DENTAL MATERIALS 35 (2019) e204–e219

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/dema

Impact of shelf-life simulation on bonding performance of universal adhesive systems

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ARTICLE INFO

Keywords:
Dentin adhesive systems
Universal adhesives
Product storage
Accelerated aging

ABSTRACT

Objectives. To evaluate the micro-tensile bond strength to dentin (μTBS), the degree of conversion (DC) and nanoleakage expression (NL) of eight dental adhesives considering their expiry date (as-received, half-life and or end of shelf-life) after shelf-life simulation.

Methods. Five universal adhesives (Single Bond Universal, SBU; Tetric Bond Universal, TBU; OneCoat Universal, OCU; OptiBond Universal, OBU; and Prime&Bond Elect, P&B), two two-step self-etch adhesives (Clearfil SE, CSE; and AdheSE, ASE) and one two-step etch-and-rinse adhesive (Adper Singlebond 2, ASB) were evaluated. Shelf-life was simulated by storing the materials in an acclimatization chamber for different periods of time. The μTBS was tested in accordance with ISO/TS 11,405. DC was evaluated by means of FTIR spectroscopy. NL was evaluated after ammoniacal silver challenge. The significance level of $\alpha = 0.05$ was used for all statistical analyses.

Results. The μTBS to dentin of TBU, P&B, ASE, and ASB adhesive systems remained stable throughout the shelf-life periods evaluated, while for SBU, OCU, OBU, and CSE, decreased significantly after evaluation in the 'half-life' or 'end of shelf-life' condition ($p < 0.05$). Except for P&B, ASE and OBU, the degree of conversion significantly decreased after the shelf-life simulation ($p < 0.05$). OCU, ASE, and CSE showed significantly increased percentage of silver deposition within the adhesive layer ($p < 0.05$).

Significance. Storing conditions and progressively longer storage time affect the performance of universal adhesives systems.

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https://doi.org/10.1016/j.dental.2019.05.023

1. Introduction

Adhesive systems are used to achieve adhesion to dental structures. The use of restorative materials in conjunction with the adhesive technique has become routine in dental practice today, especially because professionals prefer these materials because of their advantages such as aesthetics, improved adhesive properties, and conservation of dental structure, which, in turn, leads to strengthening the remaining dental structure [1].

The original multicomponent etch-and-rinse bonding systems have gradually been replaced by simplified, monocomponent self-etch adhesive systems that are more user-friendly [2]. One of the latest developments in adhesive dentistry was the introduction of universal adhesives, designed for application by means of both the etch-and-rinse technique and/or the self-etch technique using the same single bottle of adhesive solution [3]. Despite this attempt to provide more versatile and user-friendly materials, self-etch adhesives systems present a wide variety of problems related to changes in the composition of the material over the period of storage in a dental office due to hydrolysis or polymerization of the monomers, degradation of the additives (initiators/stabilizers), or evaporation of ingredients [4]. In order to minimize these effects, some manufacturers have recommended storage at low temperatures (below 10 °C), despite this, the shelf-life and stability of these materials continues to be an important concern [5].

In dental clinical practice, the shelf-life of adhesives is extremely important. The reason for failing to achieve optimal bonding performance might not only be due to poor clinical procedures but also to the limited shelf-life of the single-step self-etch adhesives [6]. Based on this, manufacturers always stipulate an expiry date (commonly 2 years), after which the material is expected to exhibit undesirable physicochemical properties for its correct application [7]. In this context, various sets of criteria have been proposed to determine which are acceptable levels of stability and how to measure them; these include mechanical, optical, surface, and biological properties [8]. These criteria are specifically applied to assess the stability of medicinal products and although these may be useful as a starting point for establishing a set of criteria for assessing the stability of dental products, other variables should be evaluated, especially because the shipment, transport, and storage conditions of the materials for use in dentistry prior to clinical application are not always ideal.

Considering this, the aim of this study was to characterize eight adhesive systems (five universal, two two-step self-etch and one etch-and-rinse) considering their expiry date after shelf-life simulation. The effect of shelf-life simulation on the micro-tensile bond strength to dentin, nanoleakage, and degree of conversion was explored. The null hypothesis tested was that the shelf-life simulation of adhesive systems would not affect the properties of micro-tensile bond strength to dentin, nanoleakage or degree of conversion of the materials evaluated.

2. Material and methods

2.1. *Study design and accelerated aging protocol*

In this study, the micro-tensile bond strength, nanoleakage, and degree of conversion of five universal adhesives systems were analyzed considering the material condition (as-received, half-life and end of shelf-life) after different periods of shelf-life simulation. Five universal adhesives (Single Bond Universal, SBU; Tetric Bond Universal, TBU; OneCoat Universal, OCU; OptiBond Universal, OBU; and Prime&Bond Elect, P&B) were evaluated. Additionally, two two-step self-etch adhesives (Clearfil SE, CSE; and AdheSE, ASE) and one two-step etch-and-rinse adhesive (Adper Singlebond 2, ASB) were evaluated for the purpose of making comparisons. The composition of the adhesive systems evaluated in this study is described in Table 1.

The dental adhesive systems were characterized as soon as they were purchased on the online market and received, by considering them to be in ‘as-received’ condition. The materials were characterized as being in the ‘half-life’ and ‘end of shelf-life’ conditions after shelf-life simulation, by storing the materials in an acclimatization chamber at 40 °C and 50% relative humidity for different periods of time. The period of time necessary to achieve the ‘half-life’ and ‘end of shelf-life’ condition was calculated individually for each adhesive system using the Arrhenius model [9], according to the following formula:

$$r = Q_{10}{}^{(RT-ET/10)}$$

where r was the accelerated aging rate; RT the storing temperature recommended by the manufacturer; ET the storage temperature in the acclimatization chamber (40 °C) and Q_{10} the reaction rate coefficient (2).

The ‘half-life’ condition was considered after simulation of half of the shelf-life of the material (approximately 1 year considering the expiry date), and the ‘end of shelf-life’ condition was considered once the adhesive reached the expiry date specified by the manufacturer (Table 2). For Single Bond™ Universal, for example, the ‘half-life’ and ‘end of shelf-life’ conditions were reached after 4 and 9 weeks of storage in the climatic chamber, respectively.

2.2. *Micro-tensile bond strength (μTBS) and failure mode analysis*

One hundred and twenty extracted bovine incisors were collected, cleansed of soft tissue, and stored in 0.5% Chloramine-T solution for seven days. Then, they were removed from the disinfectant solution, washed abundantly, and stored in distilled water at 4 °C until use [10]. For specimen preparation, the root was sectioned, and their crowns were embedded in acrylic resin, allowing the buccal enamel surface to be exposed. Then, the enamel was abraded with an orthodontic grinder until exposure of a flat medium dentin surface. The exposed dentin surface was then wet-ground with P600 silicon carbide sandpaper for 30 s to standardize the smear layer. Dentin specimens were randomly divided

Table 1 – Main components of adhesives system used.

Name	Manufacturer	Main components[a]
Single Bond™ Universal (SBU)	3M ESPE, St.Paul, MN, USA	2-Hydroxyethyl methacrylate, Bisphenol A Diglycidyl Ether Dimethacrylate, Decamethylene dimethacrylate, ethanol, Silane treated silica, water, 2-propenoic acid, 2-Methyl-, reaction products with 1,10-decanediol and phosphorous oxide, copolymer of acrylic and itaconic acid, dimethylamino ethyl methacrylate, camphorquinone, dimethylaminobenzoate, 2,6-di-*tert*-butyl-P-cresol.
Tetric® N-Bond Universal (TBU)	Ivoclar Vivadent, Schaan, Liechtenstein	2-hydroxyethyl methacrylate, Bisphenol A Diglycidyl Ether Dimethacrylate, ethanol, 1,10-decandiol dimethacrylate, Methacrylated phosphoric acid ester, camphorquinone, 2-dimethylaminoethyl methacrylate.
OneCoat 7 Universal (OCU)	Coltène/Whaledent Inc., Cuyahoga Falls, OH, USA	Ethanol, urethane dimethacrylate, 2-hydroxyehtyl methacrylate.
OptiBond® Universal (OBU)	Kerr, Orange, CA, USA	Acetone, 2-hydroxyethyl methacrylate, glycerol dimethacrylate, ethanol, glycerol phosphate dimethacrylate.
Prime&Bond Elect® (P&B)	Dentsply Caulk, Milford, DE, USA	Acetone, Urethane Dimethacrylate Resin, Dipentaerythritol pentaacrylate phosphate, Polymerizable dimethacrylate resin, Polymerizable trimethacrylate resin.
AdheSE® (ASE)	Ivoclar Vivadent, Schaan, Liechtenstein	Primer: phosphonic acid acrylate, bis-acrylamide derivative. Bond: Bisphenol A Diglycidyl methacrylate, 2-hydroxyethyl methacrylate.
Clearfil SE Bond 2 (CSE)	Kuraray Noritake Dental Inc.	Primer: 2-hydroxyethyl methacrylate, 10-Methacryloyloxydecyl dihydrogen phosphate, Hydrophilic aliphatic dimethacrylate, dl-Camphorquinone, Accelerators, Water, Dyes. Bond: Bisphenol A Diglycidyl methacrylate, 2-hydroxyethyl methacrylate, 10-Methacryloyloxydecyl dihydrogen phosphate, Hydrophobic aliphatic dimethacrylate, Colloidal silica, dl-Camphorquinone, Initiators, Accelerators.
Adper™ Single Bond 2 (ASB)	3M ESPE, St.Paul, MN, USA	Ethyl alcohol, Bisphenol A Diglycidyl methacrylate, silane treated silica, 2-hydroxyethyl methacrylate, glycerol 1,3-dimethacrylate, copolymer of acrylic acid and itaconic acids, water, diurethane dimethacrylate, diphenyliodonium hexafluorophosphate, ethyl 4-dimethyl aminobenzoate

[a] According to Manufacturers' MSDS.

into eight groups based on the adhesive system used. Subsequently, the specimens were divided into subgroups (n = 5) according to the material condition: as-received, half-life and end of shelf-life.

The adhesive systems were applied according to the manufacturers' instructions (Table 2), universal adhesives were applied in the self-etch mode. After the bonding procedures, resin composite build-ups (Filtek™ Z250, 3M ESPE, St. Paul, MN, USA) were constructed in 3 increments of 2 mm each and each layer was polymerized for 30 s. Light-curing procedures were performed using a LED photopolymerization unit Radii-cal (SDI Limited, Victoria, Australia). After immersion in distilled water at 37 °C for 24 h, the specimens were sectioned using a slow-speed diamond saw (Isomet Saw 1000 Precision, Buehler Ltd., Lake Bluff, IL, USA) to obtain resin-dentin sticks with a cross-sectional area of approximately 0.9 mm^2

After storage in distilled water at 37 °C for 24 h, the sticks were individually fixed to a tensile testing device with cyanoacrylate glue and the μTBS was tested in a mechanical universal test machine (DL 500, EMIC®, Pinhais, PR, Brazil), at a crosshead speed of 1 mm/min with a 100 N load cell. The fractured portions of the specimens were observed under a light microscope at 40× magnification to classify failures as adhesive, cohesive within dentin, cohesive within composite or mixed failures. For each tooth, the results obtained of the five sticks tested were averaged, and the mean obtained was then used for statistical purposes. Specimens with pretesting failures were included in the tooth mean value; for this purpose, the average value between zero and the lowest bond strength value obtained in each tooth was used [11].

2.3. *Nanoleakage evaluation*

Three resin-bonded sticks from each tooth were not tested in μTBS and were prepared for nanoleakage evaluation. The sticks were subjected to an ammoniacal silver nitrate solution challenge following the protocol described by Tay et al. [12]. Subsequently, silver-impregnated specimens were polished with wet 600, 1000, 1200, 1500, 2000 and 2500 grit silicon carbide sandpaper for 60 s, followed by 1 and 0.25 μm diamond paste (Buehler Ltd., Lake Bluff, IL, USA) using a polishing cloth. The sticks were ultrasonically cleaned, air dried, mounted on stubs, and coated with gold-palladium. Adhesive–dentin interfaces were analyzed by scanning electron microscopy operated in the backscattered mode (JSM - 6610LV, Jeol, Tokyo, Japan). Three images were captured of each resin–dentin bonded stick. The relative percentage of nanoleakage was measured in all images using the ImageJ software (v 1.0i, National Institute of Health, USA). The mean nanoleakage percentage of all sticks from the same tooth was averaged for statistical purposes.

Table 2 – Main information and application directions of adhesive systems used.

Material	Batch #	Shelf-life percentage[a]	Expiration date	Storing conditions	Application procedure
SBU	645031	15%	Nov-18	2 °C/25 °C	Apply with rubbing for 20 s. Air dry for 5 s. Light-cure for 10 s.
TBU	V25219	20%	Oct-18	2 °C / 28 °C	Apply with rubbing for 20 s. Air dry for 5 s. Light-cure for 10 s.
OCU	H62762	15%	Apr-19	4 °C/8 °C	Apply with rubbing for 20 s. Air dry for 5 s. Light-cure for 10 s.
OBU	6371589	25%	May-19	2 °C/8 °C	Apply with rubbing for 20 s. Air dry for 5 s. Light-cure for 10 s.
P&B	170505	15%	May-20	2 °C/8 °C	Apply with rubbing for 20 s. Air dry for 5 s. Light-cure for 10 s.
ASE	V01867 (Primer) V03476 (Adhesive)	15%	May-18 Jul-18	2 °C/28 °C	Apply primer with rubbing for 15 s and leave for other 15 s. Dry with a strong stream of air. Apply bond and disperse with a very weak stream of air. Light-cure for 10 s.
CSE	670203 (Primer) 6L0329 (Adhesive)	10%	Nov-18	2 °C/8 °C	Apply primer and leave for 20 s. Air dry with a mild air stream. Apply bond and disperse using an air stream. Light-cure for 10 s.
ASB	N855670	15%	Feb-20	21 °C/24 °C	Apply Scotchbond etchant to dentin. Leave in place for 15 s. Rinse for 10 s. Blot excess water leaving tooth moist. Apply 2 consecutive coats of adhesive. Air dry for 5 s. Light-cure for 10 s.

[a] Percentage of shelf-life considering the expiration date when the material was characterized in the ‘as-received’ condition.

2.4. *Degree of conversion*

The degree of conversion was evaluated using real-time Fourier transformed infrared spectroscopy (Prestige21; Shimadzu, Tokyo, Japan) equipped with an attenuated total reflectance device. Previously, 10 μL of each adhesive system was transferred to a small plastic receptacle and air-dried for 30 s to remove solvents. After solvent evaporation, the material was placed on the diamond crystal. A spectrum was captured before and after the polymerization process. The degree of double bond conversion was obtained considering the height of the absorption band (% of absorbance) corresponding to the υC=C aliphatic bond at 1638 cm^{-1}, and as an internal standard, the height of the absorption band (% of absorbance) corresponding to the υC=C aromatic bond at 1609 cm^{-1}. Each test was performed in triplicate.

2.5. *Statistical analysis*

Statistical analysis was performed using the Sigma Plot 12.0 software. The data were analyzed to test the assumption of normal distribution and homogeneity of variance. Two-way ANOVA was conducted to evaluate the effect of the adhesive system and shelf-life simulation on the micro-tensile bond strength to dentin. The frequency of failure mode for each adhesive system was analyzed by the Chi-Square test. For each adhesive system, independent one-way ANOVA and Kruskal–Wallis tests were performed to evaluate the effect of the period of shelf-life simulation on the nanoleakage and degree of conversion. Post hoc multiple comparisons were performed using the Tukey test. For each adhesive system, additional linear regression analyses between micro-tensile bond strength, the degree of conversion, or nanoleakage and the shelf-life period, were performed. A significance level of $\alpha = 0.05$ was used for all analyses.

3. Results

Table 3 shows the μTBS to dentin of the adhesives system used considering their period of shelf-life simulation. Two-way ANOVA revealed that there were significant differences in μTBS to dentin according to the type of adhesive system ($p < 0.001$) and the period of shelf-life simulation ($p = 0.003$). There was also a significant interaction effect between these two variables ($p < 0.001$). The bond strength to dentin of TBU, P&B, ASE, and ASB adhesive systems remained stable during the shelf-life periods evaluated ($p > 0.05$). On the other hand, the bond strength to dentin of SBU, OCU, OBU, and CSE decreased after the evaluation in the ‘half-life’ or ‘end of shelf-life’ condition. Linear regression analysis showed a significant correlation between μTBS average according to the period of shelf-life simulation for OCU, OBU and CSE ($p < 0.05$).

Fig. 1 summarizes the failure mode distribution among the adhesive systems considering their period of shelf-life simulation. The number of adhesive failure mode and pre-failure tests increased when the adhesive systems were applied in their ‘half-life’ or ‘end of shelf-life’ condition. For all materials, the variability in the frequency of different failure modes according to the period of shelf-life simulation was statistically significant (Chi-Square test, $p < 0.05$).

The degree of conversion values are shown in Table 4. Except for P&B, the degree of conversion decreased after

Table 3 – Microtensile bond strength to dentin of the adhesive systems evaluated after different periods of shelf-life simulation [mean (±SD)].

Group	Period of shelf-life simulation		
	As-received	Half-life	End of shelf-life
Single Bond^{TM} Universal	^{A} 36.48 (9.61) a	^{A} 35.01 (5.30) a	^{B} 25.90 (5.82) ab
Tetric$^{®}$ Bond Universal	^{A} 30.35 (8.58) a	^{A} 28.78 (7.23) ab	^{A} 26.67 (6.25) a
One Coat 7 Universal	^{A} 16.62 (3.18) b	^{A} 14.35 (6.12) c	^{B} 7.73 (4.72) c
OptiBond$^{®}$ Universal	^{A} 31.39 (3.81) a	^{B} 19.86 (7.23) bc	^{B} 18.59 (4.40) bc
P&B Elect$^{®}$	^{A} 14.36 (5.46) b	^{A} 17.06 (1.85) bc	^{A} 12.97 (7.89) bc
AdheSE$^{®}$	^{A} 18.00 (3.97) b	^{A} 21.38 (5.43) bc	^{A} 20.60 (5.61) bc
Clearfil SE	^{A} 36.61 (8.58) a	^{B} 22.34 (3.45) bc	^{B} 16.29 (4.46) bc
Adper^{TM} Single Bond 2	^{A} 36.54 (5.56) a	^{A} 30.00 (2.16) ab	^{A} 28.69 (6.95) a

Similar capital superscript letters (comparisons in same row) and lowercase letters (comparisons in same column) indicate no significant differences. ($p < 0.05$).

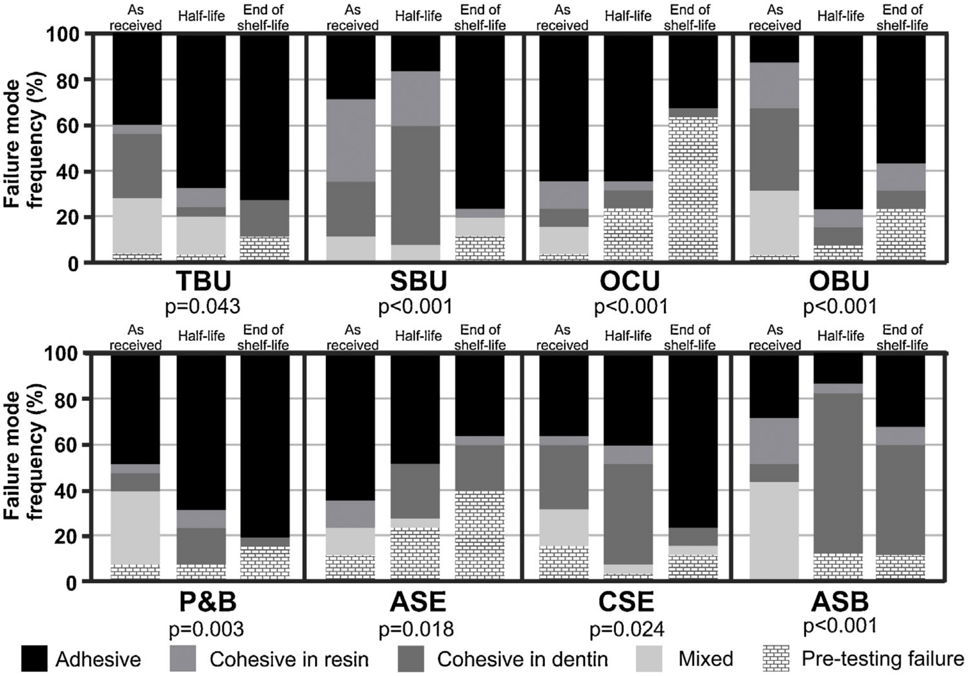

Fig. 1 – Failure mode distribution of the adhesives systems evaluated after μTBS.

Table 4 – Degree of conversion of the adhesive systems evaluated after different periods of shelf-life simulation [mean(SD)].

	Period of shelf-life simulation			Linear regression	
	As-received	Half-life	End of shelf-life	R^2	p
Single Bond^{TM} Universal	^{A} 88.29 (0.08)	^{B} 83.92 (0.34)	^{C} 64.04 (1.21)	0.876	<0.001
Tetric$^{®}$ Bond Universal	^{A} 87.10 (1.70)	^{B} 74.29 (1.43)	^{B} 76.45 (3.05)	0.523	0.028
One Coat 7 Universal	^{A} 92.41 (0.16)	^{B} 73.83 (2.57)	^{C} 65.41 (1.39)	0.934	<0.001
OptiBond$^{®}$ Universal	^{A} 74.89 (0.95)	^{B} 79.82 (0.71)	^{B} 82.36 (1.56)	0.854	<0.001
P&B Elect$^{®}$	^{A} 88.39 (1.4)	^{A} 81.88 (6.37)	^{A} 88.63 (3.35)	–	n.s.
AdheSE$^{®}$	^{A} 67.96 (4.38)	^{AB} 78.50 (0.26)	^{C} 77.38 (2.37)	0.483	0.038
Clearfil SE[a]	^{A} 63.36 (0.58)	^{A} 65.14 (4.00)	^{B} 52.01 (1.44)	0.537	0.025
Adper^{TM} Single Bond 2	^{A} 86.97 (0.35)	^{B} 82.24 (2.47)	^{B} 80.86 (1.56)	0.639	0.010

NS = not significant.

Common corresponding capital superscript letters (A–C) in a given row indicate no significant differences.

[a] Analyzed using Kruskal–Wallis test.

Table 5 – Nanoleakage of the adhesive systems evaluated after different periods of shelf-life simulation [mean(SD)].

	Period of shelf-life simulation			Linear regression	
	As-received	Half-life	End of shelf-life	R^2	p
Single Bond™ Universal[a]	^{A} 4.52 (3.76)	^{A} 6.11 (1.77)	^{A} 5.29 (3.01)	–	n.s.
Tetric® Bond Universal	^{A} 2.99 (2.62)	^{A} 8.99 (6.23)	^{A} 9.54 (2.07)	–	n.s.
One Coat 7 Universal	^{B} 1.61 (2.03)	^{AB} 6.27 (5.44)	^{A} 14.13 (0.60)	**0.763**	**0.002**
OptiBond® Universal[a]	^{AB} 6.84 (2.21)	^{B} 3.80 (1.32)	^{A} 10.34 (1.04)	–	n.s.
P&B Elect®	^{A} 5.75 (4.91)	^{A} 6.16 (2.40)	^{A} 5.44 (2.69)	–	n.s.
AdheSE®	^{B} 2.61 (0.66)	^{B} 5.09 (0.43)	^{A} 12.60 (3.31)	**0.807**	**<0.001**
Clearfil SE	^{C} 1.52 (0.15)	^{B} 5.53 (0.95)	^{A} 12.61 (0.63)	**0.962**	**<0.001**
Adper™ Single Bond 2	^{A} 0.31 (0.21)	^{A} 3.28 (2.33)	^{A} 3.70 (0.66)	**0.533**	**0.025**

NS = not significant.

Common corresponding capital superscript letters (A–C) in a given row indicate no significant differences.

[a] Analyzed using Kruskal–Wallis test.

the end of shelf-life period of shelf-life simulation ($p < 0.05$). For ASE and OBU, the degree of conversion significantly increased after the shelf-life simulation. A significant correlation between the degree of conversion and the period of shelf-life simulation was observed for all the materials, except for P&B.

With regard to nanoleakage, OCU, ASE, and CSE adhesives showed significantly increased percentage of silver deposition within the adhesive layer ($p < 0.05$) after shelf-life simulation. For these materials Linear regression analysis revealed a significant correlation between this variable and the period of shelf-life simulation (Table 5).

4. Discussion

In this study, the characterization of several adhesive systems according to a protocol of accelerated aging, simulating different shelf-life periods of the materials, was performed. The results obtained suggested that most of the evaluated properties were affected after shelf-life simulation, and these changes were material-dependent. Considering this, the null hypothesis tested was partially rejected.

The micro-tensile bond strength test is currently recommended as the best method to evaluate the bond strength of adhesive systems, and its considered useful for preliminary evaluation as a pre-clinical test [11]. According to Table 3, SBU, OCU, OBU, and CSE adhesives had a significant decrease in bond strength values after the shelf-life simulation, especially when evaluated in their end of shelf-life condition. This decrease in the bond strength values could be related to the chemical composition of these materials. According to the manufacturer's safety data sheet, SBU and CSE materials are formulated with 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), while OBU has glycerol phosphate dimethacrylate (GDMA-P) in its composition. On the other hand, although this was not specified for OCU, one of the above-mentioned monomers was probably used in its formulation. According to previous data, ester based adhesive formulations with acid pH values are very prone to undergoing hydrolysis [13,14]. Consequently, free methacrylic acid, ethylene glycol, other alcohol derivatives, and free phosphoric acid are formed [15]. This hydrolytic phenomenon changes the chemical composition of the adhesive over the period of storage in the warehouse or dental office, affecting its properties and impairing the bond strength between substrates [16].

Surprisingly, TBU and P&B universal adhesives maintained their bond strength values after simulation of the shelf-life period, even after evaluation in their 'end of shelf-life' condition. The P&B universal adhesive contains dipentaerythritol penta-acrylate phosphate monomer (PENTA-P) in its composition. The degradation mechanism of PENTA-P monomer is unknown, however, it could be hypothesized that, unlike the 10-MDP adhesive monomer, the presence of five vinyl groups within its chemical structure could make it more resistant to hydrolytic degradation. Thus, when hydrolysis occurs and breaks a vinyl group off the main structure of the monomer, four vinyl groups still remain available to maintain the connection to the phosphate group, which allows copolymerization with the other monomers, and at the same time, adhesion to the tooth structure [17]. With regard to TBU universal adhesive, since it has a relatively high pH about 3 [18], it is possible that the degradation rate of the methacrylated phosphoric acid ester on which this material is based is slower than it is in the other materials. As the hydrolysis of ester bonds into acidic aqueous media depends on how acidic the materials are, it seems that the use of self-etch adhesives with relatively higher pH could lead to materials with high shelf-life stability.

On the other hand, ASE and ASB adhesives showed bond strength stability among the shelf-life periods evaluated. This result was not surprising since ASE material is formulated with the use of patented acrylamide hydrolytically-stable monomers [19]. Because of their physical-chemical-stability, acrylamides have been proposed as an alternative to conventionally used methacrylates, mainly for purposes of increasing the shelf-life of dental adhesive formulations [20]. Amide bonds are more resistant to hydrolytic degradation since they are susceptible to hydrolysis phenomena only under circumstances of very low pH and/or temperatures above 100 °C [14,21]. On the other hand, ASB is an etch-and-rinse ethanol-based adhesive that does not contain water in its composition, and also has an elevated pH value. These conditions represent a more 'friendly' environment in which the hydrolysis of methacrylate monomers is not supposed to occur. Indeed, ethanol-based etch-and-rinse adhesives have demonstrated shelf-life stability [15].

With regard to the degree of conversion analysis, excepting for OBU and ASE, all materials showed a significant decrease in DC after shelf-life simulation. The degree of conversion is a feature that is largely influenced by the type and concentration of the photoinitiator system. Although it was not specified in some of the safety data sheets, most of the materials used in this study were based on the CQ/EDAB photoinitiation system. Some studies have demonstrated that in acidic environments, the effectiveness and stability of this photoinitiator system was low [22,23]. On the one hand, an acid base reaction occurs between the acidic monomers and the amines, preventing the amine from acting as a polymerization coinitiator [24]. On the other hand, the amine-acidic monomer interaction can neutralize the acidic functional monomer, impairing its ability to form stable bonds with the hydroxyapatite of the dentin substrate [22]. Indeed, the reduction in the bond strength values after the shelf-life simulation observed in this study, could also have occurred as a result of this neutralization process.

Contrary to results found in the remainder of the adhesive systems, ASE and OBU not only maintained stability in terms of the degree of conversion, but the values also increased when the material was evaluated in its end of shelf-life condition. As explained before, ASE contains methacrylamide monomers with a phosphonic acid moiety as functional group. Methacrylamides are more resistant to hydrolysis than esters, and maintenance of the degree of conversion values is expected. In addition, recent studies have demonstrated [25,26] that polymerization of acrylamides initiated with CQ/EDAB is enhanced when alkyl phosphonic acid moieties are added, which could also could explain the findings obtained in our study. With regard to OBU, the increase in the degree of conversion values could be attributed to the fact that as the composition of this material contains acetone as a solvent, the quantity of solvent retained in the material after the evaporation process does not interfere in the polymerization process [27]. Previous studies have demonstrated that acetone-based adhesives retain lower quantities of solvents than the ethanol-based types after air-blast evaporation [28].

Nanoleakage was used as an indirect method to evaluate the quality of the resin-dentin bonds. Nanoleakage expression represents the location of defects within the adhesive layer that might serve as the pathway for degradation, especially after any type of aging [29]. In this study, SBU and P&B, showed no increase in nanoleakage expression after shelf-life simulation. The presence of a polyalkenoic acid co-polymer within SBU composition is related to the ability to interact with calcium in hydroxyapatite [30], consequently, this feature has been used to explain the optimal long-term performance of polyakenoic-based materials [31]. Similarly, the P&B adhesive showed no increase in the nanoleakage expression, even when it was evaluated in its end of shelf-life condition. It should be highlighted that P&B was the only HEMA-free adhesive tested, and probably the absence of this monofunctional monomer enhanced the cross-linking density of the adhesive layer, decreasing water permeation [32]. On the other hand, the presence of HEMA has been related to inhibition of the nanolayering chemical bonding mechanism of the 10-MDP monomer, which could increase the nanoleakage [33]. Despite these promising results, it is worth mentioning that for both adhesives, the increase in the number of the adhesive type of failures, and the increase in the percentage of pre-testing failure after simulation of the shelf-life period, suggested some type of degradation.

This study investigated the degradation profile of universal adhesive systems simulating three different periods of shelf-life using controlled temperature and humidity conditions. Furthermore, the conditions used in this study could be considered adverse, but they may be not uncommon during transportation and storage of the product, and the manufacturers should take into account the possible effect of these variables on the stability of the materials to enable them to determine an adequate expiry date. Moreover, the present findings suggested that humidity could also play an important role in the shelf-life stability of dental adhesives. As the range of humidity in which the materials should be stored is not informed by manufacturers, more research should be conducted to determine the effect of this variable on the rate of degradation of the components on which these materials are based.

5. Conclusions

The performance of adhesives systems after shelf-life simulation was material-dependent. Apparently HEMA-free adhesive blends using methacrylamides and phosphonic acid-containing monomers, in combination with relatively high pH seemed to produce materials with higher shelf-life stability. Shelf-life simulation with controlled temperature and humidity conditions should be considered a routine procedure during the process of development, evaluation and determination of the expiration date of adhesive systems.

Formatting of funding sources

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES), Brazilian National Council for Scientific and Technological Development (CNPq; Grant # 310879/2015-9 and # 432127/2018-6), and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS; PRONEX # 16.0471-4).

Acknowledgements

Authors would like to thank The Brazilian National Council for Scientific and Technological Development (CNPq) #432127/2018-6 and 310879/2015-9 for financial support. Author Cuevas-Suárez CE wants to thank PRODEP - México for PhD scholarship (DSA/103.5/15/6615).

Appendix A.

See Figs. A1–A8.

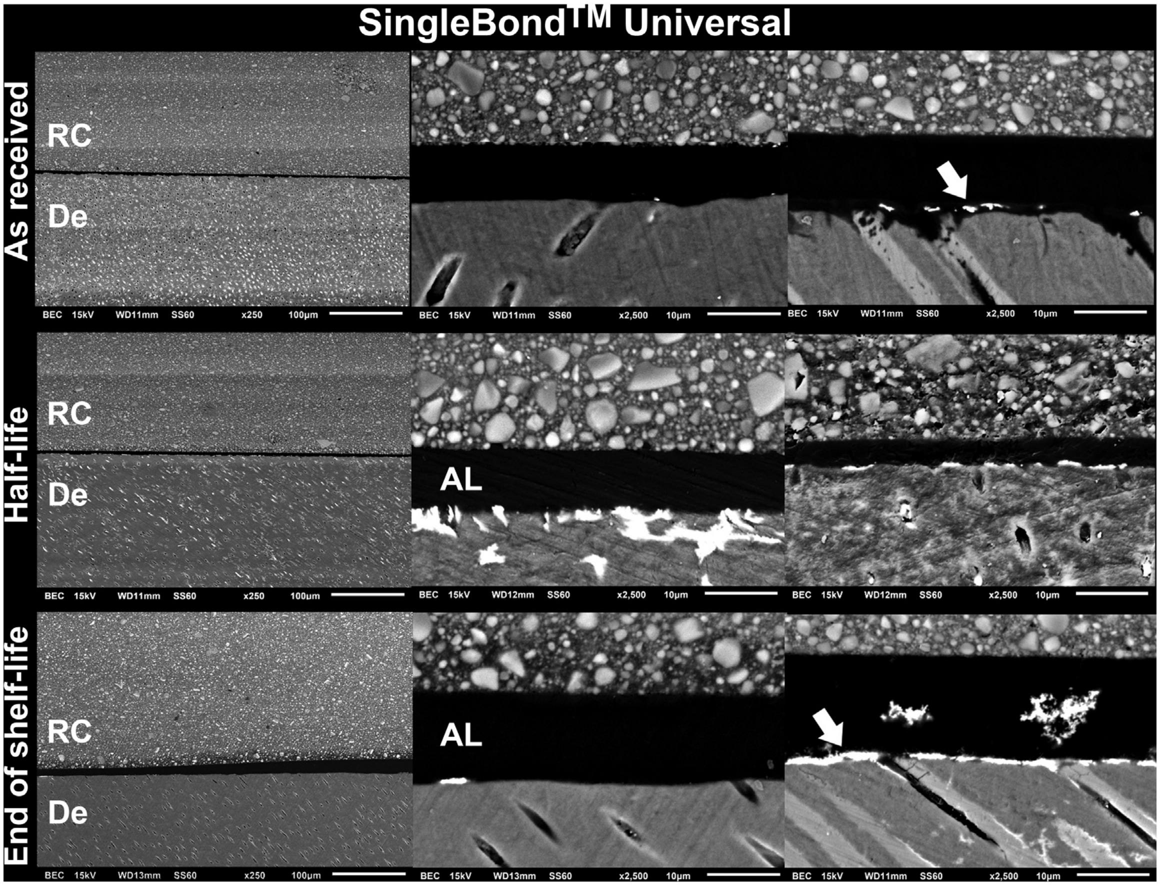

Fig. A1 – Representative backscatter SEM images of the resin–dentin adhesive interfaces of SBU. RC, resin composite. De, dentine. AL, adhesive layer. Arrows represent silver staining (nanoleakage). Pointers suggesting “water trees”.

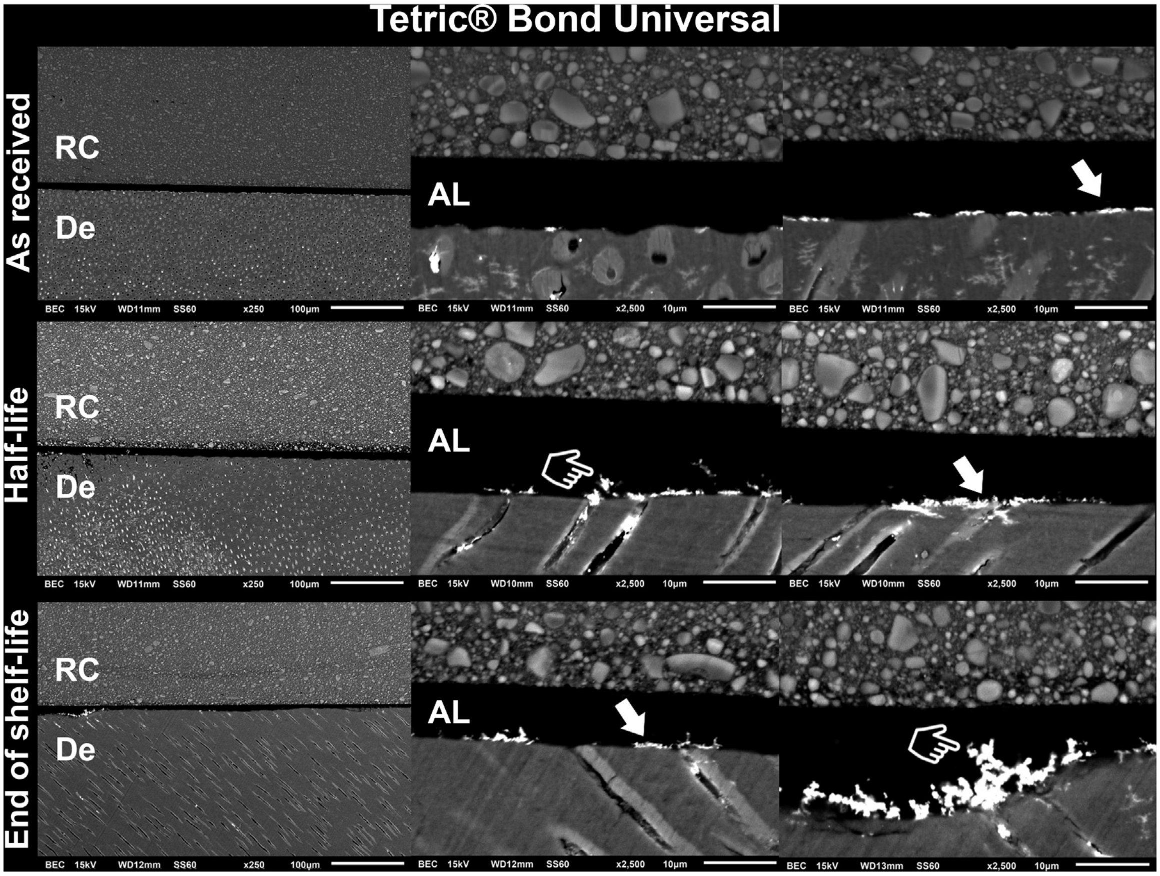

Fig. A2 – Representative backscatter SEM images of the resin–dentin adhesive interfaces of TBU. RC, resin composite. De, dentine. AL, adhesive layer. Arrows represent silver staining (nanoleakage). Pointers suggesting “water trees”.

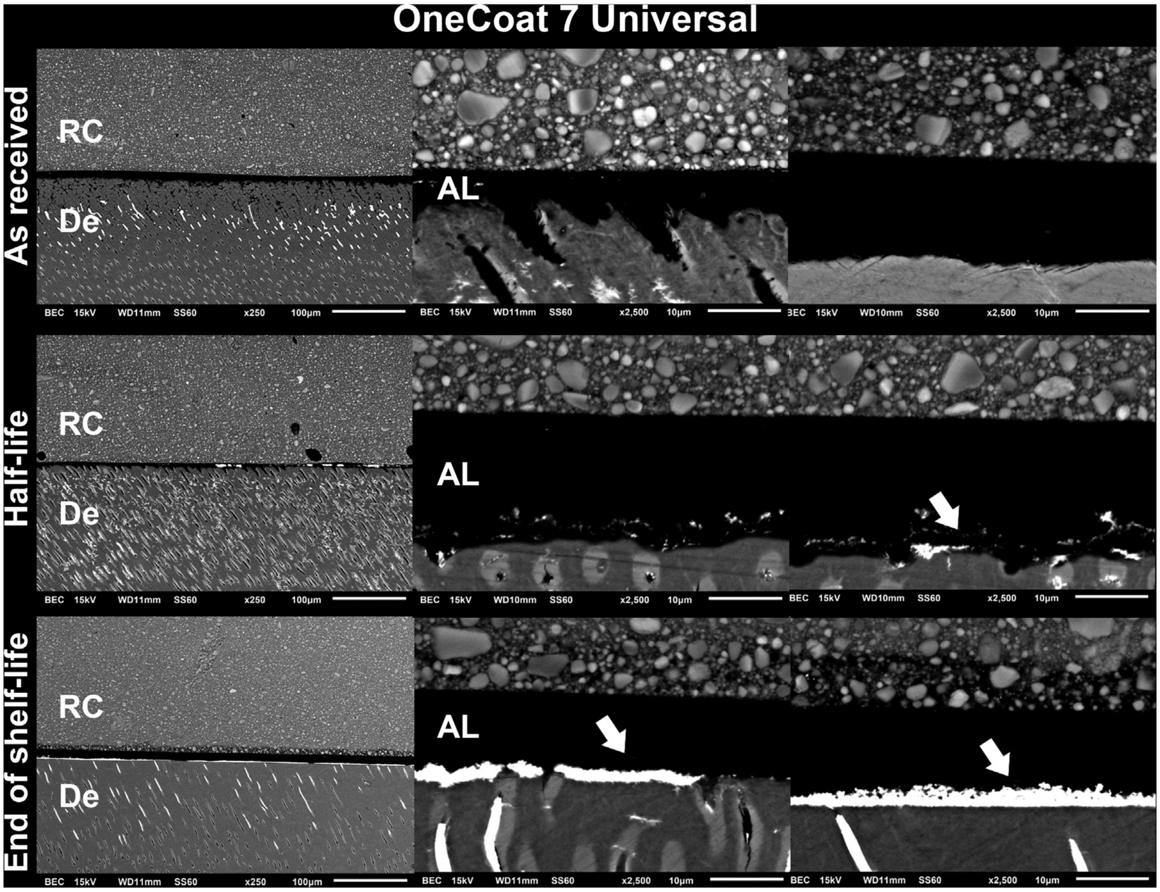

Fig. A3 – Representative backscatter SEM images of the resin–dentin adhesive interfaces of OCU. RC, resin composite. De, dentine. AL, adhesive layer. Arrows represent silver staining (nanoleakage).

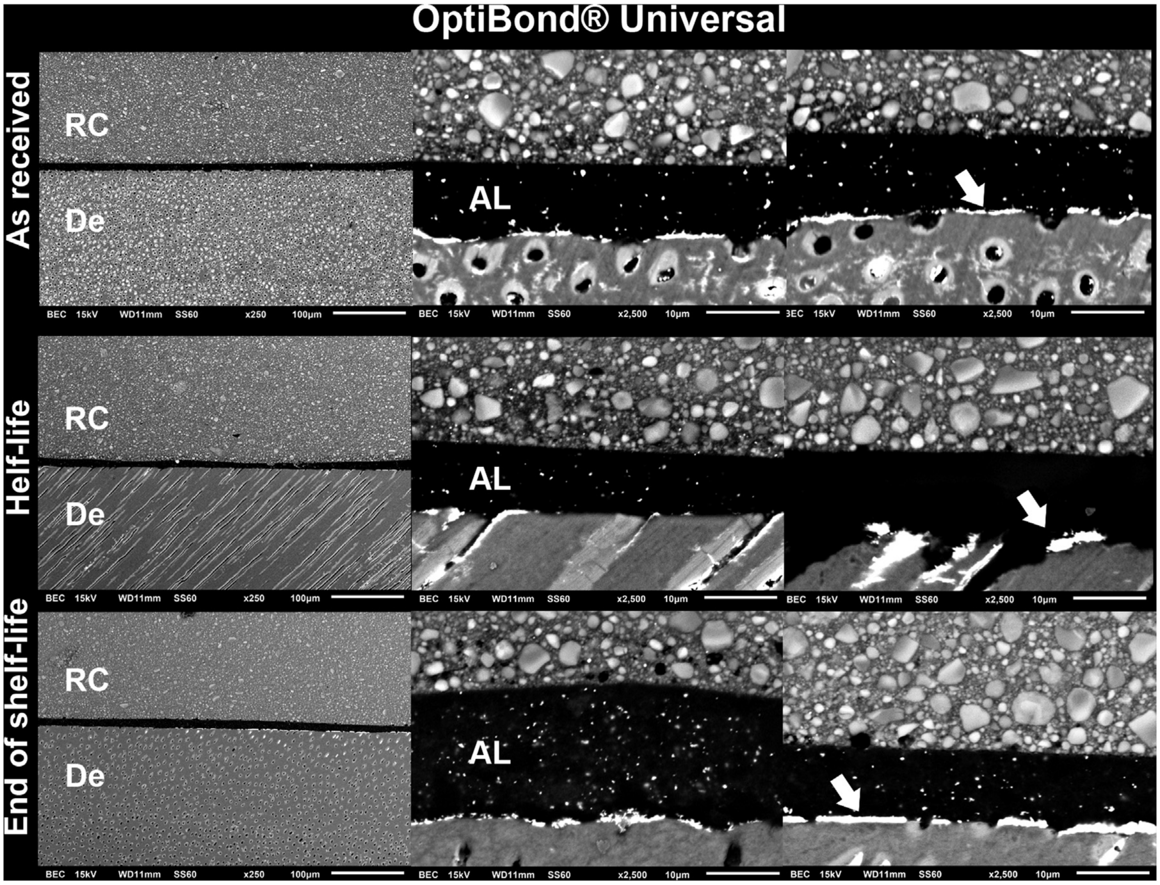

Fig. A4 – Representative backscatter SEM images of the resin–dentin adhesive interfaces of OBU. RC, resin composite. De, dentine. AL, adhesive layer. Arrows represent silver staining (nanoleakage).

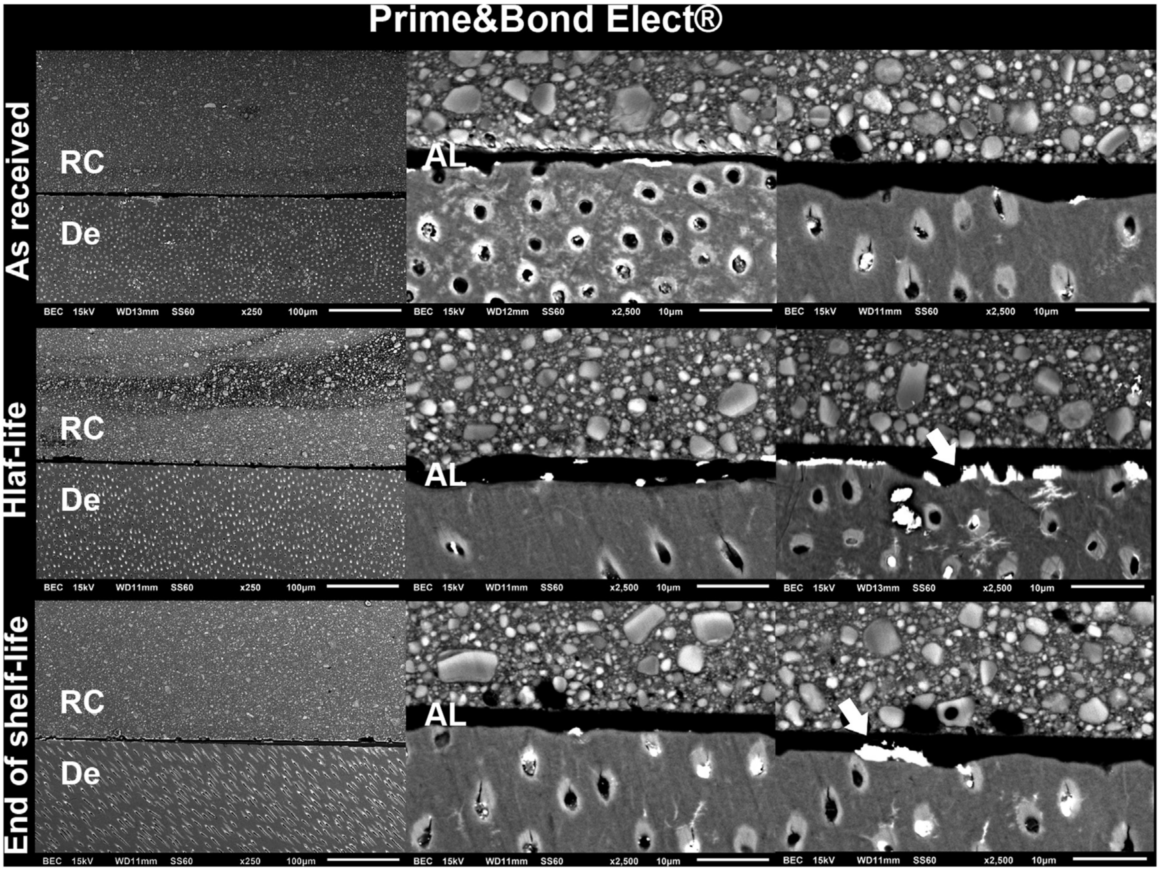

Fig. A5 – Representative backscatter SEM images of the resin–dentin adhesive interfaces of PBE. RC, resin composite. De, dentine. AL, adhesive layer. Arrows represent silver staining (nanoleakage).

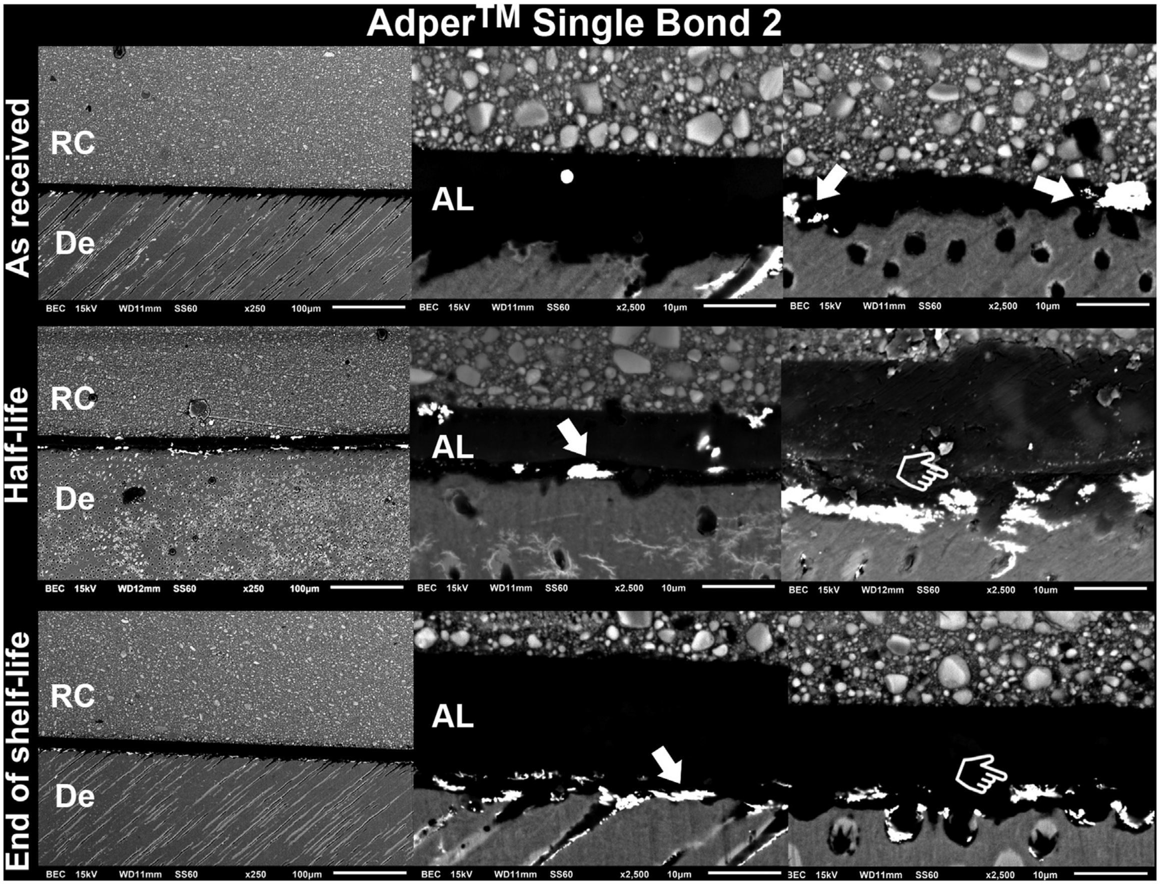

Fig. A6 – Representative backscatter SEM images of the resin–dentin adhesive interfaces of ASE. RC, resin composite. De, dentine. AL, adhesive layer. Arrows represent silver staining (nanoleakage). Pointers representing "water trees".

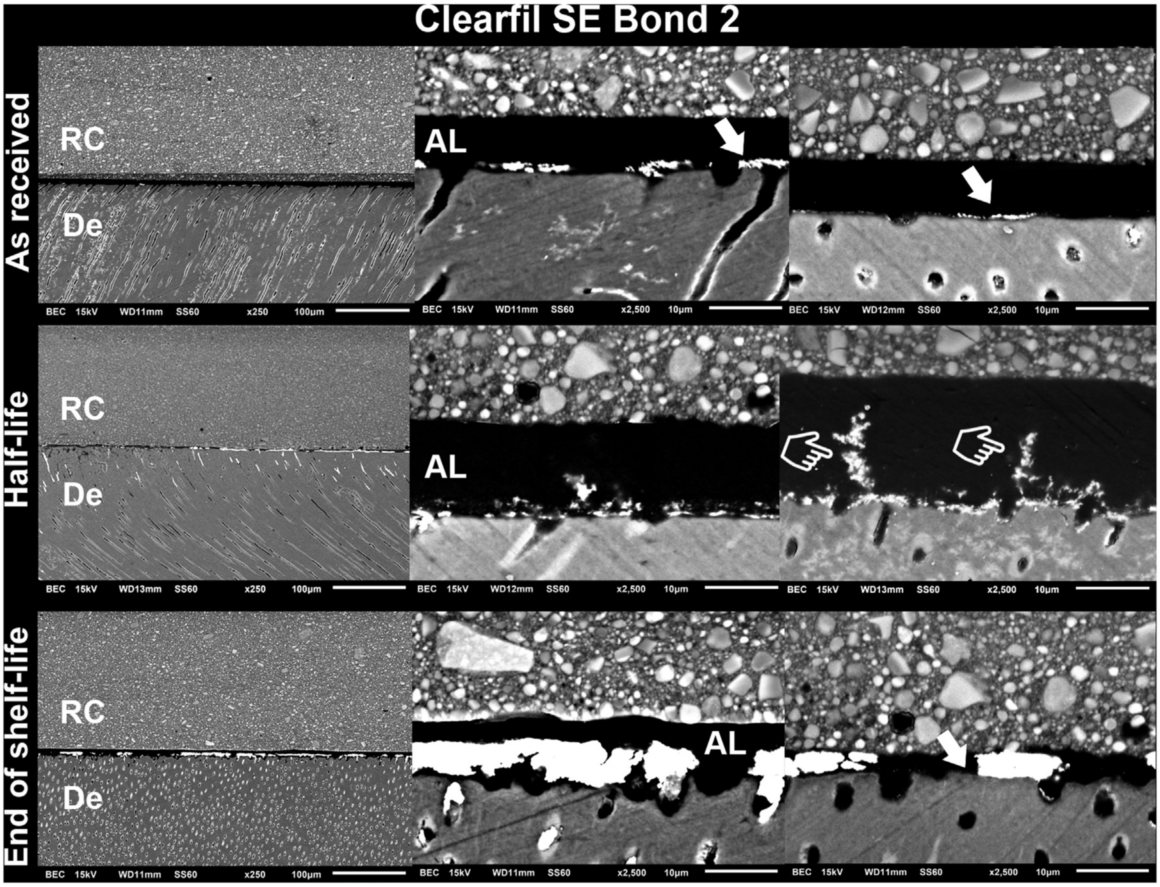

Fig. A7 – Representative backscatter SEM images of the resin–dentin adhesive interfaces of CSE. RC, resin composite. De, dentine. AL, adhesive layer. Arrows represent silver staining (nanoleakage).

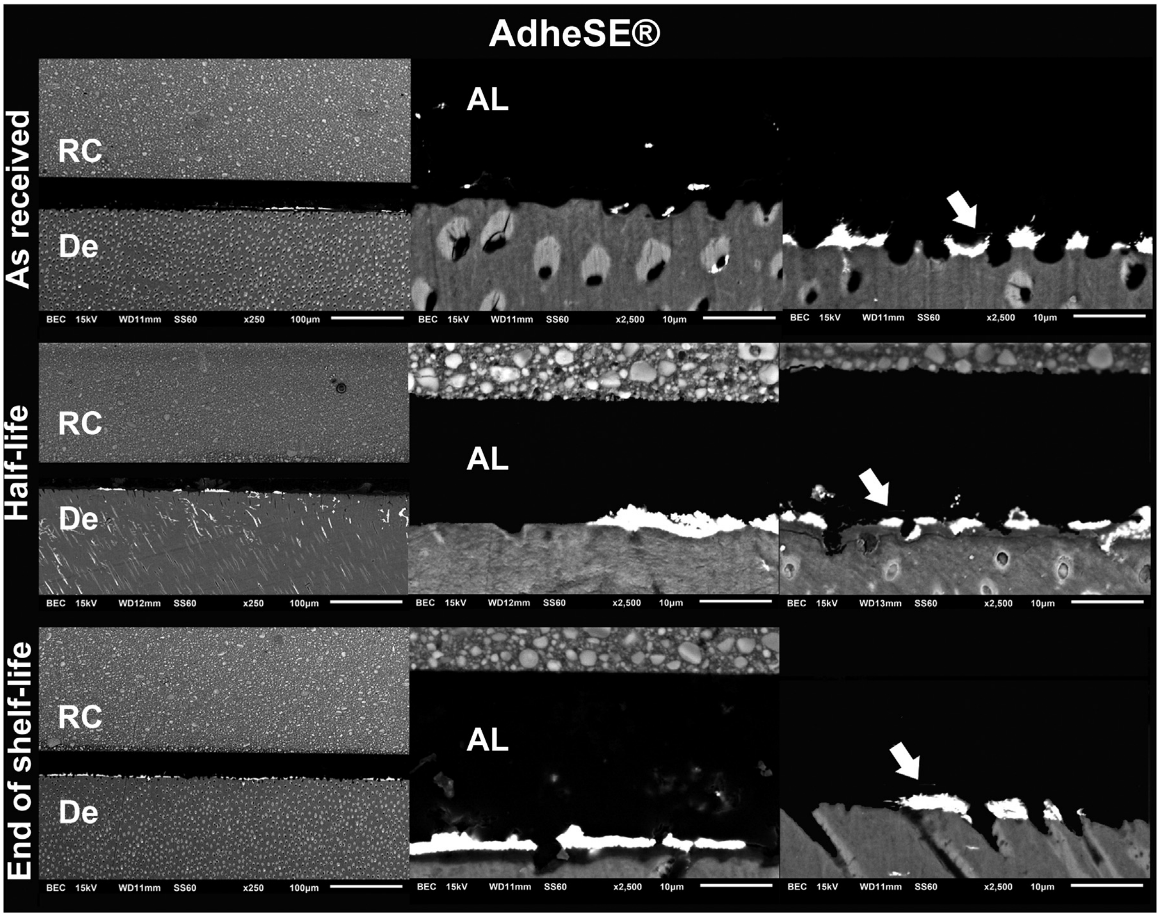

Fig. A8 – Representative backscatter SEM images of the resin–dentin adhesive interfaces of ASE. RC, resin composite. De, dentine. AL, adhesive Layer. Arrows represent silver staining (nanoleakage). Pointers suggesting "water trees".

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