Water evaporation from substrate tooth surface during dentin treatments

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The purpose of this study was to evaluate changes in the quantity of water evaporation from tooth surfaces. The amount of water evaporation was measured using Multi probe adapter MPA5 and Tewameter TM300 (Courage+Khazaka Electric GmbH, Köln, Germany) after acid etching and GM priming of enamel; and after EDTA conditioning and GM priming of dentin. The results indicated that the amount of water evaporation from the enamel surface was significantly less than that from the dentin. Acid etching did not affect the water evaporation from enamel, though GM priming significantly decreased the evaporation (83.48±15.14% of that before priming). The evaporation from dentin was significantly increased by EDTA conditioning (131.38±42.08% of that before conditioning) and significantly reduced by GM priming (80.26±7.43% of that before priming). It was concluded that dentin priming reduced water evaporation from the dentin surface.

**Keywords**: Dentin primer, GM priming, Water evaporation

**INTRODUCTION**

The bonding mechanism of dentin adhesives has been widely explained by hybrid layer formation\(^1\). Nakabayashi et al. reported that methyl methacrylate containing 4-methacryloxyethyl trimellitate anhydride (4-META) activated by tri-buthyl borane (TBB-O) penetrated and polymerized in dentin that was etched by citric acid containing ferric chloride\(^1\). Thus, a dentin bond was formed between the resin material and collagen-rich dentin. The smear layer on a ground tooth surface should be eliminated prior to the application of dentin bonding agent because it interferes with bonding. Various acid and acidic monomers such as phosphoric acid\(^2\), maleic acid\(^3\), phosphate ester monomers\(^4\) and ethylene di-amine tetra acetiac acid (EDTA)\(^9\) have been introduced in commercial bonding systems\(^5\), although the standard material for dentin conditioning is yet to be determined. In 1984, Munksgaard and Asmussen reported that pretreatment of EDTA-treated dentin with an aqueous mixture of 2-hydoxyethyl methacrylate (2-HEMA) and glutaraldehyde significantly increased the shear bond strength of the resin monomer composed of Bis-GMA and tri-ethylene glycol di-methacrylate\(^6\). Such a pretreatment between dentin conditioning and application of dentin bonding agent was clinically introduced as dentin priming. Sugizaki reported that dentin primer improved the dentin bonding, because the dentin collagen was expanded by the dentin primer and the dentin bonding agent then penetrated into the expanded collagen network\(^7\). Gwinett observed that the dentin collagen did not collapse when the dentin was not completely dried after dentin conditioning\(^8\). Therefore, they recommended wet bonding after phosphoric acid conditioning. However, Tay claimed that the water remaining in the bonding zone was a “water tree” that may have deteriorated the bonding\(^9\). To prevent degeneration of the collagen, the effect of adding chlorhexidine to dentin bonding materials was evaluated\(^10\). However, such explanations in dentin contradict the fact that resin materials exhibit high bonding efficacy to the etched enamel even though the collagen content in the enamel is extremely low.

We demonstrated that dentin bonding occurred via the interaction between inorganic components in dentin and adhesive monomers in a dentin bonding agent because the contraction gap width increased when dentin hardness was decreased by dentin conditioning or application of experimental dentin bonding agents without any adhesive monomer\(^11-13\). It has been recognized that polyethylene glycol methacrylate (PEG) possibly reduced water contamination\(^14\). Based on this mechanism, we developed an experimental dentin primer composed of aqueous solution of glycercyl mono-methacrylate (GM)\(^15\) and tri-ethylene glycol mono-methacrylate (TEGMA)\(^10\), which completely prevented contraction gap formation of light-activated resin composite in a dentin cavity. Thus, it was suggested that the GM primer altered the physical and chemical conditions of the substrate dentin surface, giving it the appearance of an acid-etched enamel surface. However, the effect of dentin primer on water evaporation from the substrate tooth surface has not been discussed in detail. The changes in the amount of water evaporation from the substrate tooth surface during dentin conditioning and dentin priming were measured in this study.

**MATERIALS AND METHODS**

Ten flat enamel and dentin surfaces of extracted human teeth were prepared using wet silicon carbide paper grit number 1000. The Showa University Ethics Committee approved the use of extracted human teeth (Approval number: 2007-31). The samples were then embedded in
self-curing methyl methacrylate resin and a needle was fixed in the apical opening of the root canal which was connected to a vinyl tube and 27 cm water pressure was applied from the pulp chamber into the dentin tubules as shown in Fig. 1. The tooth surface was covered with an adhesive tape with a center hole of diameter 3.6 mm to regulate the measuring area. The water evaporation from the tooth surface was measured using Multi probe adapter MPA5 and Tewameter TM 300 (Courage+Khazaka Electric GmbH, Köln, Germany), as shown in Fig. 2. After measuring the amount of water being evaporated from the prepared tooth surface, the enamel surface was treated with 40% phosphoric acid gel for 15 s followed by rinsing and drying. The dentin surface was conditioned with 0.5 mol/L neutralized EDTA (pH 7.4) for 60 s followed by rinsing and drying. After phosphoric acid or EDTA conditioning, the amount of water evaporation was measured again. Then the enamel and dentin surfaces were both treated with a dentin primer composed of 35% glyceryl monomethacrylate solution for 1 s and the tooth surfaces were completely dried. Immediately after drying, the amount of water evaporation was measured.

The micro-structure of the substrate dentin surface was observed by SEM. In addition, the resin adhesive surface of non-primed specimens and those primed with GM after EDTA conditioning were also observed after eliminating the dentin in hydrochloric acid and sodium hypochlorite. Specimens were dehydrated in graded alcohol solutions and critical point dried followed by ion spattering with palladium and platinum.

RESULTS

The average of consecutive measurements of 10 points in three stages (before treatment with H₃PO₄ or EDTA, after treatment with H₃PO₄ or EDTA, after GM priming) is shown in Fig. 3. The amount of water evaporation from the enamel surface was significantly smaller compared to that from the dentin (Fig. 3). The ratio when the previous stage was assumed to be 100 is shown (Table 1). The water evaporation after phosphoric acid etching hardly changed (94.66±23.17% of that before etching). The difference in water evaporation from the enamel surface between before and after etching was insignificant (Mann-Whitney U-test, \( p < 0.05 \)). The water evaporation from enamel was significantly decreased by GM priming (83.48±15.14% of that before priming).

The evaporation from dentin was significantly increased by EDTA conditioning (131.38±42.08% of that before conditioning). In addition, the evaporation was significantly reduced by GM priming (80.26±7.43% of that before priming).

Using SEM, we observed that the dentin tubules were filled with crystal-like substances in the low evaporation specimens, though tubules were open in the high evaporation specimens (Fig. 4). In addition, penetration of the dentin bonding agent into the fine lateral branches of the dentin tubules was not observed in the GM-primed dentin specimens (Fig. 5).

DISCUSSION

Clinical application of dentin bonding agents prior to resin composite filling is mandatory to prevent contraction gap formation between the resin material and cavity walls. Close adaptation of resin composite to enamel cavity walls is easily obtained by enamel etching with phosphoric acid. However, it is still difficult to achieve marginal adaptation to dentin cavity walls. It is widely known that approximately 98% of enamel is composed of inorganic substances with extremely low water content. The results obtained in this study showed that water evaporation from the enamel surface is considerably less than that from the dentin (Fig. 3). In addition, the water evaporation from enamel was hardly changed by phosphoric acid etching, though it was
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Fig. 4  For the amount of water evaporation, individual specificity was seen with individual teeth. Minimum value was below half while the maximum value (which has been indicated before) was below 60 g/hm² after EDTA was applied. Differences in the amount of water evaporation could be due to the differences in composition. a: After a hydraulic pressure of 27 cm was applied, differences were seen in the amount of water being lost from different parts of the same tooth.  b-1: In case 1, for which there was a large amount of water evaporation, a large number of dentin tubules were open in the SEM observation. b-2: In case 2, for which there was a small amount of water evaporation, there were deposits in the dentin tubules (causing them to close) in the SEM observation.

Fig. 5  a: When priming was omitted, bonding material infiltrates even further into the depths and details of dentin tubules. b: GM priming obstructed infiltration of the bonding material to side branches of the dentin tubules.
dentin conditioner in case of using phosphoric acid significantly accelerated contraction gap formation\textsuperscript{11-13}. In addition, Hashimoto et al. claimed that dentin etching caused “nano leakage” within the base of the hybrid layer where the dentin bonding agent failed to penetrate sufficiently\textsuperscript{17}. Such poor diffusion of monomers into the decalcified dentin resulted in extensive secondary caries clinically. Etching the dentin caused dentin to become porous and probably increased water evaporation from the dentin, resulting in insufficient polymerization of the adhesive monomers. It is therefore essential to remove the smear layer without possibly decalciﬁying the dentin.

The original components of the dentin primer (GLUMA primer) were 2-HEMA and glutaraldehyde. Munksgaard and Asmussen reported that glutaraldehyde was necessary to activate dentin collagen\textsuperscript{6}. However, we reported that glutaraldehyde should be omitted because the 2-HEMA solution exhibited a priming effect comparable to the GLUMA primer\textsuperscript{18}. In addition, we developed experimental dentin primers which completely prevented contraction gap formation of the resin composite. These newly developed dentin primers had the same chemical structure, etheriﬁed methacrylate with polyvalent alcohol, that may have an advantage of forming hydro-gel\textsuperscript{15, 16, 19, 20}.

It was thought that dentin primer altered the physical characteristics of the dentin surface and thus improved dentin bonding. We previously reported that the contact angle of a drop of dentin bonding agent to the dentin surface was markedly decreased by dentin priming\textsuperscript{21}. Thus, it could be speculated that the dentin primer improved the dentin bonding effect by increasing the dentin surface activity. As demonstrated in this study, the dentin primer interferes with the penetration of the dentin bonding agent into the ﬁne lateral branches of the dentin tubules and signiﬁcantly reduces the water evaporation from the dentin surface. The removal of the smear layer signiﬁcantly increased the water evaporation, probably because the dentin tubules were opened and inter-tubular dentin surface was exposed, unlike in the case of enamel. The ratio of the amount of water evaporation after GM priming compared with the former stage was almost the same as the case of enamel. However, the amount of water evaporation of the dentin was higher than that of enamel even though it was being suppressed by GM. Such relatively high water evaporation from dentin even after GM priming may be due to the water movement within the dentin tubules. The phenomenon that was observed histologically in dentin parts other than the dentin tubules appeared similar to the phenomenon that occurs in enamel. A dentin model must be made for studying this aspect in the future, and further investigations should be conducted. It is widely accepted that the water in dentin plays an important role in dentin bonding. As already mentioned, it was suggested that the water on the substrate dentin promoted bonding because the dentin collagen kept expanding under wet conditions\textsuperscript{22}. However, we demonstrated that the efﬁcacy of the total-etch blot dry method was poor because contraction gap formation was hardly prevented by wet bonding\textsuperscript{23}. There is no doubt that water on the dentin surface interferes with the polymerization of the monomer-inorganic compound resulting in deterioration of dentin bonding. Therefore, it can be easily suggested that the water on adhesive dentin surface be reduced. Such a reduction in water evaporation from the dentin surface after GM priming makes the dentin surface resemble that of the enamel surface and improves the dentin bonding.

As demonstrated in this study, water evaporation from both the enamel and dentin surface was signiﬁcantly reduced by GM priming. It was possible to conclude that dentin priming was essential for dentin bonding because it controlled water contamination in the substrate dentin.

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REFERENCES


