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Subsequent application of bonding agents to a one-step self-etch adhesive — Its effect with/without previous light-curing

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ABSTRACT

Objective. The influence of light-curing of a one-step self-etch adhesive (1-SEA) prior to the application of different bonding agents (BA) on the micro-tensile bond strength (μTBS) to dentin after 24 h and thermal cycling was investigated. Additionally, the degree of conversion was evaluated using Fourier-transform infrared spectroscopy.

Methods. Three ion-releasing BAs, BZF-21 (experimental BA), Clearfil SE Protect (CSP), and FL-Bond II (FL-II), were applied subsequently to G-Premio Bond (1-SEA, GPB). Prior to their application, GPB was either light-cured (10 s, 1000 mW/cm^2) or remained uncured. GPB was used as a control and Clearfil SE Bond 2 (CSE2) as a gold-standard 2-step reference. After resin-composite build-up and 24-h water storage (24 h), half of the specimens were subjected to 15,000 thermal cycles (TC). Then, the specimens were sectioned into beams and tested under tensile load (1 mm/min). The acquired data were analyzed using a 2-way ANOVA and Student's t-test with Bonferroni correction, and a two-parameter Weibull analysis, $\alpha = 0.05$.

Results. The μTBS of GPB increased significantly in the uncured groups with BZF-21 (24 h: $p < 0.001$, TC: $p < 0.001$) and CSP (24 h: $p = 0.039$, TC: $p < 0.001$), and in the light-cured group with CSP after TC ($p = 0.044$). The groups with FL-II were not significantly different from GPB ($p = 1$). No significant difference was found between CSE2 and the uncured groups GPB + BZF-21 (24 h: $p = 1$, TC: $p = 0.452$) and GPB + CSP (24 h: $p = 0.671$, TC: $p = 0.566$).

Significance. BZF-21 and CSP improved the μTBS of GPB to dentin both immediately and after TC. Higher μTBSs were obtained when GPB remained uncured prior to BA application.

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1. Introduction

One-step self-etch adhesives (1-SEAs) have become increasingly popular because of their simplicity of use and their ability to bond to various substrates. The mixing of the necessary compounds with different chemical properties in one bottle, however, leads to several disadvantages such as limited bond durability, increased nano-leakage and water sorption, phase separation and reduced shelf life [1]. As a result, 1-SEAs generally perform worse compared to 2-step self-etch adhesives (2-SEAs) and 3-step etch-and-rinse adhesives (3-ERAs) both in short-term [2,3] and long-term [4,5] laboratory tests, as well as in clinical studies [6,7]. However, mildly acidic 1-SEAs have been recently shown to exhibit no significant difference in the annual failure rate compared to 2-SEAs and 3-ERAs standards [8].

To improve the bonding performance of 1-SEAs, the subsequent application of a more hydrophobic bonding agent (BA) has been proposed with the objective of counteracting the 1-SEAs' excessive water sorption [9,10] and water penetration through the adhesive layer [11]. In this 2-step approach, 1-SEAs are used as separately polymerizable self-etching primers. In most previous studies, the 1-SEAs were light-cured before the application of BA [12–17]. The additional hydrophobic layer resulted in increased immediate micro-tensile bond strength (μTBS) to dentin [13–17], although this increase was not significant in some studies [12,13,17], and in a significantly higher stability of the adhesive joint after 6-month water storage [13,16,17]. An improved degree of conversion (DC) was also reported [14,15]. The results of the application of BAs to uncured 1-SEAs indicated either an increase or no significant difference in the bond strength, depending on the 1-SEA [17–19]. In addition, two clinical trials evaluated the 18-month clinical performance of the approach with light-curing [20] and without it [21] in non-carious cervical lesions. Similarly to the laboratory studies, an improvement was observed in all groups but it was only significant for a 4-MET-based 1-SEA light-cured prior to BA application [20].

To the best of our knowledge, only one study has compared the effect of 1-SEA light-curing (LC) prior to BA application [17]. The two tested 10-MDP-based HEMA-containing 1-SEAs exhibited higher μTBSs after 6-month water storage if the 1-SEAs were light-cured prior to BA application, whereas no significant improvement was obtained using the approach without LC. The purpose of this study was to further investigate the effect of 1-SEA LC prior to the application of different BAs on the bond strength to dentin. In this study, a HEMA-free 1-SEA was used and three ion-releasing BAs were chosen because their bioactivity has the potential to provide additional clinical benefits [22]. The bonding performance was evaluated using μTBS to dentin after 24-h water storage (24 h) and thermocycling (TC). In addition, DC was measured using attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR). The 1-SEA without any BA served as a control group and a standard 2-SEA was used as a reference. The null hypotheses tested were (1) the application of BA does not significantly affect the micro-tensile bond strength of the 1-SEA to dentin, and (2) there is no significant difference between the approaches with and without LC of the 1-SEA prior to the application of BA.

2. Materials and methods

A HEMA-free 1-SEA G-Premio Bond (GPB, GC Corp., Tokyo, Japan), three bioactive BAs - BZF-21 (experimental BA, GC Corp., Tokyo, Japan), Clearfil SE Protect (CSP, Kuraray Noritake Dental Inc., Tokyo, Japan), FL-Bond II (FL-II, Shofu Inc., Kyoto, Japan) - and a 2-SEA Clearfil SE Bond 2 (CSE2, Kuraray Noritake Dental Inc.) were used in this study. Table 1 provides a detailed description of the respective adhesives and the application protocols.

2.1. *Micro-tensile bond strength test*

2.1.1. *Teeth*

In this study, eighty caries-free human molars were used. The teeth were gathered according to the ethical protocol No. 2014-022 approved by the Human Research Ethics Committee of Tokyo Medical and Dental University. The teeth were stored in distilled water at 4 °C and used within six months after extraction. Mid-coronal dentin surfaces were exposed using a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) under water irrigation and ground with a wet 600-grit SiC paper (DCCS, Sankyo Fuji Star, Saitama, Japan) to create a standardized smear layer.

2.1.2. *Adhesive application*

The teeth were randomly assigned to 8 groups (n = 10) according to adhesive application protocols. Each group was later randomly divided into two subgroups (n = 5) for 24-h measurement and TC. In the experimental groups, the exposed dentin surfaces were primed with GPB and strongly air-blown using a dental air syringe to evaporate the solvent following the instructions for use. Half of the specimens were light-cured using a LED light curing unit (10 s, 1000 mWcm^{-2}, Valo, Ultradent Products Inc., South Jordan, UT, USA), and the other half remained uncured. One of the BAs was then applied, gently air-blown and light-cured (10 s, 1000 mWcm^{-2}, Valo, Ultradent Products Inc.). GPB without any BA was used as a control group and was applied according to the manufacturer's instructions, as well as CSE2 which was selected as a reference. Resin composite (Clearfil AP-X, shade A2, Kuraray Noritake Dental Inc.) build-ups were constructed on the bonded surfaces in 3 increments to a thickness of approximately 5 mm. The increments did not surpass 2-mm thickness and each of them was light-cured for 20 s (1000 mWcm^{-2}, Valo, Ultradent Products Inc.). All the procedures were performed under standard room conditions, 23 °C and a constant relative humidity. The prepared specimens were then stored in distilled water at 37 °C for 24 h.

2.1.3. *Thermocycling*

After 24 h, half of the specimens of each group (n = 5) were subjected to 15,000 thermal cycles in distilled water at temperatures of 5 °C and 55 °C. The dwell time was 30 s and the transfer time was 5 s.

Table 1 – Composition, batch number and application procedure of adhesive systems.

Adhesive system (manufacturer)	Composition (batch number)	Group	Application	
			Primer	Bonding agent
G-Premio Bond (GC Corp., Tokyo, Japan)	4-MET, 10-MDP, MEPS, methacrylate monomer, acetone, water, diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide, silica (1806201)	GPB	1. Apply GPB to the entire dentin surface using disposable microbrush and leave for 10 s. 2. Air-dry for 5 s with maximum air pressure (0.35 MPa). 3. Light cure for 10 s with an LED curing unit at 1000 mWcm^{-2}.	
BZF-21 (GC Corp., Tokyo, Japan)	*Bonding agent*: Non-acidic methacrylate monomers, zinc-fluoride silicate glass, initiator, fumed silica (180907)	GPB(LC) + BZF-21	GPB application, steps 1-3.	1. Apply BZF-21 with disposable microbrush, rub for 5 s. 2. Gently air-blow (0.1 MPa). 3. light cure for 10 s.
		GPB + BZF-21	GPB application, steps 1-2.	
Clearfil SE Protect (Kuraray Noritake Dental Inc., Tokyo, Japan)	*Bonding agent*: 10-MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, dl-Camphorquinone, silica, surface treated sodium fluoride (8M0063)	GPB(LC) + CSP	GPB application, steps 1-3.	1. Apply CSP bonding agent with disposable microbrush, rub for 5 s. 2. Gently air-blow (0.1 MPa). 3. Light cure for 10 s.
		GPB + CSP	GPB application, steps 1-2.	
FL-Bond II (Shofu Inc., Kyoto, Japan)	*Bonding agent*: UDMA, TEGDMA, HEMA, S-PRG filler, initiator (041807)	GPB(LC) + FL-II	GPB application, steps 1-3.	1. Apply FL-II bonding agent with disposable microbrush, rub for 5 s. 2. Gently air-blow (0.1 MPa). 3. light cure for 10 s.
		GPB + FL-II	GPB application, steps 1-2.	
Clearfil SE Bond 2 (Kuraray Noritake Dental Inc., Tokyo, Japan)	*Primer*: 10-MDP, HEMA, Hydrophilic aliphatic dimethacrylate, water, dl-Camphorquinone (C60080) *Bonding agent*: 10-MDP, Bis-GMA, HEMA, Hydrophobic aliphatic dimethacrylate, dl-Camphorquinone, initiators, accelerators, silica (CK0122)	CSE2	1. Apply primer to the entire dentin surface using disposable microbrush and leave for 20 s. 2. Air-dry for 6 s with mild air pressure (0.2 MPa).	1. Apply CSE2 bonding agent with disposable microbrush, rub for 5 s. 2. Gently air-blow (0.1 MPa). 3. Light cure for 10 s.

The rubbing motion during the application of bonding agents and gentle air-blowing were performed to standardize the application procedures.

Abbreviations: 10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate, 4-MET: 4-methacryloxyethyl trimellitic acid, MEPS: Methacryloyloxyalkyl thiophosphate methylmethacrylate, HEMA: 2-hydroxyethyl methacrylate, Bis-GMA: bisphenol-A-glycidyl methacrylate, UDMA: urethane dimethacrylate, TEGDMA: triethyleneglycol dimethacrylate, GPB: G-Premio Bond, CSP: Clearfil SE Protect, FL-II: FL-Bond II, CSE2: Clearfil SE Bond 2.

2.1.4. *μTBS testing*

After 24 h or TC, the specimens were sectioned into non-trimmed beams with a size of 1 × 1 mm using a low-speed diamond saw (Isomet 1000, Buehler) under water cooling. The size of the beams was measured before testing using a digital caliper Mitutoyo CD15 (Mitutoyo, Kawasaki, Japan) for bonded area calculation. The specimens were then attached to an active testing jig using command-set glue (Model Repair II Blue, Dentsply-Sankin, Tokyo Japan) and subjected to a tensile load using an EZ Test (Shimadzu Corp., Kyoto, Japan) testing machine at a crosshead speed of 1 mm/min until fracture. The recorded force in Newtons was converted to μTBS by dividing this value by the calculated bonded area of the specimens.

2.1.5. *Statistical analysis*

The μTBS values were subjected to a 2-way ANOVA (adhesive application protocol, storage condition). Multiple Student's t-test with Bonferroni correction was used for pairwise comparisons of the adhesive application protocols and a separate Student's t-test was used to compare the bond strengths after 24 h and TC. Mean μTBSs calculated for each tooth were used for the analysis, $\alpha = 0.05$ (SPSS Statistics 23.0, IBM Corp., Armonk, NY, USA).

Weibull analysis was performed using Weibull R package for R distribution (R Foundation for statistical computing, Vienna, Austria). Weibull scale and shape parameters were calculated using Wald estimation, 95% confidence intervals were calculated based on Monte Carlo simulations. The different groups were compared using the scale parameter (characteristic strength at 63.2% probability of failure).

2.2. *SEM analysis*

To determine the failure mode, fractured specimens were mounted on brass stubs, dried, sputter-coated with gold and observed using a scanning electron microscope JSM-IT100 (JEOL Ltd., Tokyo, Japan). The following failure modes were distinguished: (1) adhesive failure (within the adhesive layer or at the dentin-adhesive interface), (2) cohesive failure within substrates (dentin or resin composite), and (3) mixed failure. The failures were regarded as mixed if both (1) and (2) covered at least 20% of the bonded area. A few unfractured beams from each group were polished perpendicularly to the adhesive layer using diamond polishing pastes (6 μm, 3 μm, 1 μm and 0.25 μm) DP-Paste P (Struers, Copenhagen, Denmark) for the observation of the adhesive layer in the cross-section and the measurement of its thickness.

2.3. *Degree of conversion determination*

2.3.1. *Spectra acquisition*

The ATR-FTIR spectra were acquired using a Nicolet iS50 FT-IR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) under following conditions: wavelength range 4000–400 cm^{-1}, resolution 2 cm^{-1}, co-addition of 32 scans. To simulate the application procedures in the experimental groups, a drop of GPB was applied to a mixing dish and carefully air-blown to evaporate the solvent. Once the material stopped moving under the air pressure, it was transported to the ATR crystal using a microbrush. In the cured groups, after LC of GPB BAs were applied using a microbrush and light-cured. In total, three scans were performed in the cured groups — prior to LC of GPB, after LC of GPB, and after LC of BAs. In the uncured groups, BAs were applied to uncured GPB using a microbrush and then two scans were performed — before LC and after LC. Furthermore, spectra of all neat BAs were acquired before and after LC for reference. Polymerization of the uncured adhesives resulting from ambient light was prevented using light filters. The duration of LC steps was 10 s (1000 mWcm^{-2}, Valo, Ultradent Products Inc.). All the procedures were performed under in a dark room at 23 °C and a constant relative humidity.

2.3.2. *DC calculation and statistical analysis*

The absorbance peak at 1638 cm^{-1}, representing aliphatic carbon double bonds (C=C), was selected as the analytical band, and the peak at 1608 cm^{-1}, representing aromatic double bonds, was chosen as internal standard [23]. DC was calculated using the equation $DC = 1 - (R_C/R_U)$, where R_C represents the ratio of 1638 cm^{-1} peak intensity to 1608 cm^{-1} peak intensity of the cured specimens and R_U that of the uncured ones. The data of experimental groups and BAs were statistically analyzed using two separate one-way ANOVAs and Tukey's post-hoc test, $\alpha = 0.05$ (SPSS Statistics 23.0, IBM Corp., Armonk, NY, USA).

3. Results

3.1. *Micro-tensile bond strengths*

3.1.1. *ANOVA and Student's t-test*

The 2-way ANOVA revealed that the effects of adhesive application protocol and storage condition were statistically significant (both $p < 0.001$), whereas their interaction was not ($p = 0.162$). The mean bond strengths and standard deviations are presented in Table 2.

In the cured groups, no statistically significant difference from GPB was identified after 24 h. The μTBSs obtained with BZF-21 and CSP were slightly higher, whereas a slight decrease was observed with FL-II. After thermocycling (TC), GPB(LC) + CSP exhibited significantly higher μTBS compared to GPB ($p = 0.044$). The application of BZF-21 and FL-II did not significantly improve the μTBS after TC.

In the uncured groups, the application of BZF-21 and CSP resulted in a significant increase of the mean μTBS after 24 h (GPB + BZF-21 $p < 0.001$, GPB + CSP $p = 0.039$) as well as after TC (both $p < 0.001$) compared to GPB. No statistically significant difference was observed between GPB + FL-II and GPB after 24 h ($p = 1$) and TC ($p = 0.548$). Higher μTBSs were obtained in the uncured groups compared to the cured groups with the same BA. However, the difference was statistically significant only for BZF-21 after 24 h ($p < 0.001$).

TC resulted in a decrease in the μTBS values in all tested groups. However, the decrease was significant only for GPB ($p < 0.001$) and both groups with BZF-21 (cured $p = 0.011$, uncured $p < 0.001$). When the experimental groups were compared to CSE2, the μTBS of all cured groups and GPB + FL-II were significantly lower after 24 h ($p < 0.005$) and TC ($p < 0.01$). No significant difference was observed between CSE2 and the

Table 2 – ANOVA and Student's t-test results: Micro-tensile bond strength to dentin (Mean ± S.D.).

Storage condition	G-Premio Bond	Light-cured G-Premio Bond			Uncured G-Premio Bond			Clearfil SE Bond 2
		+BZF-21	+CSP	+FL-II	+BZF-21	+CSP	+FL-II	
24-h water storage	60.5 ± 3.5^{Aa}	63.5 ± 5.2^{ABa}	63.6 ± 5.7^{ABa}	56.1 ± 3.3^{Aa}	76.8 ± 2.9^{Ca}	68.9 ± 5.2^{BCa}	60.5 ± 5.1^{ABa}	75.2 ± 6.1^{Ca}
Thermocycling	49.9 ± 4.2^{Db}	55.8 ± 2.5^{DEFb}	59.2 ± 4.2^{EFa}	51.3 ± 2.3^{DEa}	63.6 ± 4.0^{FGb}	63.3 ± 5.9^{FGa}	56.3 ± 7.2^{DEFa}	70.3 ± 2.7^{Ga}

Same superscript uppercase letters indicate no statistically significant differences in rows. Same superscript lowercase letters indicate no statistically significant differences in columns.
Abbreviations: CSP: Clearfil SE Protect, FL-II: FL-Bond II.

Table 3 – Weibull parameters and strength at 10% probability of failure.

Storage condition	Adhesive application protocol	Scale (95% CI)	Shape (95% CI)	P10 (95% CI)
24-h water storage	GPB	64.3 (62.1–66.5)^{cdef}	6.4 (5.5–7.5)	45.1 (41.9–48.5)
	GPB (LC) + BZF-21	69.6 (66.6–72.7)^{ghi}	5.0 (4.3–5.9)	44.2 (40.2–48.6)
	GPB (LC) + CSP	71.3 (68.8–73.8)^{hij}	6.4 (5.5–7.6)	50.1 (46.4–54.0)
	GPB (LC) + FL-II	60.0 (58.0–62.1)^{bc}	6.9 (5.8–8.3)	43.3 (40.1–46.7)
	GPB + BZF-21	83.4 (80.6–86.3)^{k}	5.7 (5.0–6.7)	56.4 (52.3–60.8)
	GPB + CSP	74.2 (71.5–76.9)^{ij}	5.5 (4.8–6.5)	49.4 (45.7–53.4)
	GPB + FL-II	66.1 (63.2–69.1)^{defgh}	5.0 (4.2–6.0)	42.0 (38.0–46.5)
	CSE2	81.5 (78.6–84.5)^{k}	5.4 (4.7–6.3)	53.7 (49.6–58.1)
Thermocycling	GPB	55.2 (52.6–57.8)^{a}	4.9 (4.2–5.8)	34.7 (31.3–38.4)
	GPB (LC) + BZF-21	59.6 (57.9–61.4)^{b}	7.1 (6.2–8.4)	43.5 (40.7–46.4)
	GPB (LC) + CSP	61.7 (59.3–64.3)^{bcd}	6.0 (5.1–7.2)	42.4 (38.9–46.2)
	GPB (LC) + FL-II	54.4 (52.9–56.0)^{a}	7.9 (6.8–9.3)	40.9 (38.4–43.4)
	GPB + BZF-21	68.7 (66.5–71.0)^{fghi}	5.7 (5.0–6.5)	46.1 (43.0–49.5)
	GPB + CSP	68.6 (65.5–71.9)^{efgh}	4.8 (4.2–5.8)	43.1 (39.0–47.7)
	GPB + FL-II	63.2 (60.9–65.6)^{bcde}	5.7 (4.9–6.8)	42.6 (39.2–46.2)
	CSE2	75.2 (72.9–77.4)^{j}	7.4 (6.4–8.8)	55.5 (52.0–59.2)

Same superscript lowercase letters in α column indicate no statistically significant differences based on 95% confidence intervals.
Abbreviations: P10: strengths at 10% probability of failure, CI: confidence interval, GPB: G-Premio Bond, CSP: Clearfil SE Protect, FL-II: FL-Bond II, CSE2: Clearfil SE Bond 2, LC: Light-cured.

uncured groups GPB + BZF-21 (24 h: $p = 1$, TC: $p = 0.452$) and GPB + CSP (24 h: $p = 0.671$, TC: $p = 0.566$).

3.1.2. *Weibull analysis*

The results of the Weibull analysis are presented in Table 3 and Figs. 1–3. In the cured groups, the characteristic strengths of GPB(LC) + BZF-21 and GPB(LC) + CSP were significantly higher than that of GPB, both after 24 h and TC. Conversely, the values of GPB(LC) + FL-II were lower than those of GPB, however, the difference was not significant.

In the uncured groups, the characteristic strengths with BZF-21 and CSP after 24 h, and with all the BAs after TC were significantly higher than that of GPB. The characteristic strengths of the uncured groups were also significantly higher compared to the cured groups in both storage conditions except for CSP after 24 h.

A significant decrease in the characteristic strength after TC was observed in all the groups except for the uncured GPB + FL-II. A comparison of the experimental groups with CSE2 revealed that its characteristic strength was superior to that of the experimental groups for both storage conditions, except for the uncured GPB + BZF-21 after 24 h.

3.2. *SEM analysis*

In the majority of the groups, fractures occurred most often within the adhesive layer. Their proportion decreased with an increase of the mean μTBS because more mixed and cohesive failures were observed (GPB + BZF-21, GPB + CSP, CSE2). Failures at the dentin-adhesive interface were seldom observed except for group GPB + FL-II, in which case their proportion was 41% after 24 h and 25% after TC. No pretesting failures occurred in the study. The distributions of the failure modes are summarized in Table 4 and representative SEM images are presented in Fig. 4.

Detailed surface analysis revealed round-shaped droplets within the adhesive layer of some specimens bonded using GPB and in the cured groups (Fig. 5). These specimens usually fractured at lower μTBS values than the mean of their respective groups. Homogenously distributed contrastive round-shaped particles with sub-micrometer size were observed on the surfaces that failed within the layer of BZF-21 (Fig. 5). FL-II showed the presence of many irregular filler particles and their loosened attachment to the surrounding matrix (Fig. 5). It is also noteworthy that the fractures often propagated through the layer of FL-II in group GPB(LC) + FL-II after TC where FL-II occupied approximately 60% of the fractured surfaces.

Cross-sectional observation of unfractured specimens revealed that the thickness of the adhesive layer of GPB varied from 3 μm to 7 μm. The two-step procedures resulted in thicker adhesive layers that varied between 20 μm and 30 μm. However, their structure differed depending on whether GPB was light-cured prior to the application of BA. With previous LC, the thinner basal layer of GPB was clearly separated from the thicker layer of the BAs. In the groups where GPB remained

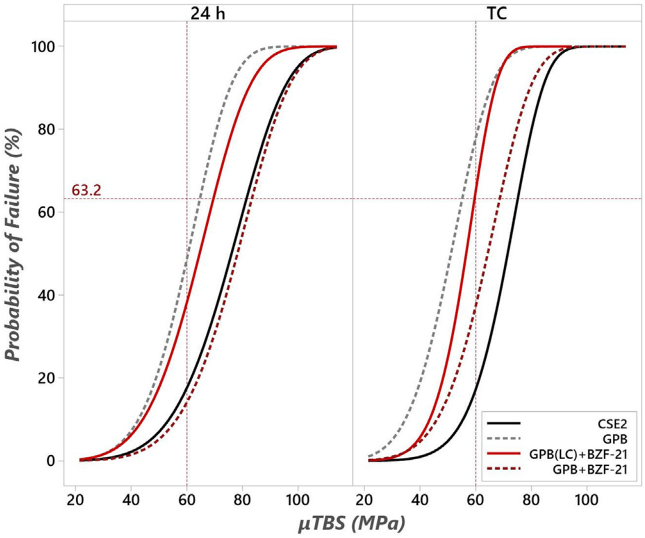

Fig. 1 – Survival graphs of the experimental groups with BZF-21 and the control groups.
The survival graphs compare the experimental groups with BZF-21 (cured group GPB(LC) + BZF-21 and uncured group GPB + BZF-21) to the control group of GPB without any BA and a standard reference CS2. The horizontal dashed line represents the 63.2% probability of failure, which was used to compare the characteristic strengths of the groups. The vertical dashed line at 60 MPa should facilitate the comparison between 24-h results and the results after thermocycling.
***Abbreviations*: TC: thermocycling, μTBS: micro-tensile bond strength, GPB: G-Premio Bond, LC: Light-cured, CSE2: Clearfil SE Bond 2.**

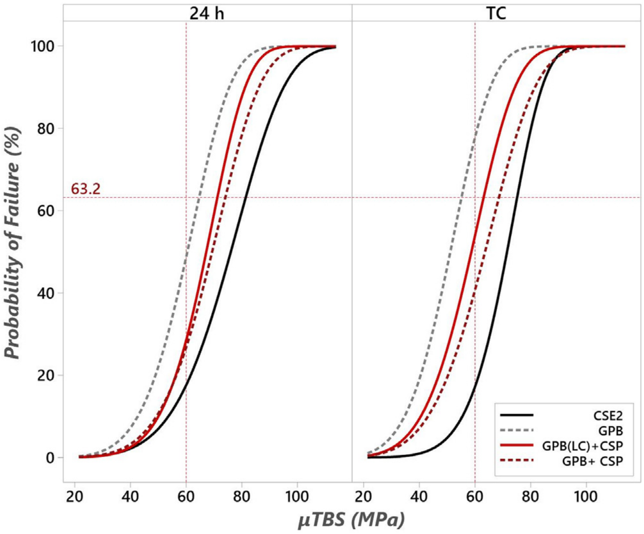

Fig. 2 – Survival graphs of the experimental groups with Clearfil SE Protect and the control groups.
The survival graphs compare the experimental groups with CSP (cured group GPB(LC) + CSP and uncured group GPB + CSP) to the control group of GPB without any BA and a standard reference CS2. The horizontal dashed line represents the 63.2% probability of failure, which was used to compare the characteristic strengths of the groups. The vertical dashed line at 60 MPa should facilitate the comparison between 24-h results and the results after thermocycling.
***Abbreviations*: TC: thermocycling, μTBS: micro-tensile bond strength, GPB: G-Premio Bond, LC: Light-cured, CSP: Clearfil SE Protect, CSE2: Clearfil SE Bond 2.**

uncured, only one apparently uniform layer of mixed GPB and BA was observed (Fig. 6).

3.3. DC

The results are presented in Table 5. The DC of neat GPB without BAs was 76.3 ± 3.9% and it increased slightly after the application and LC of BAs, however, the difference was not significant ($p > 0.05$). DC was significantly higher in the cured groups compared to the uncured groups ($p < 0.001$). DCs of uncured groups GPB + BZF-21 and GPB + CSP were not significantly different from each other, whereas the DC of GPB + FL-II was significantly lower ($p < 0.001$) compared to all other groups. Among the BAs, the highest DC was measured for FL-II (74.8 ± 3.5%), followed by CSE2, CSP and BZF-21 whose DCs were significantly lower than that of FL-II.

4. Discussion

1-SEAs provide a simple and fast application procedure that is suitable for a variety of substrates, but they are associated with inferior bonding performance. The results of previous studies have shown that the addition of a more hydrophobic BA in a second step could minimize their disadvantages and improve bond durability. However, the procedure is more elaborate, especially if the 1-SEA is light-cured prior to the application of BA. The effect of LC of the 1-SEA has not been thoroughly examined and it remains unclear whether it provides any additional benefit. Therefore, the efficiency of this two-step approach with and without LC of a HEMA-free 1-SEA GPB was evaluated in this study to clarify its effect on the bond strength and its durability. Furthermore, DC was evaluated for both the strategies. The results revealed slight improvements in the μTBS and the DC of GPB in the cured groups compared to GPB without BAs. The differences were more marked in the groups where GPB remained uncured prior to the application of BA, where the μTBS of GPB increased significantly with BZF-21 and CSP. Therefore, the null hypotheses tested in this study should be rejected.

In the groups with light-cured GPB, the Weibull analysis disclosed that the 24-h characteristic strengths with BZF-21 and CSP were significantly higher compared to that of GPB. However, when ANOVA was performed, the difference was not significant. This may be partly attributed to the relatively small sample size because the teeth (and not the beams) were regarded as statistical units. The present results corroborate previous studies where no significant difference was obtained [12,13,17] rather than those reporting significantly higher μTBSs [13–16]

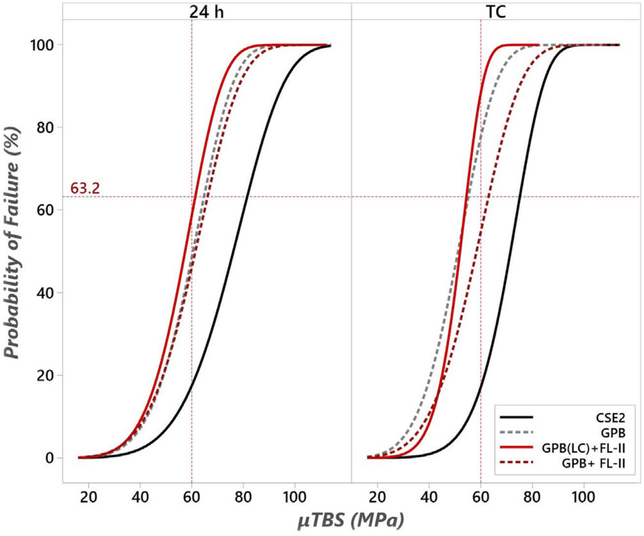

Fig. 3 – Survival graphs of the experimental groups with FL-Bond II and the control groups.
The survival graphs compare the experimental groups with FL-II (cured group GPB(LC) + FL-II and uncured group GPB + FL-II) to the control group of GPB without any BA and a standard reference CS2. The horizontal dashed line represents the 63.2% probability of failure, which was used to compare the characteristic strengths of the groups. The vertical dashed line at 60 MPa should facilitate the comparison between 24-h results and the results after thermocycling.
***Abbreviations*: TC: thermocycling, μTBS: micro-tensile bond strength, GPB: G-Premio Bond, LC: Light-cured, FL-II: FL-Bond II, CSE2: Clearfil SE Bond 2.**

The SEM observation of the adhesive layers of the unfractured specimens revealed clearly separated layers of GPB and BAs in the cured groups (Fig. 6). Furthermore, round-shaped droplets within the layer of GPB (Fig. 5), which might indicate the phase separation of its components [24,25], were present only in groups where GPB was separately light-cured. On the contrary, no such droplets were found in the uncured groups. We therefore assume that the effect of BAs in the cured groups was limited because they could not mix with the light-cured 1-SEA and thus create the desired more hydrophobic complex.

However, some previous studies reported significantly higher immediate μTBSs to dentin after the application of BAs to previously light-cured 1-SEAs [13–16]. We suggest that this discrepancy can be attributed to different compositions of the 1-SEAs and consequently to their different properties such as DC. In two of the studies, significant improvements of initially low 1-SEAs' DCs were reported after the application of BAs and associated with significantly increased μTBSs [14,15]. However, the DC of GPB was high even before the application of BAs, presumably due to the incorporation of the diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide photoinitiator (known as TPO), which is very efficient [26–28]. Therefore, its DC and consequently μTBS could not improve so markedly [29].

In the groups with uncured GPB, BZF-21 and CSP significantly increased the immediate μTBS. Similarly, the μTBS of uncured GPB combined with the BA of CSE2 was reported to be significantly higher than that of GPB [19]. An increase in μTBS was also previously reported with experimental adhesives by the GC Corp. [18]. It has been speculated that this increase could be attributed to a higher proportion of cross-linking monomers delivered by the bonding agent, resulting in improved strength and other mechanical properties of the adhesive layer, and therefore μTBS [18]. The mechanical properties of the adhesive layer could also be improved by the presence of fillers in the BAs. Furthermore, HEMA contained in BAs was probably able to revert the phase separation of GPB (Fig. 5) and the elimination of droplets could result in more uniform stress distribution in the adhesive layer [18]. However, the effect of the application of BAs without previ-

Table 4 – Failure mode distributions in percentages.

Storage condition	Adhesive procedure	Adhesive failure		Cohesive failure	Mixed failure
		Within adhesive layer	Dentin-adhesive interface		
24-h water storage	GPB	75%	1%	16%	8%
	GPB (LC) + BZF-21	57%	0%	16%	28%
	GPB (LC) + CSP	87%	0%	9%	3%
	GPB (LC) + FL-II	71%	1%	13%	14%
	GPB + BZF-21	16%	0%	60%	24%
	GPB + CSP	38%	3%	33%	26%
	GPB + FL-II	23%	41%	28%	8%
	CSE2	14%	1%	62%	23%
Thermocycling	GPB	76%	2%	18%	4%
	GPB (LC) + BZF-21	84%	0%	9%	6%
	GPB (LC) + CSP	87%	0%	11%	3%
	GPB (LC) + FL-II	92%	0%	7%	1%
	GPB + BZF-21	12%	0%	68%	19%
	GPB + CSP	50%	5%	36%	9%
	GPB + FL-II	62%	25%	9%	4%
	CSE2	26%	1%	50%	22%

Abbreviations: GPB: G-Premio Bond, CSP: Clearfil SE Protect, FL-II: FL-Bond II, CSE2: Clearfil SE Bond 2, LC: Light-cured.

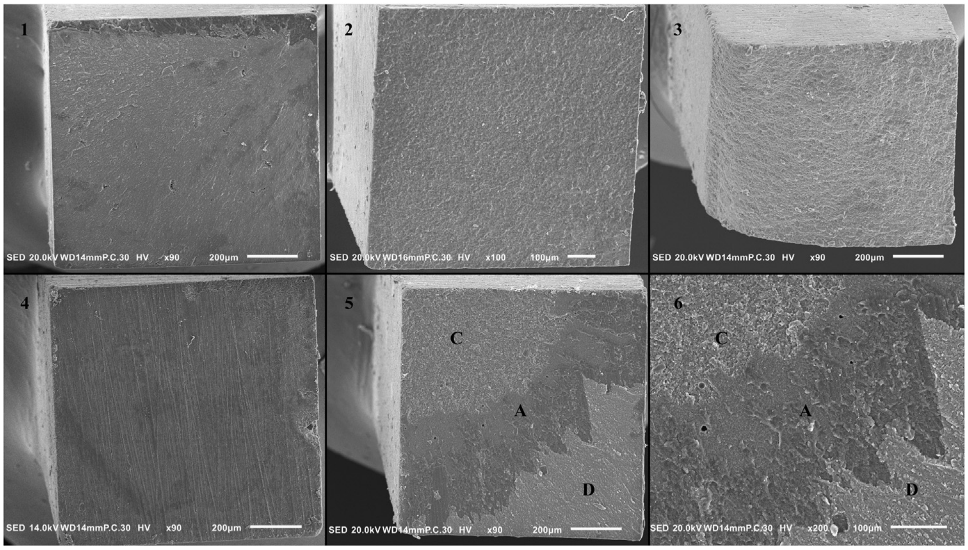

Fig. 4 – Representative images of different failure modes.
(1) Cohesive failure within dentin. (2) Cohesive failure within the adhesive layer (GPB). (3) Cohesive failure within resin composite, (4) Failure at dentin-adhesive interface (GPB + FL-II), 5) Mixed failure in dentin (D), adhesive (A), and resin composite (C). (6) Detailed view of image 5.
***Abbreviations*: GPB: G-Premio Bond, FL-II: FL-Bond II.**

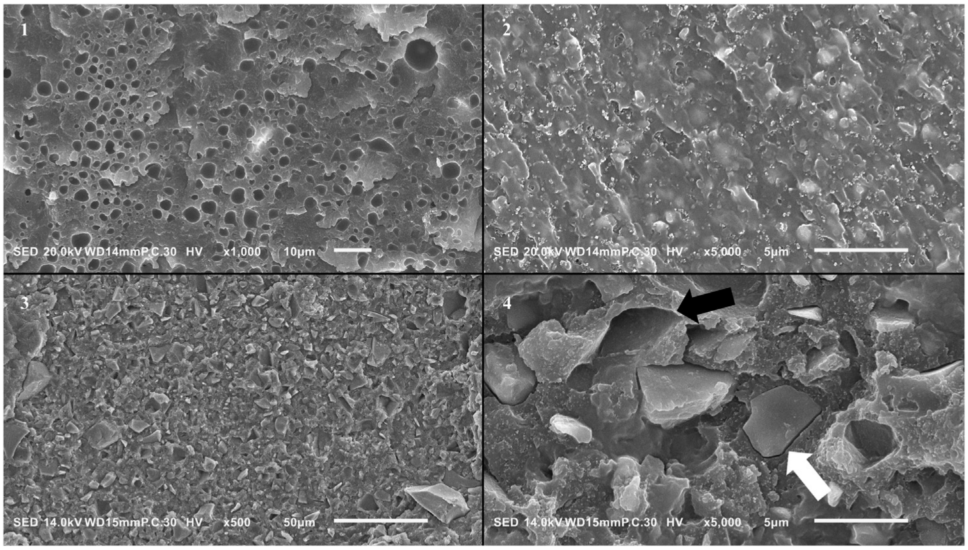

Fig. 5 – Detailed SEM images of adhesives.
(1) Droplets within the layer of GPB in group GPB(LC) + CSP, composite fragment. (2) Contrastive round-shaped particles in BZF-21, possibly containing zinc. (3) GPB(LC) + FL-II: Failure within the layer of FL-II after thermocycling. (4) Detailed view of image 3. White arrow shows a particle with loosened attachment to the matrix. Black arrow shows a defect resulting from glass particle dislocation.
***Abbreviations*: GPB: G-Premio Bond, CSP: Clearfil SE Protect, FL-II: FL-Bond II, LC: Light-cured.**

ous LC seems to be strongly dependent on the 1-SEAs because no improvement has been reported for other tested 1-SEAs [17,19].

Unlike the other uncured groups, no improvement in μTBS was observed with FL-II and the specimens often failed at the dentin-adhesive interface; 41% after 24 h and 25% after TC. Given the high DCs of both GPB and FL-II and the very low DC of their combination, we suppose that this was due to incompatibility of GPB and FL-II. The issue of compatibility seems to be of high importance when the 1-SEA remains uncured because it is mixed with the BA. Incompatibility of 1-SEAs with chemically-cured and dual-cured composites due to an adverse interaction between the acidic monomers and tertiary amines used as catalysts in the resins has been comprehen-

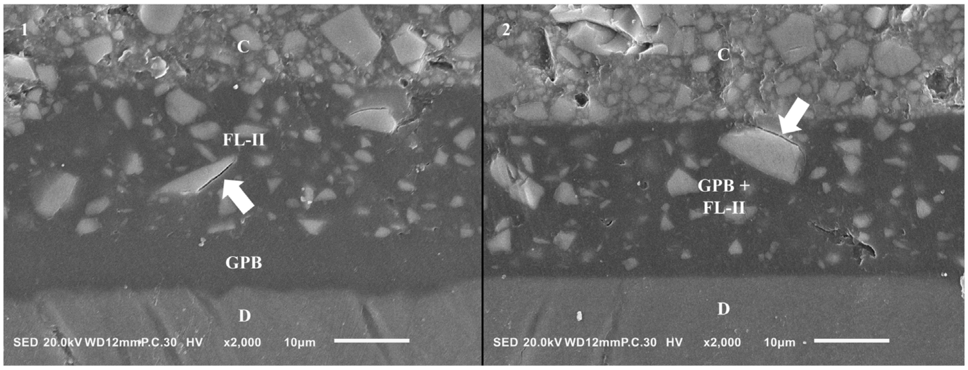

Fig. 6 – SEM observation of the adhesive layers.
(1) GPB(LC) + FL-II: Light-cured GPB formed the basal part of the adhesive layer and was clearly separated from the subsequently applied bonding agents (FL-II). 2) GPB + FL-II: Without previous light-curing, no such separation was observed and the bonding agents (FL-II) were completely intermixed with GPB. White arrows in both images show a glass particle with loosened bond to the matrix.
***Abbreviations*: GPB: G-Premio Bond, LC: Light-cured, FL-II: FL-Bond II.**

Table 5 – Degree of conversion in percentages (Mean ± S.D.).

	Experimental groups						
	GPB	GPB (LC) + BZF-21	GPB (LC) + CSP	GPB (LC) + FL-II	GPB + BZF-21	GPB + CSP	GPB + FL-II
Mean ± S.D. (%)	76.3 ± 3.9^{C}	80.2 ± 1.0^{C}	79.7 ± 1.3^{C}	80.0 ± 3.7^{C}	64.8 ± 5.5^{B}	60.3 ± 3.4^{B}	12.5 ± 0.4^{A}

	Bonding agents			
	CSE2	BZF-21	CSP	FL-II
Mean ± S.D. (%)	61.7 ± 1.4^{A}	56.2 ± 2.8^{A}	59.8 ± 1.2^{A}	74.8 ± 3.5^{B}

Same superscript uppercase letters indicate no statistically significant differences in rows.
Abbreviations: GPB: G-Premio Bond, CSP: Clearfil SE Protect, FL-II: FL-Bond II, CSE2: Clearfil SE Bond 2, LC: Light-cured.

sively described in the literature [30–32]. Tertiary amines are also used in light-cured resins with camphorquinone initiating systems. However, their polymerization is only affected if a high concentration of acidic monomers is present [31,32] or if light activation is delayed, thereby providing enough time for the reaction of the tertiary amines with acidic monomers, resulting in decreased bond strength [33].

Despite the short interval until LC in this case, we suppose that intermixing of GPB (pH = 1.5) with the BA may provide the necessary conditions for the tertiary amines to react with the acidic monomers and result in the very low DC of GPB + FL-II. The DC was presumably not so low when μTBS was tested, because this reaction should be less intensive when GPB is applied to dentin and the acidic monomers react with hydroxyapatite, thus resulting in higher DC [34]. Furthermore, post-irradiation polymerization could further improve the DC prior to testing. However, the lower μTBS and failures at the dentin-adhesive interface observed in group GPB + FL-II were apparently caused by the described incompatibility.

After 15,000 thermal cycles, a decrease in the μTBS was observed in all groups. Compared to GPB, the bond strengths after TC in the cured groups with BZF-21 and CSP were slightly higher. According to the Weibull analysis, the difference was significant for both groups, whereas ANOVA results indicated that the difference was significant only for GPB(LC) + CSP. The results corroborate previous studies that reported higher stability of the adhesive layer after six-month water storage when BAs was applied to previously light-cured 1-SEAs [13,16,17]. Given the separation of the layers of GPB and BAs, we assume that it is due to higher DC [14,15] and lower water permeability [13,16] of the adhesive layer rather than due to its higher hydrophobicity.

No significant difference was found between GPB and GPB(LC) + FL-II after TC. In this group, failures occurred more often within the layer of the BA, probably due to the poor attachment of the S-PRG (Surface Pre-Reacted Glass-ionomer) filler particles to the matrix (Figs. 5 and 6). We concluded that the particles were not treated with a silane coupling agent, which was also confirmed by the manufacturer. As shown in Figs. 5 and 6, the filler particles were commonly dislocated or their attachment to the matrix was loosened. A similar phenomenon was previously observed in an S-PRG containing composite [35].

The SEM observation also revealed more contrastive domains with a sub-micrometer diameter in the cured group with BZF-21 (Fig. 5). Using Energy Dispersive X-Ray Spectroscopy (JEOL Ltd., Tokyo, Japan), it was revealed that the domains represent areas with increased zinc concentration. The ions released by the BAs used in this study may have also partially contributed to the improved durability. However, we suppose that their effect is reduced in the cured groups, because the basal part of the adhesive layer is only formed by

GPB (Fig. 6). The effect of ion-release from the BAs used in this study should be further examined in future studies because it could be beneficial to the long-term bond stability [22,36].

Similarly to the 24-h values, significantly higher μTBSs were obtained in the uncured groups with BZF-21 and CSP after TC compared to GPB. GPB + FL-II also exhibited a higher μTBS than GPB. This may be due to post-irradiation polymerization during TC, which could improve its initially low DC. In a previous study, the addition of the BA of CSE2 to GPB resulted in a significantly improved μTBS after TC [19], which is in agreement with our results. However, no such improvement was obtained with other 1-SEAs after TC [19] and 6-month water storage [17].

In this study, a gold-standard 2-SEA CSE2 was used as a reference for the experimental groups. ANOVA revealed that there was no significant difference between CSE2 and the uncured groups GPB + BZF-21 and GPB + CSP for both storage conditions. The Weibull analysis identified no significant difference only between CSE2 and uncured GPB + BZF-21 after 24 h. The bond strengths of all the other groups were significantly lower than that of CSE2.

Similarly to GPB + BZF-21 and GPB + CSP, no significant difference from CSE2 was previously reported for uncured GPB with the BA of CSE2, both with and without TC [19]. However, other uncured 1-SEA + BA combinations underperformed the 2-SEA references [17–19]. If the 1-SEAs were light-cured beforehand, their μTBSs were significantly lower than those of the reference 2-SEAs [17], which is in accordance with this study. Unfortunately, the other studies did not include this reference.

The objective of this study was to evaluate the influence of LC of a 1-SEA prior to the application of BA. Compared to the cured groups, higher bond strengths were found in the uncured groups with all BAs. The differences were statistically significant according to the Weibull analysis and non-significant according to ANOVA. To the best of our knowledge, only one study has compared these two strategies [17]. The results in the cured groups were similar, significantly higher μTBSs were observed after aging. However, due to differences in the uncured groups, the study reported that 1-SEAs benefit from the application of BA only when light-cured beforehand [17], which is the opposite of the conclusion of this study. The μTBS of HEMA-free GPB used in this study increased significantly both after 24 h and TC, whereas no significant improvement was observed for two HEMA-containing 1-SEAs used in study of Ermis et al. [17]. Together with the results of Ahmed et al. [19], it is demonstrated that the effect of BA application to 1-SEAs cannot be generalized, especially without previous LC.

5. Conclusion

The results of the uncured groups GPB + BZF-21 and GPB + CSP were promising. Further testing of durability and adhesion to enamel should be performed before their eventual clinical application. The usage of FL-II in this study reaffirmed that different adhesives cannot be combined arbitrarily because they might be incompatible. The results also indicated that the application of BAs to previously light-cured GPB was not as effective as to uncured GPB. However, due to the low predictability and high material-dependence, we do not intend to generalize this statement to other 1-SEAs and BAs. The application of any BA in a second step lengthens the bonding procedure and when the 1-SEA is light-cured beforehand, it becomes more time-consuming than the conventional 2-step self-etch technique. Given that no combination of 1-SEA and BA outperformed the standard 2-SEAs in this or any previous studies, the usage of optimized and well-tested 2-SEAs should be preferred in clinical practice.

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REFERENCES

[1] Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater 2011;27:17–28.
[2] Van Landuyt KL, Mine A, De Munck J, Jaecques S, Peumans M, Lambrechts P, et al. Are one-step adhesives easier to use and better performing? Multifactorial assessment of contemporary one-step self-etching adhesives. J Adhes Dent 2009;11:175–90.
[3] Sadek FT, Goracci C, Cardoso PE, Tay FR, Ferrari M. Microtensile bond strength of current dentin adhesives measured immediately and 24 hours after application. J Adhes Dent 2005;7:297–302.
[4] Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. Dent Mater 2008;24:90–101.
[5] De Munck J, Mine A, Poitevin A, Van Ende A, Cardoso MV, Van Landuyt KL, et al. Meta-analytical review of parameters involved in dentin bonding. J Dent Res 2012;91:351–7.
[6] Heintze SD, Ruffieux C, Rousson V. Clinical performance of cervical restorations—a meta-analysis. Dent Mater 2010;26:993–1000.
[7] Peumans M, Kanumilli P, De Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B. Clinical effectiveness of contemporary adhesives: a systematic review of current clinical trials. Dent Mater 2005;21:864–81.
[8] Peumans M, De Munck J, Mine A, Van Meerbeek B. Clinical effectiveness of contemporary adhesives for the restoration of non-carious cervical lesions. A systematic review. Dent Mater 2014;30:1089–103.
[9] Hosaka K, Nakajima M, Takahashi M, Itoh S, Ikeda M, Tagami J, et al. Relationship between mechanical properties of one-step self-etch adhesives and water sorption. Dent Mater 2010;26:360–7.
[10] Takahashi M, Nakajima M, Hosaka K, Ikeda M, Foxton RM, Tagami J. Long-term evaluation of water sorption and ultimate tensile strength of HEMA-containing/-free one-step self-etch adhesives. J Dent 2011;39:506–12.
[11] Tay FR, Pashley DH, Suh B, Carvalho R, Miller M. Single-step, self-etch adhesives behave as permeable membranes after polymerization. Part I. Bond strength and morphologic evidence. Am J Dent 2004;17:271–8.
[12] Frankenberger R, Perdigao J, Rosa BT, Lopes M. “No-bottle” vs “multi-bottle” dentin adhesives—a microtensile bond

strength and morphological study. Dent Mater 2001;17:373–80.
[13] Reis A, Albuquerque M, Pegoraro M, Mattei G, Bauer JRdO, Grande RHM, et al. Can the durability of one-step self-etch adhesives be improved by double application or by an extra layer of hydrophobic resin? J Dent 2008;36:309–15.
[14] Muñoz MA, Sezinando A, Luque-Martinez I, Szesz AL, Reis A, Loguercio AD, et al. Influence of a hydrophobic resin coating on the bonding efficacy of three universal adhesives. J Dent 2014;42:595–602.
[15] Perdigão J, Muñoz M, Sezinando A, Luque-Martinez I, Staichak R, Reis A, et al. Immediate adhesive properties to dentin and enamel of a universal adhesive associated with a hydrophobic resin coat. Oper Dent 2014;39:489–99.
[16] Sezinando A, Luque-Martinez I, Muñoz MA, Reis A, Loguercio AD, Perdigão J. Influence of a hydrophobic resin coating on the immediate and 6-month dentin bonding of three universal adhesives. Dent Mater 2015;31:e236–46.
[17] Ermis RB, Ugurlu M, Ahmed MH, Van Meerbeek B. Universal adhesives benefit from an extra hydrophobic adhesive layer when light cured beforehand. J Adhes Dent 2019;21:179–88.
[18] Van Landuyt KL, Peumans M, De Munck J, Lambrechts P, Van Meerbeek B. Extension of a one-step self-etch adhesive into a multi-step adhesive. Dent Mater 2006;22:533–44.
[19] Ahmed MH, De Munck J, Van Landuyt K, Peumans M, Yoshihara K, Van Meerbeek B. Do universal adhesives benefit from an extra bonding layer? J Adhes Dent 2019;21:117–32.
[20] Reis A, Leite TM, Matte K, Michels R, Amaral RC, Geraldeli S, et al. Improving clinical retention of one-step self-etching adhesive systems with an additional hydrophobic adhesive layer. J Am Dent Assoc 2009;140:877–85.
[21] Sartori N, Peruchi L, Guimarães J, Silva S, Monteiro Jr S, Baratieri L, et al. Clinical effectiveness of a hydrophobic coating used in conjunction with a one-step self-etch adhesive: an 18-month evaluation. Oper Dent 2013;38:249–57.
[22] Imazato S, Ma S, Chen J-h, Xu HHK. Therapeutic polymers for dental adhesives: loading resins with bio-active components. Dent Mater 2014;30:97–104.
[23] Stansbury JW, Dickens SH. Determination of double bond conversion in dental resins by near infrared spectroscopy. Dent Mater 2001;17:71–9.
[24] Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, et al. Monomer-solvent phase separation in one-step self-etch adhesives. J Dent Res 2005;84:183–8.
[25] Van Landuyt KL, Snauwaert J, De Munck J, Coutinho E, Poitevin A, Yoshida Y, et al. Origin of interfacial droplets with one-step adhesives. J Dent Res 2007;86:739–44.
[26] Ilie N, Hickel R. Can CQ be completely replaced by alternative initiators in dental adhesives? Dent Mater J 2008;27:221–8.
[27] Cadenaro M, Antoniolli F, Codan B, Agee K, Tay FR, Dorigo Ede S, et al. Influence of different initiators on the degree of conversion of experimental adhesive blends in relation to their hydrophilicity and solvent content. Dent Mater 2010;26:288–94.
[28] Pongprueksa P, Miletic V, Janssens H, Van Landuyt KL, De Munck J, Godderis L, et al. Degree of conversion and monomer elution of CQ/amine and TPO adhesives. Dent Mater 2014;30:695–701.
[29] Sato K, Hosaka K, Takahashi M, Ikeda M, Tian F, Komada W, et al. Dentin bonding durability of two-step self-etch adhesives with improved of degree of conversion of adhesive resins. J Adhes Dent 2017;19:31–7.
[30] Tay FR, Pashley DH, Yiu CK, Sanares AM, Wei SH. Factors contributing to the incompatibility between simplified-step adhesives and chemically-cured or dual-cured composites. Part I. Single-step self-etching adhesive. J Adhes Dent 2003;5:27–40.
[31] Suh BI, Feng L, Pashley DH, Tay FR. Factors contributing to the incompatibility between simplified-step adhesives and chemically-cured or dual-cured composites. Part III. Effect of acidic resin monomers. J Adhes Dent 2003;5:267–82.
[32] Sanares AME, Itthagarun A, King NM, Tay FR, Pashley DH. Adverse surface interactions between one-bottle light-cured adhesives and chemical-cured composites. Dent Mater 2001;17:542–56.
[33] Tay FR, King NM, Suh BI, Pashley DH. Effect of delayed activation of light-cured resin composites on bonding of all-in-one adhesives. J Adhes Dent 2001;3:207–25.
[34] Hanabusa M, Yoshihara K, Yoshida Y, Okihara T, Yamamoto T, Momoi Y, et al. Interference of functional monomers with polymerization efficiency of adhesives. Eur J Oral Sci 2016;124:204–9.
[35] Minami H, Hori S, Kurashige H, Murahara S, Muraguchi K, Minesaki Y, et al. Effects of thermal cycling on surface texture of restorative composite materials. Dent Mater J 2007;26:316–22.
[36] Shinohara MS, Yamauti M, Inoue G, Nikaido T, Tagami J, Giannini M, et al. Evaluation of antibacterial and fluoride-releasing adhesive system on dentin - microtensile bond strength and acid-base challenge. Dent Mater J 2006;25:545–52.