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Measurement of shear bond strength to intact dentin

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Previously, we reported that the integrity of a resin composite restoration deteriorated when the dentin cavity wall was decalcified by conditioning. In this study, to evaluate the bonding between dentin adhesive and non-decalcified dentin surface, we experimented with a novel method of using a high-pressure water spray device to prepare smear layer-free dentin surfaces. When the smear layer was removed, shear bond strength significantly increased regardless of the removal method employed. Further, with glyceryl monomethacrylate (GM) priming, no significant differences in bond strength were observed among these smear layer removal methods: ethylenediamine tetraacetic acid (EDTA) conditioning, phosphoric acid conditioning, and removal by water spray. It was also found that GM priming was key to achieving marginal integrity, whereas contraction gap width increased with phosphoric acid conditioning. It was thus concluded that the efficacy of a dentin adhesive should be evaluated by consistently observing the contraction gap in three-dimensional cavities rather than by mere measurement of bond strength to a flat dentin surface.

Keywords: Dentin adhesive, GM priming, Shear bond strength

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INTRODUCTION

Dentin primers were first developed by Munksgaard and Asmussen in 1984[1]. They demonstrated that the shear bond strength to dentin of resin monomer composed of Bis-GMA and triethylene glycol dimethacrylate was significantly improved by dentin pretreatment with an aqueous solution of 2-hydroxyethyl methacrylate (2-HEMA) and glutaraldehyde (GLUMA) after EDTA conditioning. It was speculated that GLUMA increased the bond strength because the amino group in dentin collagen was activated by glutaraldehyde and polymerized with 2-HEMA[2]. For this reason, GLUMA was considered to be a dentin bonding agent.

However, in a previous study[3], we found that chemical activation of dentin collagen by glutaraldehyde was not essential for dentin priming because the priming efficacy of 2-HEMA solution without glutaraldehyde was also comparable to that of GLUMA. For this reason, we classified GLUMA as a dentin primer because a dentin bonding agent or resin monomer should be applied on the dentin cavity wall after GLUMA application. Following the development of GLUMA, 2-HEMA solution was frequently added to most of the commercial dentin bonding systems as the main component of dentin primers, self-etching primers, or one-step bonding agents.

In 1989, we observed that contraction gap formation of a light-activated resin composite in a cylindrical dentin cavity was completely prevented by priming with glyceryl monomethacrylate (GM) solution before the application of a commercial dentin bonding agent containing phosphate ester monomer[4]. In addition, we reported that complete marginal integrity was achieved by dentin priming with an aqueous solution of erythritol monomethacrylate, xylitol monomethacrylate, ethylene glycol, 1,6-hexanediol, triethylene glycol, and triethylene glycol monomethacrylate[5-9].

In several studies, the dentin priming effect was investigated by expanding the dentin collagen network which was exposed as a result of decalcification during dentin conditioning and which collapsed as a result of air-drying[10-13]. Consequently, the dentin adhesive easily penetrated into the primed and enlarged micro-spaces among the dentin collagen. Thus, hybrid layer formation was ensured by dentin priming and resin penetration into the expanded collagen network. However, the target of the dentin bonding agent was considered to be the inorganic component in dentin because contraction gap width increased when the dentin cavity wall was softened by the dentin conditioner[14]. Besides, contraction gap width also increased when adhesive monomers such as 10-MDP and 4-META were eliminated from the dentin bonding agents[15]. For these two reasons, it was suggested that dentin bonding is established by interaction between the adhesive monomer in dentin bonding agents and the inorganic component in dentin.

On the bond strength of dentin bonding agents to enamel, strong bonding has been speculated to be due to the extremely high inorganic content in enamel[16]. Against this background, it would be quite conflicting for dentin bonding agents to bond to both the inorganic component in enamel and the organic component in dentin within the same cavity. It is noteworthy that in previous reports, the dentin primer was applied after ethylenediamine tetraacetic acid (EDTA) conditioning of the dentin. This meant that the surface layer of the dentin substrate might already have been decalcified by EDTA conditioning, although it was generally speculated that dentin bonding agents had an effect on the inorganic component in dentin. In other words, it still remained to be confirmed whether it was the dentin primer or the dentin bonding agent which

affected the inorganic component of dentin. The purpose of this study, therefore, was to investigate the effects of GM priming on bonding to non-decalcified and smear layer-free dentin.

MATERIALS AND METHODS

Eighty extracted human teeth were embedded in an epoxy resin, and flat dentin surfaces were polished using a wet, 1000-grit silicon carbide paper. The teeth were divided into four groups based on the dentin conditioning method: (1) No conditioning; (2) Chemical conditioning with neutralized 0.5 mol/L EDTA (E-lize conditioner, Pentron Clinical, CT, USA); (3) Etching with phosphoric acid (Clearfil K-etchant gel, 40% H_3PO_4, Kuraray, Okayama, Japan); or (4) Physical conditioning with high-pressure water spray.

Chemical and physical conditioning of dentin

In the chemical conditioning groups, the dentin surface was conditioned with EDTA for 60 seconds or etched with phosphoric acid for 20 seconds. This was following by rinsing and drying.

Physical conditioning was performed using a commercial high-pressure water spray jet (Fig. 1) (Heart Pump HP-402S, Sugino Machine, Toyama, Japan). Water pressure was controlled at 4 MPa and the dentin surface was thus conditioned for 3 minutes.

Priming and dentin bonding agent application

For half of the specimens in each conditioning group, the dentin surface was primed with 35 vol% of glyceryl monomethacrylate (GM) (E-lize primer, Pentron Clinical, CT, USA) solution for 1 second, followed by drying with a gentle air spray. Then, a split Teflon mold of 3.6 mm inner diameter, 20 mm outer diameter, and 8 mm height was placed on the dentin substrate.

A commercial dual-cured dentin bonding agent (Clearfil Photo Bond, Kuraray, Okayama, Japan) was applied on the primed dentin surface. After eliminating excess bonding agent with gentle air blow, the bonding agent was irradiated for 10 seconds using a halogen lamp (White Light, Morita, Tokyo, Japan). Finally, a commercial resin composite (Palfique Estelite,

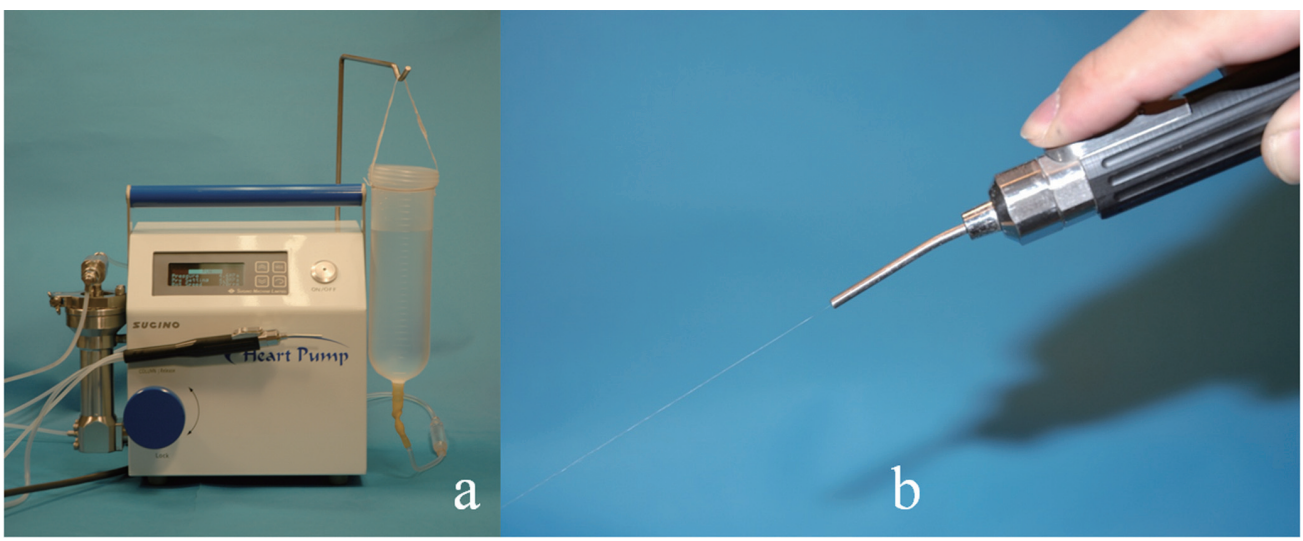

Fig. 1 (a) Commercial high-pressure water spray jet (Heart Pump HP-402S, Sugino Machine, Toyama, Japan) used in this study; (b) Water spray jet nozzle.

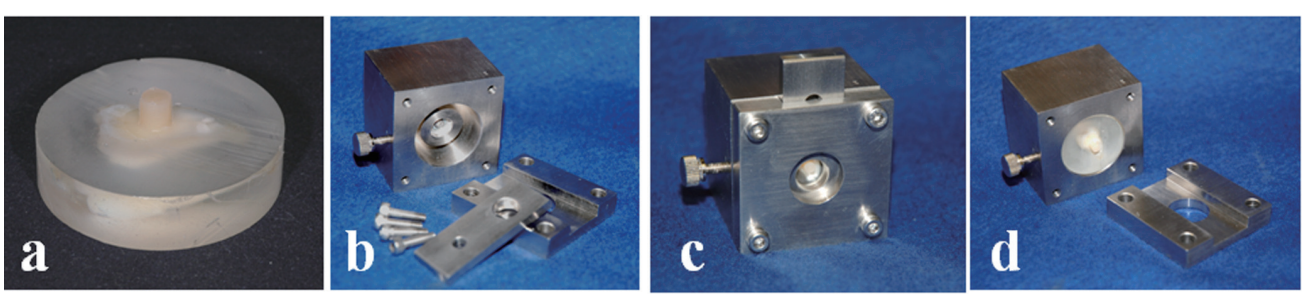

Fig. 2 (a) Specimen for shear bond strength test; (b) Specimen holder; (c) Specimen fixed in the holder; (d) Blade for shear bond strength measurement was installed and load was applied from the upper side. Upon specimen fracture, load at fracture was measured.

Tokuyama Dental, Tokyo, Japan) was placed on the dentin surface to a thickness not more than 3 mm and irradiated for 40 seconds from the top window of the center hole of the Teflon mold.

For the other half of the specimens in each conditioning group, GM priming was omitted but the other steps were carried out as per those in the GM priming group.

Shear bond strength measurement

Ten minutes after the resin composite was irradiated, the Teflon mold was removed (Fig. 2a) and the specimen was fixed in a holder as shown in Figs. 2b, 2c, and 2d. Shear bond strength of the dentin adhesive was measured using a universal testing machine (Model 4302, Instron Corp., MA, USA) with a crosshead speed of 0.5 mm/min. Fracture modes of the specimens were determined by observation under a stereomicroscope (Stemi 2000-C, Zeiss, Oberkochen, Germany).

SEM observation

To observe the dentin surfaces after their respective conditioning methods, the specimens were dehydrated in a graded series of alcohol solutions and vacuum-sputtered with palladium and platinum for SEM observation.

After resin composite application and curing, the representative dentin surface of each treatment group (*i.e.*, chemical or physical conditioning followed by absence or presence of GM priming) was observed using a scanning electron microscope (S-4700, Hitachi, Tokyo, Japan). To evaluate marginal adaptation, contraction gap widths were measured after treating the dentin surfaces with 1 N hydrochloric acid for 20 seconds. Following which, the specimens were dehydrated in a graded series of ethanol solutions (70, 80, 90, and 95% ethanol concentrations) for 30 minutes, and then in 99% for two 15-minute periods. For SEM observation, specimens were critical point-dried and sputter-coated with palladium and platinum.

Statistical analysis

Ten specimens were prepared for each treatment group (*i.e.*, n=10 per conditioning-GM priming/no priming group). Data were analyzed by Bartlett's test and one-way ANOVA. *Post hoc* multiple comparisons were done using Tukey's test.

RESULTS

Shear bond strength

Results of shear bond strength measurement are presented in Table 1. Homogeneity of variances was confirmed by Bartlett's test and one-way ANOVA. With GM priming, significantly higher bond strengths were achieved in the EDTA conditioning and water spray conditioning groups. Without GM priming, the bond strengths of the no-conditioning and phosphoric acid conditioning groups were significantly lower than the other three groups (namely, EDTA conditioning groups with and without GM priming and water spray conditioning group with GM priming). Results showed that when chemical or physical dentin conditioning was applied, shear bond strength was not influenced by GM priming in all the conditioning groups. Through fracture analysis, all specimens were found to fracture at the interface because the blade was loaded exactly at the dentin substrate surface.

SEM observation of smear layer

Through SEM observation, the smear layer observed in the no conditioning group (Fig. 3a) was removed by EDTA conditioning, and no smear plugs were seen (Fig. 3b). With phosphoric acid conditioning, the smear layer was removed completely, the dentin surface was decalcified, and the dentinal tubules appeared widely opened and funnel-shaped (Fig. 3c). With high-pressure water spray conditioning, the smear plugs were not removed but the tubules were clear and the smear layer was not visible (Fig. 3d).

SEM observation of resin-dentin adhesive interface

In the EDTA conditioning group with GM priming (Fig. 4a), the layer in which the adhesive monomer diffused into the decalcified dentin was observed to be under the bonding layer. Moreover, an extremely thin decalcified layer of approximately 0.2 μm was observed in the EDTA-conditioned specimen (Fig. 4b). However, this layer exfoliated from the adhesive interface in the EDTA-conditioned specimen without GM priming (Fig. 4c).

In the phosphoric acid group, the layer in which the adhesive monomer diffused into the decalcified

Table 1 Shear bond strengths (MPa) measured in this study

Conditioning treatment	With GM priming	Without GM priming
EDTA	8.17±1.72 [a]	7.28±1.71 [a]
High-pressure water spray	8.23±1.92 [a]	6.55±1.43 [a,b,c]
H_3PO_4 gel	7.03±1.92 [a,b]	4.89±1.51 [b,c,d]
No conditioning	4.46±1.37 [c,d]	3.37±0.65 [d]

N=10; Mean±SD.

Same letters indicate that differences are statistically significant (one-way ANOVA and Tukey's multiple comparison test, p>0.05).

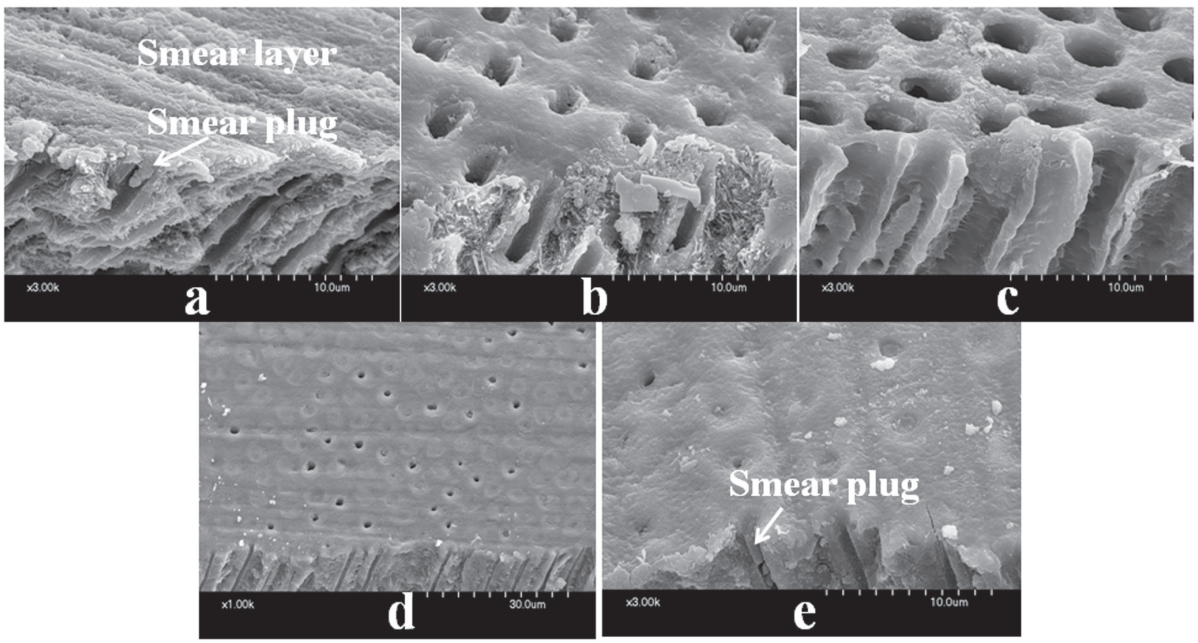

Fig. 3 Observation of the dentin surface for each conditioning treatment.
(a) Dentin surface was not conditioned.
(b) Dentin surface conditioned with EDTA.
(c) Dentin surface conditioned with phosphoric acid.
(d) Dentin surface rinsed with high-pressure water spray.
(e) Higher magnification view of Fig. 3d.

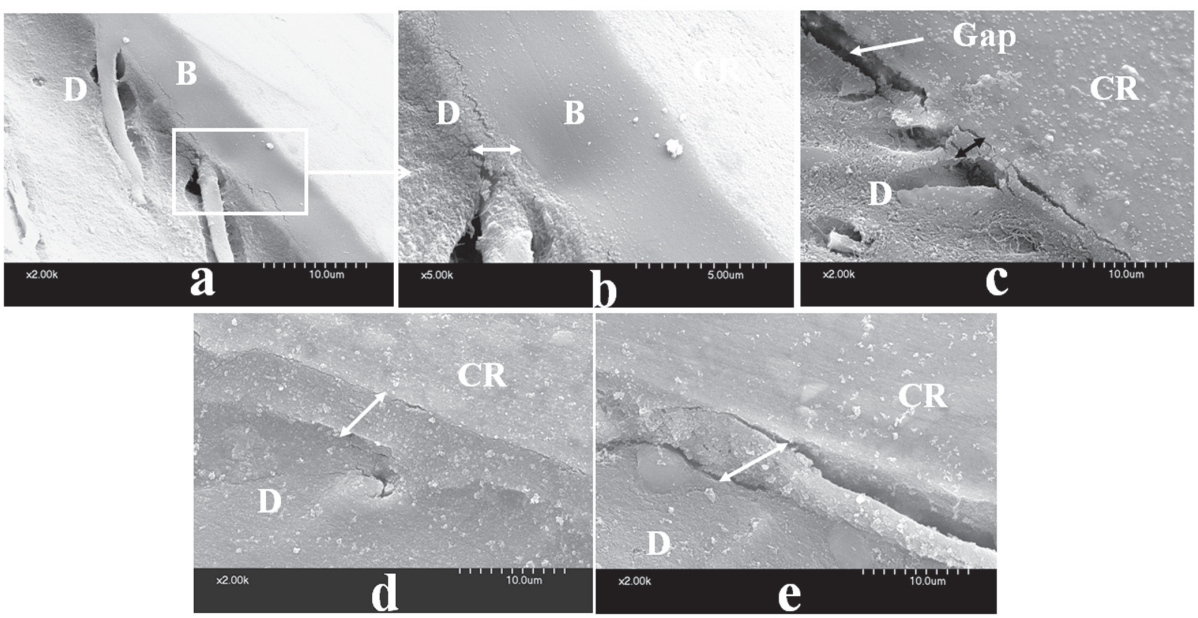

Fig. 4 SEM observation of marginal adaptation (I).
(a) Adhesive interface between resin composite and dentin conditioned with EDTA and primed with GM. CR: Composite Resin, B: Bonding Agent, D: Dentin.
(b) Higher magnification view of Fig. 4a. CR: Composite Resin, B: Bonding Agent, D: Dentin.
(c) Adhesive interface between resin composite and dentin conditioned with EDTA and not primed with GM. CR: Composite Resin, B: Bonding Agent, D: Dentin.
(d) Adhesive interface between resin composite and dentin conditioned with phosphoric acid and primed with GM. CR: Composite Resin, B: Bonding Agent, D: Dentin.
(e) Adhesive interface between resin composite and dentin conditioned with phosphoric acid and not primed with GM. CR: Composite Resin, B: Bonding Agent, D: Dentin.

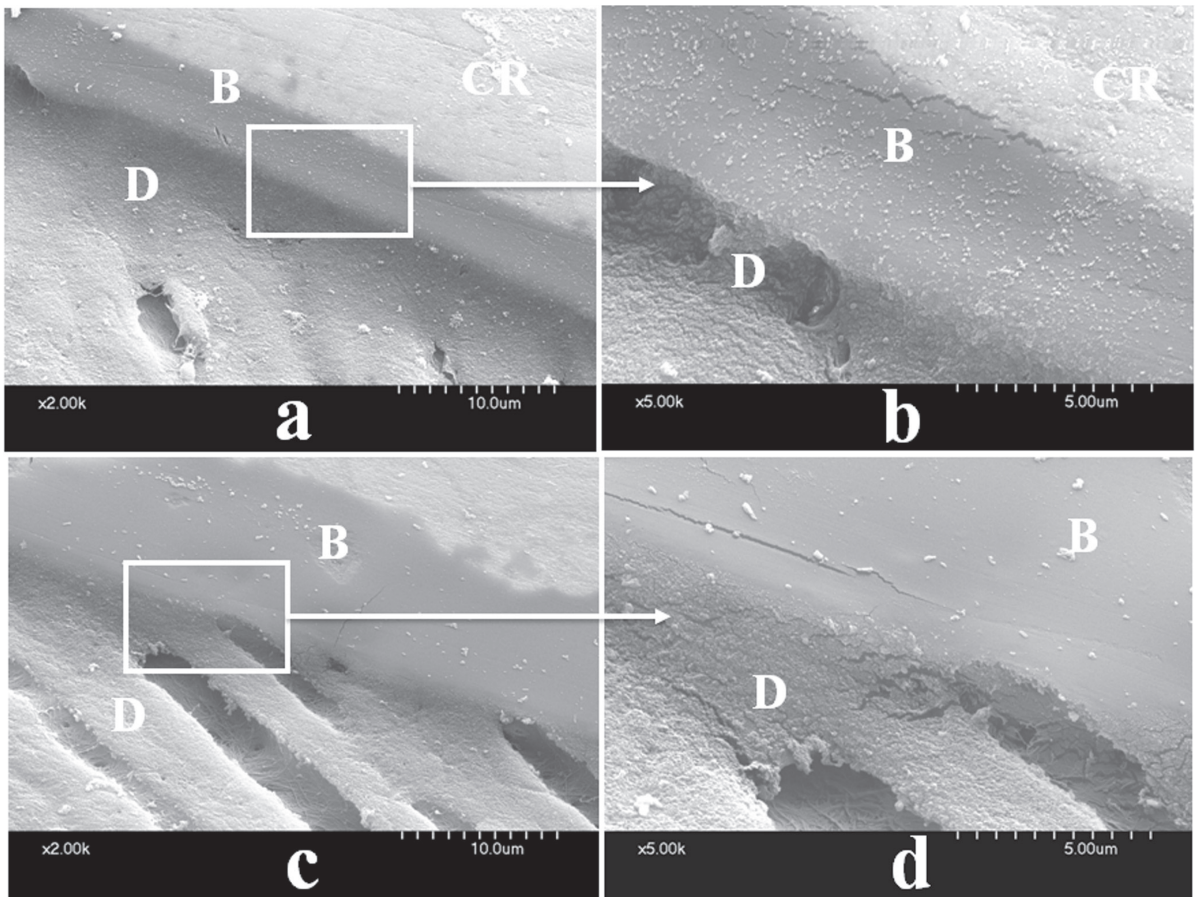

Fig. 5 SEM observation of marginal adaptation (II).
(a) Adhesive interface between resin composite and dentin rinsed with high-pressure water spray and primed with GM. CR: Composite Resin, B: Bonding Agent, D: Dentin.
(b) Higher magnification view of Fig. 5a. CR: Composite Resin, B: Bonding Agent, D: Dentin.
(c) Adhesive interface between resin composite and dentin rinsed with high-pressure water spray and not primed with GM. CR: Composite Resin, B: Bonding Agent, D: Dentin.
(d) Higher magnification view of Fig. 5c. CR: Composite Resin, B: Bonding Agent, D: Dentin.

dentin also exfoliated from the adhesive interface, with or without GM priming (Figs. 4d and 4e). Unlike EDTA or phosphoric acid conditioning, the layer in which the adhesive monomer diffused into the decalcified dentin was not clearly observed under the bonding layer in water spray conditioning groups, with or without GM priming (Figs. 5a, 5b, 5c, and 5d).

DISCUSSION

As demonstrated in this study, significantly low shear bond strength was obtained in the no-conditioning group regardless of GM priming, suggesting that the smear layer had a definite adverse effect on the bonding between the dentin substrate and the bonding agent. Conversely, when dentin conditioning was applied, be it chemical removal of the smear layer by EDTA or physical removal by high pressure water-spray, the bond strength was effectively increased. Moreover, after the smear layer was removed by any of the conditioning methods employed in this study, GM priming did not result in any statistically significant increase in bond strength — although the effect of GM priming was more pronounced for phosphoric acid etching. In light of these results, it could be suggested that the criterion of dentin collagen network expansion by dentin primers was not mandatory to effective dentin bonding

On other factors that might influence dentin bonding, it was speculated that dentin collagen exposure by dentin conditioning was not essential for dentin bonding. In the EDTA conditioning group, decalcified dentin thickness was approximately 0.4 μm (conditioned with EDTA for 60 seconds)[17]. In the phosphoric acid etching group, it was approximately 1.0 μm (etched with 40% H_3PO_4 for 20 seconds)[18]. Results of this study showed that GM priming of the intact dentin was sufficiently effective in increasing the bond

strength of the dentin adhesive, even if dentin collagen was not exposed by acidic treatment. In other words, the criterion of dentin collagen exposure by dentin conditioners was also not mandatory to effective dentin bonding. Further, as demonstrated in our previous research[14,19], phosphoric acid etching could not prevent contraction gap formation.

On contraction gap formation, both triethylene glycol (TEG) and triethylene glycol monomethacrylate (TEGMA) have been shown to be highly effective dentin primers because they completely prevented contraction gap formation by virtue of their "pegylation" effect[9]. Likewise, contraction gap formation was completely prevented with GM priming — a result applicable for both sclerotic and sound dentin. However, differing effects from dentin priming on contraction gap formation were observed for sclerotic dentin *versus* sound dentin. Interestingly, contraction gap width in sclerotic dentin cavity was significantly reduced as compared to that in sound dentin cavity when the dentin cavity wall was conditioned with EDTA without GM priming[20-22]. In sclerotic dentin, the dentinal tubules are closed by deposits of an inorganic component and thus dentin permeability is significantly reduced. This finding thus suggested that a decrease in dentin permeability was effective to improving the efficacy of dentin adhesives.

Biocompatible, biodegradable polymers such as polyethylene glycol (PEG) can form hydrogels *via* crosslinking reactions. In medical and pharmaceutical applications, PEG hydrogels absorb water and prolong the time of drug release and metabolism, thereby resulting in a decreased frequency of drug injections[23,24]. In dentistry, the PEG hydrogel technology could be leveraged and applied to help reduce dentin permeability by decreasing fluid movement through the dentinal tubules and protecting against water contamination on the dentin substrate. Consequently, polymerization of the adhesive monomer was ensured. Besides, by reducing fluid movement through the dentinal tubules, dentin primers also contributed to providing a sedative effect against dentin sensitivity according to the hydrodynamic theory[25], although details of the sedative mechanism were still not clear[26,27].

On achieving marginal integrity with resin composites, our previous study[4] showed that priming with 35 vol% GM solution completely prevented contraction gap formation of a light-cured resin composite in an EDTA-conditioned cylindrical dentin cavity, whereas contraction gaps were observed when GM priming was not performed. In the same study[4], the tensile bond strength of a commercial dentin bonding agent to flat dentin surface was also significantly increased by GM priming after EDTA conditioning. Taken together, GM priming contributed to the bonding efficacy of both the resin composite and dentin bonding agent to dentin.

However, in this study, shear bond strength was not significantly increased by GM priming. Interestingly, the effect of priming on bond strength was found to be significantly affected by the method employed for bond strength assessment. In the observation of contraction gaps, an interaction between the efficacy of the dentin bonding system and the polymerization contraction stress of the resin composite was detected consistently in the three-dimensional cavity. On the other hand, shear bond strength testing failed to detect such an interaction because the resin composite shrank in a two-dimensional direction. In light of these interesting findings in this study, it was hence recommended that the efficacy of dentin adhesives should be investigated by contraction gap observation rather than by bond strength measurement.

Based on the results of this study, it seemed to suggest that complete smear layer removal was fundamentally important to achieving good and reliable dentin bonding. However, from a clinical point of view, it is difficult to completely remove the smear layer by physical means. It must also be put into perspective that the water spray apparatus used in this study is not suitable for clinical use, as it is impossible to protect the oral mucosa or soft tissue from its extremely high water pressure. On this note, further investigations are needed to determine the interaction between the dentin adhesive and organic or inorganic components in dentin.

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