Application of a New Microtensile Bond Strength Testing Technique for the Evaluation of Enamel Bonding

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Objective: *To evaluate adhesives' enamel bonding performance utilising the traditional microtensile bond strength test (µTBST) and a new double-sided microtensile bond strength test (DµTBST) to assess the suitability of the latter.*

Methods: *A 'tug-of-war' direct encounter design was employed to compare the enamel bond strengths of two universal adhesives and their different application modes simultaneously under the same tensile load applied to double-sided bonded specimens. Clearfil Universal Bond (CU; Kuraray, Kurashiki, Japan) and Scotchbond Universal Adhesive (SB; 3M ESPE, St Paul, MN, USA) were applied in self-etch (S) and etch-and-rinse (E) mode on 110 human molar samples to perform two experiments. Experiment 1 compared the enamel bond strengths of the combinations of adhesive application modes utilising µTBST. The data were analysed using a Welch analysis of variance (ANOVA), followed by a Games-Howell test. Experiment 2 employed DµTBST to determine the suitability of the new double-sided bonded assembly and ascertain which of the adhesive application mode combinations was superior. The data were analysed using a Kaplan-Meier survival analysis, followed by pairwise comparisons with a Mantel-Cox log-rank test. The level of significance was set at $P < 0.05$.*

Results: *The µTBST results did not show significant differences for CUE vs CUS, SBE vs SBS, CUS vs SBS and CUS vs SBE ($P > 0.05$); however, from DµTBST, the survival distributions for the interventions were statistically significantly different ($\chi^2(3) = 145.130$, $P < 0.0005$), indicating the superiority of universal adhesive CU over SB and application mode E over S with certainty.*

Conclusion: *DµTBST was able to add more discerning outcomes to the µTBST results, indicating that the new technique could become a valuable adjunct to the conventional method.*

Key words: *adhesives, dental bonding, dental enamel, microtensile bond strength test, new double-sided microtensile bond strength test*

Chin J Dent Res 2021;24(3):159–166; doi: 10.3290/j.cjdr.b1965031

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This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (19K10123), National Natural Science Foundation of China (81841002), and Natural Science Foundation of Heilongjiang Province of China (YQ2019H010).

Enamel and dentine bonding can be accomplished by either removing the smear layer before applying the adhesive (etch-and-rinse approach, E) or by using an adhesive that can dissolve the smear layer and penetrate across it to achieve bonding to the hard dental tissue underneath (self-etch approach, S)[1]. Some clinical evidence suggests that in the hands of proficient operators, both etching methods have been able to confer satisfactory and durable composite resin restorations. Bonding to phosphoric acid-etched enamel (E approach) was proven durable in a 9-year clinical evaluation of cervical composite resin restorations[2]. Simultaneously, the durability of the S approach was also deemed satisfactory for enamel bonding after a 10-year clinical assessment of composite resin restorations[3]; however, another approach that involved additional etching of the enamel margins of the cavities only (selective enamel etching) resulted in an improved marginal adaptation, but this was not proven significant for the overall clinical performance of the restorations[4].

Recently, a new type of one-step adhesive categorised as 'universal' or 'multimode' has been embraced by dental practitioners worldwide because of its user-friendliness and clinical effectiveness[5,6]. As the name implies, universal adhesives can be used with multiple substrates, such as dentine, enamel, silica-based glass ceramics, zirconia ceramics and metal alloys[7,8]. Clearfil Universal Bond (CU; Kuraray, Kurashiki, Japan) and Scotchbond Universal Adhesive (SB; 3M ESPE, St Paul, MN, USA) are two of the most commonly tested universal adhesives. Their reported bonding performances indicate their clinical suitability[5-11]. With fewer steps, clinicians can save chair time, making treatment more comfortable for patients[12].

Many researchers have evaluated the microtensile bond strength (μTBS) of CU and SB in different etching modes[9-11,13,14]; however, the traditional microtensile bond strength test (μTBST) can fail to differentiate between their bonding performance, especially when their bond strength values are comparable[9,13]. Moreover, with μTBST, direct comparisons between adhesives are impossible because the traditional specimen design allows only one adhesive (or etching mode) to be tested at each sample testing, but these drawbacks could be avoided by utilising a double-sided bonded assembly. Subjecting such bonded samples to a tensile load could allow a direct comparison of two adhesive systems (or etching modes) simultaneously, analogous to a 'tug-of-war' approach.

The present study therefore applied a new double-sided bonded assembly to evaluate the enamel bonding performance of CU and SB in S and E modes simultaneously. To differentiate the new technique from μTBST, we named the former the "double-sided microtensile bond strength test" (DμTBST). We hypothesised that the DμTBST would be able to compare the bonding performance of two universal adhesives (CU and SB) and their different application modes (S and E) at the same time with a single bonded specimen subjected to a single tensile load, and that significant differences would be revealed between the bonding performance of the interventions (CUS, CUE, SBS and SBE).

Materials and methods

Selection and preparation of teeth for bond strength test

The present study was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2013. After approval from the Hokkaido University Faculty of Dentistry Ethics Committee (2013-7) and patients' informed consent, molar teeth were collected and stored in an aqueous solution of 0.5% chloramine-T at 4°C and used within 6 months of extraction. One hundred and ten extracted sound human molars free of any signs of caries, cracks or fractures were used. We divided the study into two experiments: Experiment 1 and Experiment 2.

Experiment 1

Experiment 1 aimed to evaluate the bonding performance of the adhesives utilising the μTBST. The non-trimmed bonded beam preparation procedure is schematically depicted in Fig 1. Twenty human molars were randomly allocated to four test groups (n = 5 teeth) based on adhesives (CU and SB) and application modes (S and E)[15]. The experimental groups were CUS, SBS, CUE and SBE. The adhesives and their application modes used in the experiments are shown in Table 1.

Each tooth crown was ground mesially or distally with 600-grit silicon carbide abrasive paper (SiC; Fuji Star, Sankyo Rikagaku, Okegawa, Japan) under running water for 60 seconds to expose flat enamel surfaces. For the S groups, each adhesive was then applied as per the manufacturer's instructions and light cured (Optilux 401, Demetron/Kerr, Orange, CA, USA) at ≥ 550 mW/cm^2. Composite resin of a thickness of at least 4.5 mm (Clearfil AP-X, A3, Kuraray) was then built up by being applied in three 1.5-mm increments. For the E groups, before application of the adhesive, the exposed enamel was etched for 10 seconds for the CUE group (K-ETCHANT, Kuraray) and 15 seconds for the SBE group (Scotchbond Universal Etchant, 3M ESPE). Each etched tooth was then rinsed with water for 15 seconds. The teeth were then bonded in the same manner as the S mode, followed by build-up of at least 4.5-mm-thick composite resin. After bonding, all the specimens were stored in distilled water at 37°C for 24 hours. The bonded teeth were then sectioned per-

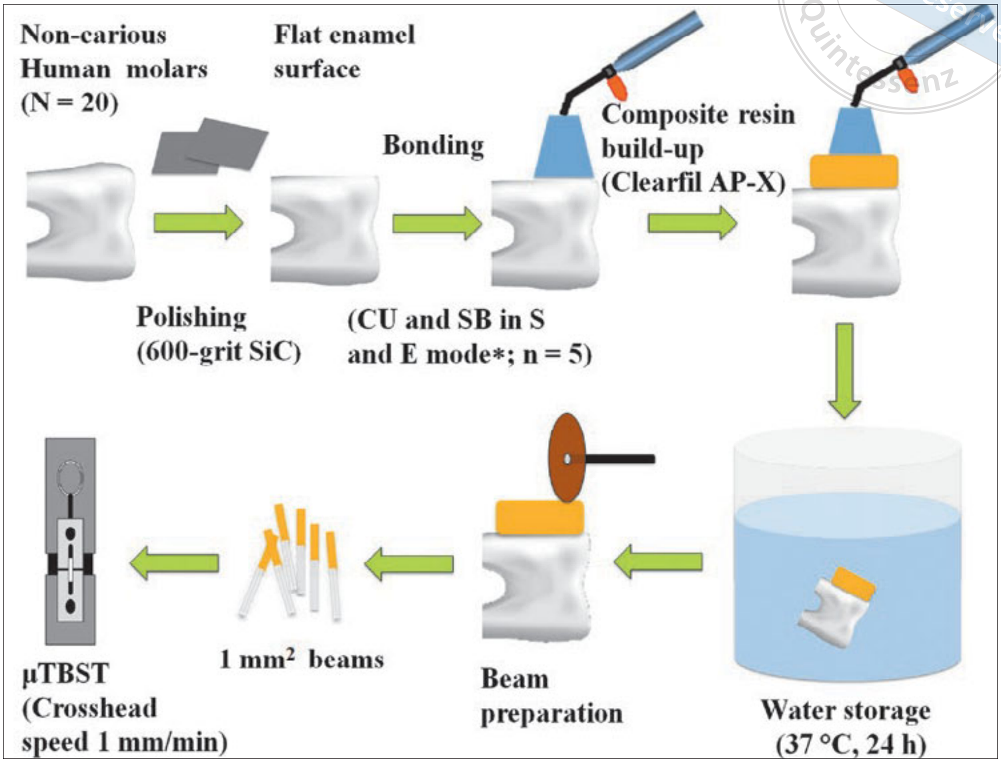

Fig 1 Schematic of Experiment 1 showing the preparation of bonded teeth and µTBST. *CU and SB were applied in S and E mode to obtain four experimental combinations (n = 5): CUS, SBS, CUE and SBE.

Table 1 Adhesives and application modes used in the present study.

Adhesive/code/lot number	Type	Composition	Application: Self-etch mode (S)	Application: Etch-and-rinse mode (E)
Clearfil Universal/ CU/000002	Universal	10-MDP, Bis-GMA, HEMA, hydrophilic aliphatic dimethacrylate, colloidal silica, silane coupling agent, dl-camphorquinone, ethanol, water	1. Apply the adhesive to the enamel surface with the applicator brush and rub it in for 10 s. 2. Dry the enamel surface sufficiently by blowing mild air for > 5 s until the adhesive does not move. 3. Light-cure for 10 s	1. Etch the enamel surface for 10 s. 2. Rinse for 15 s and then dry. 3. Apply the adhesive to the enamel surface with the applicator brush and rub it in for 10 s. 4. Dry the enamel surface sufficiently by blowing mild air for > 5 s until the adhesive does not move. 5. Light-cure for 10 s
Scotchbond Universal Adhesive/SB/572054	Universal	10-MDP, Vitrebond™ copolymer, HEMA, dimethacrylate resins, filler, silane, initiators, ethanol, water	1. Apply the adhesive on the enamel surface and rub for 20 s. 2. Gently air-dry the adhesive for approximately 5 s until it no longer moves and the solvent evaporates. 3. Light-cure for 10 s	1. Etch the enamel surface for 15 s. 2. Rinse for 15 s and then dry. 3. Apply the adhesive on the enamel surface and rub it for 20 s. 4. Gently air-dry the adhesive for approximately 5 s until it no longer moves and the solvent evaporates. 5. Light-cure for 10 s
Clearfil SE Bond 2/ SE/000013	Two-step self-etch	Primer: 10-MDP, HEMA, hydrophilic aliphatic dimethacrylate, dl-camphorquinone, water Bond: 10-MDP, Bis-GMA, HEMA, dl-camphorquinone, hydrophobic aliphatic dimethacrylate, initiators, accelerators, silanated colloidal silica	1. Apply the primer on the dentine surface and leave for 20 s. 2. Gently blow air for > 5 s. 3. Apply the bond. 4. Gently blow air to make the film uniform. 5. Light-cure for 10 s	

10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; Bis-GMA, bisphenol-A-diglycidyl methacrylate; HEMA, 2-hydroxyethylmethacrylate.

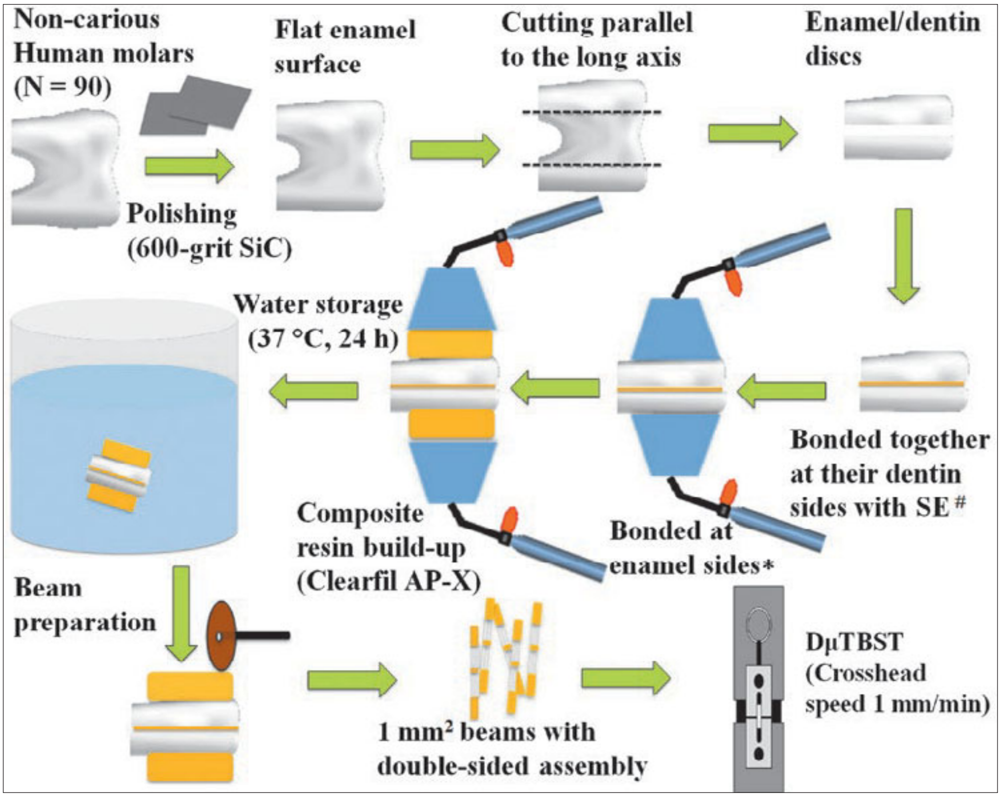

Fig 2 Schematic of Experiment 2 showing the preparation of bonded enamel-dentine discs and DμTBST. #Two enamel-dentine discs obtained from each tooth were bonded together at their dentinal sides with Clearfil SE Bond 2 (SE). *CU and SB were applied in S and E mode to obtain six experimental combinations (n = 15): CUS vs CUE, SBS vs SBE, CUS vs SBS, CUE vs SBE, CUS vs SBE and CUE vs. SBS.

pendicular to the adhesive interface to produce bonded beams (cross-sectional area 1 mm^2), using a low-speed diamond saw (IsoMet 1000, Buehler, Lake Bluff, IL, USA) under cooling water lubrication. All available bonded beams were collected. At least 29 beams per group were tested for bond strength.

Experiment 2

Experiment 2 aimed to compare the bond strength of two combinations of adhesive application modes simultaneously utilising the DμTBST. The new double-sided bonded beam preparation procedure is schematically presented in Fig 2. Ninety human molars were used. The crown of each tooth was flattened mesially and distally by grinding with 600-grit SiC for 60 seconds under running water to obtain flat enamel surfaces. Each tooth was then cut parallel to its long axis to make two discs where the flat enamel was supported by dentine (enamel-dentine discs, two discs/tooth). The discs were then bonded to each other at their dentinal sides using the gold standard two-step self-etch adhesive Clearfil SE Bond 2 (SE; Kuraray) according to the manufacturer's instructions[11]. The enamel sides were then randomly bonded with CU and SB in either E or S modes to obtain six experimental combinations (n = 15 teeth): CUS vs CUE, SBS vs SBE, CUS vs SBS, CUE vs SBE, CUS vs SBE and CUE vs SBS. There was a build-up of composite resin of a thickness of at least 4.5 mm on both ends following the adhesive application. This preparation procedure resulted in a double-sided bonded assembly comprised of two resin-enamel bonded interfaces on two sides with a resin-dentine bonded interface in the middle (Fig 2). After storage in distilled water at 37°C for 24 hours, the bonded specimens were sectioned to produce double-sided bonded beams (cross-sectional area 1 mm^2). All available double-sided bonded beams were collected. At least 48 bonded beams per experimental combination were tested for bond strength.

Bond strength tests

The bond strength test procedures for traditional and double-sided beams are shown in Figs 1 and 2. The cross-sectional area of each beam was measured using a digital caliper (DIGI-KANON, NAKAMURA MFG, Matsudo, Japan) before fixing to a Ciucchi's jig with a cyanoacrylate adhesive (Model Repair II Blue, Dentsply-Sankin, Tokyo, Japan). The bond strength test was carried out by subjecting each beam to a tensile force using

Table 2 Mean ± standard deviation (SD) of µTBS of the tested adhesive application mode combinations in Experiment 1.

Adhesive application mode combination	Number of tested beams	Mean ± SD, MPa
Clearfil Universal Bond in etch-and-rinse mode (CUE)	29	21.9 ± 4.2[b]
Clearfil Universal Bond in self-etch mode (CUS)	29	20.8 ± 5.1[a,b]
Scotchbond Universal Adhesive in etch-and-rinse mode (SBE)	30	19.0 ± 3.6[a]
Scotchbond Universal Adhesive in self-etch mode (SBS)	30	18.2 ± 4.1[a]

Values with different superscript letters indicate statistically significant differences (Games-Howell test, $P < 0.05$).

a 500-N load cell at a crosshead speed of 1 mm/minute in a desktop testing apparatus (EZ Test, Shimadzu, Kyoto, Japan) until failure occurred. µTBST and DµTBST were performed at room conditions (23°C and 30% relative humidity), and each bonded beam was tested within 5 minutes after removal from water storage to prevent the specimens from drying[16]. For µTBST, the bond strength values were obtained by dividing the force (newton) at which each beam fractured by its cross-sectional area (mm^2) and expressed in megaPascals (MPa). Similarly, for DµTBST, the bond strength value was calculated by dividing the force at which each double-sided bonded beam failed by its cross-sectional area and expressed in MPa. The 'winner' and 'failure' side were also noted.

Statistical analysis

The normality of the bond strength data obtained from the µTBST was checked with a Shapiro-Wilk test. The homogeneity of variance was evaluated with a Levene test. As the data were normal but nonhomogenous, a Welch analysis of variance (ANOVA) was conducted to determine the effects of adhesive and application mode combinations (CUS, SBS, CUE and SBE). A Games-Howell test achieved multiple comparisons at a 5% level of significance.

The DµTBST directly compared two interventions with adhesive application modes with each double-sided bonded beam, leading to a winner and a failure side. The bond strength value thus obtained represented the bond strength of the failed intervention only; therefore, for DµTBST, a Kaplan-Meier survival analysis was run to determine the differences in the survival distribution for the different interventions (CUS, CUE, SBS and SBE)[17]. For each failed double-sided bonded beam, 'failure side' was defined as the 'event' and ranked 1, and 'winner side' was defined as 'censored' and ranked 0. 'Survival time' was represented by the bond strength value (in MPa) of the relevant beam. Thus, a pair of data were generated for each beam, one for the winner side and the other for the failure side. All statistical analyses were conducted using SPSS 22.0 (IBM, Armonk, NY, USA).

Results

Experiment 1

There were no pre-test failures. A Welch ANOVA revealed that µTBS was significantly affected by the adhesive application mode combinations ($F = 4.685$, $P = 0.005$). The bond strength values obtained by µTBST in Experiment 1 are shown in Table 2.

In general, the bond strengths of CU and SB obtained with E and S application modes were similar ($P > 0.05$), but only CUE (21.9 ± 4.2 MPa) was significantly higher than SBE (19.0 ± 3.6 MPa) and SBS (18.2 ± 4.1 MPa) ($P < 0.05$). No significant differences were observed when S and E application modes were compared within each adhesive (CUE vs CUS and SBE vs SBS; $P > 0.05$).

Experiment 2

In DµTBST, there were no pre-test failures at the adhesive-enamel interfaces, and no failures were observed in the resin-dentine interface upon tensile loading. When subjected to the tensile force, each double-sided bonded beam failed at one of the resin-enamel interfaces, resulting in a 'winner' and a 'failure' intervention for that beam, meaning one intervention won over the other at that bond strength value or vice versa. The bond strength value, however, represented the bond strength value of the failed intervention. The estimated means and medians for survival time (bond strength at failure) are shown in Table 3. The estimated mean bond strength until failure was 29.7 MPa for CUE, 19.7 MPa for CUS, 25.6 MPa for SBE and 18.5 MPa for SBS. The results indicated that, in general, CU showed higher bond strengths than SB. A Mantel-Cox log-rank test revealed that the survival distributions for the four interventions were statistically significantly different ($\chi^2(3) = 145.130$, $P < 0.0005$).

The cumulative survival (or event) across the bond strength variable is shown in Fig 3. The S groups (CUS and SBS) showed lower survival probability than the E groups (CUE and SBE). A similar trend was observed

Table 3 Mean and median survival times retrieved from the results of Experiment 2.

Intervention	Mean[a]				Median			
	Estimate	Standard error	95% confidence interval		Estimate	Standard error	95% confidence interval	
			Lower bound	Upper bound			Lower bound	Upper bound
CUE	29.711	1.040	27.671	31.750	29.633	.	.	.
CUS	19.678	0.465	18.767	20.589	19.355	0.358	18.655	20.056
SBE	25.569	0.766	24.068	27.071	26.523	0.415	25.710	27.335
SBS	18.456	0.530	17.416	19.495	18.870	0.607	17.680	20.059
Overall	22.715	0.382	21.967	23.464	22.413	0.465	21.502	23.324

[a]Estimation is limited to the longest survival time if it was censored.

Table 4 Pairwise comparisons as observed from the results of Experiment 2.

Test	Intervention	CUE		CUS		SBE		SBS	
		Chi-square	Significance	Chi-square	Significance	Chi-square	Significance	Chi-square	Significance
Log-rank (Mantel-Cox)	CUE			77.001	0.000	11.182	0.001	98.309	0.000
	CUS	77.001	0.000			42.200	0.000	1.150	0.284
	SBE	11.182	0.001	42.200	0.000			62.703	0.000
	SBS	98.309	0.000	1.150	0.284	62.703	0.000		

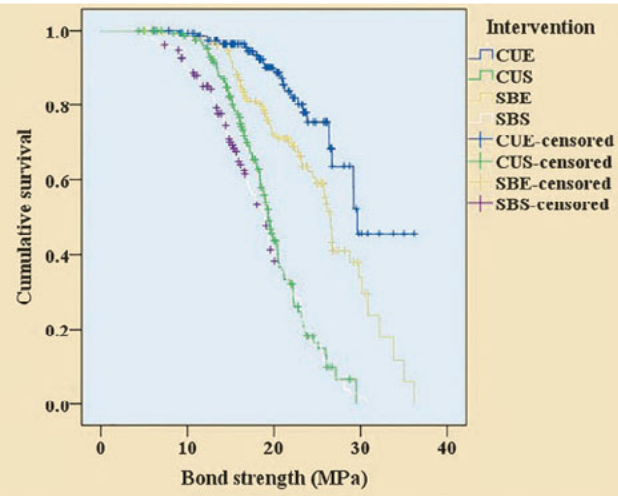

Fig 3 Survival functions graph showing cumulative survival (or event) across the bond strength variable. The different coloured lines represent the interventions: CUE, CUS, SBE and SBS.

when two different application modes were compared across the same adhesive (CUE vs CUS and SBE vs SBS).

Pairwise comparisons among the interventions are shown in Table 4. The survival probability for CUE and SBE was significantly higher than for CUS and SBS ($P < 0.05$). With each adhesive, similar trends were seen when E modes were compared to S modes, i.e., CUE > CUS and SBE > SBS ($P < 0.05$).

Discussion

Since its inception, µTBST has become the most frequently utilised in vitro bond strength testing method and has contributed significantly to the development of adhesive systems. It has a superior discriminative capability to the traditional macroshear bond test which, together with its recurrent use, makes it one of the most standard and versatile bond strength tests at present[18]. µTBST has been recommended as the most representative in vitro evaluation of composite resin restoration retention, especially after exposing the bonded specimens to a durability challenge[19]. However, with µTBST, in each test, only one adhesive and application mode can be tested with one bonded sample and, because the method is strength-based, it might not be able to differentiate between the performance of adhesives when their bond strengths are comparable[15]. Wagner et al[13] reported similar outcomes when they compared the E and S application modes of universal adhesives. The present µTBST results (Table 2) are in agreement with their report and revealed that, in general, the enamel bond strength of CU was superior to SB, and the E mode performed better than S mode. Nonetheless, multiple comparisons with the Games-Howell test revealed that except for CUE, the bonding performance of the other adhesive application mode combinations was similar ($P > 0.05$). Moreover, the differences were also nonsignificant when each adhesive's application modes were compared (CUE vs CUS and SBE vs SBS; $P > 0.05$).

In terms of chemical composition, both CU and SB are very similar (Table 1). We presume the compositional similarity resulted in their comparable bond strength values recorded with the µTBST method in the present study. Our previous report also demonstrated similarities in the bonding performance of CU and SB[9]. The outcomes of µTBST supported the rationale for adopting the new double-sided bonded assembly in the present study, aiming for more discerning results.

Contrary to µTBST, DµTBST was able to compare CU and SB and their different application modes directly through a 'tug-of-war' approach. When subjected to a tensile load, each double-sided bonded beam fractured in one of its two resin-enamel interfaces, resulting in a winner side, a failure side and a bond strength value representing the failed intervention. This observation supported our initial hypothesis that DµTBST would be able to compare the bonding performance of two universal adhesives and their different application modes simultaneously with a single bonded specimen when subjected to a single tensile load.

Moreover, the DµTBST results were not only supplementary to µTBST results but also more distinctive. The survival distributions obtained for the four interventions with the survival analysis were statistically significantly different ($P < 0.0005$). This observation supported our second hypothesis. The estimated means and medians for survival time (bond strength at failure) of E interventions were higher than S interventions (Table 3), which followed the same trend as observed in the µTBST (Table 2). The survival probability for CUE and SBE was significantly higher than CUS and SBS (Fig 3; $P < 0.05$), meaning that the S groups were less likely to survive. Moreover, within each adhesive, similar trends were seen when E modes were compared to S modes, i.e., CUE > CUS and SBE > SBS (Table 4; $P < 0.05$). These observations proved the superiority of the E mode over the S mode in the case of enamel bonding. We presume that the additional etching step before applying the adhesive might have demineralised the enamel to a greater extent, leading to improved micromechanical interlocking. Earlier studies also reported similar observations[20,21].

In the present investigation, similar to µTBST (Table 2), DµTBST results also suggested that CU generally bonded better than SB to enamel (Table 3). CU is more acidic (pH 2.3) than SB (pH 2.7)[9]; we presume that this is the reason for CU's better performance.

The double-sided specimen design for microtensile bond strength testing was first introduced by Fernandes[22], who simultaneously compared the bond strength of composite resins to superficial and deep dentine. Papacchini et al[23] later adopted the design to evaluate the contribution of silane to the repair strength of composite resin over time. The present study was the first to employ a double-sided bonded assembly to assess the bond strength of adhesives to enamel. We utilised the proximal surfaces (mesial and distal) of each tooth crown because of their smoothness compared to the occlusal surface (Fig 2). Our pilot studies revealed that a double-sided enamel-only disc is challenging to prepare and test due to the brittleness of the substrate, which resulted in many pre-test failures. We therefore adapted to the anatomically relevant enamel-dentine discs made from each tooth crown's mesial and distal sides. The discs were bonded together at their dentinal ends with the gold standard two-step self-etch system SE due to its stable and superior bonding performance over one-step systems[11,24,25].

Following previous reports[22,23], DµTBST in the present study was found to be a way of comparing two different adhesive-application interventions simultaneously, utilising a single double-sided bonded beam under the same testing conditions. On the contrary, when µTBST was employed, the bond strength data for the different interventions (adhesive application mode) had to be retrieved individually under slightly variable testing conditions. In the DµTBST, the intervention with superior bond strength to the failed intervention could be immediately determined without even considering the actual bond strength value. Thus, it might have helped to remove other potential variables, such as minor variations in the beams' alignment angulation along with the Ciucchi's jigs or the effects of variable amounts of cyanoacrylate glue applied to the ends of the specimens. We also presume that the novel design might have excluded the confounding impact of tooth variability. Because of its lookalike assembly at both ends, DµTBST might have resulted in more uniform stress distribution across the bonded assembly than µTBST. Further studies should compare the stress distribution patterns of both specimen designs under tensile stress.

Conclusion

DµTBST was able to add more discerning outcomes to the µTBST results, determining the superiority between two universal adhesives and application modes with certainty, and DµTBST could become a valuable adjunct to µTBST.

Acknowledgements

The authors thank Drs Monica Yamauti, Shinichi Kakuda, Umma Habiba and Arefin Alam for their technical support and the manufacturers for donating the materials for this study.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Dr Ji Hao SUN contributed to the investigation, original draft preparation and funding acquisition; Dr Fei CHEN contributed to the investigation and visualisation; Dr Koji KANEFUJI contributed to the formal analysis, reviewing and editing; Dr Abu Faem Mohammad Almas CHOWDHURY contributed to the data curation, formal analysis, original draft preparation and visualisation; Dr Ricardo Marins CARVALHO contributed to the conceptualisation, reviewing and editing; Dr Hidehiko SANO contributed to the conceptualisation, methodology, validation, resources, supervision, project administration, funding acquisition, reviewing and editing.

(Received Sep 25, 2020; accepted Jan 28, 2021)

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