by employing two curing conditions: “light condition” and “dark condition”. “Light condition” means that bonding procedure was performed in bright room illuminated by fluorescent light, and polymerization of the cements was performed via irradiation using a halogen light. In case of “dark condition”, the bonding procedure was performed in a photo darkroom under safe light without curing light.

Primers were dispensed onto plastic containers, mixed and applied to the dentin surfaces. Thereafter, resin cements were mixed and applied onto the dentin surfaces. Approximately, the cement was built in a thickness of 4 mm on the dentin surface in two increments. In light condition, each layer was photoactivated using a conventional quartz-tungsten-halogen light (XL 3000, 3M ESPE, St.Paul, Minnesota, USA) operating at 500 mW/cm² for 20 s. After that, the adhesive resin cements built on dentin surface were photoactivated from all quadrants which were parallel to cement-dentin interface and also form occlusal direction which were perpendicular to the interface. In dark condition, each cement was not photoactivated and left in a darkroom for 30 min. As mentioned above, the number of teeth used for each cement was ten, which ten teeth were further divided into the two conditions (light and dark). Hence, each experimental group consisted of five teeth.

**Microtensile bond strength (µTBS) test**

After storage in 37°C water for 24 h, each tooth per group was sectioned into 15 beams (cross-sectional area: 1 mm²) using an Isomet diamond saw (Isomet 1000, Buehler, Lake Bluff, Illinois, USA). Hence, each experimental group contained seventy-five beams (5 teeth×15 beams=75 beams). The specimens were then fixed to a Ciucchi’s jig with cyanoacrylate glue (Model 63)

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**Table 2: The respective manufacturer’s instructions and actual cure procedures**

<table>
<thead>
<tr>
<th>Cementing Systems</th>
<th>Manufacturer’s instructions</th>
<th>Procedures of light condition (In light room)</th>
<th>Procedures of dark condition (In dark room under safe light)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECD-89 (Tokuyama Dental, Tokyo, Japan)</td>
<td>1. Apply primer on metal/zirconium surface, air dry for 5–10 s after 10 s 2. Mix equal amounts of primer A &amp; B, apply on dentin surface, gentle air for 5 s, strong air 5 s after 10 s 3. Squeeze paste from the dispenser syringe, apply inside the prosthesis, light cure for 20 s</td>
<td>1. Mix equal amounts of primer A and B, apply on dentin, gentle air for 5 s and strong air 5 s after 10 s</td>
<td>1. Mix equal amounts of primer A and B, apply on dentin, gentle air for 5 s and strong air 5 s after 10 s</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. Squeeze paste from the dispenser syringe, apply cement to dentin, light cure for 20 s×5 from 5 directions(4 parallels to dentin surface and a occlusal), store in 37°C water for 24 h</td>
</tr>
<tr>
<td>Multilink Automix (Ivoclar Vivadent AG, Schaan, Liechtenstein)</td>
<td>1. Apply Metal/Zirconia primer on zirconium surfaces and air-dry 2. Mix equal amounts of Multilink Primer liquid A and B, apply on resin surfaces, leave for 15 s and dry 3. Squeeze MA from the dispenser syringe, apply inside the prosthesis, self cure and light cure</td>
<td>1. Mix equal amounts of Multilink Primer liquid A and B, apply on dentin surfaces, leave for 15 s and air 10 s</td>
<td>1. Mix equal amounts of Multilink Primer liquid A and B, apply on dentin surfaces, leave for 15 s and air 10 s</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. Squeeze Multilink Automix from the dispenser syringe, apply cement to dentin, light cure for 20 s×5 from 5 directions, store in 37°C water for 24 h</td>
</tr>
<tr>
<td>Panavia F2.0 (Kuraray Noritake Dental, Tokyo, Japan)</td>
<td>1. Mix one drop of each ED primer 2 liquid A and B for 5 s, apply on dentin surface, air dry gently after 30 s</td>
<td>1. Mix one drop of each ED primer2 liquid A and B for 5 s, air dry gently after 30 s</td>
<td>1. Mix one drop of each ED primer2 liquid A and B for 5 s, air dry gently after 30 s</td>
</tr>
<tr>
<td></td>
<td>2. (about the inside of the prosthesis) Etch for 5 s, rinse, dry and mix one drop of each Clearfil SE primer and Porcelain Bond Activator for 5 s and apply inside of the prosthesis. 3. Mix universal and catalyst paste for 20 s and apply inside of the prosthesis, light cure for 20 s, after removing excess cement, apply Oxyguard for 3 min</td>
<td>2. Mix universal and catalyst paste for 20 s, apply cement to dentin, light cure for 20 s×5 from 5 directions, store in 37°C water for 24 h</td>
<td>2. Mix universal and catalyst paste for 20 s, apply cement to dentin, leave in dark room for 30 min, store in 37°C water for 24 h</td>
</tr>
</tbody>
</table>
Table 3  Micro-tensile bond strength (µTBS) and fracture modes (n=75/group)

<table>
<thead>
<tr>
<th>Cements</th>
<th>µTBS mean(SD) MPa</th>
<th>Fracture mode (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>ECD-L</td>
<td>68.6(14.9)a</td>
<td>60.0</td>
</tr>
<tr>
<td>ECD-D</td>
<td>54.5(22.4)b</td>
<td>48.0</td>
</tr>
<tr>
<td>MA-L</td>
<td>39.2(18.9)b</td>
<td>46.7</td>
</tr>
<tr>
<td>MA-D</td>
<td>36.7(15.6)b</td>
<td>40.0</td>
</tr>
<tr>
<td>PF-L</td>
<td>39.4(18.5)b</td>
<td>38.7</td>
</tr>
<tr>
<td>PF-D</td>
<td>13.4(4.66)c</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Same superscript letters indicate no statistical difference (Games Howell test, p>0.05).
Fracture mode categories: A: Cohesive failure within the adhesive resin cement only with partial primer failure, where remnants of resin were observed on the dentin surface. B: Adhesive failure at the resin-dentin interface only. C: Mixed adhesive failure with cohesive failure within dentin as well as cohesive failure in dentin only.
in comparison to dark condition, though ECD and MA indicated no statistical difference between the two conditions.

The fracture mode distribution (%) as analyzed by light-microscope was also indicated in Table 3. ECD-L, ECD-D, MA-L and MA-D analysis showed a prevalence of dentin fracture pattern (A). For ECD-D and MA-D, a higher percentage of mixed fracture pattern (C) was observed compared to the light conditions. PF-L presented the high percentages of cohesive and mixed fracture pattern (A and C). However, PF-D showed higher percentage of adhesive fracture pattern (B). For three adhesives, the fracture mode had a tendency to show the higher incidence of cohesive fracture at the higher bond strength.

**SEM observation**

Figure 1 shows SEM observations of the typical primer-dentin fractured surfaces (×3,000). Figure 1a–f might show the decalcified collagen fibril network in...
inter-tubular dentin. Scratches caused by SiC paper on the dentin surface were also clearly observed in Figs. 1e and f. Figure 1f revealed similar observations to the other groups, however, remnants of primer was observed on the dentin surfaces.

TEM observation
Figures 2–4 show the TEM observation of the cement-dentin interface at low (×5,000) and high magnification (×50,000).

At low magnification, each material contained various fillers within the cement layer. ECD: very fine spherical and columnar fillers. MA: spherical and quadrangular fillers. PF: various sizes of glasses and fillers.

The thickness of primer layer of each cement were dependent on the materials: ECD-L: 8 µm, ECD-D: 16 µm, MA-L: 2 µm, MA-D: 1 µm, PF-L: 1 µm. However, the primer layer of PF-D could not be observed at high magnification. The thickness of hybrid layer was below 1.0 µm for all cements. Gap formations were observed in MA-L and PF-D. Infiltrated epoxy embedding resin was only observed in the gap formation of MA-L.

At high magnification, the thicknesses of hybrid layer were: ECD-L, ECD-D, MA-L, MA-D and PF-L: approximately 0.5 µm. However, the hybrid layer of PF-D could not be observed because of the gap formation.

The detail of the hybrid layer of ECD-L revealed a completely demineralized upper part but in the lower part, the hydroxyapatite crystals were still present. In contrast, detail of the hybrid layer of ECD-D, revealed a completely demineralized upper part, but in the lower part, the primer layer could be seen through. On the top of the dentin of MA-D and PF-L, the hydroxyapatite showed a typical needle-like shape. For PF-D, gap formation between the dentin and the primer could be observed. Some apatite crystals were contained on the edge of the separated primer in PF-D.

DISCUSSION
In this study, ECD showed statistically higher µTBS than those of MA and PF in both light and dark condition. This is also backed by both TEM and SEM observations, which agree with the high µTBS of ECD under both light and dark conditions. TEM observation showed a very thin hybrid layer, which may be caused by the structural characteristics of 3D SR monomer and the intermediate acidity of the primer as was reported previously (Figs. 2b and d). According to fractographical analysis (Figs. 1a and b), the dentinal tubules filled with tags and decalcified collagen fibril networks were observed on the dentin surface. This might indicate the effectiveness of ECD primer that permeates sufficiently to dentin and dentinal tubules. The scratches on dentin were not clearly detected. These morphologies suggested that the scratches on the dentin surface might be covered by the remnant of the primer which strongly bonded to the

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Fig. 2 TEM images of primer-dentin interface at low/high magnification from ECD.
(a); ECD-L (×5,000), (b); ECD-L (×50,000), (c); ECD-D (×5,000), (d); ECD-D (×50,000). At low magnification, approximate thickness of the primer layer of each condition was that ECD-L was 8 µm and ECD-D was 16 µm. Very fine spherical and columnar fillers can be seen within the cement layer. Several small fillers which might be nano-fillers were observed in primer layer of ECD. At high magnification, approximate thickness of the hybrid layer of each condition was that ECD-L was 0.5 µm and ECD-D was 0.5 µm. Detail of the hybrid layer, revealing an upper part which completely demineralized, and a lower part, which hydroxyapatite crystals were still present. HL: Hybrid layer, A: Adhesive layer, D: Dentin, P: Primer layer.
Quality of adhesive interface could affect bond strength of resin composite materials\(^{21}\). All three resin cements employed in the present study contain primer. The bond strength of primer of resin cements could be affected by various factors such as monomer system, filler contents, effectiveness of polymerization initiator and acidic environment\(^{22,23}\). Regarding to ECD, the primer contains multifunctional monomers, 3D SR and Bis-GMA which could reinforce polymer structure. Hence, cured polymer could form a 3D structure which might result in higher strength of polymerized primer.

Moreover, the \(\mu\)TBS of ECD showed no statistical difference under both light and dark conditions, which is not consistent with the results of conventional resin cements of previous studies\(^ {24-26}\). Several studies reported lower \(\mu\)TBS of dual cure resin cements when the light curing procedure was omitted\(^ {1,27,28}\). However, ECD produced higher bond strength in comparison with the two self-adhering systems under both light and dark conditions. The primer and the resin cement for ECD were required to be used together. The primer contains several co-initiators, and the resin cement paste contains an initiator, thus, the self-curing interaction between the primer and the resin cement may initiate conversion when they were contacted even without light curing\(^ {19}\). Free radicals released by BPO, contained in resin composite cement paste could improve polymerization of the primer layer. This unique curing system was so called “Touch and Cure”. These unique characteristic of “Touch and Cure” system could contribute to the high \(\mu\)TBS regardless of the curing condition\(^ {19}\).

ECD primer contains nano-fillers. In TEM observations, several small fillers were observed in primer layer of ECD. The fillers might also play an important role in formation of strong primer layers (Figs. 2a and c).

In case of MA, scratches caused by SiC paper could not be clearly found on the dentin surface in SEM observations (Figs. 1c and d). This characteristic is similar to those of ECD which might explain that the primer acidity of MA might be as strong as that of ECD (Table 1). In TEM observations (Figs. 3a–d), amorphous primer layers were observed above the dentin surface. These observations suggested that the primer permeability into dentin and collagen fibril networks might be weaker than that of ECD. Hybrid layer of MA compromised the amorphous primer layers which might cause a negative effect to the primer-dentin bond strength. Gap formation between the primer...
layer and the adhesive resin cement was only observed above the amorphous primer layer in MA-L (Fig. 3a). The infiltrated epoxy embedding resin was observed in the gap. We couldn't conclude the time when the gap formation was occurred. However, according to the TEM observation, it might be the result of the procedure of specimen's preparation for TEM. The only difference between MA-L and MA-D was the gap. The other morphological structures were similar. The primer layer thickness of MA-D was thinner than that of MA-L. In MA-D, curing time was longer than MA-L due to the dark cure, the load of adhesive resin cement might thin the primer layer. However, these morphological differences between MA-L and MA-D didn’t affect the μTBS of each condition.

In case of PF-D, the gap between primer layer and dentin could be observed in TEM (Fig. 4d). In addition, scratches caused by SiC paper and remnants of primer were also seen on the dentin surface after μTBS testing in SEM observation (Fig. 1f). In fracture mode analysis, interfacial fractures were observed in the dentin and cement. These observations suggested weaker acidity of primer and adhesive interface compared to ECD and MA and corresponded to pH of primers (Table 1).

ECD would show good clinical performance regardless of curing conditions compared to conventional ones. However, the long term effect of ECD is unknown.

Previous studies revealed that the characteristics of 3D SR monomer might contribute to the bond durability. Therefore, ECD might be expected to show long term durability as well. It should be focused on in further studies.

CONCLUSION

In this study, a newly developed resin cement ECD-89 showed better bonding performance in comparison with conventional ones.

Thus, the null hypothesis that there were no differences between the new adhesive cement, ECD and two commercial resin cements was rejected. In future studies, one aimed to focus on the long term bonding durability of the cement.

REFERENCES


