

# Effect of Double-layer Application on Dentin Bond Durability of One-step Self-etch Adhesives

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## Clinical Relevance

The double-application technique could be recommended for most one-step self-etch adhesives to improve their bonding capability and to improve the stability of the bond strength to dentin over time.

## SUMMARY

**Purpose:** The aim of this *in vitro* study was 1) to analyze the influence of a double-layer application technique of four one-step self-etch adhesive systems on dentin and 2) to determine its effect on the stability of the adhesive interfaces stored under different conditions.

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**Materials and Methods:** Four different one-step self-etch adhesives were selected for the study (iBondSE, Clearfil S<sup>3</sup> Bond, XenoV<sup>+</sup>, and Scotchbond Universal). Adhesives were applied according to manufacturers' instructions or with a double-layer application technique (without light curing of the first layer). After bonding, resin-dentin specimens were sectioned for microtensile bond strength testing in accordance with the nontrimming technique and divided into 3 subgroups of storage: a) 24 hours (immediate bond strength, T<sub>0</sub>), b) six months (T<sub>6</sub>) in artificial saliva at 37°C, or c) five hours in 10 % NaOCl at room temperature. After storage, specimens were stressed to failure. Fracture mode was assessed under a light microscope.

**Results:** At T<sub>0</sub>, iBond SE showed a significant increase in microtensile bond strength when the double-application technique was applied. All adhesive systems showed reduced bond strengths after six months of storage in artificial saliva and after storage in 10% NaOCl for five hours; however at T<sub>6</sub>, iBond SE, Clearfil S<sup>3</sup> Bond, and XenoV<sup>+</sup> showed significantly higher microtensile bond strength results for the

**double-application technique compared with the single-application technique. Scotchbond Universal showed no difference between single- or double-application, irrespective of the storage conditions.**

**Conclusion: The results of this study show that improvements in bond strength of one-step self-etch adhesives by using the double-application technique are adhesive dependent.**

## INTRODUCTION

Simplification and reduced technique sensitivity of adhesive systems are a continuous trend in adhesive dentistry. One approach among simpler and faster innovations is the use of one-step self-etch adhesives, which have been marketed for their ability to achieve standardized applications and durable bond strengths over time.<sup>1</sup> Initially, self-etch adhesives required two separate application steps: application of an acidic primer followed by a layer of a relatively hydrophobic and unfilled bonding agent. To achieve faster application times, manufacturers have incorporated all components of the adhesive system into one solution.<sup>2,3</sup>

Compared with the etch-and-rinse approach, one-step self-etch adhesives are supposed to etch and infiltrate dentin simultaneously, at least from a theoretical point of view.<sup>3</sup> Apart from the success of immediate bonding and sealing, initially the durability of the adhesive interface could not be ensured, especially for the first formulation adhesives in this class.<sup>4,5</sup> Indeed, water is needed to dissociate acidic methacrylates and allow one-step self-etch adhesives to permeate the smear layer and the underlying mineralized dentin.<sup>2</sup> The intrinsic hydrophilic nature of one-step adhesives leads to hydrolytic degradation,<sup>4</sup> suboptimal polymerization,<sup>6</sup> and phase separation,<sup>7</sup> which have been reported to be important factors for degradation of the simplified adhesive interface over time. In particular, because of suboptimal polymerization, the interface created by one-step self-etch adhesives may result in a semipermeable adhesive layer.<sup>8</sup>

Infiltration of adhesives into the dentin and the thickness of the adhesive layer are directly correlated to rheological and chemical characteristics,<sup>9,10</sup> but they could also be influenced by the mode of application.<sup>11</sup> Different clinical approaches have been proposed to improve monomer infiltration: use of an additional layer of hydrophobic resin agent,<sup>12</sup> multiple-layer application,<sup>13-17</sup> enhanced solvent evaporation,<sup>18</sup> and prolonged curing-time inter-

vals<sup>6,17,19</sup> are some of the modifications to the clinical protocol that showed bonding improvements.

Some authors have indicated that an active application of self-etch adhesives on dentin could expedite solvent evaporation, resulting in a higher rate of monomer impregnation into the smear layer.<sup>20</sup> The active application could carry fresh acidic monomers to the underlying dentin, causing more aggressive demineralization and allowing better diffusion of the monomers<sup>20</sup> and increased concentration of comonomers, which could finally improve the quality of the hybrid layer.<sup>9</sup>

Enhanced microtensile bond strength and reduced interfacial nanoleakage were found when the application time of one-step adhesives was prolonged as well as when a hydrophobic coating was applied after a one-step adhesive system.<sup>21,22</sup> It has been reported that double application of one-step self-etch adhesives may result in a more uniform infiltration of the adhesive into smear layer-covered dentin if a one-step self-etch adhesive is applied in two layers.<sup>15,23</sup>

Thus, the aim of the present study was to assess the effect of a double-application technique on recently formulated (Scotchbond Universal, 3M ESPE, Seefeld, Germany), storage-improved (iBond SE, Heraeus Kulzer, Hanau, Germany; XenoV<sup>+</sup>, Dentsply DeTrey, Konstanz, Germany) or well evaluated (Clearfil S<sup>3</sup> Bond, Kuraray Medical Inc, Tokyo, Japan) one-step self-etch adhesives (Table 1) on immediate and long-term stability of the adhesive interface. The null hypotheses tested in this study were that 1) a double-application technique does not improve the immediate bond strength of the four tested one-step self-etch adhesives and 2) that the double-application technique would not influence bond strength after different aging protocols.

## METHODS AND MATERIALS

Four different one-step self-etch adhesives were selected for the study: iBond SE, Scotchbond Universal, Clearfil S<sup>3</sup> Bond, and XenoV<sup>+</sup>. A total of 56 sound, recently extracted, human third molars were disinfected in 0.5% aqueous chloramine-T solution after written informed consent was obtained from the patients, as required from the local ethics committee, and stored for at least four weeks at 4°C in distilled water. All of them were cut using a low-speed diamond saw (Isomet Low Speed Saw, Buehler, Lake Bluff, IL, USA) under water irrigation until middle/deep dentin was exposed. A standardized smear layer was created on dentin by using 180-

Table 1: Components, Compositions, and Application Procedure of the Tested Self-etch Adhesives

Adhesive/Manufacturer	Batch Number	Composition <sup>a</sup>	Application Procedure	Storage
iBond SE (Heraeus-Kulzer, Hanau, Germany) pH 2.0	010110	Acetone, UDMA, TEGDMA, 4-methacryloxyethyltrimellitic anhydride, glutaraldehyde, photoinitiator	Manufacturer's instructions: 1. Scrub adhesive for 20 s on dentin 2. Air thin 3. Light cure for 20 s	Group 1: 24 h in distilled water Group 9: 6 mo in artificial saliva at 37°C Group 17: 5 h in NaOCl
			Double application: 1. Scrub adhesive for 20 s on dentin 2. Air thin 3. Scrub adhesive for 20 s on dentin 4. Air thin 5. Light cure for 20 s	Group 2: 24 h in distilled water Group 10: 6 mo in artificial saliva at 37°C Group 18: 5 h in NaOCl
Xeno V <sup>+</sup> (Dentsply DeTrey, Konstanz, Germany) pH 1.3	1101000851	Bifunctional acrylic amides, acrylamidoalkylsulfonic acid, "inverse"functionalized phosphoric acid ester, acrylic acid, ter-butanol, butylatedbenzenediol, water, camphorquinone	Manufacturer's instructions: 1. Apply adhesive agitated for 20 s on dentin 2. Air thin for 5 s 3. Light cure for 10 s	Group 3: 24 h in distilled water Group 11: 6 mo in artificial saliva at 37°C Group 19: 5 h in NaOCl
			Double application: 1. Apply adhesive agitated for 20 s on dentin 2. Air thin for 5 s 3. Apply adhesive agitated for 20 s on dentin 4. Air thin for 5 s 5. Light cure for 10 s	Group 4: 24 h in distilled water Group 12: 6 mo in artificial saliva at 37°C Group 20: 5 h in NaOCl
Scotchbond Universal (3M Espe, Seefeld, Germany) pH 2.7	Uno-VT-Bulk-0001	MDP phosphate monomer, dimethacrylate resins, HEMA, Vitrebond Copolymer, filler, ethanol, water, initiators, silane	Manufacturer's instructions: 1. Scrub adhesive for 20 s on dentin 2. Gently air thin for 5 s 3. Light cure for 10 s	Group 5: 24 h in distilled water Group 13: 6 mo in artificial saliva at 37°C Group 21: 5 h in NaOCl
			Double application: 1. Scrub adhesive for 20 s on dentin 2. Gently air thin for 5 s 3. Scrub adhesive for 20 s on dentin 4. Gently air thin for 5 s 5. Light cure for 10 s	Group 6: 24 h in distilled water Group 14: 6 mo in artificial saliva at 37°C Group 22: 5 h in NaOCl
Clearfil S <sup>3</sup> Bond (Kuraray Medical Inc., Tokyo, Japan) pH 2.6	0157 BA	MDP, bis-GMA, HEMA hydrophobic dimethacrylate, DL-camphorquinone, ethyl alcohol, water, silanated colloidal silica	Manufacturer's instructions: 1. Apply adhesive and leave undisturbed for 20 s on dentin 2. Air thin for 5 s 3. Light cure for 10 s	Group 7: 24 h in distilled water Group 15: 6 mo in artificial saliva at 37°C Group 23: 5 h in NaOCl
			Double application: 1. Apply adhesive and leave undisturbed for 20 s on dentin 2. Air thin for 5 s 3. Apply adhesive and leave undisturbed for 20 s on dentin 4. Air thin for 5 s 5. Light cure for 10 s	Group 8: 24 h in distilled water Group 16: 6 mo in artificial saliva at 37°C Group 24: 5 h in NaOCl

<sup>a</sup> Composition of the materials as provided by the manufacturers: bis-GMA, bisphenol-glycidyl methacrylate; HEMA, hydroxyethylmethacrylate; MDP, methacryloyloxydecyl dihydrogen phosphate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

grit wet silicon carbide papers.<sup>24</sup> Smear layer-covered dentin substrates were then equally randomly assigned to the different treatment groups as shown in Table 1 (N=7). Each dentin bonding system was applied as recommended by manufacturers' instructions or with a double-application step without light curing of the first layer (Table 1).

Dentin bonding systems were light cured according to the manufacturer's instructions (Table 1) with a dental halogen-curing unit (Elipar Trilight, 3M ESPE) with at least 750 mW/cm<sup>2</sup> (checked periodically with a radiometer; Radiometer 100, Demetron Research Corp, Danbury, CT, USA). Four 1-mm thick layers of Filtek Z250 (3M ESPE) were incrementally applied on the bonded dentin surface.<sup>25</sup> Each increment was light cured separately. To facilitate microtensile bond-strength testing (4 mm dentin/4 mm resin composite), the pulp chamber was bonded with Adper Scotchbond Multi-Purpose (3M ESPE, three-step etch-and-rinse adhesive) and filled with Filtek Z250, in accordance with the manufacturer's instructions.

Resin-dentin bonded specimens (0.9 mm × 0.9 mm) were sectioned for microtensile bond strength testing with a low-speed diamond saw under water irrigation in accordance with the nontrimming technique.<sup>26</sup> To convert data into megapascals (MPa), the dimension of each beam was measured using a digital caliper (accurate to ±0.01 mm). Specimens of each tooth were divided into three subgroups of storage: a) 24 hours (T<sub>0</sub>) in artificial saliva (5 mM C<sub>8</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>S, 2.5 mM CaCl<sub>2</sub>, 0.05 mM ZnCl<sub>2</sub>, and 0.3 mM NaN<sub>3</sub>, pH 7.4) at 37°C,<sup>27</sup> b) six months (T<sub>6</sub>) in artificial saliva at 37°C, or c) five hours in 10% NaOCl and then one hour in distilled water at room temperature.

After aging the specimens were fixed on a modified jig for microtensile testing (Zwicki Z 2.5, Zwick Roell, Ulm, Germany) with cyanoacrylate adhesive (Loctite 401 and Aktivator 7455, Loctite, Munich, Germany) and stressed until failure under tension at a crosshead speed of 1 mm/min. All debonded beams during specimen preparation (pretesting failure) were recorded but not included in statistical evaluation. Only values of beams that failed during storage or microtensile testing were noted and analyzed statistically.

Failures were classified as adhesive, cohesive in dentin, cohesive in composite, or mixed and were examined by a single observer using a stereomicroscope (SV11, Zeiss, Oberkochen, Germany) at 50× magnification.

Four representative specimens of fractured microtensile beams of each group that were classified as adhesive or mixed under light microscopy were evaluated under scanning electron microscopy (SEM; Leitz ISI-SR-50, Tokyo, Japan) at 50× to 500× magnification.

Bond-strength data were statistically analyzed by using SPSS 19.0 for Windows (SPSS, Chicago, IL, USA). Because groups exhibited non-normally distributed data (Kolmogorov-Smirnov test), nonparametric tests were used. Statistical differences between the groups were analyzed pairwise using the Mann-Whitney U test at a level of significance of  $p < 0.05$ .

## RESULTS

Microtensile bond-strength mean values and standard deviations are shown in Table 2. At T<sub>0</sub> (24 hours of storage in artificial saliva), the double-application technique resulted in statistically significant increased bond strength for iBond SE compared with the control application in accordance with manufacturers' instructions ( $p = 0.008$ ; Table 2); no differences were found for all other tested adhesives (Clearfil S<sup>3</sup> Bond,  $p = 0.439$ ; Scotchbond Universal,  $p = 0.593$ ; and Xeno V<sup>+</sup>,  $p = 0.902$ ; Table 2). Differences within the adhesive systems were also found at T<sub>0</sub> as XenoV<sup>+</sup> showed the statistically significant lowest bond strength among the tested adhesives irrespective of the application mode ( $p < 0.05$ ; Table 2).

At T<sub>6</sub> (after six months of storage in artificial saliva), iBond SE ( $p = 0.046$ ), Clearfil S<sup>3</sup> Bond ( $p = 0.006$ ), and XenoV<sup>+</sup> ( $p = 0.026$ ) showed statistically significant higher microtensile bond strength when applied with the double-application technique compared with application in accordance with the manufacturer's instructions (Table 2); no differences were found for Scotchbond Universal ( $p = 0.578$ ; Table 2). The effect of aging on the bond strength compared with that of the T<sub>0</sub> specimens was statistically significant for iBond SE and Xeno V<sup>+</sup> ( $p < 0.05$ ) regardless of the application technique and for Clearfil S<sup>3</sup> Bond if applied in accordance with manufacturer's instructions ( $p = 0.0015$ ). Conversely, no reduction in bond strength between T<sub>0</sub> and T<sub>6</sub> was found for Scotchbond Universal (stable bond strength regardless of the application technique,  $p > 0.05$ ; Table 2) and for Clearfil S<sup>3</sup> Bond if the double-application technique was used ( $p = 0.229$ ; Table 2).

After storage in 10% NaOCl for five hours, all adhesives showed statistically significant reduced



Table 2: Mean ± Standard Deviation (Number of Intact Sticks Tested/Number of Debonded Specimens During Cutting) of Microtensile Bond Strengths in MPa <sup>a</sup>												
Adhesive System	iBond SE			Clearfil S <sup>3</sup> Bond			Scotchbond Universal			Xeno V <sup>+</sup>		
Application Procedure	Double Application	Increase of Bond Strength	Manufacturer's Instructions	Double Application	Increase of Bond Strength	Manufacturer's Instructions	Double Application	Increase of Bond Strength	Manufacturer's Instructions	Double Application	Increase of Bond Strength	Manufacturer's Instructions
24 h artificial saliva (T <sub>0</sub> )	53.6 ± 15.4 <sup>b</sup> (49/1)	+18%	44.2 ± 15.0 <sup>a,c</sup> (49/2)	51.0 ± 14.0 <sup>b</sup> (41/1)	+6%	48.0 ± 14.2 <sup>a,b</sup> (50/1)	53.0 ± 16.8 <sup>b</sup> (45/1)	+1%	52.3 ± 16.7 <sup>b</sup> (43/1)	22.1 ± 11.7 <sup>a</sup> (64/4)	+14%	19.0 ± 12.8 <sup>a</sup> (66/5)
6 mo artificial saliva (T <sub>6</sub> )	42.7 ± 9.5 <sup>c</sup> (47/2)	+13%	37.1 ± 11.3 <sup>d</sup> (48/1)	47.6 ± 10.5 <sup>a,b</sup> (52/1)	+18%	39.0 ± 11.1 <sup>d</sup> (52/3)	48.5 ± 11.4 <sup>a,b</sup> (50/2)	+2%	47.6 ± 11.7 <sup>a,b</sup> (52/2)	9.0 ± 5.4 <sup>g</sup> (53/3)	+30%	6.3 ± 3.8 <sup>i,j</sup> (52/2)
5 h in 10% sodium hypochlorite	7.6 ± 2.9 <sup>f,g</sup> (37/1)	+70%	2.3 ± 2.3 <sup>h</sup> (27/1)	7.2 ± 4.2 <sup>f,g</sup> (40/2)	+12%	6.4 ± 3.6 <sup>i,j</sup> (40/0)	12.0 ± 4.4 <sup>i</sup> (33/2)	+8%	11.0 ± 4.8 <sup>i</sup> (32/0)	5.5 ± 2.1 <sup>j</sup> (32/1)	+60%	2.2 ± 1.4 <sup>h</sup> (25/0)
<sup>a</sup> Groups identified with same superscripted letters are not significantly different (p<0.05). Premature failures due to preparation procedures were not included in the statistical evaluation.												

bond strength ( $p<0.05$ ; Table 2), though Scotchbond Universal showed the highest results regardless of the application mode ( $p<0.05$ ; Table 2). Only Xeno V<sup>+</sup> showed failures during storage in 10% NaOCl; these five failures were recorded as 0 MPa in statistical evaluation.

Results of failure mode distribution are shown in Table 3. Analysis of the failure mode exhibited nearly 100% adhesive failures for Xeno V<sup>+</sup> regardless of the application mode or storage conditions. When stored in 10% NaOCl, Xeno V<sup>+</sup>, iBond SE, and Clearfil S<sup>3</sup> Bond showed 100% adhesive failure in all groups, and Scotchbond Universal showed 90% adhesive failures. For Scotchbond Universal and Clearfil S<sup>3</sup> Bond, a significant increase in percentage of adhesive failure was found at T<sub>6</sub> compared with T<sub>0</sub>.

The SEM examinations of fractured microtensile sticks from the dentin side are shown in Figures 1 through 4. Adhesive and mixed fracture modes are shown.

DISCUSSION

The results of the present investigation led to the partial rejection of the null hypotheses as the use of a double-application technique increased bond strength or reduced degradation over time for some of the tested adhesives (iBond SE, Clearfil S<sup>3</sup> Bond, and Xeno V<sup>+</sup>), though no difference was found for Scotchbond Universal. The most pronounced improvement of microtensile bond strength after double application in the present study was achieved for iBond SE, which confirmed previous findings.<sup>13,14,15,21</sup> We can speculate that this unfilled adhesive profits from a thicker adhesive layer

Table 3: Failure Mode Distribution in Percent <sup>a</sup>													
Adhesive System	Application Procedure	24 h in Artificial Saliva (T <sub>0</sub> )				6 mo in Artificial Saliva (T <sub>6</sub> )				5 h in 10% Sodium Hypochlorite			
		A	CC	CD	M	A	CC	CD	M	A	CC	CD	M
iBond SE	Double application	46.9	30.6	12.2	10.2	38.3	44.7	12.8	4.3	97.3	0	0	2.7
	Manufacturer's instructions	51.0	22.4	16.3	10.2	72.9	18.8	6.3	2.1	100	0	0	0
Clearfil S <sup>3</sup> Bond	Double application	70.7	24.4	2.4	2.4	86.3	11.8	2.0	0	100	0	0	0
	Manufacturer's instructions	68.0	26.0	2.0	4.0	84.6	13.5	1.9	2.4	100	0	0	0
Scotchbond Universal	Double application	22.2	60.0	8.9	8.9	52.0	42.0	6.0	0	90.9	0	9.1	0
	Manufacturer's instructions	14.7	67.6	8.8	8.8	38.5	42.3	11.5	7.7	90.6	3.1	3.1	3.1
Xeno V <sup>+</sup>	Double application	98.4	0	0	1.6	100	0	0	0	100	0	0	0
	Manufacturer's instructions	95.5	1.5	1.5	1.5	100	0	0	0	100	0	0	0
<sup>a</sup> Abbreviations: A, adhesive failure; CC, cohesive failure in composite; CD, cohesive failure in dentin; M, mixed failure.													

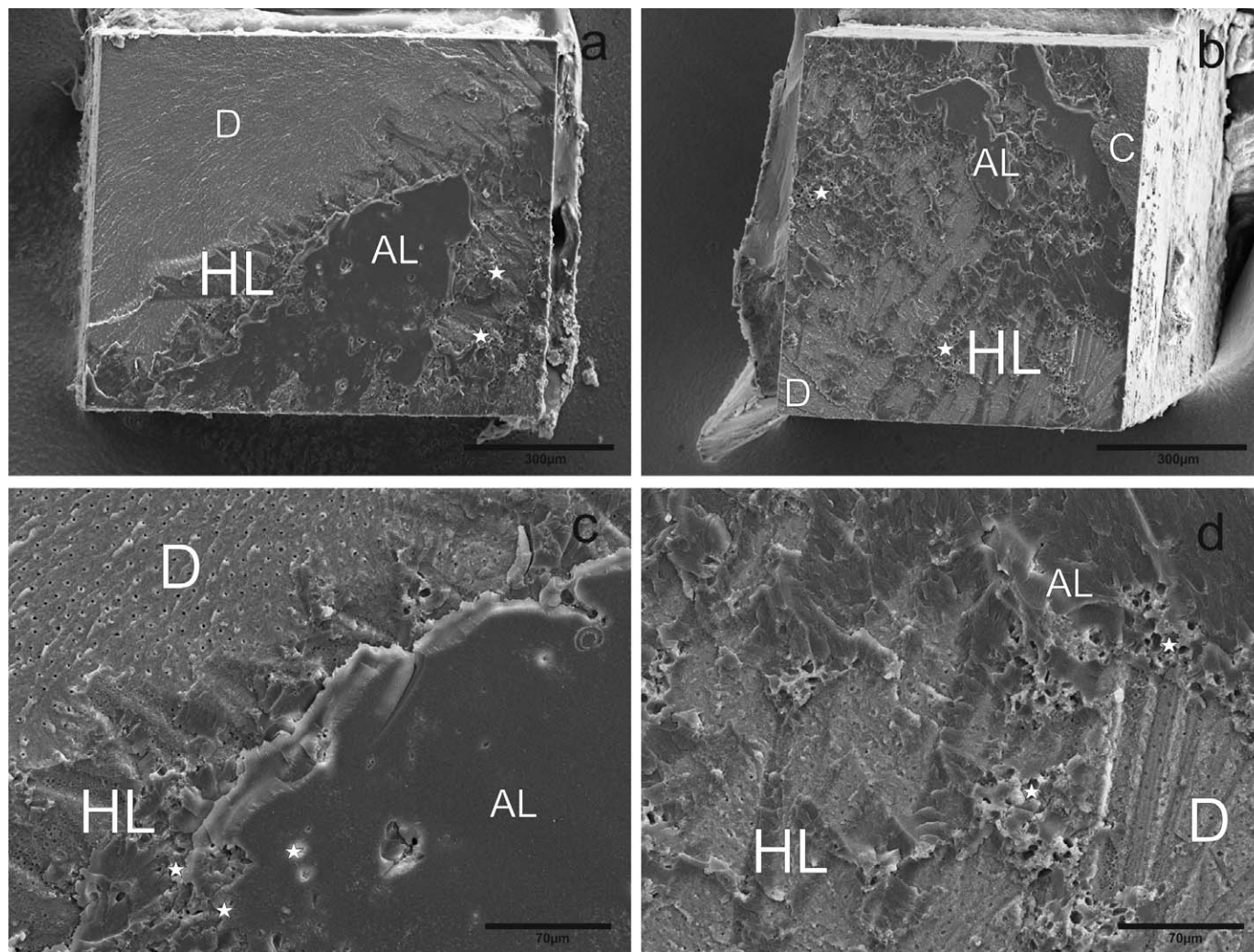


Figure 1. SEM images of debonded iBond SE specimens after six months (a, c = manufacturer's instructions; b, d = double application) from the dentin side. Abbreviations: AL, adhesive layer; HL, hybrid layer; D, dentin; C, composite. The overall area of adhesive covering the surface (AL) is considerably larger with single application (a, c). In both groups, some osmotic blistering is detectable (asterisks). Mixed-type failures with exposed intertubular dentin are evident under the SEM at larger magnifications.

resulting from the second application step. Moreover, a second application step of unpolymerized acidic monomers could improve the etching performance of iBond SE by increasing the amount of acidic reagents in direct contact with dentin and reducing the buffer capability of hydroxyapatite. This may synergistically combine with the increased adhesive thickness to explain the increase in microtensile bond strength.<sup>28</sup>

Clearfil S<sup>3</sup> Bond and Scotchbond Universal incorporate the functional monomer methacryloyl-oxydecyl dihydrogen phosphate (MDP). MDP is known for its primary chemical interaction with hydroxyapatite, which occurs within a clinically relevant time span of 20 seconds.<sup>29</sup> This kind of chemical interaction did not increase the immediate micro-

tensile bond strength, but investigations evaluating the biodegradation resistance of adhesive interfaces have shown that it could enhance long-term stability.<sup>30,31</sup>

Clearfil S<sup>3</sup> Bond showed a higher decrease in microtensile bond strength than Scotchbond Universal when used according to manufacturer's instructions. This may be related to the fact that Scotchbond Universal contains Vitrebond Copolymer (3M ESPE), which creates an additional bond to hydroxyapatite,<sup>32</sup> and furthermore, Scotchbond Universal maybe result in better bonding of the filler particles inside the adhesive.

Clearfil S<sup>3</sup> Bond is also known to exhibit debonding of silica filler particles after six months of storage in water, as described by Van Landuyt and others,<sup>33</sup>



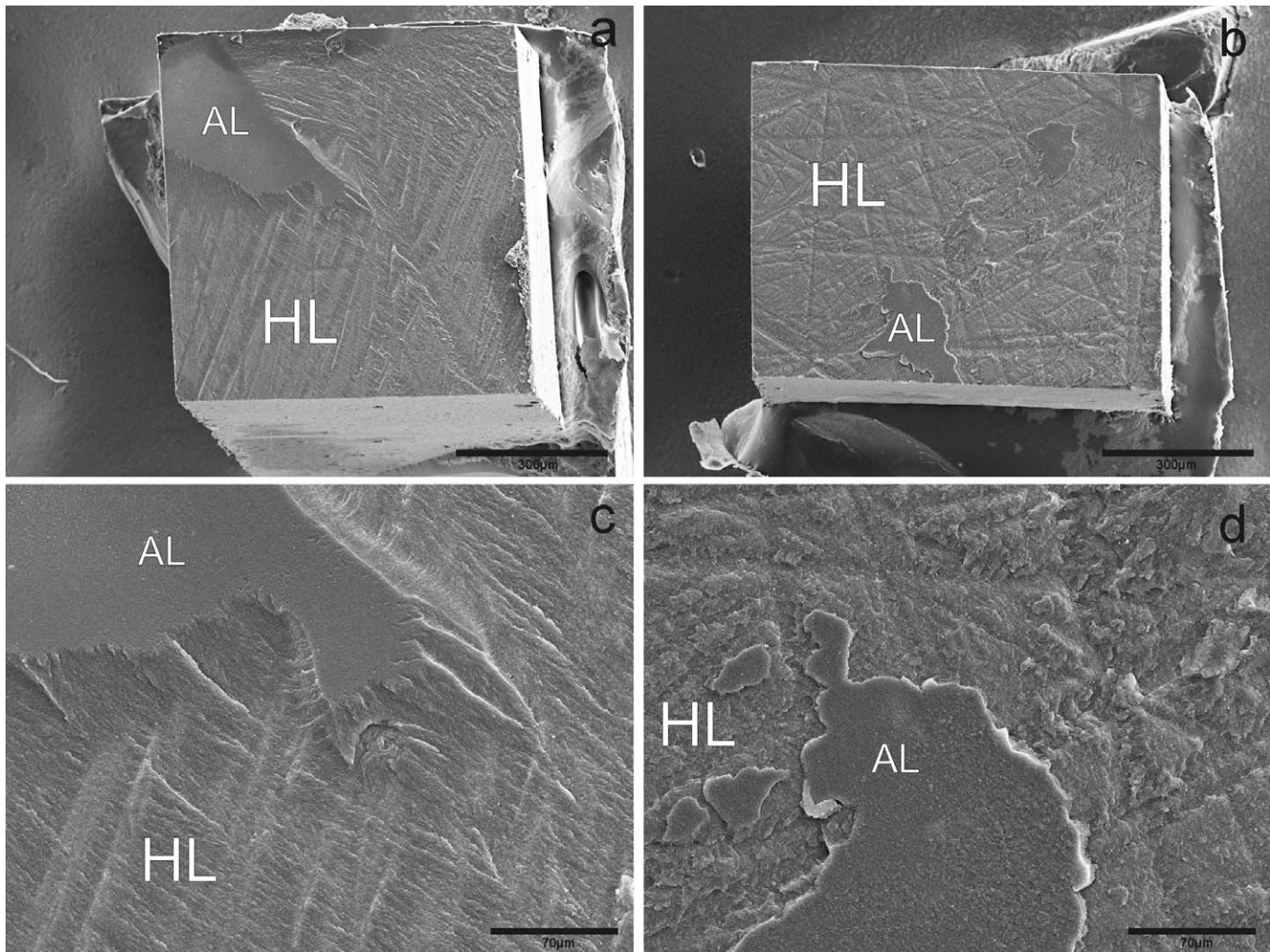


Figure 2. SEM images of debonded Clearfil S<sup>3</sup> Bond specimens after six months (a, c = manufacturer's instructions; b, d = double application) from the dentin side. Abbreviations: AL, adhesive layer; D, dentin. Dentin areas show a continuous view of the top of the hybrid layer with only few areas still covered with adhesive (AL). Mixed failures with exposed intertubular dentin structures are not found.

who suggested that water uptake into the adhesive layer leads to hydrolysis of the coupling agent, resulting in filler detachment from the resin matrix. In the present study we may speculate that this phenomenon is responsible for the better performance of Clearfil S<sup>3</sup> Bond when used with the double-application technique. We could also speculate that the observed improvement is due to a thicker adhesive layer or better impregnation of the collagen fibers when Clearfil S<sup>3</sup> Bond is applied twice.

Scotchbond Universal was the only adhesive tested in the present investigation that did not benefit from double application regardless of the aging protocol. Moreover, Scotchbond Universal also remained stable over time if applied in accordance

with manufacturer's instructions. This is probably due to its improved curing capability, which allows a high degree of polymer cross-linking, even in the thin adhesive layer created by the single-application technique.<sup>34</sup> So perhaps the filled adhesives form a sufficiently thick adhesive layer in one application step<sup>35,36</sup> resulting in its being less prone to oxygen inhibition.<sup>37</sup>

The double-application technique of self-etch adhesives also influences the etching ability due to increased application time and continuous refreshment of new acidic monomers because of agitated application. This can be of particular importance if a thick smear layer is present because it is known that the smear layer can affect the bonding ability of self-etch adhesives.<sup>38-41</sup> Clinically, the smear layer



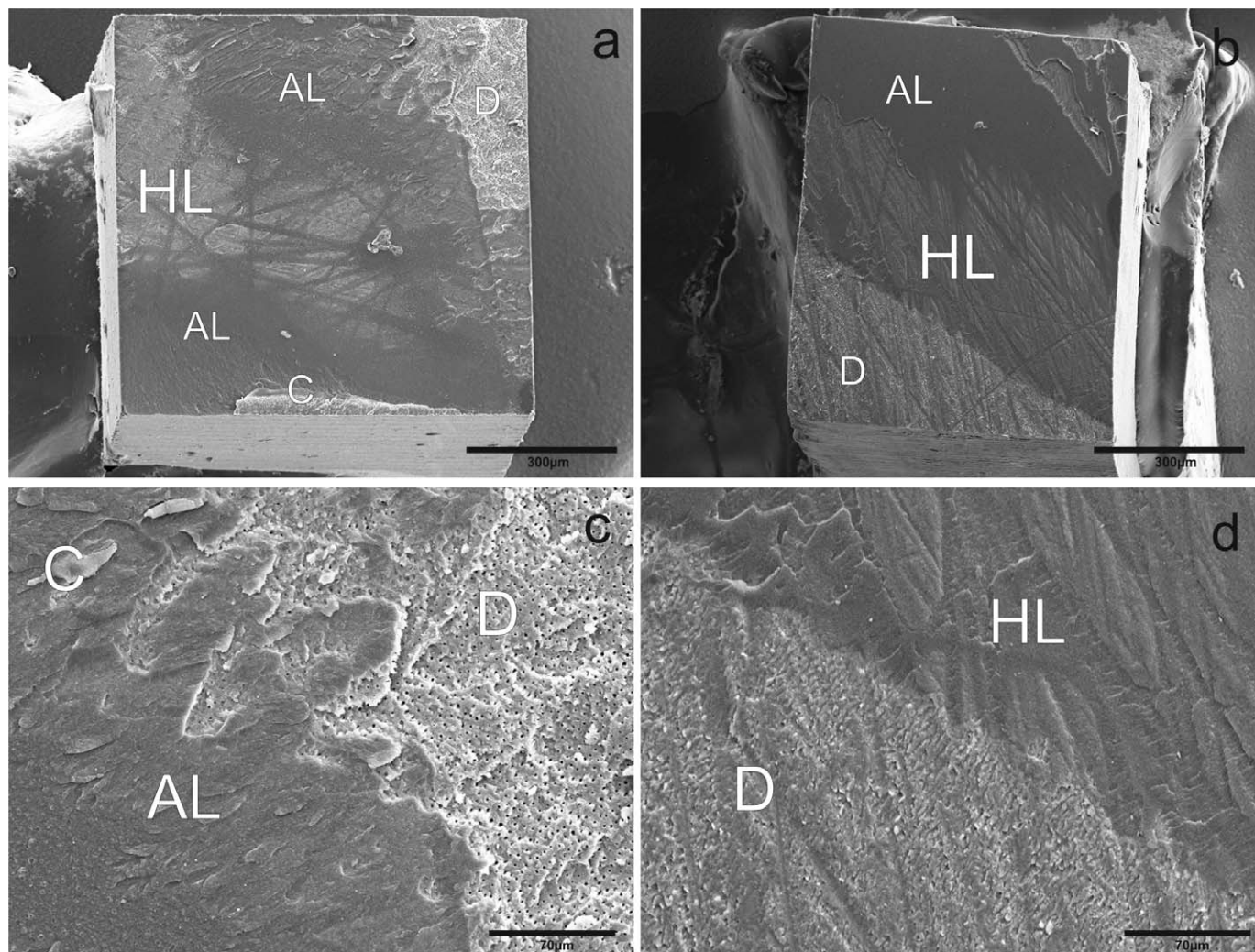


Figure 3. SEM images of debonded Scotchbond Universal specimens after six months (a, c = manufacturer's instructions; b, d = double application) from the dentin side. Abbreviations: AL, adhesive layer; D, dentin; C, composite. The failure patterns extend more into the dentin (D) when a single application was performed (a, c). The failure site distribution is more uniform when the adhesive was applied twice (b, d).

created using a medium-grit diamond bur (average particle size of 70 µm) is rougher than the smear layer produced by using 600-grit wet paper used in *in vitro* studies. Because increased smear-layer thickness and greater surface roughness correspond to lower self-etch adhesive impregnation, bond strength obtained after using a 600-grit paper (average particle size of 14.5 µm) may be overestimated due to enhanced adhesive penetration.<sup>42,43</sup> Therefore, in this study 180-grit wet paper (average particle size of 63 µm) was used to prepare a clinically relevant smear layer.<sup>44</sup>

The double-application technique may also result in overetched dentin substrates, which leads to the formation of dentin-unprotected collagen fibrils within the hybrid layer, and this can be responsible

for the degradation of the bond over time due to enzymatic degradation.<sup>45,46</sup> An easy way to challenge the durability of this unprotected collagen is to store bonded interfaces in 10% NaOCl at room temperature for five hours, according to Yamauti and others.<sup>47</sup> The potent proteolytic agent NaOCl degrades unprotected collagen due to the presence of superoxide radicals in aqueous solution. This aging method simulates aging within a short time and allows the collection of data on the ability of a dentin bonding system to infiltrate and penetrate the exposed dentin matrix appropriately. This aging protocol was used in previous studies to accelerate aging of the adhesive interface<sup>27</sup> and as an indicator of complete infiltration into the hybrid layer.<sup>48</sup> Additionally, Toledano and others<sup>49</sup> showed that beside the dentin matrix suboptimally polymerized



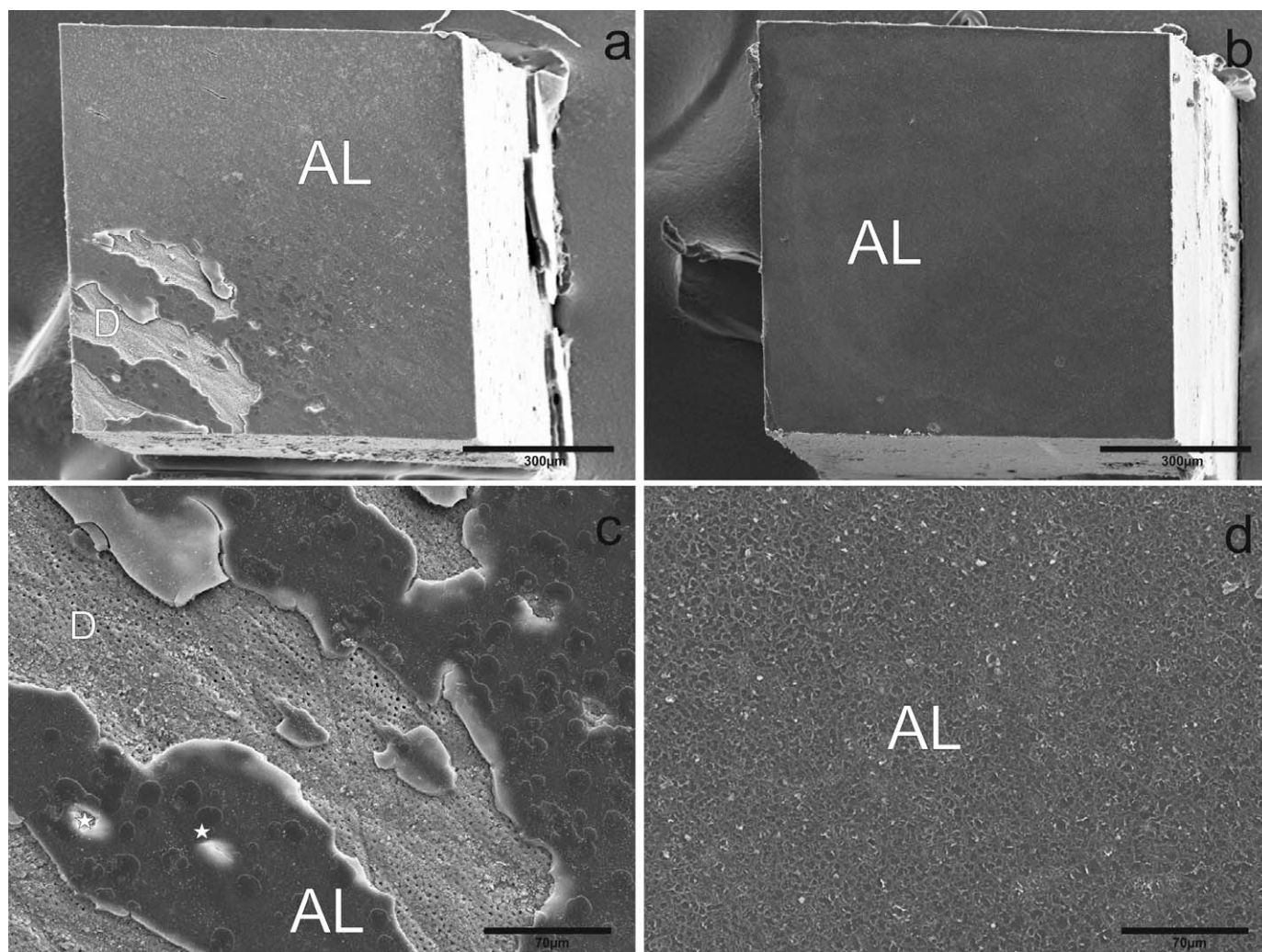


Figure 4. SEM images of debonded Xeno V<sup>+</sup> specimens after six months (a, c = manufacturer's instructions; b, d = double application) from the dentin side. Abbreviations: AL, adhesive layer; D, dentin. Some mixed-type failure patterns with exposed dentin areas (D) were detected only after single application. When applied twice, a more uniform failure pattern was evident (d). Also some distinct osmotic blistering areas can be identified (asterisks).

resin could also be degraded, depending on the adhesive system tested.

The results of this present study show that immersion in 10% NaOCl for five hours is a useful method to challenge the adhesive interface. Whereas previous findings obtained with different adhesive systems showed bond strength reductions comparable to six-month storage in artificial saliva,<sup>27,50</sup> in the present study microtensile bond strength values were lower than the those obtained after storage in artificial saliva for six months.

Overall, in the present study there is a higher tendency for adhesive failures in adhesives with lower microtensile bond strength results and for cohesive failures mainly in composite (CC) for those

with higher microtensile bond strength. This phenomenon is in accordance with results from Toledano and others,<sup>51</sup> where more adhesive failures were also observed for one-step self-etch adhesives with lower microtensile bond strength results, either immediately or after artificial ageing. Further clinical trials should investigate the influence of double application of one-step self-etch adhesives on the durability of these bonds over time.

## CONCLUSION

In conclusion, the results of this study show that a double application of one-step self-etch adhesives improves immediate bond strength and increases bond stability for some one-step self-etch adhesives.

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## Conflict of Interest

The authors of this manuscript certify that they 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.

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