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Does Shortened Application Time Affect Long-Term Bond Strength of Universal Adhesives to Dentin?

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Clinical Relevance

Although a shorter application time may be clinically appealing, higher bond strength was achieved when adhesives were applied according to the manufacturer's instructions. Therefore, clinicians should perform the bonding procedure strictly according to the manufacturer's directions.

SUMMARY

Objective: This study evaluated the effect of shortened application time on long-term bond strength with universal adhesives.

Methods and Materials: Three universal adhesives were used: Clearfil Universal Bond (CU, Kuraray Noritake Dental Inc, Tokyo, Japan), Scotchbond Universal Adhesive (SB, 3M ESPE, St Paul, MN, USA) or G-Premio Bond (GP, GC Corp, Tokyo, Japan). Sixty molars were cut to expose midcoronal dentin and prepared with a regular diamond bur. Each adhesive was applied either according to the manufacturer's instruction or with shortened time. Specimens were stored in distilled water at 37°C for 24 hours and then cut into resin-dentin sticks. Microtensile bond strength (μTBS) was tested after either 24 hours or 1 year of water storage. Data were analyzed by the three-way ANOVA and Duncan tests (α=0.05). Fracture modes were analyzed under a scanning electron microscope (SEM). One dentin stick per group was selected after fracture mode analysis and further observed using transmission electron microscopy (TEM). Six additional dentin discs were prepared and conditioned with each adhesive under the different application time to observe the adhesive-smear layer interaction by SEM.

Results: Shortened application time affected the μTBS (p<0.001) while storage time did not affect bond strength (p=0.187). A significant effect of shortened application time on μTBS was observed in the CU at 1 year and in the GP at both storage times.

Conclusions: One-year storage time had no effect on the μTBS of universal adhesives to

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DOI: 10.2341/17-205-L

bur-cut dentin. The performance of universal adhesives can be compromised when applied using a shortened application time.

INTRODUCTION

Current dental adhesives are being developed to be more user-friendly. Recently, a simplified application technique taking less time appears to be driving the attention for new product development. Universal adhesives are appealing to many clinicians because they can be applied by either self-etching or etch-and-rinse.[1,2] Previous studies have shown that bonding performance of universal adhesives was similar regardless of bonding technique both *in vitro*[2,3] and *in vivo*.[4,5] In the case of self-etching technique, they are similar to typical all-in-one self-etching adhesives. Recently, even the one-step self-etching adhesives appear to be too slow. G-Premio Bond, the newly developed product from GC Corp, claims that high bond strength can be achieved even when applied with reduced application time (optional manufacturer's recommendation from a Japanese brochure). However, a previous report has demonstrated some drawbacks of reduced application time on 24-hour resin-dentin bond strength.[6]

In general, the dentin surfaces in *in vitro* studies are prepared mainly by SiC paper and a diamond bur, although the diamond bur is more clinically relevant. In addition, due to different characteristics of the smear layer,[7,8] more impact on self-etching adhesives was observed when bonded to bur-cut dentin.[6,9] Furthermore, from a previous study, various-size pores were observed on the adhesive surface of the fractured dentin beams prepared by the diamond bur. Thus, a crucial effect on long-term adhesion was expected.[6] Regarding the longevity of restorations, long-term water storage has been used to evaluate the durability of the resin-dentin bond in the oral cavity.[10-12] This bond degradation was observed after long-term storage due to the combined effect of resin dissolution[10] and collagen degradation.[13] Therefore, the aims of this study were to evaluate the effects of application time and long-term storage time on resin-dentin microtensile bond strength (μTBS) of three universal adhesives to bur-cut dentin. The null hypotheses were that (1) the adhesive application time has no effect on the strength of the resin-dentin bonds of universal adhesives and (2) the resin-dentin bond strength of universal adhesives is not affected by storage time.

METHODS AND MATERIALS

Tooth Selection and Preparation

Sixty-six extracted noncarious human third molars were used. They were stored in an aqueous solution of 0.5% chloramine-T at 4°C and used within 6 months after extraction. The teeth were collected under a protocol reviewed and approved by the university ethics committee. The teeth were cut with a gypsum model trimmer under water cooling to expose the midcoronal dentin. A light microscope was used to confirm that no enamel remained on the dentin surface. A uniform dentin surface was prepared with the tapered regular grit diamond bur (diamond point FG, #103R, Shofu, Kyoto, Japan) in a high-speed handpiece with copious water spray and five light-pressure strokes per tooth.

Adhesive and Bonding Procedure

The teeth were randomly divided into three experimental groups according to the adhesives used: CU (Clearfil Universal Bond, Kuraray Noritake Dental Inc, Tokyo, Japan), SB (Scotchbond Universal Adhesive, 3M ESPE, St Paul, MN, USA), and GP (G-Premio Bond, GC Corp, Tokyo, Japan). Material compositions and details are provided in Table 1. The teeth assigned for each adhesive were further randomly divided into two subgroups (n=10). Each subgroup was bonded with the adhesive applied either according to the manufacturer's instructions or under a shortened application time (optional manufacturer's instructions for GP).[6] Two 2-mm layers of resin composite (Clearfil AP-X, Kuraray Noritake Dental Inc, Tokyo, Japan) were built up. Each layer was light-cured for 20 seconds using an Optilux 401 (Demetron/Kerr, Orange, CA, USA) at ≥ 550 mW/cm^2.

Microtensile Bond Strength Test and Fracture Mode Analysis

After storage of the bonded teeth in water at 37°C for 24 hours, each tooth was sectioned into beams (cross-sectional area approximately 1 mm^2) using an Isomet diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). Then three beams per tooth were randomly selected from the central portion of the crown. The mean bond strength obtained from these 3 beams was used for statistical calculation, resulting in 5 values per group. In total, fifteen beams from five teeth were tested immediately after sectioning (24-hour test), while the remaining 15 beams selected in the same manner were kept in distilled water at 37°C for 1 year (1-year test).

Table 1: *Adhesive System (Batch Number), Composition, and Application Procedures*

Adhesive (Batch Number)	pH*	Composition	Manufacturers' Instructions	Shortened Application Time
Clearfil Universal Bond (000002)	2.3	10-MDP, Bis-GMA, HEMA, ethanol, hydrophilic aliphatic dimethacrylate, colloidal silica, dl-camphorquinone, silane coupling agent, water	1. Apply the adhesive to the dentin surface with applicator brush and rub it for 10 s. 2. Dry the dentin surface sufficiently by gently blowing air for more than 5 s until the adhesive does not move. 3. Light cure for 10 s.	1. Drop the adhesive directly from the bottle on the surface. 2. Dry immediately by gently blowing air for 5 s. 3. Light cure for 10 s.
G-Premio Bond** (1411061G, 1510131)	1.5	10-MDP, 4 methacryloxyethyltrimellitate anhydrate, dimethacrylate monomer, distilled water, acetone, photo initiators, fine silica powder	1. Apply using a microbrush. 2. Leave undisturbed for 10 s after application. 3. Dry thoroughly for 5 s with oil-free air under maximum air pressure. 4. Light cure for 10 s.	1. Drop the adhesive directly from the bottle on the surface. 2. Dry immediately for 5 s under maximum air pressure. 3. Light cure for 10 s.
Scotchbond Universal (572054, 609623)	2.7	10-MDP, HEMA, silane, dimethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators	1. Apply adhesive on the surface and rub it for 20 s. 2. Gently air-dry the adhesive for approximately 5 s for the solvent to evaporate. 3. Light cure for 10 s.	1. Drop the adhesive directly from the bottle on the surface. 2. Dry immediately by gently blowing air for 5 s. 3. Light cure for 10 s.

Abbreviations: 10-MDP, 10-methacryloxydecyl dihydrogen phosphate; Bis-GMA, bisphenol A diglycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate.
** The pH for SB and CU was obtained from Ref 1. For GP, it was supplied by the manufacturer.*
*** The shortened application time is an optional application mode suggested by the manufacturer.*

The beams were fixed onto a Ciucchi's jig with cyanoacrylate glue (Model repair 2 Blue, Dentsply-Sankin, Otahara, Japan) and subjected to a tensile force at a crosshead speed of 1 mm/min in a desktop testing apparatus (EZ test, Shimadzu, Kyoto, Japan). μTBS was expressed in MPa, and data were analyzed by three-way ANOVA and Duncan tests (α=0.05). The fractured specimens were carefully removed from the jig and mounted on an aluminum stub, then coated with Pt-Pd for 150 seconds. The fracture modes were determined using SEM (S-4000, Hitachi, Tokyo, Japan) at an accelerating voltage of 10 kV with low magnification (80×). Fracture mode categories were classified and the specific features of fractured surfaces were further observed at high magnification (10,000×).[6]

TEM Observation of Resin-Dentin Interface

After fracture mode analysis, representative dentin beams from each subgroup having μTBS values close to the mean were further prepared for TEM observation.[14] Specimens were fixed overnight in 2.5% glutaraldehyde containing 0.1M sodium cacodylate buffer at pH 7.4, and then rinsed with the same buffer. Subsequently, the specimens were dehydrated in ascending grades of ethanol series and embedded in epoxy resin (Epon 812, Polysciences, Inc, Warrington, PA, USA). Sections of 75-nm to 90-nm thickness through the resin-dentin interface were obtained using a diamond knife (Diatome, Bienne, Switzerland) in an ultramicrotome (Ultracut, UCT, Leica, Vienna, Austria). The sections were observed with a transmission electron microscope (H-800, Hitachi, Tokyo, Japan) operating at 75 kV.

Observation of Dentin Surface Treated With Adhesives

To observe dentin surface characteristics after adhesives application, approximately 2-mm dentin discs of six additional teeth were prepared by using a diamond saw. The midcoronal dentin surfaces were prepared by a regular diamond bur as previously described. Each disc was assigned to one of six groups according to the adhesives (CU, SB, and GP) and application times (manufacturer's instructions vs shortened application time) involved. Immediately after applying with the specific adhesive and application time without light curing, the discs were immersed in 100% acetone for 1 minute to remove the applied adhesive,[15] dehydrated with ethanol, and dried with hexamethyldisilazane.[16] The surfaces were then examined under SEM (3,000×) to observe the morphological changes on dentin resulting from the various adhesive application protocols.

RESULTS

μTBS and Fracture Modes

There were no pretest failures in this study. There were significant effects of adhesive (F=47.740, p<0.001) and application time (F=31.199,

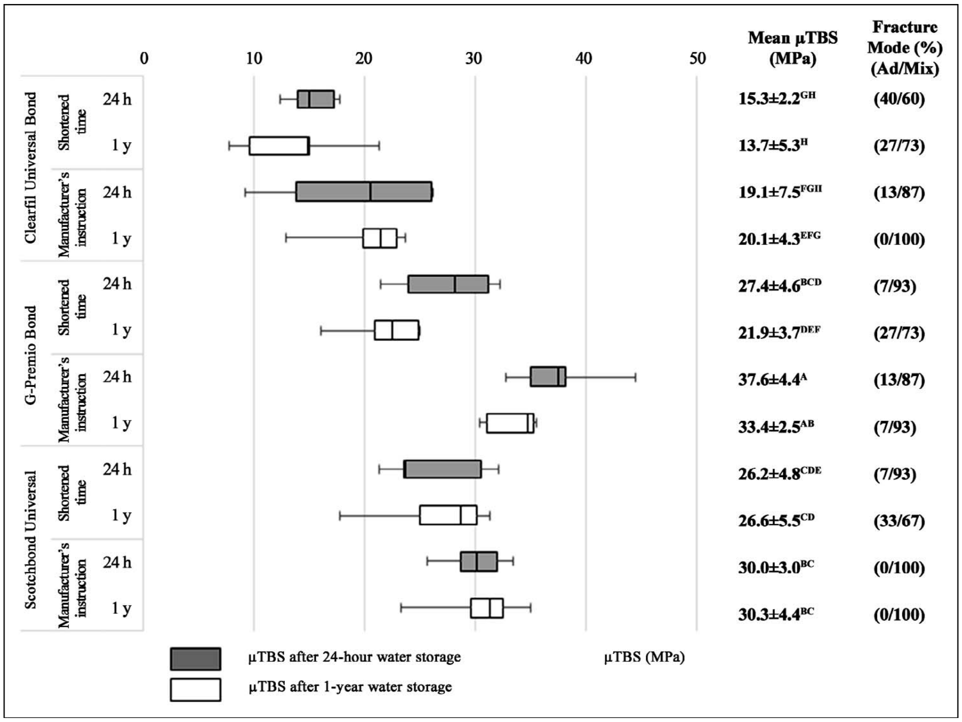

Figure 1. *Box-whisker plots of the µTBS to dentin and percentage of fracture mode. Different letters indicate statistically significant differences (*p*<0.05). S = shortened application time; MI = manufacturer's instructions; Ad = adhesive failure; Mix = mixed failure.*

$p<0.001$), but none of storage time (F=1.789, p=0.187). Only interaction between adhesive and application time was significant (F=3.387, p=0.042).

In general, higher µTBSs were observed when all the adhesives were applied according to the manufacturer's instructions for both testing periods of 24 hours and 1 year (Figure 1). At 24 hours, significantly higher bond strengths were observed for GP when applied with longer application time, and the significance was sustained after 1-year storage. Application time had no significant effect on either CU or SB at 24 hours. After 1-year storage, significant effects of application time were observed for both GP and CU, but not for SB. Storage time alone did not seem to significantly affect bond strength. When each combination of adhesive and application protocol was analyzed for the effect of storage time, no significant differences were observed. Similar bond strengths (range 23 to 36 MPa) were exhibited by GP and SB within each test time and application protocol. Conversely, CU always resulted in significantly lower bond strengths for each of the tested subgroups.

The most frequent fracture mode was classified as mixed failure wherein the failure was observed within the adhesive layer with some part of cohesive failure in dentin (Figure 1). Representative SEM images of the adhesive layer from the fractured surfaces at high magnification (10,000×) are shown in Figure 2. Various size porosities were detected in all tested groups. They were uniformly distributed on the entire surface of the adhesive layer except those in SB. A pore-free area was observed when applied with SB according to the manufacturer's instructions (Figure 2f and 2l). Greater size of porosities was detected in GP followed by CU and SB, respectively. They were predominately round, having a diameter of submicron size. Only a few of those in GP were above 1-2 µm. The porosities were smaller when applied to the adhesives having the longer application time. No difference was detected between the fractured beams at 24 hours and at 1-year storage.

TEM Observation of Resin-Dentin Interface

Hybrid layers ranging from 200 nm to 1000 nm thick were observed for all adhesives at the different testing conditions (Figure 3,4). One interesting finding was the striking difference observed in the adhesive layer when 24 hours was compared with 1-year images for all groups. The dense presence of fillers that can be observed in all images at 24 hours seemed to disappear in the specimens after 1 year of storage (comparing 24 hours with 1-year images for

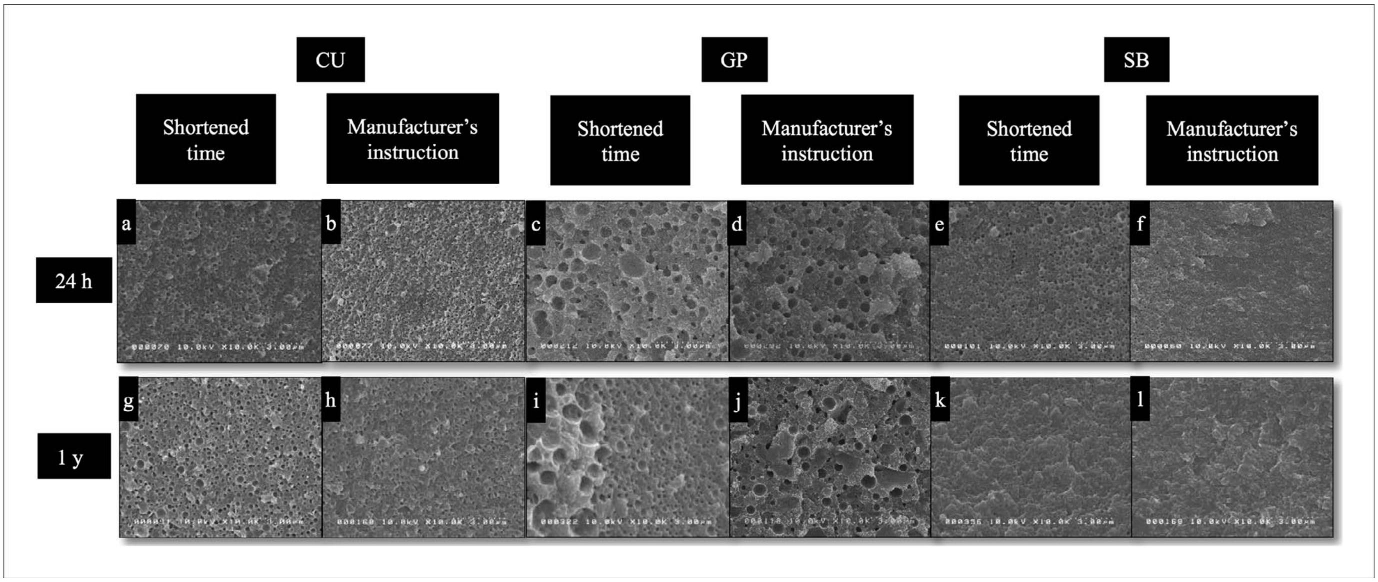

Figure 2. *Representative SEM images of adhesive layer from fractured beams at high magnification (10,000×).*

each group, Figures 3 and 4). With the exception of GP, in which the presence of some remaining fillers could be detected after 1 year, storage in water for 1 year rendered the adhesive layer devoid of fillers, and voids could be observed where the fillers were before. Some voids entrapped in the adhesive layer were detected in the GP (Figure 3e)

Observation of Dentin Surface Treated With Adhesives

Remnants of the smear layer were observed in all groups regardless of application time (Figure 5). An intact smear layer was observed when CU and SB were applied with a shortened application time (Figure 5a,c), whereas partial loss of the smear layer was detected when GP was used (Figure 5b). Conversely, application of the adhesives according to the manufacturer's directions clearly resulted in further dissolution of the smear layer and exposure of the underlying dentin, including some collagen fibrils (Figure 5d-f).

DISCUSSION

In this study, higher bond strength was demonstrated in all universal adhesives using the manufacturer's recommended time compared with those with shortened application time. However, statistically significant differences ($p<0.05$) were noticed for GP (at both storage times) and CU (at long-term storage). Therefore, the first null hypothesis that application mode would not affect bond strength of the adhesives was rejected. After 1 year storage, the μTBS of all adhesives tested were not statistically significantly different from those having 24-hour storage. Therefore, the study failed to reject the second null hypothesis that resin-dentin bond strength of universal adhesives would not be affected by storage time ($p=0.187$).

All adhesives showed higher μTBS when applied using the manufacturer's recommended time. This is in line with previous studies.[6,17] However, a significant effect of application time was observed in three out of six pairs (Figure 1). This might be explained by the different material compositions, adhesive-smear layer interaction, and method of application. According to the adhesives' composition, GP is acetone based whereas CU and SB are ethanol-water based (Table 1). Due to the high vapor pressure of acetone and the recommended burst of air, longer application time enhanced solvent evaporation in the GP, resulting in better integrity of the adhesive layer (compare Figure 2c,i vs 2d,j). This could be the reason why the significant effect of application time on μTBS was observed in the GP.

Regarding ethanol-based adhesives, when water is added as a solvent to ethanol-solvated monomer, water and ethanol can form a hydrogen bond both with each other and with the monomers.[18] In addition, the vapor pressure of ethanol is lower than that of acetone.[19] Therefore, it is likely that the shortened application time was insufficient for the solvent to evaporate from the ethanol-water–based adhesive. Nevertheless, the significant effect of reduced application time on the bond strength was

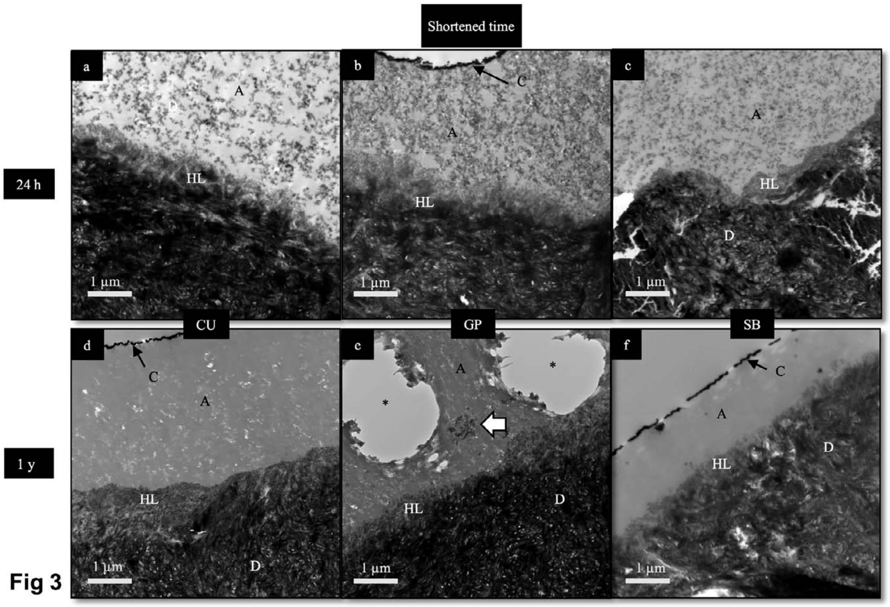

Fig 3

Figure 3. *Representative TEM images of dentin beams with shortened application time (10,000×). Pointer indicates remaining fillers in GP after box-whisker plots storage. Asterisks indicate voids entrapped in adhesive layer in GP. A = adhesive resin; HL = hybrid layer; D = dentin; C = coated Pt-Pd.*

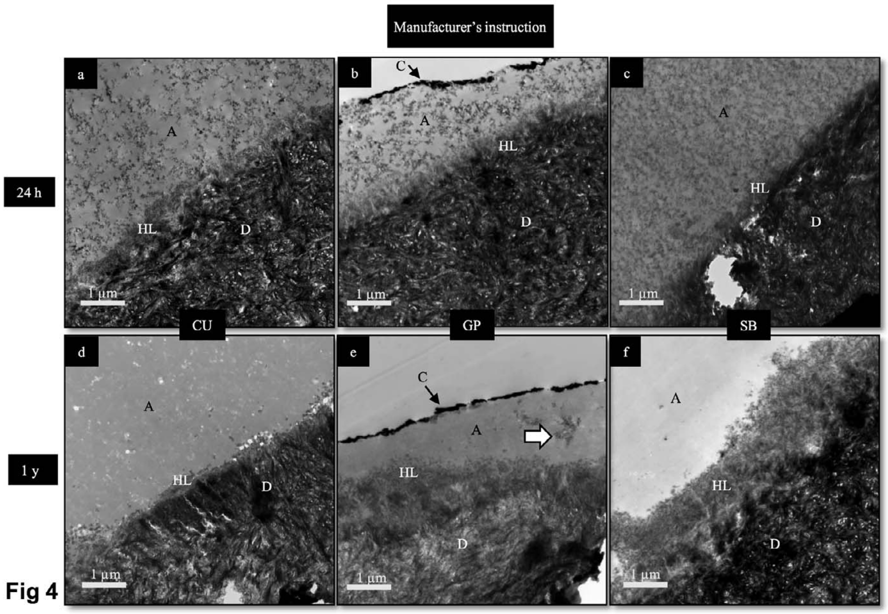

Fig 4

Figure 4. *Representative TEM images of dentin beams with manufacturer's recommended time (10,000×). Pointer indicates remaining fillers in GP after box-whisker plots storage. A = adhesive resin; HL = hybrid layer; D = dentin; C = coated Pt-Pd.*

revealed after 1 year in CU. Probably, the polymerization was compromised by residual water[20] and solvent,[21,22] which then could have accelerated the degradation of the resin-dentin bond over time.

In the case of SB, the µTBS was not affected by shortened application time or long-term storage. Since nano-layering formation of 10-MDP-Ca salts at the adhesive interface has been demonstrated with commercially 10-MDP-containing adhesives,[23,24] this nano-layering is thought to enhance a water-stable interface and contribute to bond durability.[23-26] So far, SB is the only universal adhesive for which the presence of nano-layering has been reported.[23] Conversely, it has been reported that nano-layering can be inhibited by the presence of 2-HEMA.[27] Moreover, the presence of nano-layering has not being identified at the resin-dentin interface created by most of the latest generation of universal adhesives.[28] Therefore, the claim that nano-layering of 10-MDP-Ca salts contributes to bond durability of the resin-dentin interface has recently been questioned.[28,29] However, from a previous report, the nano-layering formation in SB has been observed in a location

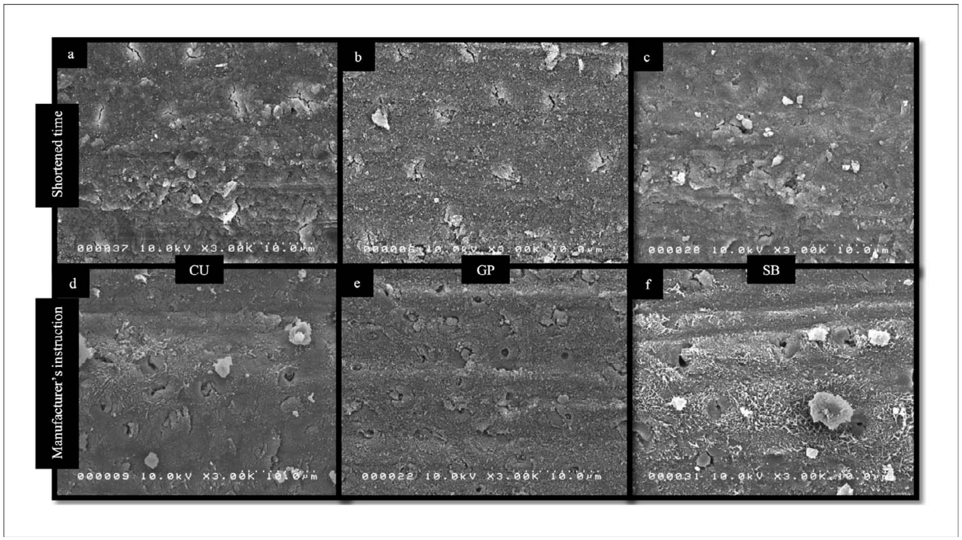

Figure 5. *Representative SEM images (3000×) of bur-cut dentin conditioned with each adhesive when applied with shortened time (a-c) and according to manufacturer's instructions (d-f).*

above the hybrid layer.[23] We speculate that this nano-layering might be responsible for improving the cohesive strength of the adhesive layer itself rather than prevent the degradation of the resin-dentin interface. In addition, chemical bonding between polyacrylic acid monomer (Vitrebond copolymer) and hydroxyapatite has also been reported.[30] Vitrebond copolymer is incorporated in SB (Table 1), and a recent study has demonstrated the chemical bond between Vitrebond copolymer containing adhesives and synthetic hydroxyapatite.[31] Therefore, it is possible that these two chemical bonding mechanisms might have contributed to the stable bond strength of SB in this study.

Another study demonstrated an increase in mineral dissolution from dentin when the application time of GP was increased to 10 seconds, compared with those of no waiting time.[17] This also supported the result of the current study since all adhesives demonstrated the ability to dissolve the smear layer more efficiently when applied with a longer time (Figure 5). However, the smear layer remained in all groups regardless of application time. This indicated that the smear layer was incorporated into the hybrid layer when this adhesive was applied in self-etching mode. Based on the pH (Table 1), GP was classified as an intermediately strong self-etching adhesive.[32] The etching ability of GP was more pronounced (Figure 5b) than the others, which are mild, self-etching adhesives (Figure 5a,c). Conversely, when adhesives were applied according to the manufacturer's instructions, the collagen fibrils were observed in CU (Figure 5d) and SB (Figure 5f) compared with GP (Figure 5e). This is possibly due to the different method of adhesive application. The GP was left undisturbed for 10 seconds according to the manufacturer's directions, whereas CU and SB were recommended to be rubbed for 10 and 20 seconds, respectively (Table 1). Previous studies demonstrated that rubbing application enhanced smear layer removal[33] and improve water evaporation, which led to less porosity in the adhesive layer[34-36] and improves resin monomer infiltration into dentin.[37] In the previous study,[6] resin tags had penetrated more deeply into the dentin when these adhesives were applied for the longer period, thus supporting the current findings. Regarding the smear layer, Pashley and Carvalho[38] suggested that the dentin smear layer impeded adhesion of the self-etching primer. Moreover, as the compromised bond strength from the bur-cut smear layer has already been reported,[6-8,15,39] the longer adhesive-smear–layer interaction time, combined with the rubbing application, could have induced the acidic primer to dissolve and penetrate through the smear layer to form a stronger bond to the underlying dentin.

In the present study, μTBS beams were cut and exposed directly to distilled water for 1 year to accelerate aging,[40] although this did not affect the dentin bond strength of the tested adhesives. Furthermore, microporosities of various sizes were observed on the fractured surfaces by SEM at high magnification (Figure 2). As previously discussed,[6] the porosities represent entrapped solvent and water that could not evaporate due to the limited amount of time allowed. Although porosities were observed for all adhesives in the SEM analysis, they were detected in GP only during TEM observation (Figure 3e). This might be due to the limited area of TEM observation and the larger-size porosities encountered with GP.

The presence of the porosities was confirmed from both SEM and TEM observations, and the porosity sizes matched (Figure 2c,d ,j and Figure 3e). In the current study, less porosity at the interface due to solvent evaporation was observed when the adhesives were applied for the longer time. For SB, however, the amount of residual porosity and related amount of residual solvent did not appear to have affected the resultant bond strength. A previous study[41] showed that bond strengths were also not different when SB was applied and experimentally had its solvent evaporated 50% versus 100%, suggesting that residual solvent had no effect on bond strength, such as were the findings of this study.

According to TEM observation, de-bonding of silica particles were detected in long-term storage groups (Figure 3d-f and Figure 4d-f). Some remaining filler can be observed in GP (Figure 3e and 4e). Filler debonding of hydrophilic adhesives was caused by hydrolysis of silane coupling agent.[14,42] GP is HEMA-free adhesive, thus expected to be less hydrophilic than the others and therefore, the filler detachment was less pronounced when compared to SB and CU (Figure 3d, f and Figure 4d, f). Surprisingly, according to μTBS test (Figure 1), filler de-bonding after long-term storage appears not to affect adhesive bond strength. Further study on the matter is warranted.

Our previous study[6] was the first to demonstrate the presence of such porosities within the adhesive layer of these universal adhesives. Because that study tested bond strength only after 24 hours, the findings prompt us to speculate that such a porous adhesive layer could favor water sorption and promote quick degradation of the adhesive, adversely affecting bond strength. Accordingly, it was expected in this follow-up study that the μTBS of the universal adhesives would be significantly decreased after long-term storage.[6] Nevertheless—and surprisingly—the 1-year storage did not influence the bond strength of the adhesives tested, regardless of the reduced application time of the experimental conditions. It is unclear whether the porosities in the adhesive layer actually increased water sorption, and if it did, why the expected softening and consequent weakening of the adhesive polymer did not become evident with resultant compromise of the bond strength. The higher percentage of mixed failures indicates that the bond to dentin was strong, even after the 1-year storage. Previous studies also evaluated the effect of aging on the bonding performance of universal adhesives and found that the bond strengths were stable after both 6-month storage[11] and 5000 cycles of thermocycling.[3] Based on the limitations of this study, one might not expect that 1-year storage would be sufficient to demonstrate the effect of bond degradation on universal adhesives. Further studies should be performed with a longer term to observe the effect of long-term storage.

CONCLUSIONS

Reduced application time caused significant reduction of bond strength of some adhesives and, therefore, is not warranted. Water storage for 1 year did not affect the bond strength of any of the adhesives to bur-cut dentin.

Acknowledgements

We thank Dr J De Munck and Assoc Prof Yoshihara for their advice on TEM. All materials in this study were supported by Kuraray Noritake Dental, Inc, and GC Corp. The study was partially supported by UBC Start-Up Funds (RMC) and Grants-in-Aid for Scientific Research 26293403.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Hokkaido University ethics committee. The approval code for this study is 2013-7.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

(Accepted 2 October, 2017)

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