Effects of remaining dentin thickness, smear layer and aging on the bond strengths of self-etch adhesives to dentin

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This study evaluated the effects of remaining dentin thickness (RDT), different smear layers, and aging on the microtensile bond strength (µTBS) of universal adhesives to dentin when applied in self-etch mode. Ninety-six human third molars were randomly allocated to 12 groups (n=8) based on adhesives: Clearfil SE Bond 2 (SE, control), Clearfil Universal Bond (CU) and ScotchBond Universal Adhesive (SB); smear layers: prepared either with 600-grit SiC paper (P) or regular diamond bur (B); and aging: stored in distilled water at 37°C for 24 hours (24h) or 1 year (1y). µTBS was significantly affected by the type of adhesives, smear layers, and aging (p<0.001). A statistically significant and positive linear relationship was also observed between µTBS and RDT (p<0.05) in all the tested groups, except for SEB1y and CUB24h (p>0.05). RDT, smear layer types, and aging can influence the bonding performances of universal adhesives when applied in self-etch mode.

Keywords: Dentin, Smear layer, Aging, Bond strength, Self-etch Adhesive

INTRODUCTION

The development of the dentin bonding technique has already brought positive changes in clinical success¹, and the newer adhesive systems are likely to result in even more². The conventional multicomponent etch-and-rinse adhesives have gradually been replaced by more user-friendly, simplified, monocomponent self-etch adhesives³. A more simple yet versatile version—the universal adhesive that can be used in both etch-and-rinse and self-etch mode—is now available for clinical use. With these systems, clinicians are enjoying the liberty of choosing their adhesive strategy based on the dental substrates⁴ and their preferences⁵,6. Moreover, the elimination of a separate priming step reduces the application time and minimizes the possibility of restorative failure that can result from technique sensitivity⁶,7. As a result, universal adhesives are quickly gaining popularity among the clinicians⁸, and their bonding performance to dentin has become the center of attraction of in vitro studies, employing different modifying factors such as mode of application⁹,10, the type of substrates⁴, their smear layers⁵,10, and aging¹,12.

The bonding of universal adhesives to enamel showed reliable outcomes⁴. However, the humid and organic nature of dentin puts inherent challenges to bonding, primarily due to an increase in the number of tubules and consequent increase in dentin wetness and permeability¹³,1⁴, when deep dentin (i.e., thin remaining dentin thickness) is approached. In coronal dentin, the number of tubules per area varies from 8,000 (superficial or thick remaining dentin) to 58,000/mm² (deep or thin remaining dentin). The regional and thickness variability of dentin not only relates to the bond strength¹²,16-18, but also to the biologic reactions from the pulp¹⁵ to restorative procedures. In clinical situations, it is not unusual that young patients fracture teeth or even develop carious lesions, exposing deep dentin. Universal adhesives must contain water for dissociation of the acidic functional monomers, that makes self-etching possible¹⁹. The residual water after the drying step could further affect the bonding outcomes. Besides, resin-based materials (e.g., adhesive systems) release toxic components, which can diffuse to the pulp through the dentinal tubules²⁰ and negatively affects the cells’ metabolism and pulpal reaction. Dentin thickness impacts the amount of adhesive components and by-products that penetrates pulpal tissues²¹.

According to Van Meerbeek et al.²³, despite the high-product dependency, both etch-and-rinse and self-etch mode have performed successfully for dentin bonding in both laboratory and clinical research. However, the current evidence has pointed out that adequate bonding to dentin can be achieved with the self-etch mode²², indicating that a more technique sensitive etching step is redundant for dentin bonding. Therefore, recent studies aiming to evaluate the dentin bonding performance of new adhesive systems have mostly employed self-etch mode²⁴. However, to date, no study has assessed the effect of RDT on the bond strength of universal adhesives. Moreover, all previous studies that evaluated the effects of RDT have employed 600-
griit silicon carbide paper (P) for creating standardized dentin smear layers\(^2,^{16,23}\). Therefore, clinicians are often comparing these results with clinical substrates, which are markedly different\(^10,^{24}\). So far, no study has evaluated the effect of RDT of bur-prepared (B) dentin.

Within the oral cavity, degradation of bonding may result from biofilm attack, hydrolytic degradation of adhesives, enzymatic degradation by matrix metalloproteinases (MMP), and adhesive fatigue\(^25\). The effects of hydrolytic degradation and MMP can be more detrimental, particularly in deep dentin\(^26\). Hence, determination of the durability of the universal adhesive, especially when bonded to bur-prepared deep dentin, is of paramount importance to assume a firsthand idea about their clinical success. In laboratory settings, this could be done by prolonging water-storage\(^27\).

Therefore, this study aimed to evaluate the effects of remaining dentin thickness (RDT) and different smear layers on the microtensile bond strength (µTBS) of universal adhesives to bur-prepared dentin after 24 h and long-term water-storage and to compare their performance with a two-step self-etch adhesive. The null hypotheses tested were: (1) RDT, (2) type of smear layers, and (3) aging would not affect the µTBS of the tested adhesives to dentin.

### MATERIALS AND METHODS

#### Tooth collection and preparation

Extracted non-carious human third molars (n=96) were used in this study employing eight teeth per tested group (n=8)\(^28\). The teeth were collected after patients' informed consent, stored immediately in 0.5% chloramine-T at 4°C, and used within six months of extraction. The Ethical Committee of Hokkaido University Faculty of Dentistry approved this study (#2013-7).

#### Bonding procedures for µTBS test

The adhesive systems used in this study are listed in Table 1. Flat dentin surfaces were exposed by using a gypsum model trimmer (Model Trimmer, MT-7, J Morita, Tokyo, Japan) under water coolant. All teeth were then randomly allocated to two groups according to the dentin surface preparation methods: dentin surfaces prepared manually using 600-grit SiC (P; Sankyo-Rikagaku, Saitama, Japan) for 60 s under running water or with regular grit diamond bur (B; Shofu, Kyoto, Japan) in a high-speed handpiece with copious water using five light-pressure strokes\(^9\). Then, each group was further divided into three subgroups according to the adhesives used: Clearfil™ SE Bond 2 (SE; control; Kuraray Noritake Dental, Okayama, Japan), Clearfil™ Universal Bond (CU; Kuraray Noritake Dental, Okayama, Japan) and ScotchBond™ Universal Adhesive (SB; 3M ESPE, St. Paul, MN, USA). The adhesives were then applied as per the manufacturer's instructions. Then, approximately 4-mm thick resin composite build-up (Clearfil™ AP-X, Kuraray Noritake Dental) was done, followed by light curing (Optilux 401, Demetron/Kerr, Orange, CA, USA) at 600 mW/cm\(^2\) for 40 s. All the bonded teeth were then stored in distilled water at 37°C for 24 h.

#### RDT measurement and µTBS test

After water-storage, following the procedure described by Pegado \textit{et al.}\(^29\), the pulp chamber of each bonded tooth was exposed from apical direction and filled with resin composite (Clearfil™ AP-X) employing SE as the bonding agent. This procedure was done to increase the

### Table 1  Materials used in the study

| Material, (Code, Manufacturer/ Lot No.) | Adhesive type | Composition | Manufacturer’s instructions |
|---------------------------------------|---------------|-------------|----------------------------|
| Clearfil™ SE Bond 2 (SE; Kuraray Noritake Dental, Okayama, Japan/000014) | Two-step self-etch | Primer: 10 MDP, HEMA, Hydrophilic aliphatic dimethacrylate, dl-camphorquinone, Water. Bond: 10 MDP, Bis-GMA, HEMA, Hydrophobic aliphatic dimethacrylate, dl-camphorquinone, Initiators, Accelerators, Silanated colloidal silica. | 1. Apply the primer and leave for 20 s. 2. Gentle air-blowing for >5 s. 3. Apply the bond. 4. Gentle air-blowing to make the film uniform. 5. Light-cure for 10 s. |
| Clearfil™ Universal Bond (CU; Kuraray Noritake Dental, Okayama, Japan/000002) | Universal | 10-MDP, Bis-GMA, HEMA, Hydrophilic aliphatic dimethacrylate, Colloidal Silica, Silane coupling agent, dl-camphorquinone, Ethanol, Water. | 1. Apply adhesive and rub it in for 10 s. 2. Gently air-dry for >5 s for the solvent to evaporate. 3. Light-cure for 10 s. |
| ScotchBond™ Universal Adhesive (SB; 3M ESPE, St, Paul, MN, USA/609889) | Universal | 10-MDP, Vitrebond™ Copolymer, HEMA, Dimethacrylate resins, Filler, Ethanol, Water, Initiators, Silane. | 1. Apply adhesive and rub it in for 20 s. 2. Gently air-dry for approximately 5 s for the solvent to evaporate. 3. Light-cure for 10 s. |

10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; Bis-GMA: bisphenol-A-glycidyl methacrylate.
beam lengths and to ease the fixation with the jig for the µTBS test. Each bonded tooth was sectioned into 1×1 mm² beams using a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) under running water. At least 10 resin-dentin beams were selected from each tooth for the µTBS test. Half of the beams from each tooth were tested after 24 h of water-storage (24h) at 37°C, while the rest of the beams were kept for aging in distilled water at 37°C for 1 year (1y) and then tested. During 1 year of storage time, the water was changed weekly. This resulted in 12 experimental groups (n=8) based on adhesive, dentin surface preparation, and storage time. Before the µTBS test, the RDT of each beam was measured with a digital caliper (Absolute Digimatic, Mitutoyo, Kanagawa, Japan) with 10 µm accuracy. The average RDT of each beam was determined following the procedures as described by Ting et al.10. The measured beams were categorized into either deep dentin (D) when RDT was 0.5–1.50 mm or superficial dentin (S) when RDT was 1.51–3.00 mm.

For the µTBS test, each bonded beam was attached to a Ciucci’s jig with a cyanoacrylate adhesive (Model Repair II Blue, Dentsply-Sankin, Tokyo, Japan) and was subjected to a tensile force at a crosshead speed of 1 mm/min in a universal testing apparatus (EZ test, Shimadzu, Kyoto, Japan), until failure occurred. To prevent drying during the µTBS test, each beam was tested within 5 min after removal from water-storage. The fractured beams during the µTBS test, each beam was tested within 5 min in a universal testing apparatus (EZ test, Shimadzu, Kyoto, Japan), until failure occurred. To prevent drying during the µTBS test, each beam was tested within 5 min after removal from water-storage. The fractured specimens were then removed from the jigs, and their cross-sectional area at the dentin halves was measured. The tensile load recorded at the failure of each beam was then divided by the cross-sectional area of that beam to retrieve the µTBS in MPa. The mean µTBS of at least 5 beams derived from each tooth represented the µTBS of that tooth, generating 8 values for each group.

SEM observation of the fractured beams
For SEM observation of the failure mode, the fractured specimens were prepared following a protocol described by Saikaew et al.9. Both halves of the fractured beams were room dried for 24 h. They were then fixed on aluminum stubs and coated with Pt-Pd alloy (E-1030, HITACHI, Tokyo, Japan) for 150 s. Failure modes were determined by using a scanning electron microscope (SEM; S-4000, HITACHI) at an accelerating voltage of 10 kV. First, all the surfaces were examined at lower magnification (×80) to classify the mode of failure. Special features were further observed at ×800 and ×8,000 to classify the mode of failure. Failure modes were classified as: A, adhesive failure; CC, cohesive failure within resin composite; CD, cohesive failure within dentin; or M, mixed failure.

Statistical analysis
The Shapiro-Wilk test confirmed the normality of all µTBS data. Levene’s test was done to verify the homogeneity. A three-way ANOVA was done to determine the effects of adhesives, smear layers, and aging on the µTBS. Multiple comparisons were made with Duncan’s post-hoc test. Pearson’s correlation test was done to determine the correlation between µTBS and RDT values. All statistical analysis was done using IBM SPSS version 22.0 (SPSS Statistics 22.0, SPSS, Chicago, IL, USA), and the significance was set at α=0.05.

RESULTS
Three-way ANOVA revealed that µTBS was statistically significantly affected by the types of adhesives (F=97.762, p<0.001), smear layers (F=29.033, p<0.001) and aging (F=179.916, p<0.001). The interaction between these factors was also statistically significant (F=4.008, p<0.05).

The µTBS results were shown in Table 2. Overall, bonding with superficial dentin yielded higher bond strength values than deep dentin, although significantly only in SE24hP, CU24hP, CU1yP, and CU24hB. Regardless of aging and RDT, bond strengths of the tested adhesives were higher when bonded to SiC-prepared dentin than their bur-prepared counterparts.

| Adhesive | Superficial dentin (S) | Deep dentin (D) | Superficial dentin (S) | Deep dentin (D) |
|----------|------------------------|-----------------|------------------------|-----------------|
|          | SiC-prepared (P)       | Bur-prepared (B) | SiC-prepared (P)       | Bur-prepared (B) |
|          | 24h | 1y | 24h | 1y | 24h | 1y | 24h | 1y |
| SE       | 61.4±3.2(17/0/0/83)    | 47.3±1.7(25/0/75) | 52.2±2.0(17/0/0/83) | 43.0±1.7(36/0/0/64) | 56.2±3.0(33/0/0/67) | 42.8±2.3(46/0/0/54) | 52.9±6.4(25/0/75) | 40.1±2.1(42/0/0/58) |
| CU       | 50.0±6.5(26/11/0/63)   | 35.8±2.6(25/0/75) | 44.2±3.6(30/0/0/70) | 28.0±1.9(36/0/0/64) | 38.9±4.5(14/5/0/81) | 33.6±3.1(42/0/0/58) | 34.4±1.9(37/0/0/63) | 31.0±3.5(45/0/0/55) |
| SB       | 46.9±4.9(33/6/0/61)    | 37.0±3.7(27/0/73) | 41.7±3.3(36/0/0/64) | 31.2±1.5(48/0/0/52) | 41.4±4.7(29/0/0/71) | 31.2±2.4(33/0/0/67) | 36.6±3.6(31/0/0/69) | 27.1±1.0(45/0/0/50) |

Values with different lowercase superscripts indicate statistically significant difference (Duncan’s post-hoc test, p<0.05).

*A: Adhesive failure; CC: Cohesive failure within resin composite; CD: Cohesive failure within dentin; M: Mixed failure.
The relation between µTBS and RDT

Pearson’s correlation test demonstrated a linear relationship between µTBS and RDT (Fig. 1) in all the tested groups. The direction of the relationship was positive, meaning greater RDT was associated with greater µTBS. Except for SEB1y and CUB24h, the relationships were significant ($p<0.05$), and the magnitudes of the associations were moderate to strong ($r=0.3–0.8$).

SEM observation of the fractured beams

SEM images taken at ×80 revealed that after 24 h, the fracture mode was mainly mixed, irrespective of RDT, smear layers, and adhesives (Figs. 2 and 3). The percentage of fracture modes were summarized in Table 2. After 1 year, though the percentage of mixed failure was more prevalent, the percentage of adhesive failures increased, especially in the case of bur-prepared dentin.

At ×800, open and occluded dentinal tubules could be seen (Fig. 2(a-f)-2D(a-f) and Fig. 3A(a-f)-3D(a-f)). The number of open tubules seemed to increase with the increasing depth of dentin (Fig. 2C-a) and aging (Figs. 2D-a, c; 3D-a, e) and decreased with bur-prepared dentin (Figs. 3A-a, c, e; 3B-c, e). At ×8,000, collagen structures could be seen (Figs. 2A-b, d, f; 2B-d; 3A-b, d, f; 3B-b, d, f), which were clearer in deep dentin (Figs. 2C-b, d, f; 3C-b, d, f) and became less conspicuous with aging. The presence of more open dentinal tubules indicated failure at the bottom of the hybrid layer. In contrast, more occluded tubules would indicate that failure has taken place at the top of the hybrid layer. After 1 year water-storage, loss of resin resulted in an increased number of open dentinal tubules, and exposure of more collagen fibrils, particularly with increasing depth of dentin.

**DISCUSSION**

Several previous studies focused on the effects of RDT on the bond strength of one-step self-etch adhesives when applied in self-etch and etch-and-rinse modes. However, the observations of these reports are contradictory, probably because of compositional differences of the materials leading to material-dependency on their bonding effectiveness. Pereira et al. evaluated the effects of RDT and intrinsic wetness on the bond strengths of two two-step adhesives, applied in etch-and-rinse and self-etch mode with or
Fig. 2 Representative SEM images of the failure modes of adhesives bonded to SiC-prepared dentin (×80–8,000). Row A—failure modes with superficial dentin after 24 h; row B—superficial dentin after 1 y; row C—deep dentin after 24 h; row D—deep dentin after 1 y. Column a, c and e show images were taken at ×800 with the inset images taken at ×80. Column b, d, and f show images taken at ×8,000. A predominance of mixed failures can be seen at ×80. A-a, c: failure at the top of the hybrid layer (rectangle mark); A-e: failure at the bottom of the hybrid layer (oval mark); A-b, d, f and C-b, d, f and D-d. f. collagen is surrounded by resins (arrow); A-c, D-a, D-c, D-e: tubules are partially or entirely occluded with resin (zigzag mark).

Fig. 3 Representative SEM images of the failure modes of adhesives bonded to bur-prepared dentin (×80–8,000). Row A—failure modes with superficial dentin after 24 h; row B—superficial dentin after 1 y; row C—deep dentin after 24 h; row D—deep dentin after 1 y. Column a, c and e show images at ×800 with the inset images taken at ×80. Column b, d, and f show images were taken at ×8,000. A predominance of mixed failures can be seen at ×80. A-a, c: failure at the top of the hybrid layer (rectangle mark); A-e, C-a: failure at the bottom of the hybrid layer (oval mark); A-b, d, f and B-b, d, f and C-b, d, f and D-d: collagen was probably pulled from the overlying resin and recoiled back after debonding (arrow), A-a, c and B-c, d and C-a, b, d and D-a, b, d: dentinal tubules were occluded, and resin tags are also found (zigzag mark).
without pulpal pressure and dentin dried in a desiccator. Interestingly, they found that in the etch-and-rinse mode, the tested adhesive showed a significant decrease in bond strength with dentin at the pulp horn region. However, no significant difference was found in the self-etch mode. Toledano et al.\textsuperscript{34}, on the other hand, observed significantly higher bond strength with deep dentin by using a two-step adhesive in etch-and-rinse mode.

Contradictory to these results, Yoshikawa et al.\textsuperscript{25} reported that in the case of Clearfil SE Bond (two-step self-etch adhesive) in self-etch mode, \( \mu \text{TBS} \) increased with the increase of RDT, whereas, Single Bond (two-step) in etch-and-rinse mode showed no significant difference in \( \mu \text{TBS} \) for any RDT. Pegado et al.\textsuperscript{29} and Zhang et al.\textsuperscript{30} demonstrated that bond strength obtained with superficial dentin was significantly higher than that of deep dentin for both one-step and two-step systems when applied in etch-and-rinse and self-etch mode. However, Ting et al.\textsuperscript{12,16} demonstrated that bond strengths of the tested one-step self-etch adhesives were affected by RDT, but the bond strength of Clearfil SE Bond was independent of RDT. Moreover, thermocycling had a more detrimental effect on bonding with deep dentin. But so far, similar evaluations are not available for universal adhesives.

Furthermore, only 600-grit silicon carbide paper was used for smear layer preparation in all these previous studies. Their results showed that the bond strengths of the adhesives were affected by RDT when dentin was prepared by 600-grit SiC\textsuperscript{12,16,17}. However, clinical substrates are different than SiC-prepared dentin. Therefore, in the current study, we evaluated the bond strengths of universal adhesives by bonding with 600-grit SiC-prepared dentin and compared them with more clinically relevant regular diamond bur-prepared dentin\textsuperscript{16,24}. Our results revealed that irrespective of the type of smear layer, in general, superficial dentin showed higher bond strengths (Table 2). Pearson correlation test confirmed that the correlation between RDT and \( \mu \text{TBS} \) was positive in all the combinations tested (Fig. 1). The relationships were significant \((p<0.05)\) and moderate to strong in all the tested conditions except for SEB\textsubscript{1y} and CUB\textsubscript{24h} \((p>0.05)\). These results rejected our first null hypothesis that the thickness of the remaining dentin would not affect the \( \mu \text{TBS} \) of the tested adhesives.

All self-etch adhesives contain water as an ionizing medium to eliminate a separate etching step. The amount of water and solvent has been further increased in single component systems\textsuperscript{9}. Too much water can degrade the chemistry of these systems leading to decreased shelf-life. Moreover, as complete removal is difficult to achieve during the air-drying step, the residual water contributes to phase separation of monomers, incomplete adhesive polymerization, and increased hydrolysis after polymerization leaving a generally compromised adhesive interface\textsuperscript{16}. Besides, these adhesives’ decreased viscosity can increase the degree of oxygen diffusion and reduce the degree of monomer conversion at the uppermost surface\textsuperscript{30}. Furthermore, one-step self-etch adhesives are sensitive to the wetness of deep dentin\textsuperscript{20}. Due to the increased permeability of deep dentin, pulpal fluids could compromise the degree of conversion of adhesives\textsuperscript{49}, jeopardizing the bonding to dentin. Also at deep dentin, water sorption becomes aggravated because the incompletely polymerized resin is not able to block the osmotic effect. The consequences are the plasticization of polymers, increased solubility, and decreased modulus of elasticity\textsuperscript{41}. Eluted monomers and adhesive by-products, especially the low-weight molecules, can diffuse into dentin tubules and reach the pulp tissue\textsuperscript{20}. Those substances can trigger mild to severe pulpal inflammation, alter the metabolism of pulp cells, interfere in cell growth, and alter cell morphology, among other detrimental effects\textsuperscript{20,21,42}. Thinner remaining dentin is more prone to diffuse molecules, and less prone to protect the pulp against toxic substances\textsuperscript{21,49}.

In the current study, our results have proven that the type of smear layers \((F=29.033, p=0.000)\) and aging \((F=179.916, p=0.000)\) also exerted significant effects on the \( \mu \text{TBS} \) values. These observations reject our second and third null hypotheses. Previous studies have also demonstrated the impact of different types of smear layers (SiC and bur-prepared) on the bond strengths of self-etch adhesives\textsuperscript{41,49}. However, the current study has pioneered in evaluating these effects against the RDT and aging with universal adhesives. Regardless of aging and RDT, in our study, the bond strengths of the tested universal and two-step adhesives were higher with SiC-prepared dentin than their bur-prepared counterparts, though significantly higher in the case of SE\textsubscript{24hP}, SE\textsubscript{1yP}, CU\textsubscript{24hP} and CU\textsubscript{24hB} \((p<0.05)\). Moreover, the correlations between RDT and \( \mu \text{TBS} \) were comparatively stronger in 600-grit SiC-prepared groups than their bur-prepared counterparts (Fig. 1). The use of SiC paper produces a looser smear layer, tends to have more open dentinal tubules than that provided by a dental bur\textsuperscript{49}, and also helps resin infiltration. However, in the clinical situations, bur-prepared dense smear layers might hinder the penetration of the acidic monomer of self-etch adhesive, thus compromising the bond strength\textsuperscript{40}. Saikawet al.\textsuperscript{8} also reported similar results when they compared the bonding performance of universal adhesives with SiC and bur-prepared dentin. The results of the present investigation complemented their observations and proved the rationale for utilizing bur-prepared dentin, ensuring the clinical relevance in our bond strength testing.

According to the Academy of Dental Materials guidance on in vitro testing\textsuperscript{28}, the clinical effectiveness of adhesives can be predicted at laboratory settings by the \( \mu \text{TBS} \) test, especially after subjecting the specimens to aging challenges\textsuperscript{48}. Previously in similar studies, the effects of aging on the bond strength of adhesives have been evaluated employing thermocycling\textsuperscript{12,17}. We opted for water-storage to evaluate the durability of bond with dentin in our study\textsuperscript{11,12}. Our results also confirmed the significant effects of aging irrespective of the type of dentin preparation. The effects of aging were more marked when deep dentin was concerned. This finding is in agreement with the previous report by Ting et al.\textsuperscript{12}.  

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*References* 

1. Toledano et al.\textsuperscript{34} 
2. Yoshikawa et al.\textsuperscript{25} 
3. Pegado et al.\textsuperscript{29} 
4. Zhang et al.\textsuperscript{30} 
5. Ting et al.\textsuperscript{12,16} 
6. Saikawet al.\textsuperscript{8} 
7. Ting et al.\textsuperscript{34} 
8. In vitro testing\textsuperscript{28}
Hydrolysis of resin and collagen fibril is considered the main reason for resin degradation within the hybrid layer47. If resin monomers do not completely infiltrate demineralized dentin, the uninfiltred water-rich collagen fibrils degrade over 1–2 years59.

According to our results, three-way ANOVA revealed a significant effect of the type of adhesives ($F = 27.762, p = 0.000$) on the $\mu$TBS values, indicating the materials' dependency. With SB, significant differences were observed for aging but not for smear layers. According to the manufacturer's instruction, SB needs to be actively applied. SB contains 10 MDP as the functional monomer. It is plausible that the active application of SB might have contributed to better chemical interaction with hydroxyapatite (HAp), leading to the formation of nanolayered structures. Previous reports have suggested the improvement of SB's bond strengths in these mechanisms48,49). The increased application time of SB (20 s) than CU (10 s) might have also contributed to better water removal50 and chemical interaction with HAp51). Besides, Vitrebond copolymer incorporated in SB might have also contributed to improved bonding performance, where during demineralization, already existing polyalkenoic-acid polymer with multiple functional groups can attach the polymer backbone and can take Ca++ at different and remote sites3). These might be the reasons for SB's stable data despite smear layer variations.

In this study, the two-step self-etch adhesive, SE, did not show a significant difference between bond strengths with superficial and deep dentin, except only for SEP24h. Conceivably, its separate demineralization and bonding steps contribute to satisfactory bonding outcomes by ensuring better removal of water. As a result, the suboptimal cure of the hydrophilic monomers contained in its formulation is prevented52). SE is the result, the suboptimal cure of the hydrophilic monomers outcomes by ensuring better removal of water. As a and bonding steps contribute to satisfactory bonding for SEP24h. Conceivably, its separate demineralization strengths with superficial and deep dentin, except only did not show a significant difference between bond layer variations.

The percentage of failure modes and SEM images of this study showed that the number of adhesive failures increased after aging for all tested groups, especially when bonded with bur-prepared dentin. This observation substantiates the effect of aging and the type of smear layer. It is plausible to extrapolate that the impaired resin flow through the more compact smear layer of bur-prepared dentin, together with the residual water, incomplete polymerization, and increased permeability within the adhesive layer might have contributed to more adhesive failures over time60.

Routinely, in clinical situations, teeth from children, young, adult, or elderly patients need to be restored due to caries, fracture, abrasion, or erosion. Regardless of the source of the problem, the resulting cavity preparation, either prepared with steel or diamond burs may present different types and thicknesses of dentin. Although the universal adhesives were developed to facilitate and speed the clinical practice, it is disturbing to know that the same adhesive material must face different challenges to bond dentin. Therefore, it is advisable to understand the adhesive material's limitations. According to the present study, the clinically-simulated smear layer hampers the bond strength to dentin. The bond strength results from a laboratory-simulated smear layer should be taken with attention and care. It is also recommended to apply an indirect pulp capping agent on the deepest dentin portions, where the RDT is very thin. With all the steps involved in the restorative procedure, the biologic factors of the stomatognathic system and the behavior of dental materials cannot be dissociated. As a consequence, aging is unavoidable and must be addressed not only by the professionals but also by the patients. Even though the self-etch adhesives presented a decrease in bond strength after 1 year storage (except for CUBS and CUBD), patients can contribute to the longevity of their restorations by maintaining good oral hygiene. Further studies should be aimed to validate our findings with transmission electron microscopy and to employ other clinically relevant substrates, such as carious and fractured dentin.

**CONCLUSION**

Within the limitations of this in vitro study, it can be concluded that although material dependent, RDT, dentin smear layer, and aging can influence the bonding performances of self-etch adhesives. Therefore, it would be reasonable to evaluate whether the bond strength of the new adhesive to dentin is depth-dependent in association with other variables.

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