Effect of Scrubbing Technique with Mild Self-etching Adhesives on Dentin Bond Strengths and Nanoleakage Expression

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Purpose: To evaluate the effect of a scrubbing technique with one-step self-etching adhesives on bond strengths and nanoleakage expression at the resin/dentin interface.

Materials and Methods: Flat human dentin surfaces bonded with one of two mild self-etching adhesives, SE One (SE) or Scotchbond Universal (SU) applied either with scrubbing or without scrubbing technique, were prepared (n = 5). The microtensile bond strengths (μTBS), SE micrographs of morphological changes on treated dentin surfaces, and expression of nanoleakage along the bonded dentin interfaces as shown with TEM were evaluated. μTBS data were analyzed using two-way ANOVA and the post-hoc t-test at the significance level of 0.05.

Results: The scrubbing technique had a significant positive effect on the μTBS of SU ($p < 0.05$), while it produced no significant difference for SE ($p > 0.05$). Morphological evaluation of the treated dentin surfaces demonstrated that SU with scrubbing showed the highest etching ability, followed by scrubbing SE > nonscrubbing SE > nonscrubbing SU. In the nonscrubbing groups, nanoleakage formation using SU exhibited a reticular pattern throughout the hybridized complex, whereas with SE, water-tree nanoleakage was only found in the adhesive layer at dentinal tubule orifices. The scrubbing groups of both adhesives did not exhibit any nanoleakage expression.

Conclusion: Using a scrubbing technique when applying mild self-etching adhesives could improve resin monomer infiltration into dentin, chase water on adhesive surfaces, and facilitate smear layer removal.

Keywords: scrubbing, one-step self-etching adhesive, dentin, bond strength, nanoleakage, smear layer, water chasing.

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One-step self-etching adhesives were developed to simplify the bonding procedure, reducing chair time and minimizing technique sensitivity.[7] Combining acidic resin monomers, multifunctional monomers, solvent, and water into one mixture allows the bonding procedure to be accomplished in one application, and theoretically results in complete resin impregnation into demineralized dentin[26] due to simultaneous demineralization and monomer infiltration into the dentin substrate. Recently, "universal adhesives" with multimode application techniques (etch-and-rinse and self-etching) have been developed as one-step self-etching adhesives to provide equally effective bonding to all tooth

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Table 1 Materials used in the study

Materials	Manufacturer	Batch number	Composition	Application (manufacturer's instructions)		
				Apply	Air blow	Light cure
SE One (SE)	Kuraray Noritake; Tokyo, Japan	0039AA	MDP, bis-GMA, HEMA, hydrophilic aliphatic dimethacrylate, hydrophobic aliphatic methacrylate, ethanol, initiator, filler, accelerators, distilled water, NaF, CQ (pH = 2.3)	10 s	5 s	10 s
Scotchbond Universal (SU)	3M ESPE; St Paul, MN, USA	482153	MDP, dimethacrylate resins, bis-GMA, HEMA, Vitrebond copolymer, silane, ethanol, water, filler, initiator (pH=2.7)	20 s with scrubbing	5 s	10 s

Abbreviations: 10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate; bis-GMA: 2,2-bis[4-(2-hydroxy-3-methacryloyloxy propoxy) phenyl]propane; HEMA: 2-hydroxyethyl methacrylate; CQ: camphorquinone.

substrates (sound, sclerotic dentin, as well as enamel).[8] Moreover, they contain additional components to promote bonding to various other substrates (eg, glass ceramics and metals).[4] Water is an essential component, which enables dissociation of the acidic monomers responsible for etching, while solvents are added to enhance monomer miscibility into one solution and accelerate water elimination from the adhesive surface.[17,34] Ideally, excess water/solvent should be removed after application to adhesive substrates, otherwise their residue will result in deterioration of the resin-dentin bond by interfering with the polymerization reaction,[12] causing blister formation,[25] or intensifying permeability of the adhesive layer.[5] Therefore, one-step self-etching adhesives require strict air blowing prior to light curing. In addition to the water which is a constituent of the adhesive agent, water is present within the adhesive-dentin subsurface and dentinal tubules at the adhesive interface.[27,28] This surface water may prevent complete resin impregnation into demineralized dentin, leading to nanoleakage within the hybrid layer and water-tree nanoleakage in the adhesive layer.[31] The term water-tree nanoleakage describes water-filled channels originating from the surface of the hybrid layer, passing through the adhesive layer to reach the adhesive/composite interface. Importantly, this could be a pathway for hydrolytic degradation and jeopardize the longevity of the resin-dentin bond.[18] However, complete water/solvent evaporation from the adhesive layer by air blowing seems to be impossible to achieve, due to the relatively low vapor pressure of the water/solvent when mixed with hydrophilic monomers, leaving the last portion of solvent in the resin mixture.[20] Therefore, investigating the strategies to enhance the elimination of water from the dentin surface during application of the adhesive would be of clinical interest.

Active adhesive application using a scrubbing action is a practical technique which has been reported to increase the bonding performance to enamel and dentin of one-step self-etching adhesives, by facilitating removal of the smear layer.[1,3,14] A recent study on enamel bonding revealed that active adhesive application of universal adhesives increased the degree of conversion of the adhesive at the interface due to enhancement of solvent evaporation, and improved the enamel bond strengths.[13] Additionally, a scrubbing action on the dentin surface promotes monomer infiltration into the substrate[24] and water chasing from the adhesive dentin surface, which could reduce the nanoleakage at the adhesive/dentin interface.[13,22] However, there have been few studies published to date on whether application using scrubbing results in morphological alterations of the adhesive/dentin interface of one-step self-etching adhesives.

Therefore, the aim of this study was to evaluate the effect of a scrubbing technique on nanoleakage at the resin/dentin interface by using transmission electron microscopy (TEM) and dentin bond strengths using the microtensile bond strength (μTBS) test. In addition, the etching ability of each adhesive application technique was investigated by examining the morphology of the adhesive-treated dentin surface using a scanning electron microscope (SEM). The null hypothesis tested was that there were no differences in μTBS, etching ability, or nanoleakage expression of one-step self-etching adhesives bonded to dentin when applied with or without scrubbing.

MATERIALS AND METHODS

Specimen Preparation

Freshly extracted human third molars from patients ages 18 to 25 were collected after obtaining approval from the Ethics Committee of Tokyo Medical and Dental University, protocol number 725. The teeth were stored in distilled water with 0.1% thymol solution at 4°C for no more than 6 months prior to the experiments. The occlusal enamel was removed perpendicular to the long axis of the tooth using a model trimmer under water lubrication to expose midcoronal dentin, and then polished with 600-grit SiC paper under running water for 30 s to create standardized smear layers. The specimens were divided into 2 groups of one-step self-etching adhesives: SE One ([SE], Kuraray Noritake Dental; Tokyo, Japan) or Scotchbond Universal ([SU], 3M ESPE; St Paul, MN, USA). The bonding procedure was performed either by following the manufacturer's instructions (with nonscrubbing application in SE and scrubbing application in SU; Table 1) or with scrubbing application in

SE and nonscrubbing application in SU. After air blowing for 5 s and light curing for 10 s with a light-curing unit at 830 mW/cm^2 (Optilux 501, Kerr; Orange, CA, USA), three 2-mm-thick increments of a resin composite (Clearfil AP-X; Kuraray Noritake) were built up on the dentin surface, curing each increment for 20 s. The light intensity was checked every 5 specimens by a built-in radiometer attached to the light-curing unit.

Microtensile Bond Strength (µTBS) Test

Twenty bonded specimens (5 in each group) to be subjected to the microtensile bond strength test were stored in water at 37°C for 24 h. Subsequently, the bonded specimens were sectioned parallel to the long axis of the teeth, creating 1-mm-thick slabs, which were further cut into beams with a bonded area of 1 ± 0.1 mm^2. The samples were fixed with cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin; Ohtawara, Japan) onto a flat jig, and then tested for µTBS in a universal testing machine (EZ test; Shimadzu, Kyoto, Japan) at a crosshead speed of 1 mm/min. The µTBS data were analyzed using two-way ANOVA (due to normal distribution of the results) to examine the effect of the factors (materials and application technique), and the t-test to determine differences between mean values, with a significance level of 0.05. Statistical analysis was carried out using SPSS version 22.0 (SPSS; Chicago, IL, USA).

Failure Mode Analysis

After the µTBS test, both the dentin and composite sides of the fractured specimens were observed using a scanning electron microscope (SEM; JSM-5310LV, JEOL; Tokyo, Japan). A 10 × 10-square table was superimposed on the image of the entire surface of each fractured specimen in order to determine the mode of failure in each particular area. Failure modes were classified as cohesive failure in resin composite, adhesive failure at the resin composite/adhesive interface, adhesive failure at the adhesive/dentin interface, or cohesive failure in dentin. Failure modes were analyzed for statistically significant differences using the nonparametric Pearson chi-square test at a significance level of 0.05.

SEM Examination of Dentin-surface Morphology

In order to evaluate the etching pattern of each group, additional prepared dentin surfaces (2 teeth per group) were treated according to the bonding protocols described above, followed by rinsing with 50% acetone for 5 min to remove the applied adhesive. The dentin specimens were subsequently dehydrated in ascending concentrations of ethanol, immersed in hexamethyldisilazane (HMDS) for 10 min, and then left for 24 h on filter paper placed in a covered glass vial at room temperature. After gold-sputter coating (SC-701AT, Elionix; Tokyo, Japan), the treated dentin surfaces were examined using SEM.

Nanoleakage Evaluation by TEM

Three bonded teeth per group were additionally prepared as for the µTBS test using a low-viscosity composite (Protect Liner F; Kuraray Noritake). Nanoleakage evaluation with a transmission electron microscope (TEM, H-7100, HITACHI; Tokyo, Japan) was performed according to the protocol previously described.[29] After 24 h of water storage of the bonded teeth, two 0.9-mm-thick slabs perpendicular to the bonded interface were obtained from the center of each tooth using a low-speed diamond saw (Isomet, Buehler; Lake Bluff, IL, USA) with water cooling. Each slab was coated with two layers of nail varnish, leaving a 1-mm window around the bonded interface. They were immersed in the ammoniacal silver nitrate solution for 24 h, rinsed thoroughly with distilled water, and then immersed in photodeveloping solution for 8 h under a fluorescent light. Specimens were fixed in Karnovsky's solution, post-fixed in osmium tetroxide, dehydrated in an ascending series of ethanol (50%-100%) and embedded in epoxy resin.[23] The 70-nm-thick ultrathin sections were prepared with an ultramicrotome and a diamond knife (Diatome; Bienne, Switzerland), and then collected on 150-mesh copper grids. The nanoleakage expression of the bonded interface was examined using a TEM operating at 75 kV.

Table 2 Mean and standard deviations of the microtensile bond strengths (n = 20)

Adhesive	Application technique	
	Scrubbing	Nonscrubbing
SE	60.46 (9.55)^{Aa}	60.23 (9.47)^{Ac}
SU	66.26 (10.13)^{Bb}	57.25 (9.29)^{Cc}

Different superscript capital letters indicate statistically significant differences between application technique within adhesive ($p < 0.05$). Different superscript lowercase indicate statistically significant differences between adhesives with the same application technique ($p < 0.05$). SE: SE One; SU: Scotchbond Universal.

RESULTS

µTBS Test

The results of µTBS testing are summarized in Table 2. Two-way ANOVA revealed a significant difference in µTBS among the application techniques ($p = 0.004$), but no significant difference among the adhesives ($p = 0.374$). There was a significant interaction between the application technique and adhesive ($p = 0.006$). For SU, the scrubbing group exhibited significantly higher µTBS than that of the nonscrubbing group ($p < 0.05$), whereas for SE, scrubbing did not affect the µTBS ($p > 0.05$).

Failure Mode Analysis

The frequency of the failure modes in each group was calculated as %, as shown in Fig 1. There were significant differences in failure mode among groups ($p < 0.05$). In the nonscrubbing SU group, adhesive failure at adhesive interface was predominant, and this percentage was significantly larger than in the other groups ($p < 0.05$).

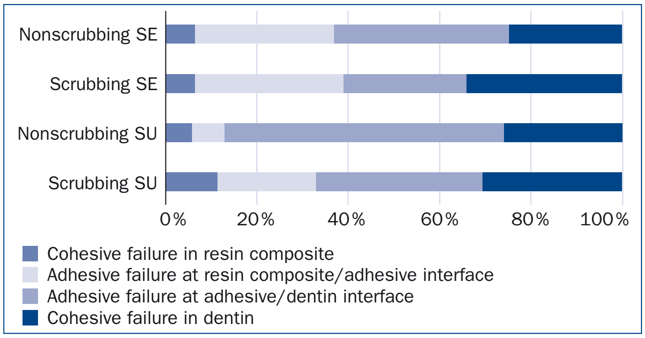

Fig 1 Bar graph shows percentage of failure mode in each group. The chi-square test revealed the significant differences in failure mode distribution among the groups ($p < 0.05$). Note that failure at the adhesive/dentin interface was the most common mode in the nonscrubbing SU group. SE: SE One; SU: Scotchbond Universal.

Dentin Morphology

SEM observation of the treated dentin surfaces revealed different etching patterns among the groups (Fig 2). SU applied with scrubbing had the highest etching ability, in which the smear layer was completely removed, exposing collagen fibrils and the dentinal tubule orifices, with peritubular dentin distinctly visible. In contrast, SU applied with a nonscrubbing technique had the lowest etching ability, in which the smear layer was largely left on the dentin surface, with many occluded dentinal tubules (Figs 2c and 2d). For SE applied with the scrubbing technique, intertubular dentin was observed to be slightly demineralized with some smear plugs still remaining in the dentinal tubules. whereas with the non-scrubbing technique, the smear layer partially remained with some dentinal tubules closed (Figs 2a and 2b).

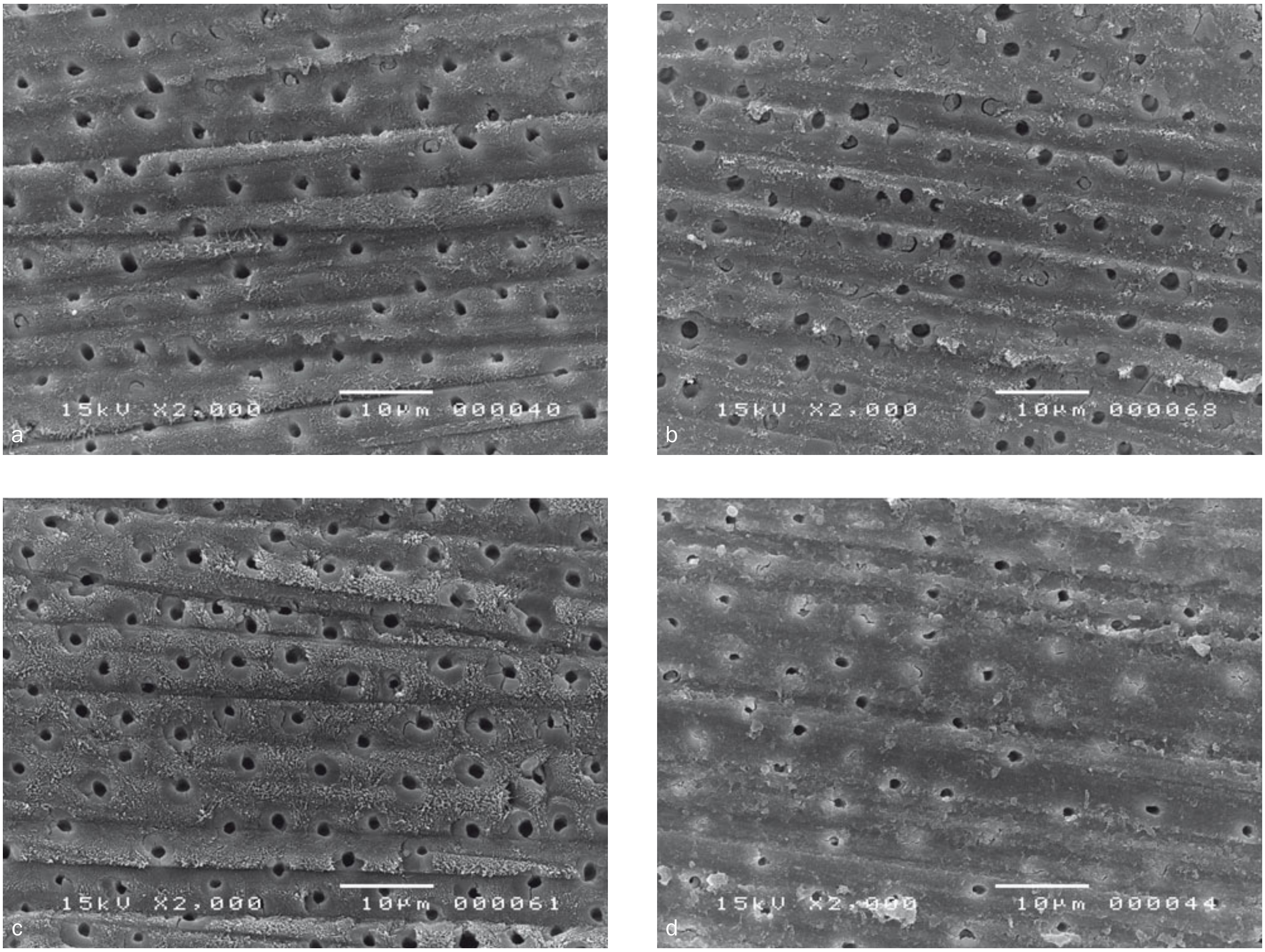

Fig 2 SEM micrographs show etching patterns of each adhesive with different application techniques. (a) SE with scrubbing: intertubular dentin was partially, slightly demineralized, but some smear plug remained. (b) SE with the nonscrubbing technique: smear layer partially remained with some dentinal tubules closed. (c) SU with scrubbing: smear layer was completely removed with exposure of collagen fibrils, disclosure of dentinal tubule orifices, and with peritubular dentin distinctly visible. (d) SU with the nonscrubbing technique: smear layer was mostly left on dentin surface with many occluded dentinal tubules. SE: SE One; SU: Scotchbond Universal.

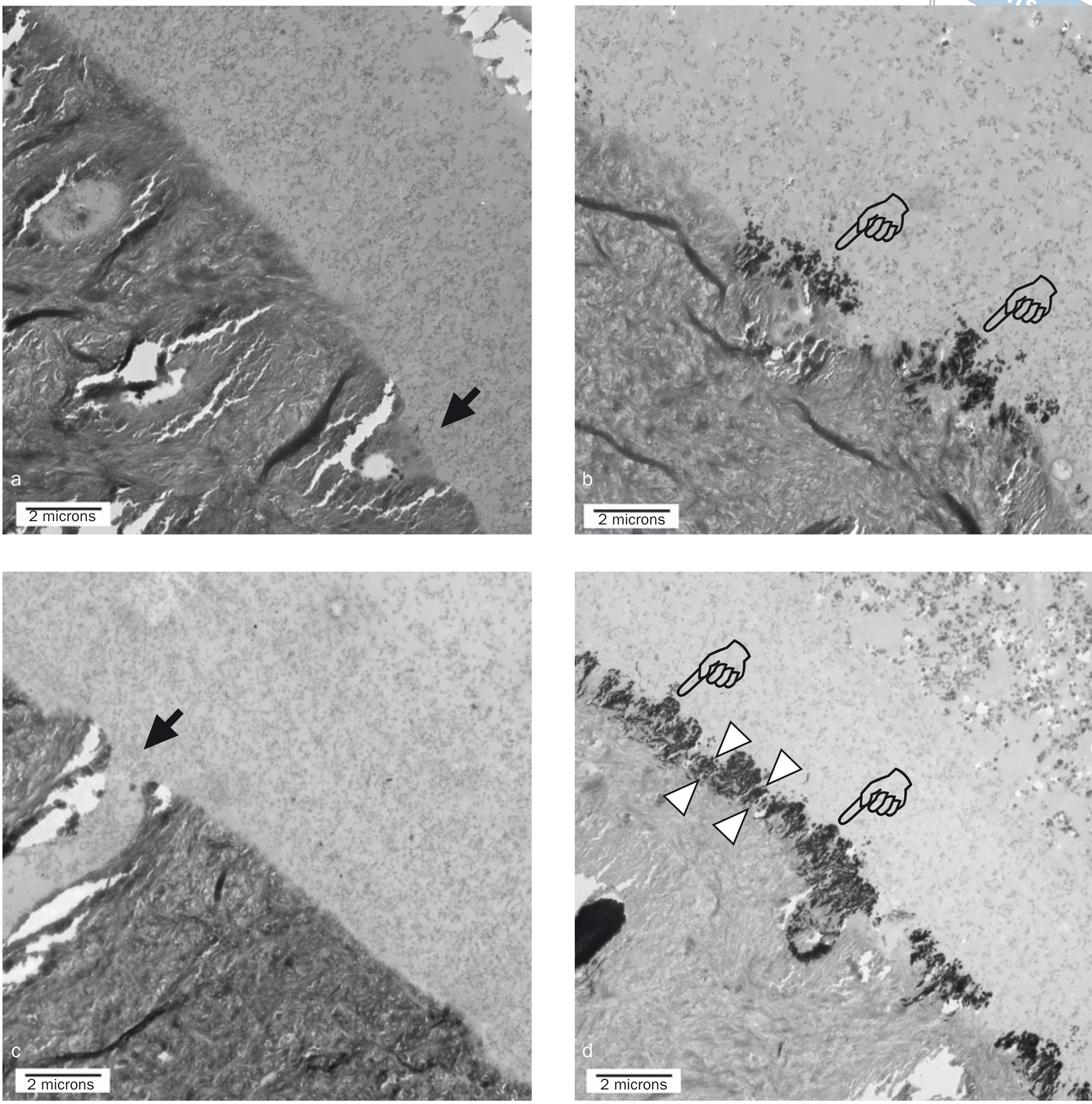

Fig 3 Undemineralized, silver-stained TEM micrographs (7000X magnification) of resin/dentin interfaces of each adhesive with different application techniques. (a) SE and (c) SU with scrubbing did not possess any silver deposits along the interface, even in the dentinal tubule orifices (arrows). (b) Nonscrubbing SE group: silver deposit islands were only found in the adhesive layer at the orifice of dentinal tubules (pointers). (d) Nonscrubbing SU group: silver deposits were found dispersed within the hybrid layer (between arrow heads) and on the adhesive surface (pointers). SE: SE One; SU: Scotchbond Universal.

Nanoleakage Evaluation

TEM images of nanoleakage expression by silver nitrate staining are shown in Fig 3. Both adhesives applied with scrubbing were apparently free of nanoleakage formation in the bonded interfaces (Figs 3a and 3c). On the other hand, in the nonscrubbing groups, SU exhibited reticular nanoleakage within the hybrid layer and water-tree nanoleakage on the adhesive surface (Fig 3d), but no water-tree nanoleakage in the area of dentinal tubule orifices. SE exhibited water-tree nanoleakage at the dentinal tubule orifices without nanoleakage formation within the hybrid layer (Fig 3b).

DISCUSSION

One-step self-etching adhesives can simultaneously demineralize and infiltrate into dentin, but cannot completely remove the smear layer due to the higher pH of the adhesive agent. Both SE and SU, the one-step self-etching adhesives used in this study, contain HEMA and MDP functional monomers, and water/ethanol as solvents. However, there is a difference in the pH of the adhesives, which are 2.3 and 2.7 for SE and SU, respectively. This might be the reason for the different recommended modes of application and different application durations, because the pH of the adhesive is a critical factor contributing to the smear layer removal.[30] For SU, it is strongly encouraged to use scrubbing during the application time of 20 s, while for SE, the application technique is not specifically addressed in the shorter time of 10 s. Using a scrubbing technique when applying a self-etching adhesive was reported to effectively disrupt the smear layer, keeping fresh monomers in contact with tooth surface.[24,37] This effect not only improved the immediate μTBS, but also increased the stability of a one-step self-etching adhesive bond to dentin.[14] In the present study, scrubbing contributed to a significant improvement in the μTBS of SU but not of SE. This may be the result of the scrubbing action having different effects on smear layer removal in SE compared to SU.

SEM observations of the treated dentin surface showed that scrubbing SU was effective for removing the smear layer and demineralizing the dentin surface, whereas when SU was applied with no scrubbing, the treated dentin surface was still covered with a smear layer. On the other hand, for SE, there was little difference in smear layer removal with vs without scrubbing. These results indicate that using a scrubbing technique was more effective in enabling SU, with a higher adhesive pH and longer application time, to dissolve and remove the smear layer compared to SE, with a lower pH and shorter application time. SU is an ultra-mild self-etching adhesive, and failing to scrub would insufficiently remove the smear layer for resin monomer infiltration into the underlying dentin (residual smear layer on the adhesive surface can hamper infiltration of resin monomer).[19,32,33] Moreover, it has been demonstrated that remnants of the smear layer on the dentin surface can create a so-called hybridized smear layer on the authentic hybrid layer.[30] This layer is regarded as a weak point of the resin/dentin interface due to the discontinuity of the smear layer and underlying dentin,[32] which may have rendered this interface prone to fracture, thus accounting for the lower μTBS in the nonscrubbing SU group. Failure mode analysis confirmed this speculation, as only the nonscrubbing SU group exhibited failures predominantly in the adhesive/dentin interface. Presumably, in the case of SE, smear layer removal during the 10-s application time may have been sufficient to form a strong bond to dentin, regardless of application technique, leading to similar μTBS results between the scrubbing and nonscrubbing groups. Thus, the null hypothesis regarding μTBS and etching ability were partially accepted only for SE.

In order to reveal the nano-sized porosities within the hybrid layer and the adhesive layer, a nanoleakage evaluation technique using silver nitrate staining was introduced.[9] Silver particles are deposited in areas of incomplete monomer penetration and incomplete polymerization within the hybrid layer, which is manifested as a reticular nanoleakage pattern, and/or in residual water droplets in the adhesive layer and on the adhesive surface, which is manifested as water-tree nanoleakage.[2,26,29] In the present study, nanoleakage expression differed between the scrubbing and nonscrubbing groups of SE, so this null hypothesis had to be rejected. The nonscrubbing SE group showed only water-tree nanoleakage in the adhesive layer at dentinal tubule orifices. This would indicate that perfused water from the dentinal tubules pooled at the orifices during application with the adhesive agent. On the other hand, in the scrubbing SE group, water-tree nanoleakage was absent. These results emphasize the difficulty in removing pooled water at the dentinal tubule orifices with the air-blowing procedure in a nonscrubbing technique[36] and the water-chasing effect of scrubbing action. That is, scrubbing might spread the pooled water at dentinal tubule orifices into small droplets and chase these water droplets to the outermost surface,[24] leading to elimination of water-tree nanoleakage expression. Interestingly, SE did not demonstrate nanoleakage within the hybrid layer regardless of the scrubbing procedure. These results would indicate that the smear layer was sufficiently eliminated in the 10-s application time to infiltrate resin monomer into the underlying dentin.

SU is a “universal adhesive”, which can be applied to enamel and dentin with multimode application techniques (etch-and-rinse or self-etching), and also promote bonding to ceramics and metals. The nonscrubbing SU group exhibited substantial nanoleakage formation throughout the adhesive interface, but in the SU scrubbing group, nanoleakage formation was not observed. This might be because the undissolved and remaining smear layer acts as a physical barrier preventing monomer infiltration into the underlying dentin[19,32,33] and serves as water reservoir to impede monomer polymerization.[36] On the other hand, in the nonscrubbing SU group, despite nanoleakage in the adhesive interface, water-tree nanoleakage in the adhesive layer at dentinal tubule orifices was not observed, unlike the nonscrubbing SE group. This might be due to residual smear plugs which may eliminate water perfusion from the dentinal tubules. Using a scrubbing technique with SU may improve resin monomer infiltration into dentin and enhance water chasing on the adhesive surface, facilitating smear layer dissolution and removal. Presumably, the presence of Vitrebond copolymer in SU might require a scrubbing procedure, because without scrubbing, Vitrebond copolymer might be retained on the smear layer. Vitrebond copolymer is a methacrylate-modified polyalkenoic acid copolymer of high molecular weight, which can chemically bond to calcium in hydroxyapatite.[16] It was reported that Vitrebond copolymer in the two-step etch-and-rinse adhesive, Single Bond Plus, enhanced the bonding ability to dentin,[21] with more consistent bonding performance under varying dentin moisture conditions. However, there is no evidence of hybridization of Vitrebond copolymer in the dentin substrate, even when the dentin is acid etched.[6,35] Using TEM, a dark electron-dense amorphous phase was observed at outer surface of the hybrid layer with the Scotchbond Multi-Purpose system using Vitrebond copolymer.[35] This phase was reported to represent the formation of Ca-polyalkenoic acid complexes by reaction of the carboxylic groups from the Vitrebond copolymer with residual calcium.[35] Therefore, in nonscrubbing application, Vitrebond copolymer in SU might create a thin coating on the smear layer and interfere with the infiltration of the other monomer components in the adhesive agent into underlying dentin. On the other hand, a layer of Vitrebond copolymer on smear layer/plugs might contribute to prevention of water contamination from dentinal tubules in the adhesive layer. It was reported that Vitrebond copolymer in the one-step self-etching adhesive, Easy Bond, did not improve bonding ability to dentin, which was speculated to be due to a low concentration of Vitrebond copolymer.[21] It is presumed that Vitrebond copolymer is effective for bonding to moist dentin in the etch-and-rinse mode of SU. Further research is necessary on the effect of Vitrebond copolymer in one-step self-etching adhesives with etch-and-rinse and self-etching modes on the initial and long-term quality of the resin/dentin interface.

Previous studies have reported that hydrostatic pulpal pressure could compromise the resin-dentin bond, because perfused water from dentinal tubules contaminated the adhesive dentin surface.[10,11,15] Therefore, when hydrostatic pulpal pressure is present during the bonding procedure, the water-chasing effect on the dentin surface when using a scrubbing technique might play a significant role in bond strength to dentin. Further research is necessary to investigate effect of scrubbing technique with one-step self-etching adhesives on dentin bond strength and nanoleakage expression under hydrostatic pulpal pressure.

CONCLUSIONS

Within the limitations of this study, it was concluded that employing a scrubbing technique when applying a mild self-etching adhesive could enhance resin monomer infiltration of dentin, water chasing on the dentin surface, and smear layer dissolution and removal, which improved the quality of the resin/dentin interface.

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Clinical relevance: Using a scrubbing technique when applying mild self-etching adhesives is recommended to improve the quality of the resin/dentin interface.