Degradation of Adhesive-Dentin Interfaces Created Using Different Bonding Strategies after Five-year Simulated Pulpal Pressure

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Purpose: To compare after five-year simulated pulpal pressure (SPP) the degradation of adhesive-dentin interfaces created using two simplified adhesives applied with different bonding strategies.

Materials and Methods: A two-step self-etch (CSE: Clearfil SE Bond) adhesive was used as a control multistep adhesive. The tested experimental materials were two simplified adhesives, a one-step self-etch (CS3: Clearfil S3 Bond) and a self-priming etch-and-rinse adhesive (SB2: Adper Single-Bond 2). Half of the bonded specimens were submitted to microtensile bond strength (μTBS) testing after 24 h. The other half submitted to SPP for five years before μTBS testing. Nonfractured sticks were evaluated using transmission electron microscopy (TEM). Scanning electron microscopy (SEM) was used to evaluate silver-nitrate nanoleakage within the interface. Data were statistically analyzed by two-way ANOVA and Tukey's test ($p < 0.05$).

Results: Prolonged SPP induced bond-strength reduction for both SB2 and CS3. All bonding approaches showed increased nanoleakage after aging. The two simplified adhesives showed severe degradation at the resin-dentin interface. TEM revealed that the main degradation patterns for the etch-and-rinse adhesive SB2 was collagen breakdown, while polymer hydrolysis along with filler debonding was mainly observed in CS3.

Conclusions: Simplified adhesives applied in etch-and-rinse mode are mainly characterized by hydrolysis and collagen degradation. In self-etch mode, simplified adhesives may principally show hydrolysis of the polymeric matrix and/or at the interface of fillers and coupling agent. The use of multistep self-etching adhesives may guarantee greater dentin bond durability compared to simplified adhesives.

Keywords: aging, dentin, bond strength, electron microscopy, pulpal pressure.

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The adhesive-dentin interface has been considered for many years as the "weak link" in the longevity of composite restorations. The hybrid layer is considered the key structure at the composite-dentin interface; it is prone to degrade over time through hydrolytic processes.[2,4] Such a degradation is a consequence of the heterogeneity of the dentin substrate, as well as the unreliable stability of relatively highly hydrophilic polymers formed after light-curing procedures (eg, 10-20 s) in etch-and-rinse and self-etch simplified adhesives.[23,34] Polymer hydrolysis and collagen degradation within the resin-dentin interface may be trigged by water seepage.[2,27] Indeed, after polymerization, nanoleakage assays have revealed demineralized collagen poorly infiltrated by the resin monomers, and zones with residual solvent within the adhesive layer.[35] Such areas with resin-sparse collagen are more vulnerable to hydrolytic degradation.[4,33] Unprotected collagen may additionally suffer from accelerated biodegradation induced by activated host-derived enzymes such as matrix metalloproteinases (MMPS) and cysteine cathepsins.[4,21,33] However, such enzymes may be inhibited by several compounds[3,32] that are often incorporated in dental adhesives to increase the durability of resin-dentin bonds.[1,42] Along with collagen degradation, polymer hydrolysis may also occur upon contact with water from different sources (eg, dentin wetness, saliva, and intratubular fluids). Moreover, this type of degradation may be accelerated by enzymes (eg, esterases) found in saliva, as well as those produced by different bacterial species.[18] Thus, the degradation of collagen and resin polymers represents the main cause of reduced resin-dentin bond longevity. However, this phenomenon depends on the hydrophilicity of the adhesives, which in turn directly determines the bonds' water sorption.[16]

The longevity of adhesive-dentin bonds is conventionally evaluated in vitro through storage of stick-shaped specimens (cross-sectional area = 0.9-1.0 mm^2) in aqueous solutions for a period of 3 to 12 months.[39] However, the aging of composite restorations in a real clinical scenario may differ compared to that tested in vitro.[3,8] Indeed, recent studies suggested that in vitro aging of adhesive-dentin bonds may underestimate the in vivo results, as clinical reports depicted high longevity of dental adhesives.[5,7,12,22,26]

The aim of this study was to analyze the degradation after five-year simulated pulpal pressure (SPP) of two simplified adhesives applied onto dentin with different bonding strategies and compare the results to those obtained with a gold-standard multi-step self-etch adhesive. This was accomplished by evaluating the microtensile bond strength, performing a TEM ultrastructure analysis of the resin-dentin interface and SEM nanoleakage evaluation after five years under aging with simulated pulpal pressure (SPP). The first null hypothesis was that the bond strength durability of simplified adhesives applied in dentin would not depend on the bonding approach (etch-and-rinse vs self-etching). The second null hypothesis was that simplified adhesives applied onto dentin in etch-and-rinse or self-etching mode would present the same type of degradation at the resin-dentin interface.

MATERIALS AND METHODS

Sample Preparation

One hundred five extracted human third molars, obtained after approval of the institutional ethics committee (protocol 167/2009), were stored in distilled water and used within two months after extraction. Flat, deep dentin surfaces were prepared by cutting the crown of each tooth 2 mm below the cemento-enamel junction (CEJ) using a diamond saw (Isomet, Buehler; Lake Bluff, IL, USA), followed by a second parallel cut 3 mm above the CEJ.[11,31] The coronal dentin was then abraded using 320-grit SiC papers under continuous water irrigation in order to obtain a standardized remaining dentin thickness (approximately 0.9 mm).[12] Pulp tissue was carefully removed using small tweezers, avoiding altering or scratching the pre-dentin surface along the wall of the pulpal chamber. The flat dentin surface of each specimen was ground with wet 600-grit SiC paper for 30 s to create a standardized smear layer.

Experimental Design

Dentin samples were randomly divided into three main groups (n = 35) based on the dental adhesive employed in this study: 1. control group: two-step self-etch adhesive Clearfil SE Bond (CSE, Kuraray Noritake; Tokyo, Japan); 2. experimental group: one-step self-etch adhesive Clearfil S3 Bond (CS3, Kuraray Noritake); 3. experimental group: two-step self-priming etch-and-rinse adhesive Adper Single Bond 2 (SB2, 3M Oral Care; St Paul, MN, USA). Table 1 describes in detail the compositions and application protocols of each adhesive. After bonding procedures, a standard nanofilled restorative composite, Filtek Z350 (3M Oral Care), was applied on each bonded specimen in six 1-mm-thick increments to attain a 6-mm buildup. Adhesives and composite were light cured according to the manufacturers' instructions using the halogen light-curing unit XL-2500 (3M Oral Care).[10,11,31] Light irradiance was kept at 600 mW/cm^2 by regular monitoring with a radiometer (Optilux Radiometer 100, SDS Kerr; Orange, CA, USA).

The specimens were finally stored in distilled water for 24 h or subjected to SPP. The latter specimens were attached to the lid of a cylindrical container filled with a distilled water column 20 cm high and turned upside down.[9-12,31] They were stored for 5 years at 37°C. The water was replaced every 30 days.[10]

Microtensile Bond Strength (μTBS) and Failure Mode

The specimens were sectioned to obtain composite-dentin sticks of 1 mm^2 cross-sectional area suitable for μTBS testing. The cross-sectional area of each stick was measured using a digital caliper (Mitutoyo; Tokyo, Japan). Sticks with residual enamel and pulp exposure were excluded. For the bond strength evaluation, the sticks were attached to jigs using cyanoacrylate glue (Superbonder gel, Loctite, Henkel; Rocky Hill, NY, USA) and tested until failure in a universal testing machine (EZ-test, Shimadzu; Kyoto, Japan) using a 500-N load cell and 1 mm/min crosshead speed. The bond strength was calculated and expressed in MPa. The bond

Table 1 Materials, batches, chemical compositions, and application protocols

Materials	Composition	Application procedure	Batch
Clearfil S3 Bond	MDP, bis-GMA, HEMA, dimethacrylates, photoinitator	Apply adhesive for 20 s. Air dry for 5 s to evaporate solvent. Light cure for 10 s.	127A
Clearfil SE Bond	Primer: MDP, HEMA, water, photoinitator Bond: MDP, bis-GMA, HEMA, TEG-DMA, hydrophobics dimethacrylates, photoinitator	Apply primer for 20 s, gently air dry; apply bond. Light cure for 10 s.	896A 1321A
Adper Singlebond 2	Etchant: 37% phosphoric acid Adhesive: HEMA, bis-GMA, TEG-DMA, polyalkenoic acid copolymer, dimethacrylates, ethanol, water and camphorquinone	Acid etch for 15 s, rinse with water for 15 s, leaving the dentin moist. Bond was applied in two coats and gently air dried. Light cure for 10 s.	7KK 9WP

* Bis-GMA: bisphenol-A-diglycidylmethacrylate; HEMA: hydroxyethylmethacrylate; MDP: 10-methacryloyloxydecylphophate; TEG-DMA: triethylene glycol dimethacrylate.

strengths obtained from sticks of the same tooth were averaged, and the mean was used as statistical unit. The μTBS data were statistically analyzed with two-way ANOVA (adhesive and aging) and Tukey's test ($p < 0.05$).

Subsequent to μTBS, the failure mode of each fractured stick was analyzed using a stereomicroscope (X100, Olympus SZ 40-50; Tokyo, Japan). Five representative fractured sticks exhibiting the most frequent failure mode and a μTBS close to the mean were processed for fractographic analysis using SEM. In brief, the fractured sticks were paired, mounted on aluminum stubs, dehydrated overnight, and gold-sputter coated (Balzers SCD 50; Balzers, Liechtenstein) prior to examination using a JSM-5600LV SEM (JEOL; Tokyo, Japan) operated at 15 kV at a 20 mm working distance. Fractures were classified as adhesive, mixed, cohesive in composite, and cohesive in dentin.[9]

Nanoleakage Assessment

Three central sticks were selected from each bonded tooth ($n = 10$) and processed for silver nanoleakage evaluation, as described in previous studies.[35] Briefly, the sticks were immersed in 50% ammoniacal silver nitrate solution in the dark for 24 h. These were subsequently rinsed with distilled water to remove excess silver solution, then immersed in photo-developing solution for 8 h under fluorescent light to reduce silver ions into metallic silver grains along the adhesive-dentin interface. The silver-impregnated sticks were embedded in epoxy resin, wet ground using SiC papers (600-, 1200- and 2000-grit) and polished using polishing cloths in combination with 6-, 3- and 1-μm diamond suspensions (Buehler). The specimens were cleaned in an ultrasonic bath for 20 min between each polishing step and at the end of the procedure. They were then dehydrated and coated with carbon. The SEM evaluation of nanoleakage was performed in backscattered electron mode.[10]

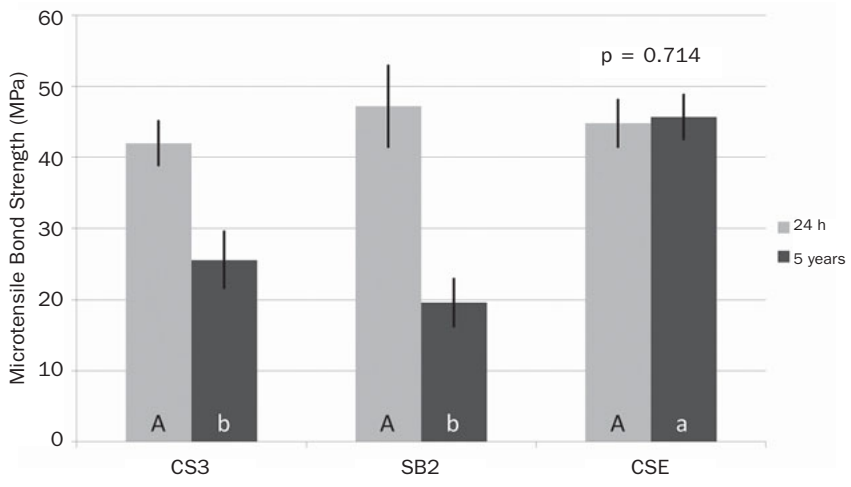
Ultrastructural Transmission Electron Microscopy (TEM)

After 5 years of SPP aging, three sticks were selected for TEM ultrastructural interface evaluation. These specimens were processed for TEM following a protocol described in previous studies.[40] Briefly, the selected specimens were cut (70-90 nm thick) using a diamond knife (Diatome; Bienne, Switzerland) in an ultra-microtome (Ultracut UCT, Leica; Vienna, Austria). The specimens were analyzed and imaged unstained or positively stained (5% uranyl acetate for 20 min followed by saturated lead citrate for 3 min) using a JEOL JEM-1200EX II microscope (JEOL) at 80 kV.

RESULTS

Two-way ANOVA revealed a significant interaction between the adhesive treatments and aging regimes ($p < 0.001$). Tukey's test indicated significant differences between all the tested groups ($p < 0.05$) after 5-year SPP except for the control gold-standard multi-step adhesive (CSE) ($p = 0.714$). In particular, the one-step self-etch adhesive CS3 revealed a significant bond strength reduction after 5-year SPP ($p = 0.006$). A greater reduction in μTBS was attained with the two-step etch-and-rinse adhesive SB2 ($p < 0.001$), reaching the lowest bond strength obtained in this study. Mean μTBS and standard deviations for the different experimental groups are presented in Fig 1. Failure mode analysis predominantly presented mixed failures for all groups at 24 h and adhesive fractures upon SPP aging.

Representative nanoleakage patterns are depicted in the SEM images in Fig 2. After 24 hours, silver uptake within the adhesive-dentin interface for the simplified self-etching adhesive (CS3) was comparable to that observed for the control self-etch adhesive (CSE), while SB2 showed clearly more silver deposits within the hybrid layer (Fig 2b). Considerably greater silver uptake was observed after 5-year SPP for all adhesives, especially within the adhesive and hybrid layers. In addition, the adhesive-dentin interfaces created with the simplified etch-and-rinse adhesive SB2 and the one-step self-etch adhesive CS3 failed upon 5-year SPP with the formation of an interfacial gap (Figs 2d and 2e).



	CS3	SB2	CSE
24 h	42.0 (3.1)^{A,a}	47.2 (5.7)^{A,a}	44.8 (3.3)^{A,a}
5years	25.6 (3.9)^{B,b}	19.6 (3.3)^{B,b}	45.7 (3.1)^{A,a}

Same superscript capital letters in rows and lowercase letters in colums indicate statistical similarity (p>0.05)

Fig 1 Microtensile bond strength (μTBS) of the three adhesives investigated to dentin at 24 h and after 5-year simulated pulp pressure (SPP). The same capital letters indicate absence of statistically significant differences in 24 h μTBS; different lower-case letters indicate statistically significant differences in μTBS after 5-year SPP. The horizontal bar indicates absence of statistically significant differences in μTBS at 24 h vs after 5-year SPP.
A table with numerical results is presented at the bottom.

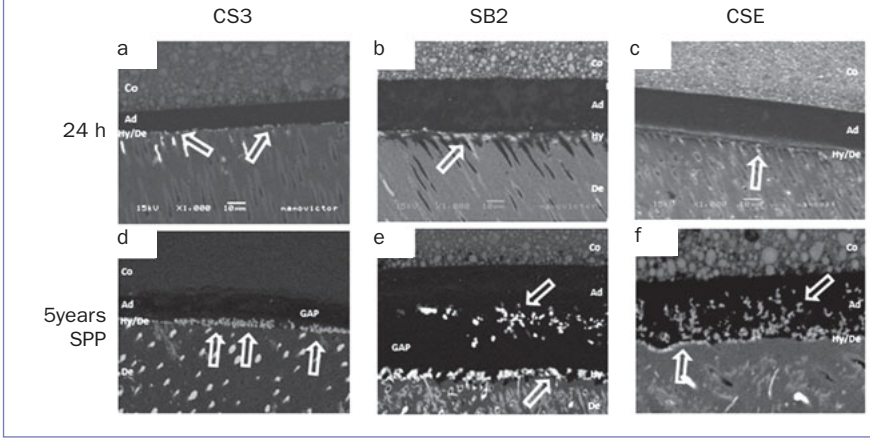


Fig 2 Representative SEM photomicrographs of nanoleakage at adhesive-dentin interfaces produced by the three adhesives investigated at 24 h and after 5-year SPP. The open white arrows demonstrate silver deposition as sign of nanoleakage at the interface or within the adhesive layer. After 5-year SPP, the adhesive-dentin interface of the one-step self-etch adhesive CS3 and the two-step etch-and-rinse adhesive SB2 debonded with the formation of an interfacial gap. Ad = adhesive layer; Co = composite; De = dentin; Hy = hybrid layer.

TEM images (Figs 3 to 5) highlighted adequate interaction of all three adhesives with dentin after 5-year SPP. They indicated different patterns of degradation related to the different adhesives. In summary, Clearfil S3 mainly presented filler debonding and voids within the adhesive layer (Figs 3a to 3c) as well as some hydrolytic degradation of the polymer at the hybrid and adhesive layer (Fig 3d). Interfaces of SB2 after 5 years of SPP are presented in Fig 4. Degradation of SB2 was mainly identified at the hybrid layer with signs of collagen breakdown and with water trees in the adhesive layer. Regarding the control adhesive CSE, only filler debonding due to hydrolysis of the silane coupling agent (Figs 5a and 5b) was observed; the hybrid layer remained largely unaffected after aging (Fig 5c). The presence of nanolayers of 10-MDP-Ca salts within the CSE-dentin interfaces (Fig 5d) after such prolonged aging was noteworthy.

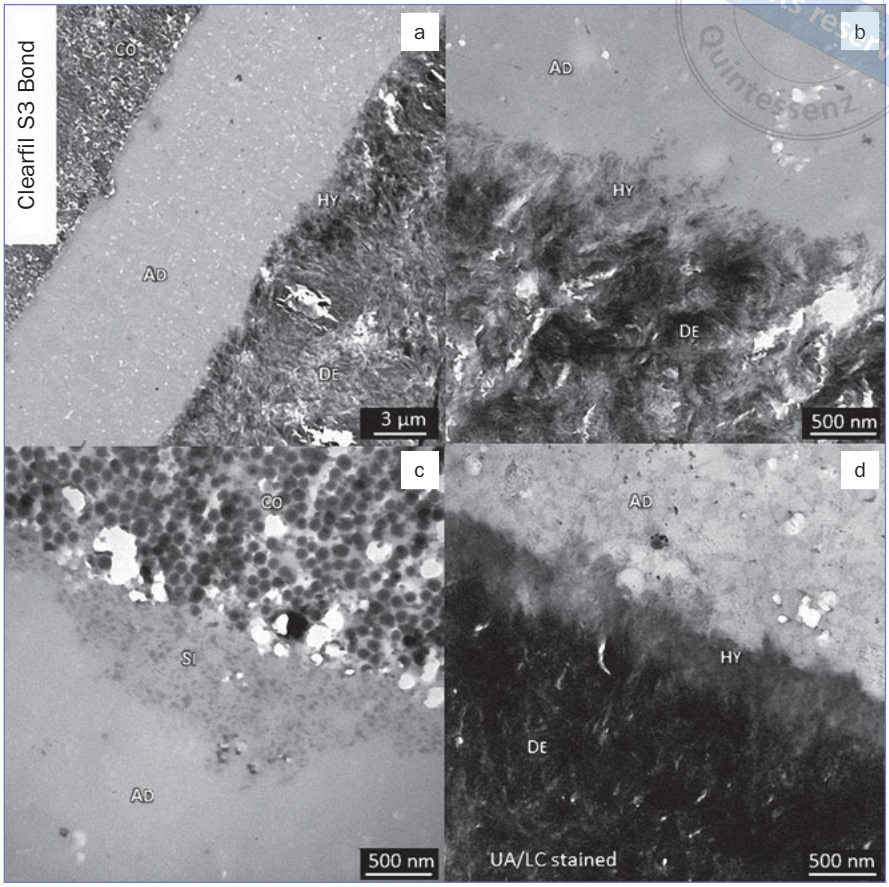


Fig 3 Representative TEM photomicrographs of adhesive-dentin interfaces of CS3 after 5-year SPP. The most prominent degradation indication was the loss of silica filler within the whole adhesive layer (a); this has created a very smooth layer full of small white holes (b), probably indicating that silica filler particles were chipped out during TEM preparation/sectioning. In (c), the top of the adhesive layer contains some remaining filler particles in the area where the water from SPP reached last. Stained sections represented in (d) indicate a dense hybrid layer with some mineral-rich areas (dark grey color) and some with signs of degradation with less intense mineral areas and white lines at the bottom. This might highlight some sub-hybrid layer degradation or sectioning artifacts due to TEM preparation. Ad = adhesive layer; Co = composite; De = dentin; Hy = hybrid layer; Sp = smear plug.

DISCUSSION

The results of this study demonstrated that the two simplified adhesives investigated have different degradation patterns at the adhesive-dentin interface compared to the control adhesive CSE. The latter was the only group to show no significant drop in bond strength after 5-year SPP aging. Therefore, both null hypotheses tested in this study must be rejected.

Our results are in agreement with the recent literature,[13] confirming that with simplified etch-and-rinse adhesives, degradation starts when the smear layer is removed by acid conditioning using 37% ortho-phosphoric acid. This removes 5-8 µm of the underlying intact mineral, thereby exposing type I collagen in the dentin matrix. Such a collagen network forms a template for the diffusion of adhesive monomers which, upon polymerization, generate the hybrid layer. However, it is well known that monomers are not often able to fully infiltrate such a demineralized zone, leaving some exposed poorly resin-infiltrated dentin collagen.[34,35,38]

These unprotected collagen fibrils are prone to rapid hydrolysis, which is the first degradation threshold with etch-and-rinse adhesives and contributes to bond-strength reduction over time.[14] However, some authors have also described the presence of unprotected collagen after the use of self-etch adhesives; this is represented by several degrees of silver deposition (water-rich zones) observed principally at the bottom of the hybrid layer.[35,44] Moreover, polymer degradation at the adhesive layer also occurs with self-etch adhesives, especially under SPP.[11] These findings corroborate with the present observations during SEM evaluation (Fig 2), which depicted nanoleakage in the adhesive layer of the tested adhesives. In particular, the two simplified adhesives SC3 and SB2 suffered intense degradation and showed a drastic decrease in bond strength ($p < 0.05$), as well as strong deposition of silver in the adhesive layer when compared to the control CSE. However, no significant difference was observed between the two simplified adhesives ($p > 0.05$).

It is important to remember that the purpose of this study was to compare the degradation of adhesive-dentin interfaces created using two simplified adhesives applied with different bonding strategies after five-year simulated pulpal pressure. Hence, the multi-step self-etch adhesive (CSE) was used only as a gold-standard control with high longevity. The choice of including only a multi-step self-etch adhesive (CSE) and not a etch-and-rinse multi-step adhesive was dictated by the fact that both a multi-step self-etch and an etch-and-rinse adhesive would in any case act as the gold-standard high-longevity control.[3,29,44]

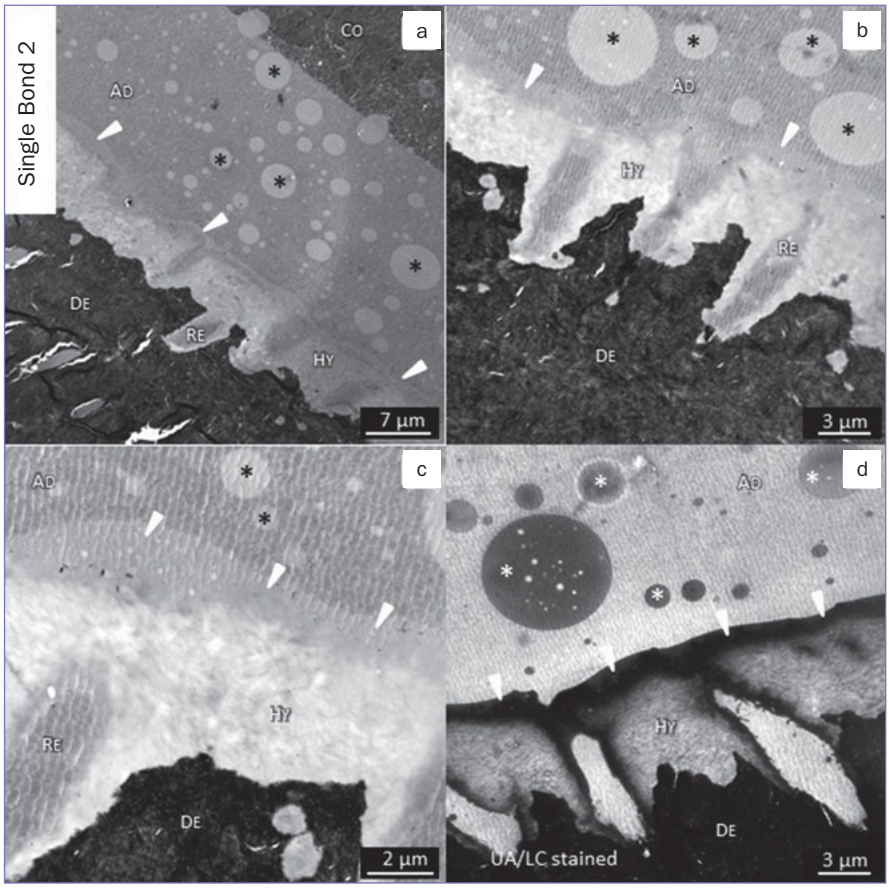


Fig 4 TEM photomicrographs of adhesive-dentin interfaces of SB2 after 5 years of SPP. White arrowheads indicate a dense polyacrylic copolymer layer atop the hybrid layer. Black asterisks mark polyacrylic copolymer globules within the adhesive layer. The less electron-dense collagen in the hybrid layer in the stained section (d) might be correlated with signs of collagen degradation upon aging. The crumbled aspect of the adhesive layer may also represent some polymer hydrolysis. Ad = adhesive layer; Co = composite; De = dentin; Hy = hybrid layer; Sp = smear plug; Re = resin tag.

A suitable explanation for this outcome may be the fact that simplified adhesives are commonly more hydrophilic and may behave as permeable membranes due to suboptimal polymerization, allowing water movement through the bonding interface. In addition, retention of residual water in dentin and adhesives may result in zones of incomplete polymerization, which further enhance polymer degradation.[2,11,27,31] Such degradation may translate clinically into gaps between composites and dental substrates (Figs 2d and 2e). The application of simulated pulpal pressure markedly increases convective fluid movement and reveals water channels through some interfaces. These water-filled channels are potential sites of hydrolytic degradation that may adversely affect the longevity of restorations bonded with these adhesives.[29] Furthermore, the density of water-filled dentin tubules increases with dentin depth, and this may reduce the bond strengths and the longevity of the restoration. Several investigations have already demonstrated the sensitivity of various adhesives to pulpal pressure and dentin depth.[24,28,30,36]

That is why simulated pulpal pressure is well suited in this regard to for obtaining degradation.[11,29,44] It has been established[6,29,30] that pulpal pressure should be 19.6 cm H_2O in order to reproduce the effect of the local vasoconstrictor in local anesthetics. Moreover, it is also imperative to state that pulpal pressure, which is under sympathetic control, may increase due to other factors, such as the presence of specific proteins which influence the osmotic pressure, or dilated lymphatic vessels in inflamed dental pulp, particularly with deep carious cavities. Therefore, the pulpal pressure is high in inflamed pulps, independent of the presence of anesthetics and vasoconstrictors. Pulpal pressure should be implemented during the bonding procedures as well as during storage/aging periods.[15,17] Accordingly, the simulation of the pulpal pressure employed in this study was set to 20 cm H_2O for investigating the degradation of the resin-dentin interface of simplified adhesives over a period of 5 years.

With TEM, it is possible to ultrastructurally analyze the interface, encompass a thicker zone, and generally provide more details than surface observation with SEM. Apart from degradation of exposed resin-sparse collagen and polymer hydrolysis, TEM (Figs 3 and 5) revealed that degradation may occur via hydrolysis of the silane coupling agent with consequent debonding/release of the filler particles, as especially observed with self-etch adhesives used here. This degradation pattern was reported in previous studies[16,37] both for adhesive resins and at adhesive-dentin interfaces

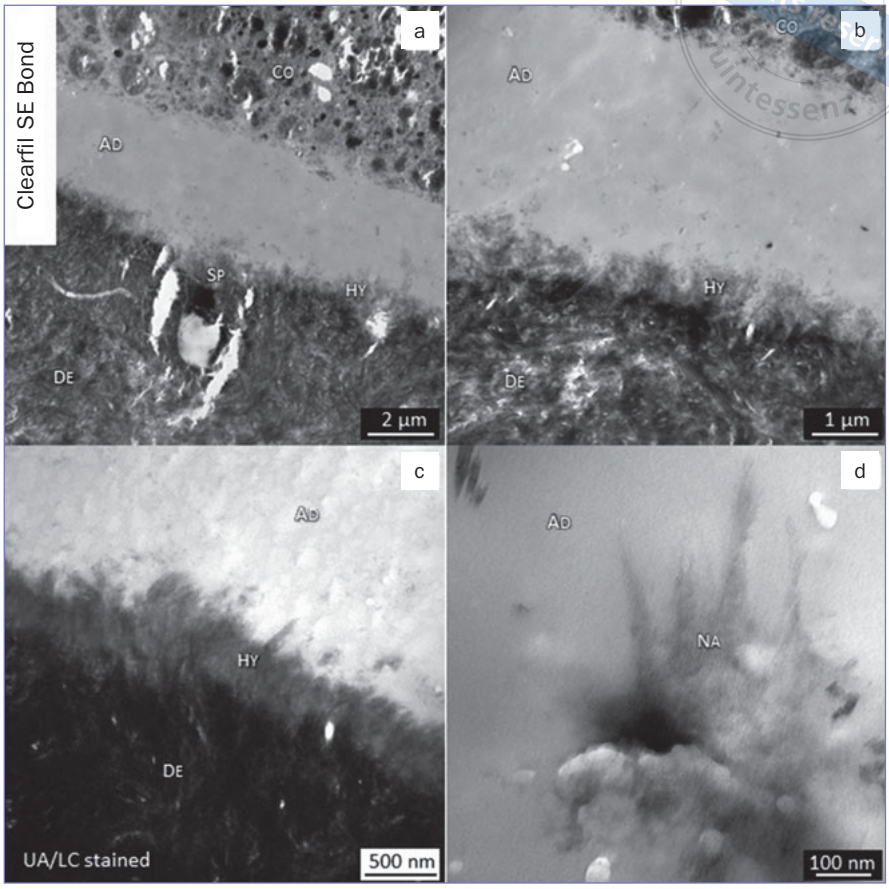


Fig 5 TEM photomicrographs of adhesive-dentin interfaces of CSE after 5 years of SPP. Noteworthy signs of degradation were again the loss of silica fillers within the adhesive layer (a), represented by white holes (b). Such filler debonding might result from TEM preparation/sectioning. Nevertheless, a homogeneous, compact hybrid layer was observed in the stained sections (c). Furthermore, nanolayering of MDP-Ca salts was found at the adhesive layer, which likely provided greater stability to the bonds. Ad = adhesive layer; Co = composite; De = dentin; Hy = hybrid layer; Na = nanolayers of 10-MDP/Ca; Sp = smear plug.

created by self-etch adhesives. It is hypothesized that the mildly acidic pH might induce degradation via released protons, resulting in silane debonding from filler particles.[37] However, Brackett et al[2] showed very similar in vivo outcomes with an etch-and-rinse adhesive upon aging of the adhesive-dentin interfaces. Therefore, filler debonding and release of nanofillers from the adhesive layer of both self-etch and etch-and-rinse adhesives is a recently discovered pattern of degradation of adhesive-dentin interfaces.

The present results show that both self-etch adhesives demonstrated high filler debonding/release (Figs 3a and 5b), involving almost the entire adhesive layer. The present outcomes emphasize the need to gather further evidence on the necessity of water/acid resistant silane coupling agents for nanofillers in adhesives. Indeed, the negative influence of filler debonding/release is based on the resulting water deposits at the adhesive layer, which might indeed accelerate polymer hydrolysis and eventually diminish the durability of composite restorations.[27,37] For this reason, some new commercial self-etch adhesives, for instance All-bond Universal (Bisco; Schaumburg, IL, USA), are free of filler particles in order to reduce this type of potential degradation.

Simulated pulpal pressure over a very long period attains degradation similar to an in vivo scenario.[10,11] The method employed to simulate pulpal pressure has been described in several other studies,[9-12] and may yield reliable water seepage to achieve interface hydrolysis. Several important observations about the degradation of different adhesives may be deduced from present outcomes. However, the high stability of bond strength with specimens created using CSE must also be highlighted. Despite the signs of degradation observed in this study for Clearfil SE Bond bonded to dentin, its bond durability to dentin has been proven in many other in vitro and in vivo investigations.[9,20] Such results are typically attributed to relative low hydrophobicity (ie, less water sorption) of the interface created when using this adhesive, as well as the optimal chemical bonding of the 10-MDP acidic functional monomer to calcium ions in hydroxyapatite.[25] In the present TEM observations, a 10-MDP-Ca nanolayer was identified within the resin-dentin interface (Fig 5d); it is proposed as a "finger print" for the optimal bonding of 10-MDP functional monomer to calcium ions.[41]

The chemical interaction of this acidic functional monomer is enhanced principally by the presence of 10-MDP in the adhesive solution, rather than only in the primer. Acidic monomer in the primer mainly acts as a conditioning agent, partially demineralizing the collagen mesh for monomer infiltration.[20] Conversely, the further supply of acidic mono-

mers with the adhesive may be responsible for the encapsulation of calcium and the actual formation of most chemical bonds.[19,43] This may explain, in part, why the chemical interaction of 10-MDP in the two-step self-etch adhesive CSE provided stable bond strength, while the one-step self-etch adhesives CS3 was less effective.

CONCLUSION

The adhesive-dentin interfacial aging resulting from 5-year SPP yielded important information on degradation patterns for the different adhesives investigated. The simplified etch-and-rinse adhesive interface may chiefly degrade by collagen breakdown within the hybrid layer with signs of polymer hydrolysis within the adhesive layer, while self-etch adhesives may be mainly characterized by degradation of polymer and silane coupling, resulting in filler debonding/release, but less collagen breakdown. Since the two-step self-etch adhesive accomplished stable bond strength after five years, a separate application of a hydrophobic solvent-free adhesive resin should be clinically recommended to enhance durability of restorations.

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Clinical relevance: Resin-dentin interfaces created with simplified adhesives undergo hydrolysis and collagen degradation over time. Multi-step self-etching adhesives may promote greater dentin bond durability.