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 37ºC 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 The conventional multicompontent etch-and-rinse adhesives have gradually been replaced by more user-friendly, simplified, monocomponent self-etch adhesives³.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 sensitivity⁷.⁸. As a result, universal adhesives are quickly gaining popularity among the clinicians⁹, and their bonding performance to dentin has become the center of attraction of in vitro studies, employing different modifying factors such as mode of application¹⁰,¹¹, the type of substrates¹², their smear layers¹³,¹⁴, and aging¹,¹².

The bonding of universal adhesives to enamel showed reliable outcomes¹⁶. 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 permeability¹³,¹⁵, 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.²⁰, 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 mode²⁰. 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-
Table 1 Materials used in the study

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

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 (31). During 1 year of storage time, the water was changed weekly (30, 31). This resulted in 12 experimental groups (n = 8) based on adhesives, 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. (12) and recorded. 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 (32). 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. (10). 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 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 magnifications to confirm the failure modes (33).

Failure modes were classified as: A, adhesive failure; CC, cohesive failure within resin composite; CD, cohesive failure within dentin; or M, mixed failure (33).

**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**

µTBS test

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.

Table 2  Mean µTBS±SD in MPa of the tested groups (n=8) and percentage of fracture modes (A/CC/CD/M)*

<table>
<thead>
<tr>
<th>Adhesive</th>
<th>Superficial dentin (S)</th>
<th>Deep dentin (D)</th>
<th>Superficial dentin (S)</th>
<th>Deep dentin (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SiC-prepared (P)</td>
<td>Bur-prepared (B)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24h 1y</td>
<td>24h 1y</td>
<td>24h 1y</td>
<td>24h 1y</td>
</tr>
<tr>
<td>SE</td>
<td>61.4±3.2a</td>
<td>47.3±1.7b,cd</td>
<td>52.2±2.0k,l,m</td>
<td>43.0±1.7b,cd</td>
</tr>
<tr>
<td></td>
<td>(17/0/0/83)</td>
<td>(25/0/0/75)</td>
<td>(17/0/0/83)</td>
<td>(36/0/0/64)</td>
</tr>
<tr>
<td>CU</td>
<td>50.0±6.5a,b,c</td>
<td>35.8±2.6d,e</td>
<td>44.2±3.6b,cd</td>
<td>28.0±1.9b</td>
</tr>
<tr>
<td></td>
<td>(26/11/0/63)</td>
<td>(25/0/0/75)</td>
<td>(30/0/0/70)</td>
<td>(36/0/0/64)</td>
</tr>
<tr>
<td>SB</td>
<td>46.9±4.0a,b,c,d,e</td>
<td>37.0±3.7a,b,c</td>
<td>41.7±3.3g,h</td>
<td>31.2±1.5b</td>
</tr>
<tr>
<td></td>
<td>(33/6/0/61)</td>
<td>(27/0/0/73)</td>
<td>(36/0/0/64)</td>
<td>(48/0/0/52)</td>
</tr>
</tbody>
</table>

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 direction of all the relationships is positive, meaning greater RDT is associated with greater µTBS. The relationships were significant \((p<0.05)\) and moderate to strong in all the tested conditions except for SEB1y\(-d\) and CUB24h\(-e\) \((p>0.05)\).

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 (Figs. 1\(-d\), \(-e\)), the relationships where 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 \(\times 80\) 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 \(\times 800\), open and occluded dentinal tubules could be seen (Fig. 2\(A(a-f)\)-2D\(a-f\)) and Fig. 3\(A(a-f)\)-3D\(a-f\)). The number of open tubules seemed to increase with the increasing depth of dentin (Fig. 2\(C-a\)) and aging (Figs. 2\(D-a\), \(c\); 3\(D-a\), \(c\)) and decreased with bur-prepared dentin (Figs. 3\(A-a\), \(c\), \(e\); 3\(B-c\), \(e\); 3\(C-a\), \(c\), \(e\) and 3\(D-a\), \(c\)). At \(\times 8,000\), collagen structures could be seen (Figs. 2\(A-b\), \(d\), \(f\); 2\(B-d\); 3\(A-b\), \(d\), \(f\); 3\(B-b\), \(d\), \(f\), which were clearer in deep dentin (Figs. 2\(C-b\), \(d\), \(f\); 3\(C-b\), \(d\), \(f\)) 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\(^{12,16,34}\) and two-step\(^{23}\) self-etch adhesives when applied in self-etch\(^{23}\) and etch-and-rinse modes\(^{17}\). However, the observations of these reports are contradictory, probably because of compositional differences of the materials leading to material-dependency on their bonding effectiveness\(^{35,36}\). Pereira et al.\(^{17}\) 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 B-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. 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. 25 reported that in the case of Clearfil SE Bond (two-step self-etch adhesive) in self-etch mode, µTBS increased with the increase of RDT, whereas, Single Bond (two-step) in etch-and-rinse mode showed no significant difference in µTBS for any RDT. Pegado et al. 29 and Zhang et al. 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. 22,16 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 12,16,17. However, clinical substrates are different then 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 20,24. 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 µTBS 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 SEB1y and CUB24h (p>0.05). These results rejected our first null hypothesis that the thickness of the remaining dentin would not affect the µTBS 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 8,9. 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 16. Besides, these adhesives’ decreased viscosity can increase the degree of oxygen diffusion and reduce the degree of monomer conversion at the uppermost surface 20. Furthermore, one-step self-etch adhesives are sensitive to the wetness of deep dentin 25. Due to the increased permeability of deep dentin, pulpal fluids could compromise the degree of conversion of adhesives 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 31. Eluted monomers and adhesive by-products, especially the low-weight molecules, can diffuse into dentin tubules and reach the pulp tissue 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 20,21,42. Thinner remaining dentin is more prone to diffuse molecules, and less prone to protect the pulp against toxic substances 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 µTBS 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 41,45. 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 SE24hP, SE1yP, CU24hP and CU24hB (p<0.05). Moreover, the correlations between RDT and µTBS 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 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 40. Saikawa et al. 9 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 28, the clinical effectiveness of adhesives can be predicted at laboratory settings by the µTBS test, especially after subjecting the specimens to aging challenges 40. Previously in similar studies, the effects of aging on the bond strength of adhesives have been evaluated employing thermocycling 12,37. We opted for water-storage to evaluate the durability of bond with dentin in our study 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. 12.
Hydrolysis of resin and collagen fibril is considered the main reason for resin degradation within the hybrid layer\(^{41}\). If resin monomers do not completely infiltrate demineralized dentin, the uninfiltated water-rich collagen fibrils degrade over 1–2 years\(^{59}\).

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 mechanisms\(^{48,49}\). The increased application time of SB (20 s) than CU (10 s) might have also contributed to better water removal\(^{50}\) and chemical interaction with HAp\(^{51}\). Besides, Vitrebond\(^{39}\) 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 sites\(^{3}\). 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 prevented\(^{52}\). SE is the new, improved version of Clearfil Mega Bond. Reports suggested that SE’s newly incorporated additional photo-initiators promote improved polymerization of both hydrophilic and hydrophobic domains by generating more free radicals during curing, leading to higher monomer conversion rates and stronger bond strength immediately\(^{20}\) and after aging\(^{53}\).

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 time\(^{60}\).

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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