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Diffusion of GM primer and dentin adhesive into EDTA-conditioned dentin

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The aim of this study was to investigate the diffusion of red-stained experimental GM primer and blue-stained dentin bonding agent into EDTA-conditioned dentin and the effect of GM priming on contraction gap formation. After GM priming at 0, 35, 70, or 100 vol%, marginal adaptation was evaluated by measuring the wall-to-wall contraction gap width of a light-activated resin composite (Palfique Estelite, Tokuyama Dental, Japan) mediated with a commercial dentin bonding agent (Clearfil Photo Bond, Kuraray Medical, Japan). Contraction gap formation was completely prevented when treated with 35 vol% GM primer. Moreover, after treatment with 35 vol% GM, the red and blue dyes of stained GM primer and dentin bonding agent diffused up to a depth of 40 µm into the dentin surface. The diffusion of primer and bonding agent into EDTA-conditioned dentin was essential for bonding efficacy although SEM observation revealed an absence of hybrid layer formation.

Keywords: Dentin bonding, Monomer diffusion, Dentin primer

INTRODUCTION

Prior to the introduction of the dentin primer in 1984, dentin bonding mechanism was either explained by resin tag formation within the dentinal tubules when the dentin surface was etched by phosphoric acid, or attributed to resin-infiltrated layer formation in superficial dentin when the dentin surface was etched by citric acid containing ferric chloride[1,2].

In 1984, the dentin primer was introduced by Munksgaard and Asmussen[3]. After EDTA conditioning, pretreatment with GLUMA primer — which was composed of an aqueous mixture of 5 vol% glutaraldehyde and 35 vol% 2-hydroxyethyl methacrylate (2-HEMA)— enabled the shear bond strength of the resin monomer to be remarkably improved. It was then speculated that the amino group in dentin collagen was chemically activated by glutaraldehyde and polymerized with 2-HEMA. However, we disputed such a suggestion because of the minuscule content of glutaraldehyde in GLUMA primer. In counter argument, we demonstrated that 35 vol% 2-HEMA solution exhibited comparable priming efficacy as the GLUMA primer[4].

In many published studies, improvement in dentin bonding with primer application was usually ascribed to an expanded dentin collagen network which was exposed after surface treatment with dentin conditioner and which collapsed after air-drying[5-7]. It is generally reported that the dentin bonding agent penetrated the enlarged microspace in the dentin collagen network, resulting in hybrid layer formation and hence improved efficacy of dentin adhesives. However, there is a weakness in this argument because the dentin bonding agent showed high bonding efficacy to the enamel rather than to dentin —although only narrow microspaces were created in etched enamel because of extremely low collagen content in enamel.

In 1989, we demonstrated that priming with 35 vol% glyceryl mono-methacrylate (GM) solution after EDTA conditioning completely prevented contraction gap formation for a light-activated resin composite in a cylindrical dentin cavity[8]. However, without exception, contraction gap was formed between the dentin cavity wall and resin composite. Therefore, it was conjectured that bonding was established between the top surface of dentin cavity wall and the dentin bonding agent. However, the diffusion process of GM primer and dentin bonding agent into EDTA-conditioned dentin remains to be clarified. The aim of this study, therefore, was to investigate the diffusion of GM primer and dentin bonding agent into the prepared dentin surface after EDTA conditioning.

MATERIALS AND METHODS

Fifty-two extracted human teeth were used in this study, which had obtained approval from the IRB committee (No. 2007-31) of the Showa University.

Wall-to-wall polymerization contraction gap value measurement after GM priming

The priming effect of GM primer at 35, 70, and 100 vol% was evaluated by measuring the wall-to-wall contraction gap width[9] of a commercial resin composite (Palfique Estelite, Tokuyama Dental, Tokyo, Japan) in cylindrical dentin cavities.

With each extracted human tooth, the proximal enamel was eliminated and the exposed flat dentin surface was ground wet on 600-grit silicon carbide paper. A cylindrical cavity of approximately 3 mm diameter and 1.5 mm depth was thus prepared in the exposed dentin. The cavity surface was conditioned for 60 seconds with 0.5 mol/L ethylene diamine tetra acetic acid (EDTA) neutralized to pH 7.4 by sodium hydroxide solution, followed by rinsing with tap water and

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drying.

After EDTA conditioning, the cavity surface was primed with GM primer for 60 seconds and dried thoroughly. A commercial dual-cure dentin bonding agent (Clearfil Photo Bond, Kuraray Medical, Tokyo, Japan) was applied in the cavity and irradiated for 10 seconds (Grip Light II, Shofu, Kyoto, Japan) after removing any excess bonding agent with a gentle air blast. The cavity was slightly overfilled beyond the cavity margin with the commercial light-cured resin composite (Palfique Estelite, Tokuyama Dental, Japan), and then the composite surface was gently pressed on a glass plate mediated with a plastic matrix. After irradiating the resin composite for 40 seconds, the specimen was stored in water at room temperature for 10 minutes.

The overfilled composite was removed with a wet silicon carbide paper, and the exposed cavity margin was polished with a linen cloth mediated with an alumina slurry with a grain size of 0.05 µm. The marginal adaptation of the resin composite was inspected under a light microscope (Orthoplan, Leitz, Wetzlar, Germany), and contraction gap width was measured using a micrometer (Eyepiece Digital, Leitz, Wetzlar, Germany) mounted on the ocular lens of the microscope at a magnification of 1024. Gap width measurement was performed at eight points (every 45 degrees) along the cavity margin. The contraction gap value was presented by the sum of diametrically opposing gap widths in percentage to the cavity diameter. The maximum of four gap values was recorded as the contraction gap value of the specimen.

For negative control, no GM priming was performed on the cavity surface although other steps were carried out in the same way as the GM priming groups. Ten specimens were prepared for each GM primer concentration (35, 70, and 100 vol%) and the no-priming control group, such that a total of 40 specimens were prepared. The results of maximum wall-to-wall contraction were analyzed statistically using one-way ANOVA and Fisher's PLSD test (p<0.05).

Observation of the diffusion of GM primer and dentin bonding agent into dentin

To observe the diffusion of GM primer and dentin bonding agent into dentin, both the primer and bonding agent were stained by adding a small amount of red dye (Acid Red, MW: 621.6) and blue dye (Brilliant Blue, MW: 72.85) respectively.

A flat dentin surface was prepared by wet-grinding on 600-grit silicon carbide paper. The dentin surface was conditioned with 0.5 mol/L EDTA (pH 7.4) for 60 seconds, followed by rinsing and drying. After EDTA conditioning, the dentin surface was primed with GM solution (w/wo red dye) for 60 seconds, and then the surface was dried completely. After GM priming, the dentin bonding agent (w/wo blue dye) was applied on the cavity surface and irradiated for 40 seconds after removing any excess bonding agent with a gentle air blast. Finally, a commercial flowable resin composite (Clearfil Majesty LV, Kuraray Medical, Japan) was placed on the dentin bonding agent with a thickness not more than 1.5 mm and irradiated for 40 seconds.

When the stained GM primer was applied, non-stained dentin bonding agent was used instead. Likewise, when the stained dentin bonding agent was applied, non-stained GM solution was used instead. In the negative control group (with red dye), Acid Red was dissolved in distilled water.

At 10 minutes after the resin composite was irradiated, the specimen was sectioned perpendicular to the dentin-adhesive interface. The diffusion of the stained GM primer or dentin bonding agent was observed under a light microscope at a magnification of 225.

SEM observation of the adhesive interface

After light microscopic observation, the sectioned adhesive interface was polished on a wet silicon carbide paper and etched with 10% hydrogen chloride for 10 seconds. The microstructure at the adhesive interface was then observed with SEM (S-4700, Hitachi, Tokyo, Japan) at a magnification of 1000 after sputter-coating with platinum and palladium.

RESULTS

Wall-to-wall polymerization contraction gap value

Table 1 presents the wall-to-wall contraction gap values measured in this study. Complete marginal integrity was obtained when the dentin cavity was primed with 35 vol% GM primer. According to

Table 1 Wall-to-wall polymerization contraction gap value in cylindrical dentin cavities

	Contraction gap value	Gap-free specimens
35 vol% GM	0	10
70 vol% GM	0.015±0.032	8
100 vol% GM	0.016±0.034	8
No priming (negative control)	0.167±0.225*	1

n=10. Contraction gap value in percentage to the cavity diameter is presented as mean±SD.
Number of gap-free specimens out of a total of 10 specimens.
GM: 35 vol% of glyceryl monomethacrylate.
*: A significantly small value statistically.

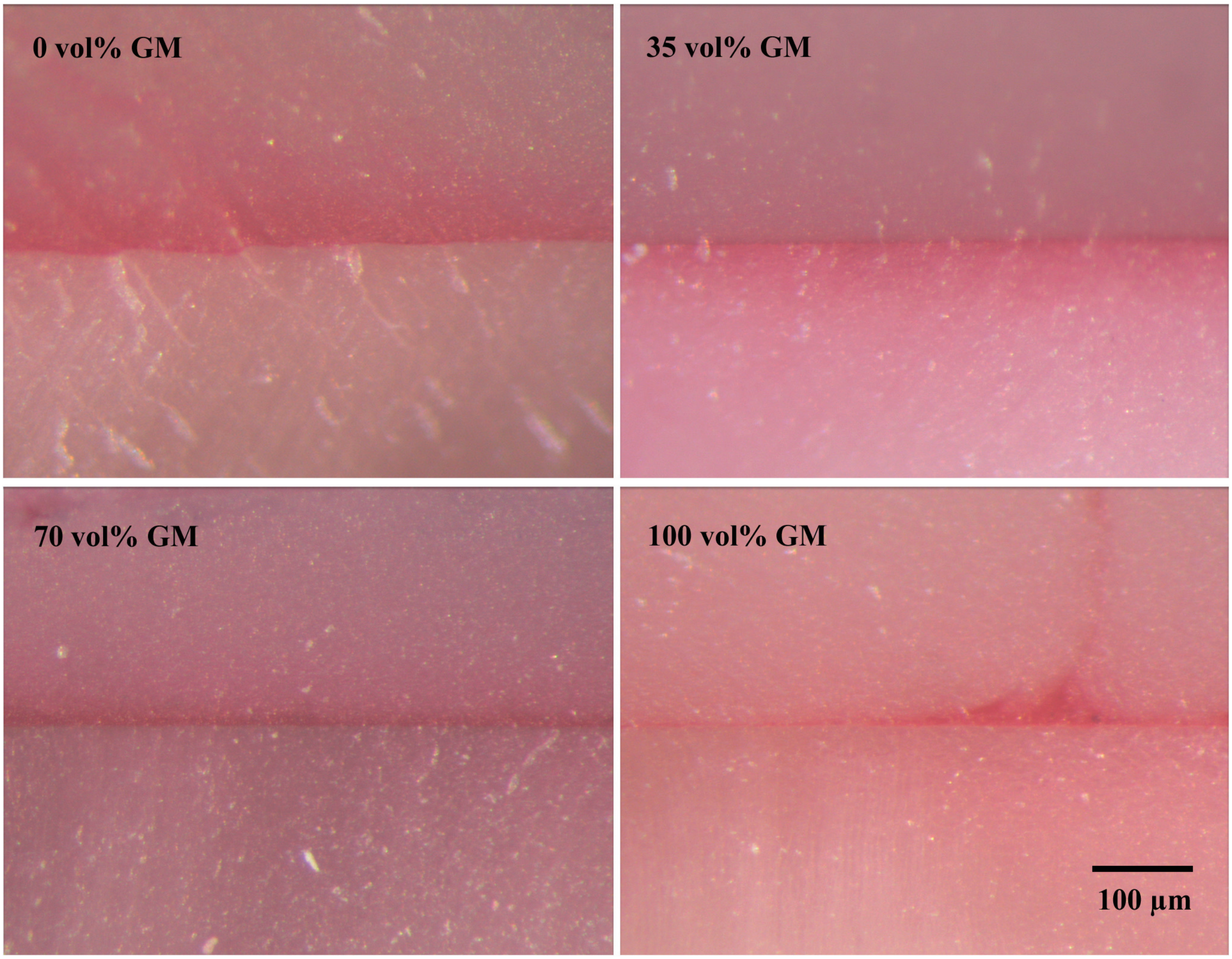

Fig. 1 0, 35, 70, and 100 vol% GM primer solutions stained by Acid Red. Only the red dye of 35 vol% GM diffused into dentin, whereas the red dye did not penetrate the dentin surface in all the other GM concentrations.

statistical analysis by one-way ANOVA and Fisher's PLSD test, no statistically significant differences were observed between the GM priming groups (35, 70, and 100 vol% GM) and negative control group (no priming).

Diffusion of GM primer and dentin bonding agent into dentin

Figures 1 and 2 show the diffusion results of red-stained GM primer and blue-stained dentin bonding agent into the dentin surface. In the negative control group where distilled water stained with Acid Red was applied to the dentin surface, the red dye did not penetrate the dentin surface (Fig. 1). In 35 vol% GM group, red-stained GM primer diffused into the dentin. However, in 70 and 100 vol% GM groups, red-stained GM primer remained at the adhesive interface.

In Fig. 2, it could also be seen that the blue-stained dentin bonding agent penetrated the dentin surface after priming with 35 vol% GM. However, in 70 and 100 vol% GM groups as well as in the negative control group, the blue-stained dentin bonding agent remained at the adhesive interface. Moreover, the red and blue dyes of 35 vol% GM and dentin bonding agent diffused up to a depth of 40 µm into the dentin surface.

SEM observation of the adhesive interface

SEM images revealed that monomer diffusion did not occur in all the specimens (Fig. 3).

DISCUSSION

The bonding mechanism to conditioned dentin is a micromechanical one with the formation of a hybrid layer and resin tags —which occurred due to the penetration of dentin primer into the decalcified dentin. Besides, a dentin primer which could enhance the diffusion of adhesive monomers into the dentin surface, and hence facilitate the formation of a hybrid layer, would also improve the bonding efficacy of dentin adhesives[10].

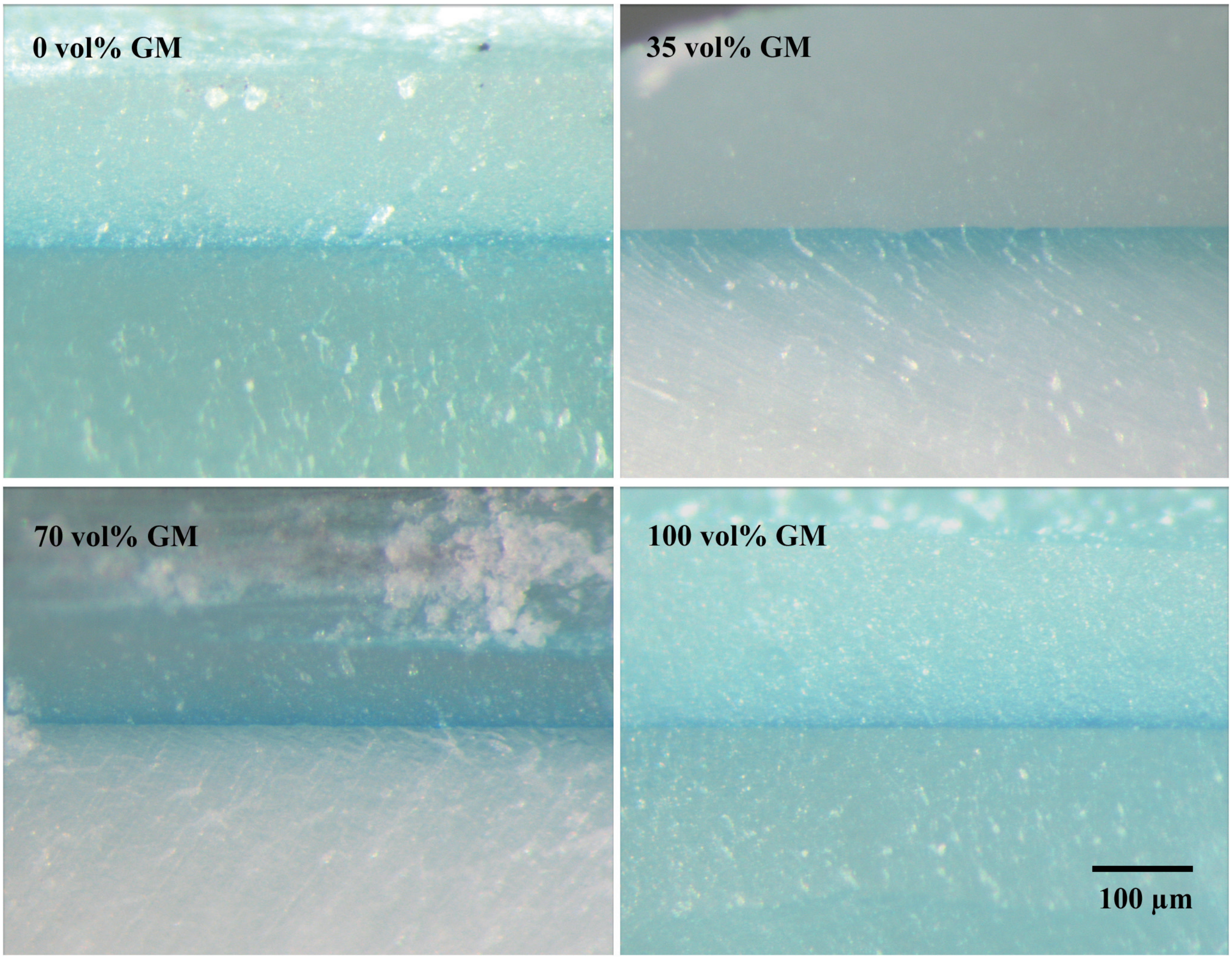

Fig. 2 Dentin bonding agent stained by Brilliant Blue. The blue dye of dentin bonding agent diffused into dentin only when it was primed with 35 vol% GM. With all other GM concentrations (0, 70, and 100 vol%), the blue dye remained at the adhesive interface.

However, it should be mentioned that in the occurrence of contraction gaps between the resin composite and dentin cavity wall, contraction gaps usually form at the adhesive interface. In an experiment by Chigira *et al.*, it was reported that contraction gap formation was completely prevented when dentin cavity wall was primed with 35 vol% GM solution after conditioning with 0.5 mol/L EDTA[8]. In another study by Chiba *et al.*, it was reported that contraction gap width increased with decrease in the Vickers hardness of the dentin surface after dentin cleaning, *i.e.*, when the dentin surface was softened by acidic solutions[11]. Therefore, to improve marginal adaptation, the focus should be on the high calcium content of the dentin surface as opposed to a decalcified dentin surface for hybrid layer formation.

Further on the importance of high calcium content in the dentin surface to improving marginal integrity, we demonstrated that contraction gap formation was completely prevented in sclerotic dentin even when no GM priming was performed on the dentin cavity wall[12]. Nonetheless, gap formation was completely prevented in both sclerotic and sound dentin when GM priming was performed. This finding thus presented a countersuggestion that limited monomer diffusion into sclerotic dentin was beneficial to dentin bonding.

Apart from GM priming, Kusunoki *et al.* reported that contraction gap formation was also completely prevented with a dentin primer that was composed of triethylene glycol monomethacrylate (TEGMA). This was probably because water movement was prevented by triethylene glycol (TEG) which has a similar molecular weight as GM[13]. They also reported that TEG prevented infiltration of the bonding monomers into the branches of the dentinal tubules. By virtue of its water absorption properties, TEG is employed in medical clinics to prolong drug metabolism time and reduce the frequency of injections.

Another important factor to the bonding efficacy of dentin adhesives is the surface-activation after primed

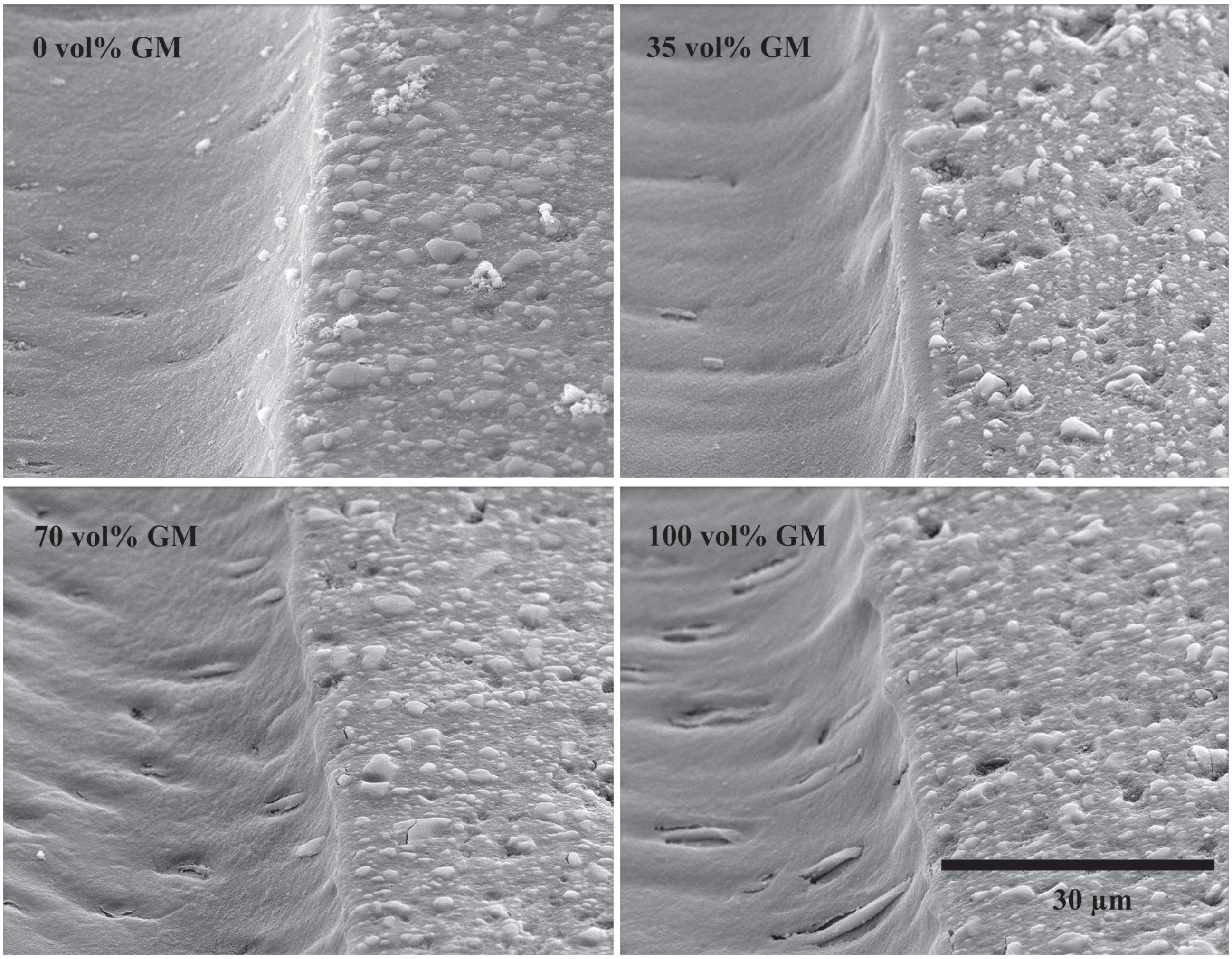

Fig. 3 SEM images of the dentin cavity margin treated with 0, 35, 70, and 100 vol% GM. In 0, 70, and 100 vol% GM priming groups, the bonding layer could hardly be observed. In the 35 vol% GM priming group, only a thin bonding layer was observed. Monomer diffusion was not observed in all groups.

dentin surface. Tani *et al.* reported that the contact angle of a commercial dentin bonding agent to the primed dentin surface was smaller than with the unprimed dentin[14]. It also reported that the commercial dentin bonding agent did not form a hemispherical shape because the former spread rapidly over the EDTA-conditioned dentin surface[14]. These results indicated that dentin priming increased the surface free energy of the dentin surface, whereby improved wettability of the primed dentin surface would lead to improved bonding.

In a study by Sauro *et al.*, it was also suggested that water from within the dentinal structure led to bond strength reduction[15]. This was because water flow from within the dentinal structure not only impeded the diffusion of hydrophobic monomer into dentin, but also hindered the curing of adhesive monomer at the adhesive interface. In the present study, red-stained GM did not penetrate the dentin surface in the cases of 70 and 100 vol% GM and Acid Red solution (negative control); diffusion into dentin was observed only for 35 vol% GM primer. Similarly, the blue dye of dentin bonding agent diffused into the dentin surface only when 35 vol% GM primer was applied. These results indicated that priming not only served to disrupt the water flow from within the dentinal structure, but also to increase surface-activation.

In light of the results and conclusions reported in published studies and based on the findings of the present study, it was thus concluded that 35 vol% GM primer was effective in preventing wall-to-wall contraction gap formation because it was able to increase surface-activation and disrupt water flow from within the dentinal structure.

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