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Prolonged Curing Time Reduces the Effects of Simulated Pulpal Pressure on the Bond Strength of One-step Self-etch Adhesives

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Clinical Relevance

Prolonged light-curing procedures improve the bond strength of simplified self-etch adhesives, in particular when bonded to deep dentin in the presence of physiological pulpal pressure.

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SUMMARY

The aim of this study was to evaluate the effects of extended light-curing procedures on the microtensile bond strength (μTBS) of one-step self-etch adhesives (1-SEAs) submitted to simulated pulpal pressure. Coronal deep-den-

tin specimens were bonded using Clearfil S3 Bond (S3), Adper Easy Bond (EB), or G-Bond Plus (GB) following the manufacturers' recommendations and light-cured for 10 seconds or 40 seconds. The dentin-bonded specimens were stored in distilled water for 24 hours without pulpal pressure (control) or submitted to 20 cm H_2O simulated pulpal pressure for 24 hours. The specimens were cut into matchsticks and subjected to μTBS testing. The data were statistically analyzed using the three-way analysis of variance and Tukey's tests (*p*<0.05). Debonded sticks were investigated through scanning electron microscopy. EB obtained higher bond strengths than GB and S3. However, prolonged light activation (40 seconds) provided higher μTBS for all adhesives when submitted to pulpal pressure. Conversely, pulpal pressure caused a drop in μTBS in EB and S3 when light-cured for 10 seconds. A mixed failure mode was mainly attained for the control groups, whereas the specimens submitted to pulpal pressure failed in the adhesive mode. The μTBS of GB was not affected by pulpal pressure when light-cured for 10 seconds. Adhesive was the most prevalent failure mode, except when light-cured for 40 seconds, which showed predominantly cohesive failure. Extended curing times improved the resistance of 1-SEAs to simulated pulpal pressure.

INTRODUCTION

One-step self-etch adhesives (1-SEAs) and two-step self-etch adhesives can be considered more user-friendly and less technique sensitive than classic three-step etch-and-rinse adhesives because there are fewer clinical application steps. Nevertheless, some of these simplified adhesives are affected by severe water permeability, especially when bonded in the presence of simulated pulpal pressure.[1] Conversely, multistep dentin bonding agents (DBAs) have shown lower permeability and bond strength reduction because of the application of a more hydrophobic solvent-free adhesive resin.[2,3]

Direct water aging is considered a suitable degradation-promoting strategy to challenge resin-dentin bonds.[4] However, in the presence of simulated physiological pulpal pressure, the water transudation through hybrid and adhesive layers may be increased and provide faster reduction in bond strength.[5,6] This may also provide more polymer hydrolysis and plasticization, jeopardizing the long-term durability of resin-dentin interfaces.[7,8] Hence, the aging strategy based on the use of pulpal pressure is considered a reliable and an effective approach to challenge the resin-dentin bonds in a more relevant clinical situation.[9,10]

It has been demonstrated that simplified 1-SEAs behave as permeable membranes[11,12] because they contain large amounts of hydrophilic monomers and solvents to prevent phase separation and maintain the mixture in a stable solution over time. Nevertheless, because of their hydrophilic nature, these types of DBAs are more prone to water transduction,[3] which compromises the polymerization ratio and the final degree of conversion.[13-15]

Researchers have suggested alternative strategies to improve the bonding performance of 1-SEAs, such as double adhesive application and/or the use of a more hydrophobic resin-bond layer.[2,16,17] Although these procedures have shown great improvements in bonding, they convert the simplified DBAs into multistep bonding systems. Further clinical procedures to improve the performance of 1-SEAs are 1) agitation during application to improve the monomer infiltration,[18] 2) use of a warm airstream to remove more solvent and water during the drying procedure,[19] and 3) extended drying time to increase solvent evaporation.[20] However, there is little information on the effects of prolonged light-curing on the microtensile bond strength (μTBS) of simplified DBAs applied on deep dentin in the presence of simulated pulpal pressure.

The objective of this *in vitro* study was to evaluate the bonding performance through μTBS and scanning electron microscopy (SEM) failure pattern analysis of three 1-SEAs submitted to 24 hours of simulated pulpal pressure (20 cm H_2O) and light-cured in accordance with the manufacturer's recommendations (10 seconds) or with extended exposure time (40 seconds). The null hypothesis to be tested was that extended light-curing procedures have no effect on the μTBS and failure pattern of 1-SEAs applied in the presence of simulated pulpal pressure.

MATERIALS AND METHODS

Sample Preparation

Sixty extracted caries-free human third molars (from patients aged 18–35 years) extracted for periodontal reasons under approval of the appropriate Research Ethics Committee (protocol 167/2009) were used in this study. The teeth were stored (4°C) in a 0.5% chloramine water solution for a period not exceeding two months.

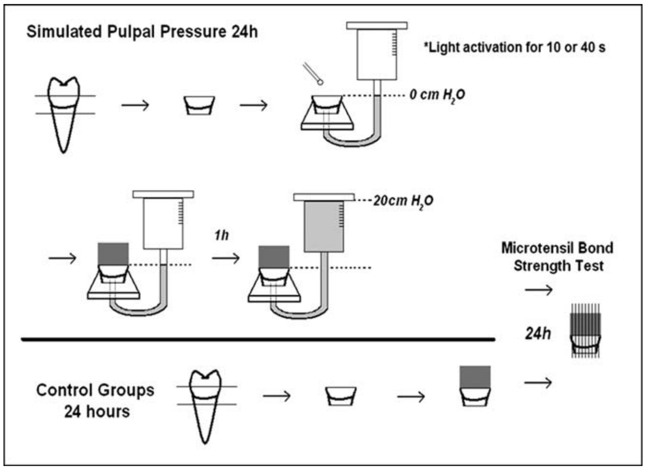

Figure 1. *Experimental design (simulated pulpal pressure and control groups). Both groups were bonded without hydrostatic pulpal pressure. For pulpal pressure groups, the pressure was undertaken during 24 hours by using the classic method described by Sauro and others.*[11]

The roots were removed 1.5 mm below the cement-enamel junction (CEJ), and a parallel cut was made on the occlusal surface 1.5 mm above CEJ using a slow-speed water-cooled diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) to expose a deep dentin with a remaining dentin thickness (the dentin thickness between the flat occlusal surface and the pulpal wall on the highest pulp horn) of 0.9-1.0 mm. The dentin surface was wet-polished for 30 seconds with 600 grit SiC papers to create a flat surface with a standardized and more clinically relevant smear layer.

Subsequently, the specimens were randomly divided into 12 groups according to light-curing time (10 seconds or 40 seconds), DBA (Clearfil S3 [S3], Kuraray Medical Inc; G-Bond Plus [GB], GC Corporation; or Adper Easy Bond [EB], 3M-ESPE), and simulated pulpal pressure (no pulpal pressure for the control or 20 cm H_2O pulpal pressure) (Figure 1). The compositions, application procedures, and batch numbers are summarized in Table 1.

Bonding Procedures and Simulated Pulpal Pressure

The DBAs selected for this study were applied on the entire exposed dentin surface per manufacturers' recommendations (Table 1) and light-cured for 10 seconds (manufacturer's recommendation) or 40 seconds (prolonged time) at a standardized distance of 3 mm. A quartz-tungsten-halogen lamp (XL2500, 3M ESPE, St Paul, MN, USA) with 600 mW/cm^2 irradiance and emission in the 400–500 nm range was selected for the light-curing procedures as the optimum wavelength for polymerization of an ideal light-curing system should lie within the 450–490 nm range for light-curable resin materials containing camphorquinone as the initiator.[21] The irradiance was measured periodically with a calibrated Demetron Radiometer (Model 100, Demetron Research, Danbury, CT, USA). Composite build-ups (Filtek Z350, batch N124853, 3M-ESPE) were made in three or four layers (each layer 1–2 mm thick) to a height of 6 mm; each layer was light-cured for 40 seconds. Subsequently, half of the specimens were stored in distilled water at 37°C for 24 hours, and the remaining half of the specimens was submitted to simulated pulpal pressure (24 hours). All samples were bonded and restored with 0 cm H_2O water pressure.[11] The pulpal pressure was delivered one hour after the bonding procedures to simulate a clinical situation where local anaesthesia induces vasoconstriction and decreases the pulpal pressure.[22,23]

The simulation of the pulpal pressure was undertaken following a previously published protocol.[11] Briefly, the crown segments were fixed using cyanoacrylate glue to a Plexiglas surface perforated by an 18-gauge stainless steel, which permitted communication between the pulp chamber and the hydraulic pressure device. For samples in simulated pulpal pressure groups, the hydraulic pressure device was filled with water to reproduce a pressure of 20 cm H_2O at the bonded dentin surface (Figure 1).

Microtensile Bond Strength

The samples were serially sectioned into slabs approximately 1-mm thick with a diamond saw (Isomet, Buehler) after the storage period. Each slab was further sectioned to produce resin-dentin matchsticks with approximately 1 mm^2 in cross-section. The matchsticks from the most peripheral area showing remnant enamel were excluded from the test.[24]

The specimens were fixed to a jig using a cyanoacrylate glue (Super Bonder gel, Loctite, Henkel, Rocky Hill, CT, USA) in a universal testing machine (EZ-test, Shimadzu, Kyoto, Japan) and tested until failure under tensile tension (0.5 mm/minute). The cross-sectional area of each tested stick was measured with a digital micrometer after debonding. Means and standard deviations were calculated and expressed in megapascals. Five restored teeth (experimental unit, n=5) were evaluated in each group, the bond strength of the matchsticks from the same restored tooth were averaged, and the mean bond strength value was used for the statistical analysis. The μTBS data were

Table 1: *Materials Composition and Application Procedures Used in Restorations*

Materials	Composition	Application Procedure	Batch No.	Manufacturer
Clearfil S3 Bond (S3)	10-MDP, BisGMA, HEMA, dimethacrylates, photoinitiator	Apply adhesive for 20 s.	127A	Kuraray Medical, Tokyo, Japan
		Air-dry for 5 s to evaporate solvent.		
		Light cure for 10 s.		
Adper Easy Bond (EB)	HEMA, BisGMA, Vitrebond copolymer, methacrylated phosphoric esther, nanofiller, ethanol, water, photoinitiator	Apply adhesive for 20 s.	376899	3M ESPE, St Paul, MN, USA
		Air-dry for 5 s to evaporate solvent.		
		Light cure for 10 s.		
G-Bond Plus (GB)	4-MET, phosphate ester monomers, UDMA, acetone, water, microfiller, photoinitiator	Apply adhesive for 10 s.	1007061	GC Corporation, Tokyo, Japan
		Air-dry for 5 s to evaporate solvent.		
		Light cure for 10 s.		

Abbreviations: 4-MET, 4-methacryloxyethyl trimellitic acid; 10-MDP, 10-methacryloyloxydecyldihydrogenphosphate; BisGMA, bisphenol A glycidyl methacrylate; HEMA, hydroxyethyl methacrylate; UDMA, urethane dimethacrylate.

statistically analyzed using three-way analysis of variance (ANOVA; DBA, pulpal pressure, and light-curing time) to identify differences among groups. Statistical differences were compared using Tukey's test ($p<0.05$).

Analysis of Fracture Pattern

The failure pattern was verified after the μTBS test using a stereomicroscope at 60× magnification. Ten representative fractured dentin and composite surfaces from each group, those exhibiting the most frequently observed failure pattern and the μTBS close to the mean, were processed for SEM. The specimens were placed on aluminum stubs, gold-coated (Balzers model SCD 050 sputter coater, BAL-TEC Aktiengesellschaft, Balzers, Liechtenstein), and examined using an SEM (JEOL JSM-5600LV, Tokyo, Japan) operated at 15 kV in secondary electron mode. The failures were classified as follows: adhesive failure (A); mixed failure (M); cohesive failure in composite (C); cohesive failure in dentin (D).

RESULTS

Three-way ANOVA showed that the three factors (dentin bonding agent, pulpal pressure, light-curing time) were statistically significant ($p<0.001$). The interactions were also statistically significant ($p<0.001$), but not for the interaction of the three factors ($p=0.211$). Mean values of μTBS (in megapascals) and standard deviations are shown in Table 2. Each bonded tooth yielded 15–20 matchsticks for the μTBS survey. The specimens that received extended light-curing time (40 seconds) attained significantly higher bond strengths than those light-cured for 10 seconds ($p<0.001$). The only exception was when S3 and EB were not subjected to simulated pulpal pressure. Simulated pulpal pressure induced bond-strength reductions for S3 and EB when light-cured for 10 seconds ($p=0.002$), but not for S3 and EB when light-cured for 40 seconds ($p=0.113$). The bond strength of specimens bonded using GB and light-cured for 10 seconds were not affected by pulpal pressure ($p=0.207$).

The percentages of failure pattern from debonded specimens are shown in Table 3, and some representative images are presented in Figures 2, 3, and 4. Groups without simulated pulpal pressure presented a predominance of mixed failures (Figures 2A,B and 3A,B), and groups with simulated pulpal pressure showed more adhesive failures for the hydroxyethyl methacrylate (HEMA)–rich adhesives EB and S3. For GB, the predominant fractures were adhesives (Figure 4A,B), except in the group of GB light-cured for 40 seconds and subjected to simulated

Table 2: *Means (Standard Deviations) of Microtensile Strength (MPa) of DBA Photoactivation Time[a]*

DBA	No Pulpal Pressure	20 cm H_2O Pulpal Pressure
S3 (10 s)	41.4 (3.9)[A,a]	34.7 (4.5)[B,b]
S3 (40 s)	45.9 (4.3)[A,a]	44.7 (6.3)[A,a]
EB (10 s)	52.1 (4.2)[A,a]	42.2 (3.4)[B,b]
EB (40 s)	52.5 (4.7)[A,a]	49.6 (3.5)[A,a]
GB (10 s)	31.2 (5.6)[B,a]	29.9 (3.0)[B,a]
GB (40 s)	49.3 (4.3)[A,a]	45.6 (4.7)[A,a]

[a] Different capital letters in columns represent statistically significant differences between the light activation times ($p<0.05$). Different lowercase letters in rows represent statistically significant difference between absence and presence of simulated pulpal pressure ($p<0.05$). The differences among DBAs are presented only in the Results section to facilitate the comparison of photoactivation times and presence of simulated pulpal pressure. Abbreviation: DBA, dentine bonding adhesive; EB, Adper Easy Bond; GB, G-Bond Plus; S3, Clearfil S3 Bond.

pulpal pressure in which the predominant failures were cohesive in composite (Figure 4C,D). Regarding groups submitted to hydrostatic pulpal pressure bonded with S3 and EB (10 seconds and 40 seconds), failures in 10-second groups mainly occurred between the adhesive layer and resin composite (Figures 2C and 3C). Conversely, the 40-second groups mostly presented fractures between the hybrid layer and adhesive layer or into the hybrid layer (Figures 2D and 3D).

Table 3: *Fracture Type After Microtensile Bond Strength*

Groups	Fracture Type (%)[a]			
	A	M	C	D
S3 + 10 s + no pulpal pressure	30	37	26	7
S3 + 10 s + pulpal pressure	46	31	23	0
S3 + 40 s + no pulpal pressure	23	47	24	6
S3 + 40 s + pulpal pressure	55	39	5	1
EB + 10 s + no pulpal pressure	38	55	7	0
EB + 10 s + pulpal pressure	61	39	0	0
EB + 40 s + no pulpal pressure	29	57	14	0
EB + 40 s + pulpal pressure	53	41	6	0
GB + 10 s + no pulpal pressure	64	36	0	0
GB + 10 s + pulpal pressure	78	22	0	0
GB + 40 s + no pulpal pressure	42	34	15	9
GB + 40 s + pulpal pressure	25	31	39	5

[a] Type A, adhesive failure; type M, mixed failure; type C, cohesive failure in resin composite; type D, cohesive failure in dentin. Abbreviations: EB, Adper Easy Bond; GB, G-Bond Plus; S3, Clearfil S3 Bond.

DISCUSSION

It is well known that simplified 1-SEAs have an intrinsic ability to absorb water in wet environments because of the high amount of hydrophilic monomers (ie, HEMA) and solvents (eg, water, ethanol, or acetone) contained within their composition.[2,16] This particular ability to absorb a drastic amount of water also induces an important fluid transudation within the resin-dentin interface, which contributes to degradation of the hybrid layer.[3,25,26] Indeed, hydrophilic monomers such as HEMA are able to imbibe large amounts of water, which jeopardizes the mechanical properties of the polymers,[27] such as the modulus of elasticity,[28] and the ultimate tensile strength.[29] Additionally, the degree of conversion of HEMA-containing 1-SEAs is reduced because of the intrinsic water entrapped within the polymer network during evaporation procedures.[14] These factors may justify the drop in bond strength attained in the specimens created with the EB and S3 light-cured for 10 seconds and submitted to pulpal pressure. In contrast, the specimens created with GB (HEMA-free adhesive) and light-cured for 10 seconds showed no bond strength reduction after 24 hours of pulpal pressure. The absence of HEMA within the composition of GB may have provided less immediate water sorption/transudation,[30] greater polymerization,[14] and more cross-linked polymer chains[26] along with the usage of the more volatile solvent acetone; this may have reduced the negative effects of pulpal pressure on the bond strengths.

The extrinsic water uptake is more evident in deep dentin, which is a highly permeable substrate and may supply excessive amounts of water to polymerized adhesives after the vasoconstrictive effect of local anesthetic solutions.[31] Therefore, deep dentin

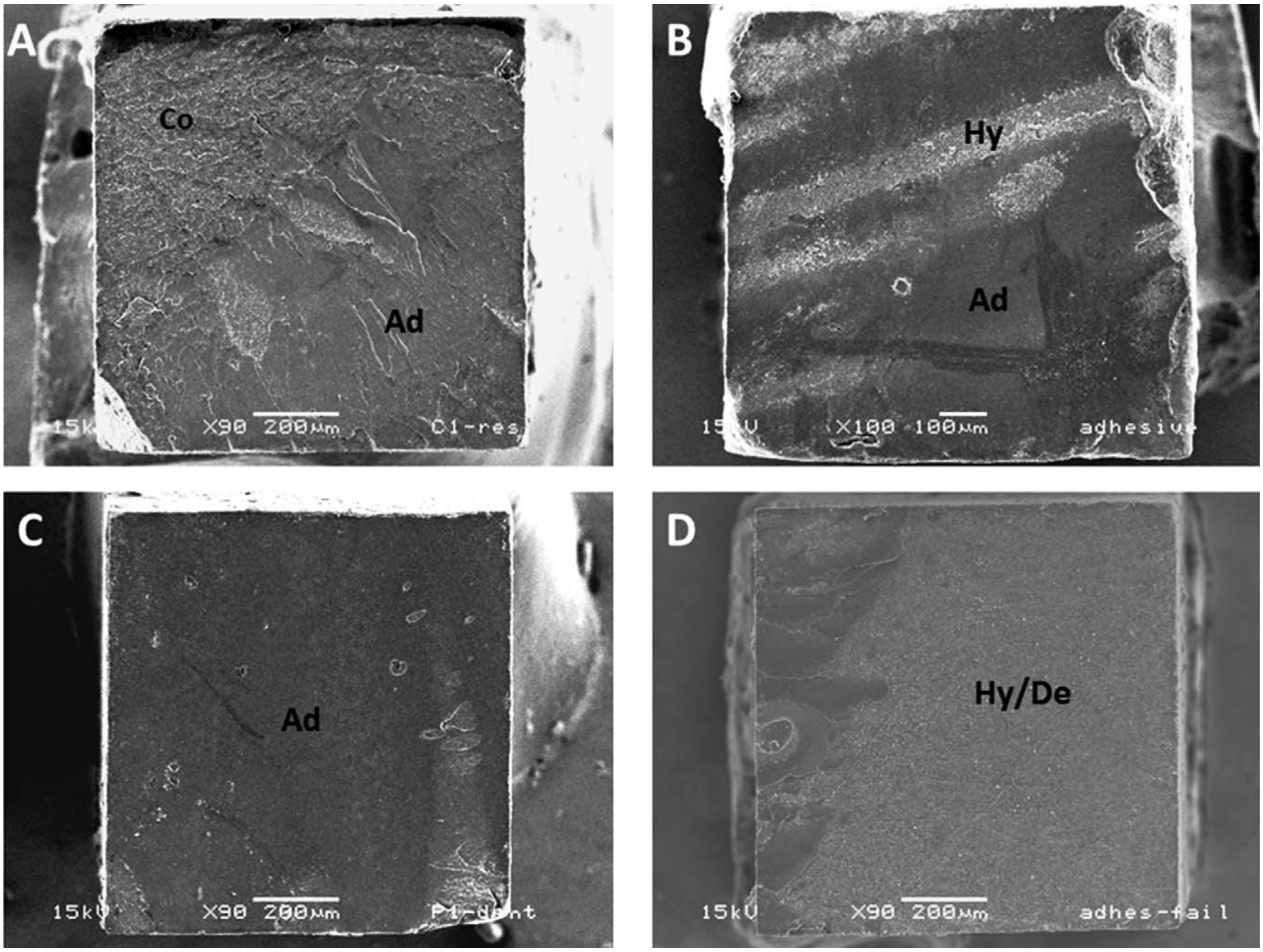

Figure 2. *Representative SEM images showing the most frequent failure modes of S3. (A): Group control with 10-second light activation. A mixed failure among hybrid layer, adhesive layer, and resin composite may be observed. (B): Group control with 40-second light activation. The micrograph shows a mixed failure between hybrid layer and adhesive layer (the predominant failure mode). (C): Group with 10-second light activation subjected to simulated pulpal pressure. The image shows an adhesive failure between the adhesive layer and resin composite (failure often observed in this group). (D): Group subjected to pulpal pressure and 40-second light activation. Note the adhesive failure between the dentin and hybrid layer, with slight vestiges of adhesive layer (predominant failure observed in this group). Abbreviations: Ad, adhesive resin; Hy, hybrid layer; Co, resin composite; De, dentin.*

with a mean thickness of 0.9 mm is currently chosen as the remaining dentin thickness for permeability and pulpal pressure investigations; it results in a suitable substrate for testing more permeable DBAs.[3,11,32-35]

The results of this study showed that the prolonged light-curing time (40 seconds) may improve bonding performance of 1-SEAs and provide higher bond strength in presence of pulpal pressure (Table 2). These findings are in accordance with previous studies that showed a positive correlation between pulpal pressure and μTBS.[3,11,21]

In Table 2, it is possible to observe (control groups) that the bond strength of S3 and EB did not increase with prolonged curing time, whereas the bond strength of GB significantly increased. This may be explained with the different solvents in the adhesive composition.[13,15] For S3 and EB, the solvents are water/ethanol, while for GB they are acetone/ethanol. Indeed, with a prolonged light-curing, the evaporation of solvents (components that jeopardize the polymerization) is improved for acetone-based adhesive, which has a higher vapor pressure, but not for the ethanol-based adhesives with lower vapor pressure. For the latter adhesives, a longer time may be necessary to achieve more evaporation of solvents and an increase in bond strength.

Furthermore, the improvements observed when extended light-curing time was used also corroborate with previous findings[15,36] that showed that pro-

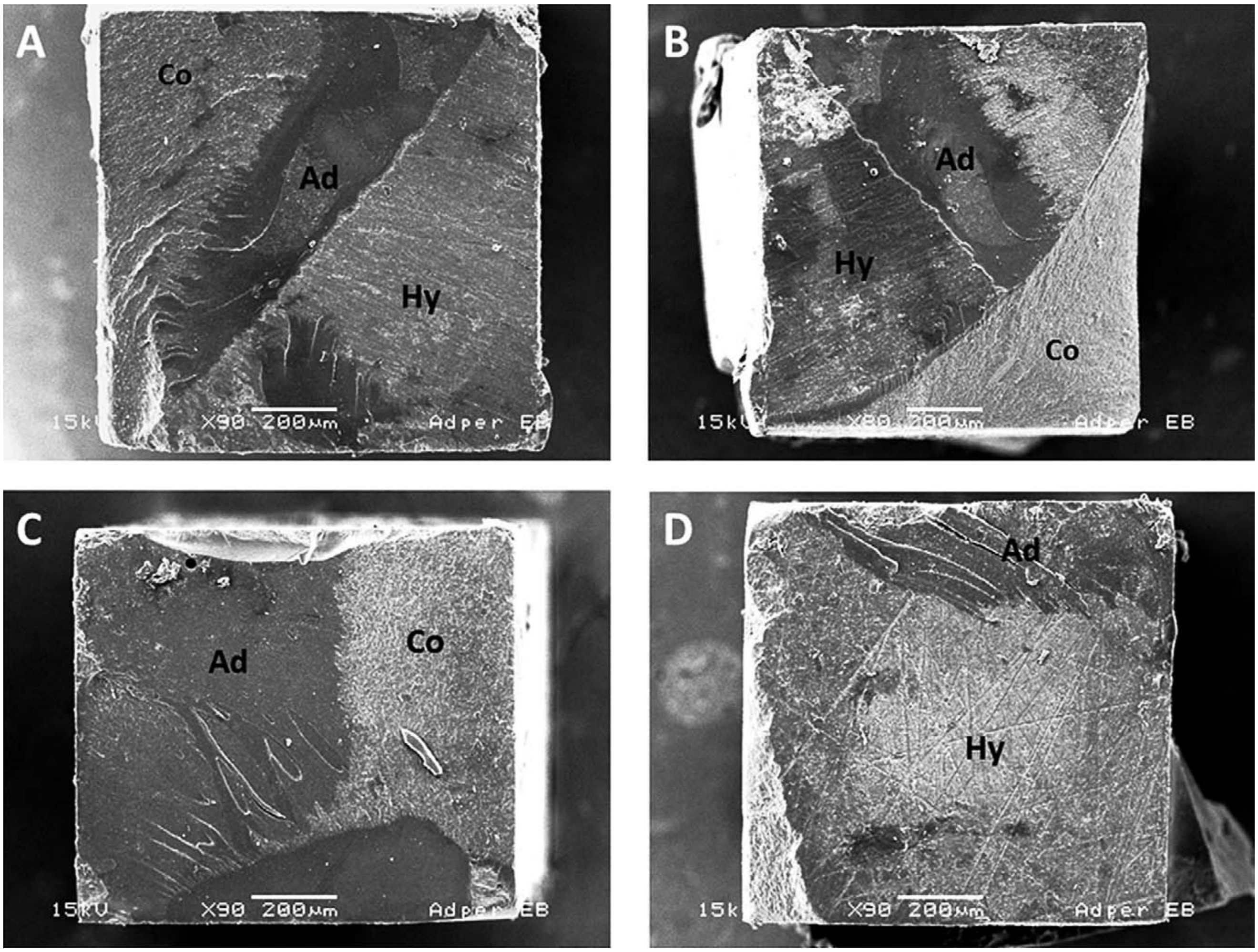

Figure 3. *Representative SEM images showing the most frequent failure modes of EB. (A): Group control with 10-second light activation. A mixed failure among hybrid layer, adhesive layer, and resin composite may be observed. (B): Group control with 40-second light activation. The micrograph reveals the resin side of fractured stick, showing mixed failure between hybrid layer, adhesive layer, and partial cohesive failure in resin composite. (C): Group with 10-second light activation subjected to simulated pulpal pressure. The image shows an adhesive failure between adhesive layer and composite resin with slight vestiges of resin composite (failure often encountered in this group). (D): Group subjected to pulpal pressure and 40-second light activation. Note the adhesive failure between the dentin and hybrid layer, with vestiges of adhesive layer. Abbreviations: Ad, adhesive resin; Hy, hybrid layer; Co, resin composite.*

longed photopolymerization exceeding 40 seconds improves the degree of conversion, creates a more homogeneous and less porous polymer network, and decreases the adhesive permeability and interface nanoleakage of 1-SEAs.[15,36] Indeed, prolonged curing times may improve the physicochemical properties (such as modulus of elasticity, water sorption, and solubility) because of the higher degrees of conversion and more cross-linked polymeric structure.

The results of this study also showed that subjecting specimens to pulpal pressure had a detrimental effect on bond strength for EB and S3, in particular when light-cured for 10 seconds. Pulpal pressure may have promoted water seepage through the adhesive layer to the composite interface (Figures 2C and 3C) because of the osmotic pressure created by uncured monomers at the interface.[37] This may explain the predominance of failures between the adhesive layer and resin composite for EB and S3 in groups light-activated for 10 seconds and submitted to pulpal pressure (Figures 2A and 3A,B).

Conversely, HEMA-rich adhesive systems (EB and S3) light-cured for 40 seconds and subjected to pulpal pressure showed, under SEM analysis, a few failures between adhesive layer and resin composite (Figures 2D and 3D). This confirms that an extended photoactivation of 1-SEAs may diminish the adhesive permeability and the fluid accumulation be-

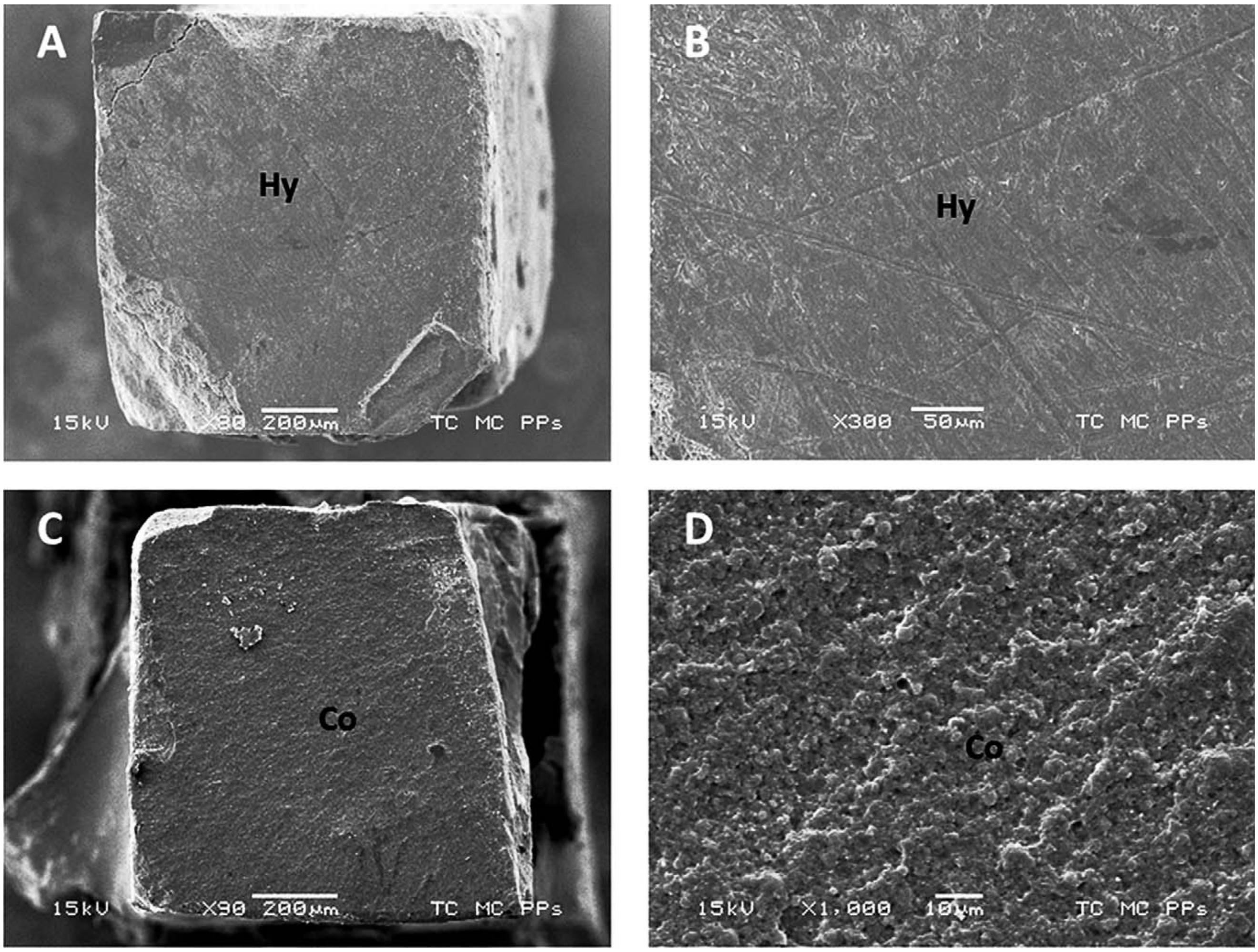

Figure 4. *Representative SEM images showing the most frequent failure modes of GB. (A): Debonded stick from group control with 40-second light activation. A mixed failure among the hybrid layer, adhesive layer, and resin composite may be observed. (B): High magnification of A. The micrograph reveals the resin side of fractured stick, showing adhesive failure in the hybrid layer. Note the vestiges of SiC abrasion undertaken before the bonding procedures. (C): Debonded stick from the group with 40-second light activation subjected to 24 hour simulated pulpal pressure. The image shows a cohesive failure in composite resin (predominant failure in this group). (D): High magnification of C. Note the absence of adhesive resin and hybrid layer vestiges. Abbreviations: Hy, hybrid layer; Co, resin composite.*

tween the adhesive layer and resin composite.[15,36] However, remarkable differences were found between adhesives, light-curing times, and presence of pulpal pressure; therefore, the study hypothesis has to be rejected.

CONCLUSION

In conclusion, under the conditions of this study, 24 hours of simulated pulpal pressure had an adverse effect on μTBS with 10 seconds of photoactivation time, especially for the HEMA-containing adhesives. No difference was encountered when the adhesives were light-cured for 40 seconds. Extended light-curing should be recommended to improve bonding performance of 1-SEAs, especially when bonded in vital deep dentin and in presence of simulated pulpal pressure.

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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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