# 博 士 論 文

Is the double-sided microtensile bond strength test appropriate to assess the performance of adhesives to dentin?
（両側微小引張試験は象牙質歯質接着性を評価する際に妥当か?）

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**Original Research**

**Title:** Is the double-sided microtensile bond strength test appropriate to assess the performance of adhesives to dentin?

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**Abstract**

[Objectives] To assess the bond strength of self-etch adhesives to dentin and to verify the appropriateness of double-sided compared to the conventional microtensile bond strength test in dentin. [Material and Methods] Sixty non-carious human molars and four current self-etch adhesives were used: Clearfil™ SE Bond2 (SE), Clearfil™ Universal Bond (CU), Scotchbond™ Universal (SB) and G-Premio Bond (GP). The adhesives were applied to 600 SiC paper-flat dentin surfaces according to manufacturer's instructions. In EXP-1: Teeth were sectioned in the middle portion of dentin and bonded with self-etch adhesives: SE, CU, SB and GP for conventional μTBS test. In EXP-2: Teeth were sectioned and different dentin discs thicknesses (0.5, 1.0, 1.5, 2.0 mm) were obtained and bonded with SE and CU for the double-sided μTBS test. Both adhesives were tested simultaneously in the same beam. In EXP-3: 1-mm dentin discs were used for double-sided μTBS test and bonded with CU, SB and GP (six combinations) either on coronal or pulpal side of dentin disc. Samples were stored in distilled water (24 h, 37°C), sectioned into 1×1 mm beam, and μTBS test was performed. Failure mode was observed using stereoscope and SEM. Data were statistically analyzed by ANOVA and post hoc tests ($p$<0.05). [Results] EXP-1: Differences were found on μTBS conventional test for all adhesives. EXP-2: Dentin thickness of 1 mm showed to be the most appropriate substrate for the double-sided μTBS test of tested adhesives to dentin. EXP-3: Differences on bond strength were found for adhesive's combinations. SB-CU combinations showed the highest and no significant different μTBS values (43.86 and 41.20 MPa). [Conclusions] Within the limitations of this study, double-sided μTBS test is not always applicable to assess the performance of adhesives to dentin. It will applicable when if two different adhesives shown almost similar bond strength values on conventional μTBS test.

## 1. Introduction

Different types of mechanical tests are employed to evaluate the bonding performance of adhesives to tooth substrates. The common methods are shear bond strength test, tensile bond strength test, microtensile bond strength (μTBS) test and microshear bond strength (μSBS) test[1-5]. Conventional tensile and shear bond strength tests demonstrated to be very sensitive due to non-uniform stress distributions[5], to large bonded surfaces 3-6 mm in diameter[6], that could generate defects in the materials and underestimate bond strength values[7], and to usual occurrence of cohesive failure of dental substrate and composite, that could compromise an accurate assessment of the interfacial bond strength[8].

In the mid 90's, Sano *et al.* (1994) introduced the microtensile bond strength (μTBS) test to overcome the limitations of shear and tensile strength test[7]. This test introduced specimens with smaller bonding areas (i.e., below 1 $mm^2$)[5,6] and has since been widely used across all over the world[7,9]. The μTBS test have a long list of advantages, i.e., less chance of defects generated by fractures in the substrate, the possibility of assessing bond strengths of specific areas of the substrates[4,5,10], it reduces the heterogeneity of stress distribution[10]. Despite all advantages, the results of μTBS test still presents huge variation among researches and laboratories, even when the same bonding system is compared across similar experimental circumstances[11].

Partially, those differences are related to substrates variability, that is especially critical in dentin, and to the materials themselves. The bond strength of resin to dentin fluctuates due to its intrinsic structure as morphology, histology, chemical and physical properties, age of teeth, condition of teeth[12,13]. Though universal adhesive systems became very popular, their composition is very complex and not stable[14].

Critics concerning the conventional μTBS technique have been drew over years. μTBS test usually the assembly of the beams have one adhesive interface with resin composite and dentin on two opposite side; but their modulus of elasticity is not same[9,10,15], and errors in the alignment of the specimen could also occur during testing[5,9,13]. Failure mode and bond strengths could be affected by the rate of stress concentration[13]. In addition, during the test, the weakest part of the beam assembly should be fractured. Under this circumstance, assessment of mechanical strength of components is correlated to the bond strength. Furthermore, expressing bond strength in terms of average of stress had been questioned due to the heterogeneity of the stress distribution at the bonded interface[6]. Also, when comparing the bond performance of two different adhesive systems using conventional μTBS testing method, different dentin surfaces are necessary. It is not possible to directly and simultaneously compare those adhesives[14,15].

Nevertheless, in this current study an attempt has been performed to simultaneously test different self-etch adhesives under the same experimental conditions. The double-sided μTBS testing design has previously been described to evaluate the composite repair strength[16]. The null hypotheses tested were: (1) There will be statistically significant differences between bond strength of adhesives by using conventional μTBS method, (2) There will be no effect of dentin thickness on μTBS values of adhesives by using double-sided μTBS method and (3) The conventional μTBS method and the double-sided μTBS method will show dissimilar results.

## 2. Materials and methods

*2.1 Sample preparation:*

The adhesive materials used in this study are shown in Table 1. The teeth were collected under a protocol reviewed and approved by the University Ethics Committee (2014-1). After extraction they were stored in an aqueous solution of 0.5% chloramine-T at 4°C. This study was designed into three experiments.

*2.2 Experiment-1 (EXP-1):*

In this EXP-1, the first hypothesis was tested. The bond strength to dentin of four self-etch adhesive systems was assessed by means of conventional microtensile bond strength test. Tooth was considered as the sample unit (n=4). Sixteen human molars were randomly divided into four adhesive groups: Clearfil™ SE Bond 2, (SE, Kuraray Noritake Dental Inc., Kurashiki, Japan), Clearfil™ Universal Bond, (CU, Kuraray Noritake Dental Inc., Kurashiki, Japan), Scotchbond™ Universal (SB, 3M ESPE, St Paul, MN, USA), G-Premio Bond (GP, GC Corporation, Tokyo, Japan). Teeth were sectioned on the mid portion of dentin using a diamond saw mounted in low-speed cutting machine (IsoMet 1000, Buehler, Lake Bluff, IL, USA) under copious water. Exposed dentin surfaces were polished with 600-grit SiC paper (Sankyo-Rikagaku Co., Saitama, Japan) for 60 s. The adhesives systems were applied on dentin surfaces according to manufacturer's instructions. Composite resin blocks were built-up (Clearfil AP-X, shade A2, Kuraray Noritake Dental Inc., Kurashiki, Japan.). Prepared teeth were stored in distilled water at 37 °C for 24 h.

*2.3 Experiment-2 (EXP-2):*

The second hypothesis was tested in this EXP-2. The bond strength values of SE and CU different adhesive systems were assessed using the double-sided test. The effect of dentin thickness on the bond strength was verified. Tooth was considered as the sample unit (n=8). Thirty-two human molars were randomly divided into four dentin thickness groups (n=8): 0.5, 1.0, 1.5 and 2.0 mm. The dentin discs were prepared by removing the dentin from the pulp side, that is, 0.5 mm of dentin refers to the superficial dentin. Both adhesives were bonded simultaneously on the same dentin disc. They were used alternately, that is, when SB as applied to the coronal side of the dentin disc, CU was applied to the pulpal side and vice-versa. Teeth were prepared and stored as described above in EXP-1.

*2.4 Experiment-3 (EXP-3):*

EXP-3 was employed to test the third hypothesis. The double-sided test was used to assess the bond strength of CU, SB and GP to dentin. The effect of dentin thickness on the bond strength was verified. Tooth was considered as the sample unit (n=4). 1-mm thick dentin discs were used, and both surfaces (coronal and pulpal) were simultaneously and alternately bonded with the combination of pairs of adhesives. In total, there were six combinations of adhesives systems. Teeth were prepared and stored as described in EXP-1.

*2.5 μTBS test:*

After storage, each sample was section in both, “x” and “y” directions, to obtain beams (cross sectional area 1 $mm^2$), using an Isomet diamond saw (Isomet 1000, Buehler, Lake Bluff, Illinois, USA). The beams were fixed to a Ciucchi’s jig with cyanoacrylate glue (Model repair 2 Blue, Dentsply-Sankin, Otahara, Japan) and subjected to a tensile force at a crosshead speed of 1 mm/min in a desktop testing apparatus (EZ test, Shimadzu, Kyoto, Japan).

μTBS results were expressed in MPa. Data were tested for normality and homoscedasticity ($p$<0.05). When the both assumptions were met, parametric tests were used. When the assumptions were violated, non-parametric analyses were performed. Therefore, in case of EXP-1 and EXP-3, data was statistically analyzed using One-way ANOVA followed by Tukey’s *post hoc* test. For EXP-2 Two-way ANOVA followed by Tukey’s *post hoc* test was used. Statistical analyses were performed with SPSS software (SPSS statistics version 20.0, SPSS, Chicago, IL, USA) ($p$<0.05).

*2.6 Failure mode analysis:*

The failure mode was classified as A: adhesive failure (failure occur within the adhesive layer); B: cohesive failure (failure occur within the dentin or composite resin only); C: mixed failure (failure within adhesive layer and cohesive failure within dentin and/or composite resin)[12]. In addition, in case of EXP-2 and EXP-3, depending on the exact location of the failure, five types of failure sites were distinguished: Adhesive (A), Adhesive (B), Cohesive in resin composite (C), Cohesive in dentin (D), Mixed (E) (Figure 1B). Failure modes of the specimens were observed using the stereoscope (20x; Asone, Osaka, Japan). Further, the fractured specimens were mounted on an aluminum stub, then coated with Pt-Pd for 120 s (Ion sputter, E-1030, Hitachi, Tokyo, Japan). Coated beams were observed using a scanning electron microscope (SEM, S-4000, Hitachi, Tokyo, Japan) at an accelerating voltage of 10 kV. Fracture and specific features on dentin surfaces were examined at lower magnification (80×) to categorize the mode of fractured.

## 3. Results

### *3.1 Bond strength results:*

The results are displayed in Fig. 2. There were no pre-test failures in this study. In case of EXP-1, One-way ANOVA analysis revealed significant difference in μTBS means of tested adhesives to dentin ($p<0.05$). The pairwise comparison using Tukey's *post hoc* test (Fig. 2A) showed that SE presented the highest bond strength values (63.98 MPa) followed by SB>CU> GP.

In EXP-2, the Two-way ANOVA analysis revealed that dentin disc thickness significantly affected the μTBS of adhesives ($p<0.05$) when the double-sided μTBS test was used (Fig. 2B). The Tukey's *post hoc* analysis showed that using 1.5 mm (37.23 MPa) and 2.0 mm (37.84 MPa) dentin thickness produced the lowest bond strength results.

In EXP-3, the One-way ANOVA analysis showed that differences on bond strength were found for adhesives ($p<0.05$). According to the *post hoc* pairwise comparisons, there was no significant difference between mean microtensile bond strength values from paired-adhesives ($p<0.05$). SB-CU and CU-SB groups showed the highest bond strength values and there was no significant difference between these values (43.86 and 41.20 MPa, respectively when SB was at coronal and pulpal side) (Fig. 2C).

*3.2 Failure mode analysis:*

The failure modes were mainly categorized as adhesive failure, cohesive failure and mixed failure. The result of the failure modes percentage of EXP-1 is shown in Table 2. High μTBS values were associated with cohesive failure in dentin or in composite resin.

The percentages of failure mode of EXP-2 is shown in Table 3. The bonded dentin beams always failed on CU side. In this case, SEM observation confirmed that most failures were mixed (Fig. 4). Depending on the exact location of the failure, five types of failure sites were distinguished and shown their bond strength on Table 3.

The result of the failure mode percentage of EXP-3 is shown in Table 4. There was no cohesive failure. SEM observation confirmed that most failure were mixed (Fig. 4). The percentages of failure side are also shown in Table 4. Observing the failure sites in all adhesive combinations, most failure occurred on GP side followed by SB and CU sides. In those cases, mixed failures were predominant. Depending on the exact location of the mixed failure, five types of failure sites were distinguished and shown their correlated bond strength on Table 4.

## 4. Discussion

When bonded to dentin, the four self-etch adhesives examined in EXP-1 differed significantly in their μTBS. Therefore, the first null hypothesis was accepted. SE is two-step self-etch adhesive and SB, CU, GP universal adhesives. The better performance of SE over universal adhesives has been reported previously in the literature[12,14,17-21]. One of the reasons attributed to the good performance of SE is that two-step self-etch adhesives contain hydrophilic self-etch primer and hydrophobic adhesive resin. This makes the interface more hydrophobic and thus better seals it to the direct advantage of bonding durability[10,12,14]. In addition, two-step self-etch adhesives may perform better than one-step self-etch adhesives as it has longer shelf life[14,19,20]. According to SEM observation most cohesive failure occurred on the SE fractured surface (Fig. 3), it is possible to observe a smooth and uniform adhesive surface of SE.

Universal adhesives could be considered one-step self-etch adhesives. They present very complex chemical composition, even though maximum adhesive systems include the similar components, they may vary significantly[22]. Our results corroborate this assertation as bond strength of universal adhesives significantly different from each other (Fig. 2A). Simplified one-step self-etch adhesives are more hydrophilic and more water absorbent than two-step self-etch adhesive. Evaporation of water is difficult from the one-step self-etch adhesives, even if water evaporate successfully, rapidly water diffuses back into the adhesive resin from the bonded dentin again. Water bubbles, resin tags, blister formation and voids are also aspects which effects bond strength of adhesives[18]. Adhesive and mixed failure were predominant of one-step self-etch adhesives in this EXP-1 (Table 2).

Concerning EXP-2, dentin thickness affected the bond strength of adhesives, therefore, the second null hypothesis was rejected. The density of dentinal tubule is usually 30,000 tubules per square millimeter in mid coronal dentin, and the quantity of the tubules per square millimeter increases from the dentin-enamel junction towards the pulp chamber[18,23]. Effectiveness of bonding in superficial dentin is generally better than deep dentin because quantity of dentinal tubules more in deep dentin[7,12]. In this study, 0.5 mm dentin (superficial dentin) showed higher bond strength than other thicknesses (Table 3), and no significant difference was found between coronal side and pulpal side of dentin in each thickness (Fig. 2B). On the other hand, 1.5 mm and 2.0 mm thickness showed lower bond strength than 0.5 mm and 1.0 mm thickness (Table 3). However, 1.0 mm dentin thickness showed no significant difference with other three thickness (Fig. 2B). As a result, 1.0 mm showed to be the most appropriate substrate for the double-sided μTBS test of tested adhesives to dentin. The EXP-2 design allowed that bond strength from 2 distinct adhesives were tested using a single dentin beam under similar testing conditions and probably with more uniform stress distribution. This testing assembly serves to remove variables such as minor variations in the alignment angulations of the bonded sticks along the fixtures of a universal testing machine, or the effect of variable volumes of cyanoacrylate glue along the ends of different samples[16)].

When 1-mm dentin thickness discs were used to test with six combinations of three different universal adhesives (EXP-3), the results revealed that significant differences on bond strength were found for adhesive's combinations but there was no significant difference between the same simultaneously tested materials combination as shown in Fig. 2C. Unfortunately, it was not perfectly possible to recognize which adhesive system was the strongest one against the other. Most fracture occurred on GP side followed by SB and CU sides (Table-4). In case of EXP-1, most fracture occurred at the sides of adhesives on GP followed by SE, SB and CU (Table 2). Therefore, the third null hypothesis was also rejected. In EXP-3, both SB-CU combinations showed the highest bond strength then other combinations (CU-GP and SB-GP). According to EXP-1, among these three adhesives, SB and CU showed significantly higher bond strength than GP (Fig. 2A). Similarly, in EXP-3, adhesive combinations with GP showed lower bond strength values, and more failure occurred on GP side (Table 4).

In recent years, conventional microtensile bond strength testing was considered as a technique to assess the true bond strength of an adhesive system to different dental substrates[8,9)]. Ideally, the design of double-sided μTBS testing could deliver direct comparison between different adhesives simultaneously using the same dentin beam and it could be performed under the same testing condition[16)]. Nevertheless, the analyses from all 3 experiments of the present study did not confirm the expectations of the double-sided μTBS test as similar results were obtained with conventional test. Further studies on the double-sided technique should be conducted to improve this method. The findings of our study should be either confirmed or the double-sided technique should be improved to verify the viability of getting more homogeneous stress distribution across the dentin, becoming feasible to truly simultaneously compare two adhesive materials.

## 5. Conclusion

Conventional μTBS testing was still considered a good technique to assess the bond strength of adhesives to dentin. Within the limitations of this study, double-sided μTBS test is not always applicable to assess the performance of adhesives to dentin. Further investigations should be conducted to better explore the double-sided μTBS test.

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Table 1 Description of the materials used in the study

| **Materials, manufacturers, lot numbers** | **Adhesive system classification** | **Basic composition** | **Application mode** |
|---|---|---|---|
| Clearfil™ SE Bond 2,<br>Kuraray Noritake Dental Inc., Japan,<br>LOT- 000013 | Two-step self-etch | **Primer:** 10-MDP, HEMA, Hydrophilic aliphatic dimethacrylate, di-camphorquinone, Water.<br>**Bond:** 10-MDP, Bis-GMA, HEMA, Hydrophobic aliphatic dimethacrylate, di-camphorquinone, Initiators, Accelerators, Silarated colloidal silica. | 1. Apply Primer for 20 s to the dentin surface<br>2. Mild air blowing for 10 s<br>3. Apply the adhesive to the dentin surface with the applicator brush and rub it in for 10 s<br>4. Dry the dentin surface by mild air blowing for 5 s<br>5. Light cure for 10 s |
| Clearfil™ Universal Bond,<br>Kuraray Noritake Dental Inc., Japan,<br>LOT- 000002 | Universal | **Bond:** 10-MDP, Bis-GMA, HEMA, Hydrophobic aliphatic dimethacrylate, colloidal silica, Silane coupling agent, di-camphorquinone, Ethanol, Water. | 1. Apply the adhesive to the dentin surface with the applicator brush and rub it in for 10 s<br>2. Dry the dentin surface sufficiently by blowing mild air for more than 5 s until the adhesive does not move<br>3. Light cure for 10 s |
| Scotchbond™ Universal adhesive,<br>3M ESPE, USA,<br>LOT- 609889 | Universal | **Bond:** 10-MDP, HEMA, Silane, Phosphate monomer, Dimethacrylate resins, Vitrebond™ copolymer, Filler, Ethanol, Water, Initiators, Silica | 1. Apply adhesive on the surface and rub it in for 20 s<br>2. Gently air-dry the adhesive for approximately 5 s for the solvent to evaporate<br>3. Light cure for 10 s. |
| G-Premio Bond,<br>GC Corporation, Japan,<br>LOT- 1701111 | Universal | **Bond:** 10-MDP, 4-META, 10-methacryoyloxydecyl dihydrogen thiophosphate, methacrylate adic ester, distilled water, acetone,photo initiators, silica fine powder. | 1. Apply using a micro brush<br>2. Leave undisturbed for 10 s after application<br>3. Dry thoroughly for 5 s with oil free air under maximum pressure<br>4. Light cure for 10 s. |

methacryloxydecyldihydrogen phosphate; HEMA: 2-hydroxysthyl methacrylate; Bis-GMA: Bisphenol A diglycidyl methacrylate; 4-META: 4-methacryloyloxyethyl trimellitate anhydrate, MDTP: 10-methacryoyloxydecyl dihydrogen thiophosphate.

Table 2 Failure mode percentage of Experiment 1

| Percentage of failure (%) | | | |
|---|---|---|---|
| Failure mode | | | |
| Groups | Adhesive | Cohesive | Mixed |
| SE | 3.1 | 65.6 | 31.3 |
| CU | 37.5 | 9.4 | 53.1 |
| SB | 28.1 | 25 | 46.9 |
| GP | 81.3 | - | 18.7 |

Table 3 Mean of microtensile bond strength values of Experiment 2 with double-side failure mode and failure mode (%) by SEM

| | | | Failure mode of double-side test: mean (SD)MPa | | | | Failure mode (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Adhesive | | Cohesive | Mixed | Adhesive | Cohesive | Mixed |
| Dentin thickness (mm) | Adhesives combinations | μTBS mean (SD)MPa | A | B | C | E | | | |
| 0.5 | SE-CU | 43.62(13.92) | - | 51.62(12.23) | - | 38.82(12.76) | 37.5 | 0 | 62.5 |
| | CU-SE | 44.11(13.06) | 39.25(10.52) | - | 65.19(5.28) | 43.84(12.78) | 17.5 | 5 | 77.5 |
| 1.0 | SE-CU | 41.54(9.42) | - | 42.64(9.65) | 67.10(0) | 39.79(7.85) | 37.5 | 2.5 | 60 |
| | CU-SE | 40.86(10.98) | 28.04(4.30) | - | - | 42.28(10.57) | 10 | 0 | 90 |
| 1.5 | SE-CU | 37.23(10.96) | - | 36.83(11.61) | 53.64(0) | 36.79(10.42) | 40 | 2.5 | 57.5 |
| | CU-SE | 39.54(10.66) | 42.89(10.62) | - | 60.93(0) | 38.14(10.09) | 17.5 | 2.5 | 80 |
| 2.0 | SE-CU | 37.84(9.61) | - | 31.26(7.76) | - | 41.39(8.66) | 35 | 0 | 65 |
| | CU-SE | 37.68(9.94) | 33.40(5.78) | - | 65.94(0) | 37.61(9.32) | 15 | 2.5 | 82.5 |

SE-CU: SE was applied on coronal dentin and CU was applied on pulpal dentin. CU-SE: CU was applied on coronal dentin and CU was applied on pulpal dentin.

Table 4 Mean of microtensile bond strength values of Experiment 3 with double-side failure mode and percentages of failure side and failure mode by SEM

| | | Failure mode of double-side test: mean (SD)MPa | | | Failure mode (%). | | Failure side (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Adhesive | | Mixed | | | | | |
| Group | μTBS mean (SD)MPa | A | B | E | Adhesive | Mixed | SB side | CU side | GP side |
| SB-CU | 41.76(4.60) | 42.06(0) | 47.07(9.20) | 41.12(3.98) | 15 | 85 | 30 | 70 | - |
| CU-SB | 40.01(4.83) | 41.86(0) | - | 39.91(4.94) | 5 | 95 | 40 | 60 | - |
| SB-GP | 36.34(4.12) | - | 37.02(2.36) | 36.27(4.31) | 10 | 90 | 5 | - | 95 |
| GP-SB | 35.24(3.96) | 34.95(1.79) | - | 35.27(4.17) | 10 | 90 | 10 | - | 90 |
| CU-GP | 29.94(4.90) | 32.88(8.18) | 28.85(3.50) | 30.57(5.95) | 60 | 40 | - | 10 | 90 |
| GP-CU | 29.63(3.20) | 28(2.51) | - | 30.71(3.24) | 40 | 60 | - | 15 | 85 |

SB-CU: SB was applied on coronal dentin and CU was applied on pulpal dentin. CU-SB: CU was applied on coronal dentin and SB was applied on pulpal dentin. SB-GP: SB was applied on coronal dentin and GP was applied on pulpal dentin. GP-SB: GP was applied on coronal dentin and SB was applied on pulpal dentin. CU-GP: CU was applied on coronal dentin and GP was applied on pulpal dentin. GP-CU: GP was applied on coronal dentin and CU was applied on pulpal dentin.

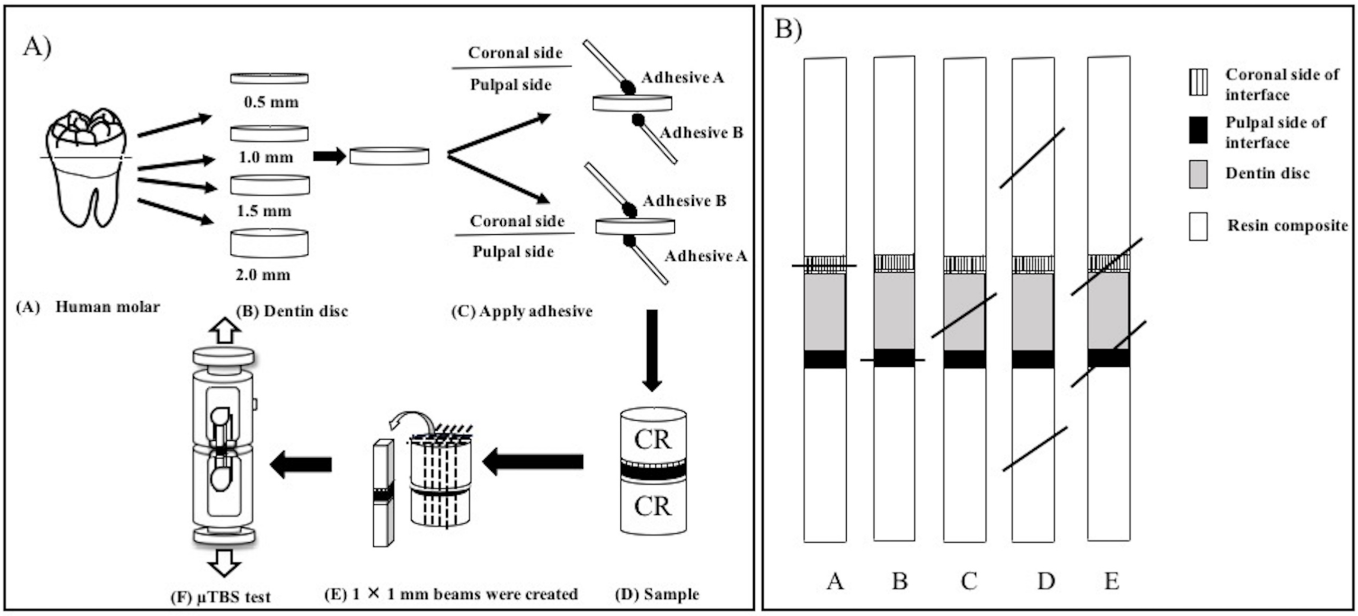

A)
0.5 mm
1.0 mm
1.5 mm
2.0 mm
Coronal side
Pulpal side
Adhesive A
Adhesive B
Coronal side
Pulpal side
Adhesive B
Adhesive A
(A) Human molar
(B) Dentin disc
(C) Apply adhesive
CR
CR
(F) µTBS test
(E) 1 × 1 mm beams were created
(D) Sample
B)
Coronal side of interface
Pulpal side of interface
Dentin disc
Resin composite
A
B
C
D
E

**Fig. 1**

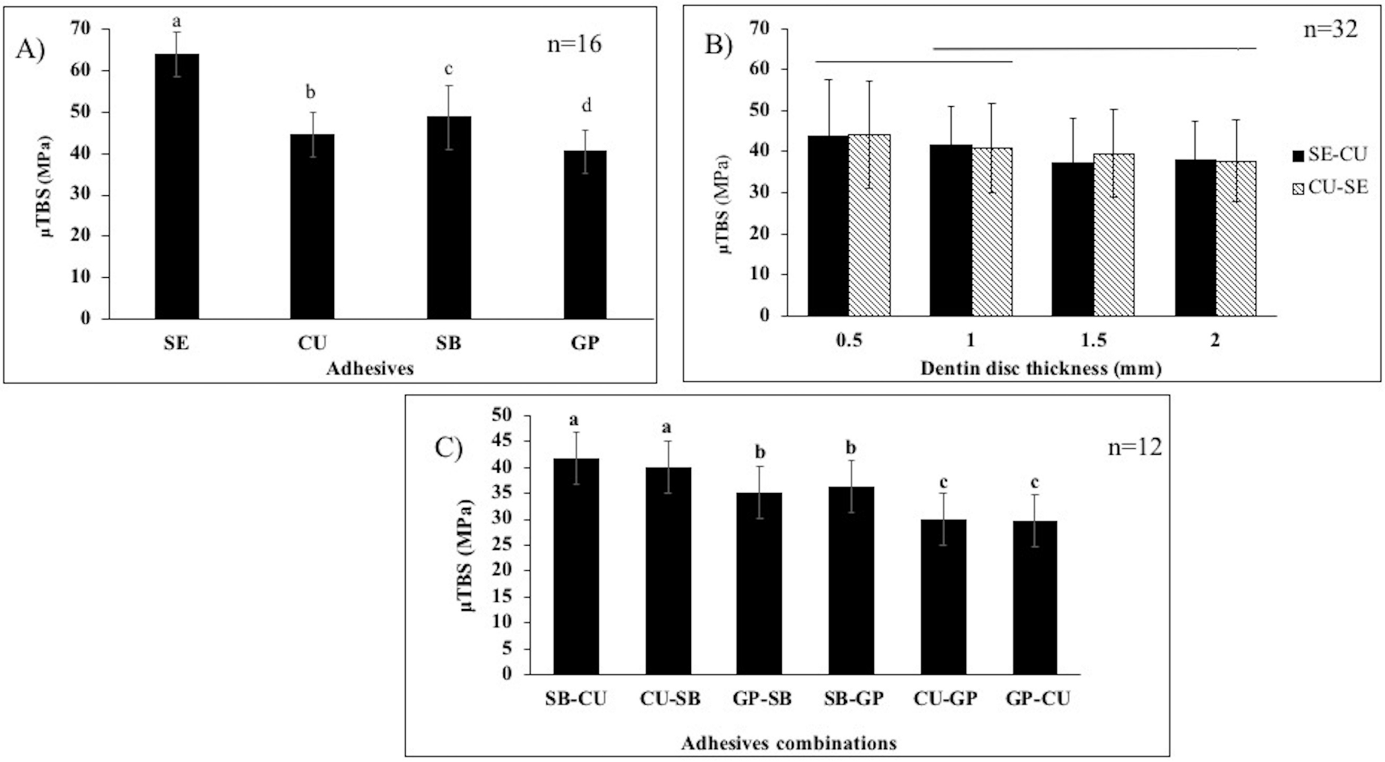

A)
n=16
μTBS (MPa)
70
60
50
40
30
20
10
0
a
b
c
d
SE
CU
SB
GP
Adhesives
B)
n=32
μTBS (MPa)
70
60
50
40
30
20
10
0
SE-CU
CU-SE
0.5
1
1.5
2
Dentin disc thickness (mm)
C)
n=12
μTBS (MPa)
50
45
40
35
30
25
20
15
10
5
0
a
a
b
b
c
c
SB-CU
CU-SB
GP-SB
SB-GP
CU-GP
GP-CU
Adhesives combinations

**Fig. 2**

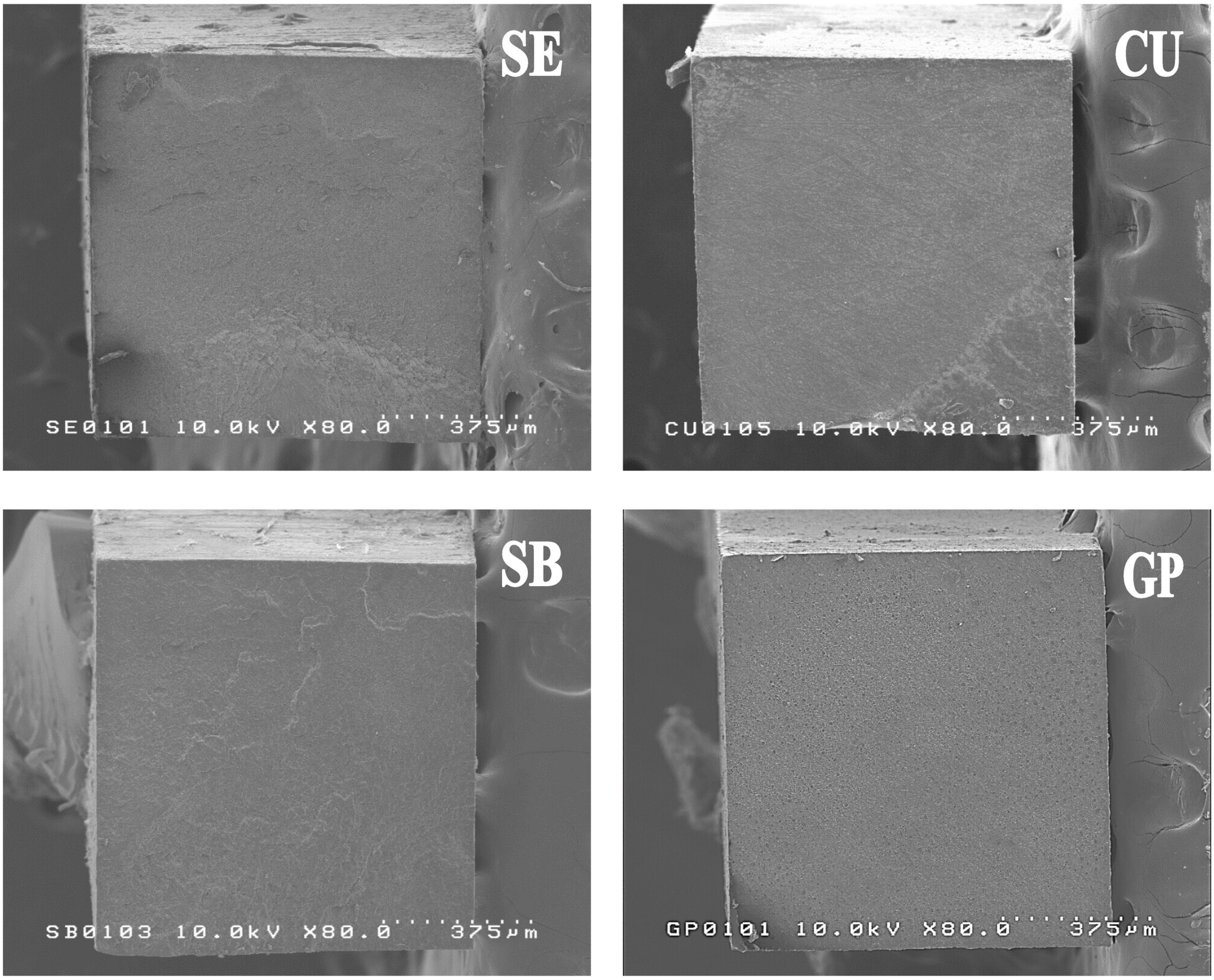
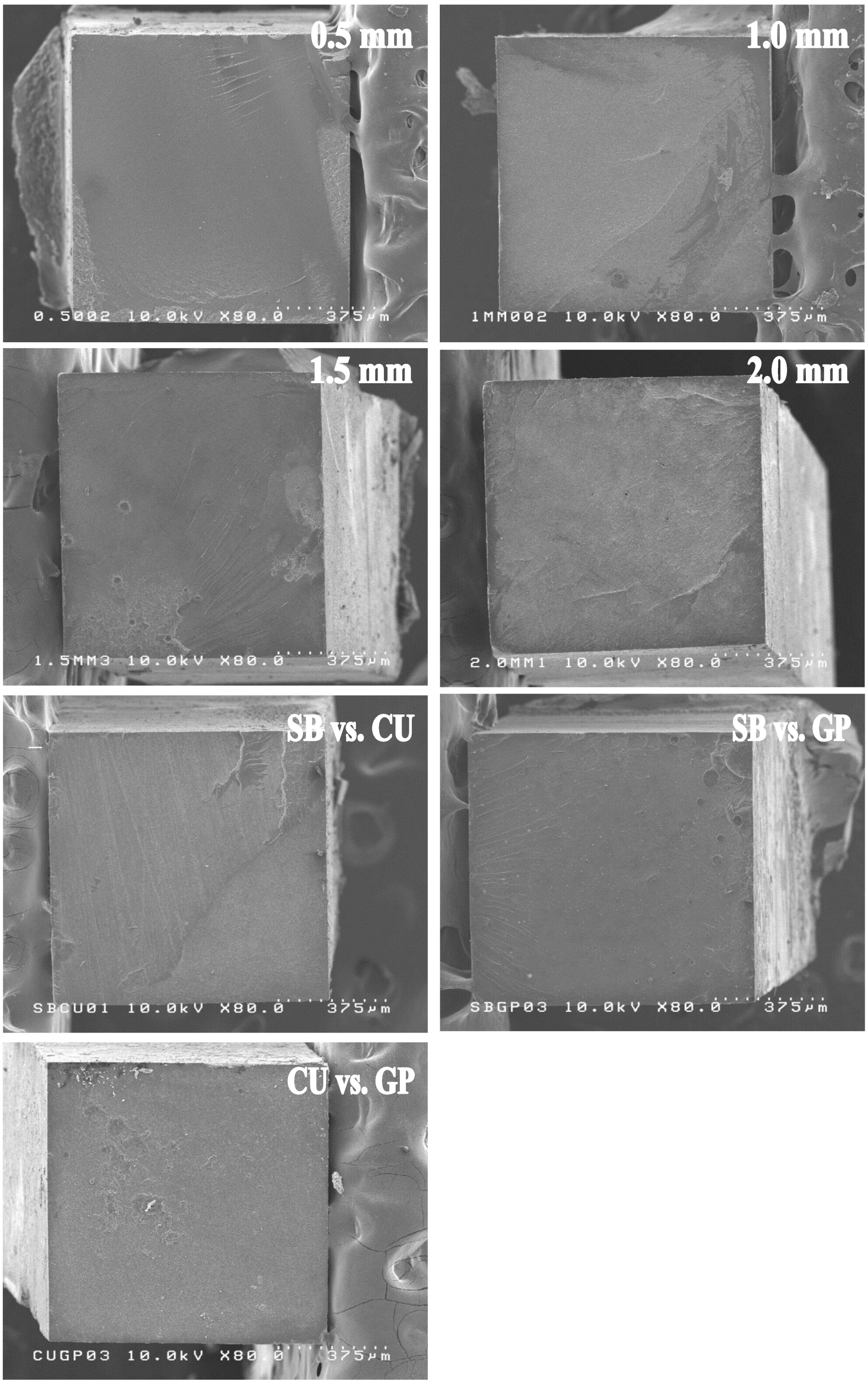

SE
SE0101 10.0kV X80.0 375µm
CU
CU0105 10.0kV X80.0 375µm
SB
SB0103 10.0kV X80.0 375µm
GP
GP0101 10.0kV X80.0 375µm

**Fig. 3**



0.5 mm
0.5002 10.0kV X80.0 375μm
1.0 mm
1MM002 10.0kV X80.0 375μm
1.5 mm
1.5MM3 10.0kV X80.0 375μm
2.0 mm
2.0MM1 10.0kV X80.0 375μm
SB vs. CU
SBCU01 10.0kV X80.0 375μm
SB vs. GP
SBGP03 10.0kV X80.0 375μm
CU vs. GP
CUGP03 10.0kV X80.0 375μm


**Fig. 4**

**Figure Legends:**

Fig.1: [A] In order to compare the new technique with the conventional μTBS procedure, sample preparation and application methods of Experiment 2 and 3 of double-sided technique; [B] Schematic representation of the site of failure for the double-sided microtensile specimen. Site A: Failure involving at the coronal side of the interface, Site B: Failure involving at the pulpal side of the interface, Site C: Failure involving within the dentin, Site D: Failure involving within the composite, Site E: Failure along sites that were not included in the previous four classifications. Sites of failure may be further classified by the mode of failure into adhesive (A, B), Cohesive (C, D) or mixed failure E.

Fig. 2: [A] Microtensile bond strength (μTBS) results of Experiment 1 (n=16). The conventional microtensile bond strength test was used in this Experiment. Different small letters indicate statistical difference ($p<0.05$); [B] Microtensile bond strength (μTBS) results of Experiment 2 (n=32). Columns united by bars showed no significant difference ($p>0.05$); no significant difference between SE-CU and CU-SE ($p>0.05$); [C] Microtensile bond strength (μTBS) results of Experiment 3 (n=12). Same superscript letters indicate no statistical difference, but different superscript letters indicate statistical difference ($p<0.05$).

Fig. 3: Representative SEM images of failure modes of four different adhesives after μTBS testing of conventional method on Experiment 1 at low magnification (80x). In general, the fracture modes were mainly categorized as adhesive failure, cohesive failure and mixed failure. There was a clear tendency that most of them had adhesive and mixed failure. High μTBS values were associated with a higher tendency to fail within dentin or composite resin, especially for SE, SB and CU groups.

Fig. 4: Representative SEM images of failure modes of four different dentin disc thickness (0.5, 1.0, 1.5, 2.0 mm) and three different adhesives comparison (SB vs CU, SB vs GP, CU vs GP) after μTBS testing of new method on Experiment 2 and Experiment 3 at low magnification (80x). In general, the fracture modes were mainly categorized as adhesive failure, cohesive failure and mixed failure. There was a clear tendency that most of them had adhesive and mixed failure.