Gradual dehydration affects the mechanical properties and bonding outcome of adhesives to dentin

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This study evaluated the effects of dehydration on the mechanical properties of adhesive resins and dentin, and on the microtensile bond strength (μ TBS) of adhesives. Third molars were randomly bonded with Clearfil Mega Bond (MB) or Clearfil SE Bond 2 (SE). After water-storage (37°C; 24 h), μ TBS was obtained in ‘wet’ (tested after 5 min of removal from storage) and ‘dehydrated’ (tested after 10, 15 min and 24 h) conditions by a universal tester (crosshead speed: 1 mm/min). Data were analyzed by two-way ANOVA and Duncan’s test. Hardness (H), Elastic modulus (E) and weight-loss of dentin beams and adhesive-resin discs were also monitored over time and analyzed by one-way repeated measures ANOVA and Bonferroni’s test ($\alpha=0.05$). Significant differences in bond strength were observed for adhesives and for conditions. Except for dentin’s E, dehydration caused significant gradual changes in the H, E and weight of adhesive resins and dentin ($p<0.05$).

Keywords: Dehydration, Two-step self-etch adhesive, Bond strength, Hardness, Elastic modulus

INTRODUCTION

Among the current adhesive systems, self-etch adhesives are preferred by the clinicians for their ease of application, less technique-sensitivity and proven clinical performance^{1–4}. Currently, the one-step self-etch systems are growing increasingly popular due to their ability to further reduce the clinical application time and technique-sensitivity. However, most academics and researchers still prefer the two-step self-etch systems because of their better bonding performance compared to one-step systems^{5,6}.

The superior bonding efficiency of ‘mild’ two-step self-etch adhesive systems has been proven extensively, *in vitro*^{1,7–9} and *in vivo*^{10–12}. Nevertheless, the quest for further improvement of self-etch systems is constant. Recently, in addition to the extensively employed camphorquinone (CQ)¹³, a new photo-initiator has been introduced to one system. This photo-initiator significantly enhances monomer conversion rates, leading to stronger bonds¹⁴.

Regardless of the method, bond strength testing implies that the load applied to break the joint will generate stresses that will be distributed across the substrates that form the joint, commonly resin composite, adhesive and dentin. It is known that the mechanical properties, such as strength, hardness and elastic modulus of the substrates that compose the

joint can significantly affect the outcome of the bond strength test¹⁵. Dentin’s mechanical properties are affected by its hydration status^{16–18} and water sorption of the adhesive resins can cause significant reduction of their properties¹⁹. Moreover, superior properties of the adhesive resins have been associated with increased durability of the bonds^{20–22}. Particularly in the case of microtensile bond strength (μ TBS) testing method, the specimens are of smaller dimensions, usually having a cross-sectional area of 1 mm² or less and approximately 6–8 mm in length. When testing μ TBS, researchers remove their specimens from the storage solution and glue or attach them to the grips of the testing machine. What is not reported in the articles is the time taken between the removal of the specimen from the storage solution and the actual testing. In order to facilitate the bond of the specimens to the testing grips, some researchers blot-dry the specimen, others use air syringe, or simply let the specimens dry on the bench before bonding them to the grips. Some of these drying procedures can quickly dehydrate the small specimens, and as a consequence, affect the properties of the bonded substrates and possibly the outcome of the bond strength test.

Therefore, the purpose of this study was to investigate the effects of gradual dehydration on the mechanical properties of cured two-step self-etch adhesive resins and dentin, as well as on the bonding performance of adhesives to dentin. The null hypotheses tested were: (1) gradual dehydration of bonded dentin

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beams does not affect the adhesives' μ TBS to dentin, (2) there is no significant difference between the μ TBS of the tested adhesives at the tested conditions, and (3) dehydration does not affect the hardness (H) and elastic modulus (E) of cured adhesive resins and dentin.

MATERIALS AND METHODS

Teeth preparation and bonding procedures for μ TBS test

The composition and application instructions of the adhesives used in this study are shown in Table 1. Clearfil Mega Bond (Kuraray Noritake Dental, Tokyo, Japan) is identical to Clearfil SE Bond (was marketed in USA) and was commercially available in Japan. Clearfil SE Bond 2 (Kuraray Noritake Dental) is the new improved version of Clearfil SE Bond. According to the material's technical profile, its new integrated photo-initiator generates more free radicals during curing leading to higher monomer conversion rates and stronger bonds (Clearfil SE Bond 2 Brochure).

This study was approved by the local Ethical Committee (# 2013-7). All the teeth were collected after the patient's informed consent, stored in an aqueous solution of 0.5% Chloramine-T at 4°C and used within 6 months of extraction. The teeth were free from any signs of caries, cracks or fractures. Forty flat, occlusal dentin surfaces of third molars were exposed by using a gypsum model trimmer under water coolant. The teeth were then subsequently polished with 600-grit SiC paper (Sankyo-Rikagaku, Saitama, Japan) under running water for 60 s to produce standardised smear layers. Then, the teeth were randomly bonded with either Clearfil Mega Bond or Clearfil SE Bond 2 (20 teeth/adhesive) and light cured (Optilux 401, Demetron/Kerr, Orange, CA, USA) at ≥ 550 mW/cm². Following composite resin (Clearfil AP-X, Kuraray, Osaka, Japan) build-up, the bonded specimens

were stored in distilled water at 37°C for 24 h, and then, resin/dentin beams (cross-sectional area: 1 mm²) were prepared by a low-speed diamond saw (IsoMet 1000, Buehler, Lake Bluff, IL, USA).

μ TBS test

Three beams from the center of each tooth were randomly selected and allocated to eight groups: Clearfil SE Bond 2 tested at 5 min (SE5m), 10 min (SE10m), 15 min (SE15m), 24 h (SE24h) and Clearfil Mega Bond tested at 5 min (MB5m), 10 min (MB10m), 15 min (MB15m) and 24 h (MB24h). A total of 15 beams per group were tested for μ TBS. Another 30 bonded beams (15/adhesive and 1/tooth) were collected and kept for weight-loss test (see below).

A pilot study established that it takes approximately 3 min to remove each bonded beam from the storage medium, wipe off water, measure the cross-sectional area and attach to the Ciocchi's jig with a cyanoacrylate adhesive (Model Repair II Blue, Dentsply-Sankin, Tokyo, Japan). In the 5 min groups (MB5m and SE5m), each beam was tested after 2 min of fixing to the grips of the testing device to allow adequate setting of the cyanoacrylate adhesive and to prevent glue failure. During this period, a small piece of wet paper (Kimwipe S-200, Nippon paper Crecia, Tokyo, Japan) was used to cover the beams to prevent dehydration. In the rest of the groups, after fixing to the jig, each beam was kept on the bench until testing without wet paper covering. All tests were conducted at room conditions (23°C and 30% RH). Therefore, the 5 min groups (MB5m and SE5m) that were tested at 5 min after removal from the storage medium and the beams were kept wet until testing were considered as 'wet'; and for the rest of the groups, after removal from the distilled water and fixation with the jigs, the beams underwent free ambient dehydration

Table 1 Composition and application instructions of the adhesives tested

Adhesive (Code/Manufacturer/ Lot number)	Type	Composition	Application instructions
Clearfil Mega Bond (MB/Kuraray Noritake Dental, Tokyo, Japan/000040)	Two-step self-etch	Primer: 10-MDP, HEMA, Hydrophilic aliphatic dimethacrylate, dl- Camphorquinone, N, N-diethanol-p-toluidine, Water Bond: 10-MDP, Bis-GMA, HEMA, Hydrophobic aliphatic dimethacrylate, dl- Camphorquinone, N, N-diethanol-p-toluidine, Colloidal silica	1. apply the primer and leave for 20 s 2. gentle air-blowing 3. apply the adhesive for 10 s 4. gentle air-blowing 5. light-cure for 10 s
Clearfil SE Bond 2 (SE/Kuraray Noritake Dental/000013)	Two-step self-etch	Primer: 10-MDP, Bis-GMA, HEMA, Hydrophilic aliphatic dimethacrylate, dl- Camphorquinone, Water Bond: 10-MDP, Bis-GMA, HEMA, Hydrophobic aliphatic dimethacrylate, dl-Camphorquinone, Initiators, Accelerators, Silanated Colloidal silica	1. apply the primer and leave for 20 s 2. gentle air-blowing for >5 s 3. apply the adhesive 4. gentle air-blowing to make the film uniform 5. light-cure for 10 s

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

and hence considered as ‘dehydrated’. The μ TBS test was carried out at a crosshead speed of 1 mm/min in a desktop testing apparatus (EZ-S, Shimadzu, Kyoto, Japan) until failure occurred. The tensile load at failure of each beam was divided with the cross-sectional area to achieve the μ TBS in megaPascals (MPa). The mean bond strength of 3 beams derived from each tooth represented the μ TBS value of that tooth, generating 5 values per group.

Fracture mode analysis

After μ TBS test, for the ease of determination of the fracture modes the two halves of each fractured specimen were coated with Pt-Pd using an ion sputter (E-1030, Hitachi, Tokyo, Japan), and were observed using a field emission scanning electron microscope (FE-SEM; S-4000, Hitachi) at an accelerating voltage of 10 kV. Failure modes at the dentin sides of the specimens were taken into consideration and classified into the following categories²⁹: A, Adhesive failure; CD, Cohesive failure in dentin; CC, Cohesive failure in composite resin; M, Mixed failure.

Specimen preparations for hardness (H) and elastic modulus (E) tests

Ten additional dentin slabs (approximately 6×10×1.5 mm) were prepared from five third molars by cutting parallel to their long axis with IsoMet. The slabs were then sequentially finished with no. 1000-, 1200-, and 2000-grit waterproof SiC paper (Sankyo-Rikagaku) under running water; and polished with 6, 3, and 1- μ m particle-sized diamond pastes (DP-Paste, Struers, Ballerup, Denmark) for a period of 1 min each. The slabs were then further cut to prepare 1 mm² beams of uniform thickness and smooth surfaces essential for the precision of the H and E measurement. The specimens were cleaned in an ultrasonic unit (Fine ultrasonic cleaner, model FU-2H, Gao Hui Mechanical and Electrical International Trade, Nanjing, China) with phosphate buffered saline solution (PBS; Wako Pure Chemical Ind., Osaka, Japan) for 3 min after every finishing and polishing step. A total of 15 beams (3 beams/tooth) were preserved in PBS and tested within three days after preparation.

Adhesive resin discs (15 discs/adhesive) measuring 1.5±0.2 mm in thickness and 10±0.2 mm in diameter were produced from plastic ring molds. The plastic rings were glued to a glass slide and filled with the bonding resins in one drop layers that were air blown for 5 s and cured individually for 10 s. The last layer to fill the ring was covered with a polyester matrix strip and a glass slide, pressed for 10 s to ensure a uniform smooth surface of the specimen and to prevent formation of the oxygen inhibition zone on the top. Following removal of the glass slide, the bonding resin was light cured for 10 s and the polyester matrix strip was removed followed by additional 30 s light-curing from both sides. The discs were then stored at room temperature for 24 h and then the plastic frames were removed. They were then stored in distilled water at 37°C for 24 h before testing.

Hardness (H) and elastic modulus (E) test

Pilot studies were done for establishing indentation procedures with material-specific settings. Sequentially, 15 polished dentin beams were removed from PBS, blotted of excess water (Kimwipe S-200, Nippon paper Crexia), fixed on glass slides and tested with a dynamic ultra micro hardness tester (DUH-211, Shimadzu) having a triangular pyramidal diamond indenter with a tip angle of 115° and radius 0.1 μ m. Samples were tested in the range of ambient temperatures 22–24°C with a maximum humidity of 30%. The dentin at the center of each beam was targeted. If any part of the indentation mark occurred on a dentinal tubule (lumen and peritubular dentin), the data was discarded and retaken. Indentations were performed at 5, 10, 15, 20 min, 1 and 24 h after removal from the PBS, at a constant speed of 0.2926 mN/s, with a 45 s holding at peak load. The maximum depth of indentation was 0.683 μ m which corresponded to the maximum loads of 5.04 mN.

In a similar sequential manner, 15 discs for each adhesive resin were tested at 5, 10, 15, 20 min, 1 and 24 h after removal from distilled water at a constant speed of 0.2926 mN/s, with a 45 s holding time at peak load. The maximum depth of indentation was 1.3832 μ m, which corresponded to the maximum loads of 5.03 mN. H and E values were obtained from the default software of the testing device. At least a 10 μ m distance between adjacent indentations was maintained for all materials. Poisson’s ratio assumed for both dentin and adhesive resin was 0.30.

Weight-loss measurement tests for dentin beams and adhesive resin discs

Sequentially, bonded dentin beams (15/adhesive, 1/tooth) were removed from distilled water, quickly blotted dry and placed on the stage of a digital balance (AB204-S Analytical Balance, METTLER TOLEDO, Greifensee, Switzerland). The weight-loss of the specimens was monitored over time and the weight recorded after 5, 10, 15, 20 min, 1 and 24 h of free, ambient dehydration. To confirm weight-loss phenomena separately in both dentin and adhesive resins, polished dentin-only beams (cross sectional area: 1mm²; prepared from additional 5 third molars; n=15, 3/tooth) and additional adhesive resin discs (15 discs/adhesive; prepared in the same way as before) were also removed from the distilled water and weighed in the same sequence as bonded dentin beams.

Statistical analysis

The normality of all data was tested using the Shapiro-Wilk test. The μ TBS data were analyzed by two-way ANOVA to demonstrate the effects of adhesive (*i.e.* MB or SE) and condition (*i.e.* ‘wet’ or ‘dehydrated’), followed by Duncan’s test at 5% level of significance. H, E and weight-loss data of dentin and adhesives were subjected to one-way repeated measures ANOVA, followed by Bonferroni’s *post-hoc* at a 5% level of significance. All statistical analysis was done by using SPSS 22.0 for Windows (SPSS, Chicago, IL, USA).

RESULTS

 μ TBS

μ TBS results are summarized in Fig. 1. Bond strength was significantly affected by the type of the adhesives ($F=10.761$; $p=0.003$) and test conditions ('wet' vs. 'dehydrated'; $F=12.463$; $p<0.001$). The interaction between the factors was not significant ($F=0.993$; $p>0.05$). Owing to dehydration, MB's μ TBS became significantly increased ($p<0.05$) at 10 min (74.0 ± 7.5 MPa), 15 min (74.7 ± 10.0 MPa) and 24 h (81 ± 9.3 MPa) from the baseline (MB5m: 56.1 ± 7.6 MPa). However, in the case of SE, μ TBS became significantly increased ($p<0.05$) at 15 min (81.7 ± 4 MPa) and 24 h (85 ± 3.5 MPa) from the baseline (SE5m: 70.7 ± 10 MPa). SE5m showed significantly higher μ TBS than MB5m ($p<0.05$).

Fig. 1 Mean μ TBS of the adhesives (MB and SE) tested at wet (5 min) and dehydrated (10, 15 min and 24 h) conditions. Different lowercase letters indicate statistically significant differences (Duncan's test; $p<0.05$).

Fracture modes

SEM observations of the debonded surfaces revealed a predominance of cohesive dentin failure in all the groups except for MB5m, where the mixed failure prevailed over other failure patterns (Fig. 2). A clear trend of increased number of cohesive failures with increased dehydration of the tested beams was apparent. Higher μ TBS results corresponded with an inclination to fail cohesively in dentin or composite resin.

Hardness (H) and elastic modulus (E) of cured adhesive resins and dentin

Our results indicated that gradual dehydration caused significant differences between mean H values of MB ($F=154.340$; $p<0.001$), SE ($F=67.260$; $p<0.001$) and dentin ($F=11.260$; $p<0.001$). Bonferroni's *post-hoc* tests revealed that, MB's H values became significantly different at 10 min, SE's at 15 min and dentin's at 24 h ($p<0.05$; Table 2) from the baseline (5 min).

One-way repeated measures ANOVA also showed

Fig. 2 Failure modes of the adhesives (MB and SE) tested at wet (5 min) and dehydrated (10, 15 min and 24 h) conditions.

Table 2 Mean hardness (standard deviations) of dentin and bonding resins obtained at wet (5 min) and dehydrated (10, 15 min and 24 h) conditions (*)

Material	Hardness (SD) in MPa					
	5 min (Baseline)	10 min	15 min	20 min	1 h	24 h
Dentin (n=15)	403.0 (120.0) ^a	436.6 (93.0) ^a ($p=1.000$)	443.7 (59.5) ^a ($p=1.000$)	457.5 (101.4) ^a ($p=1.000$)	522.3 (121.0) ^{a,b} ($p=0.373$)	664.1 (140.1) ^b ($p=0.002$)
MB (n=15)	96.0 (6.9) ^a	106.0 (7.0) ^b ($p<0.001$)	114.8 (7.8) ^c ($p<0.001$)	119.8 (6.5) ^d ($p<0.001$)	137.3 (13.3) ^e ($p<0.001$)	170.1 (13.6) ^f ($p<0.001$)
SE (n=15)	126.1 (15.2) ^a	133.6 (12.7) ^{a,b} ($p=0.114$)	138.4 (12.7) ^b ($p=0.019$)	142.6 (10.3) ^b ($p=0.001$)	156.9 (13.5) ^c ($p<0.001$)	181.0 (8.4) ^d ($p<0.001$)

(*) Comparisons are valid between different testing points of each material. Different superscript letters indicate statistically significant differences (Bonferroni's test, $p<0.05$).

Table 3 Mean elastic modulus (standard deviations) of dentin and bonding resins obtained at wet (5 min) and dehydrated (10, 15 min and 24 h) conditions (*)

Material	Elastic modulus (SD) in MPa					
	5 min (Baseline)	10 min	15 min	20 min	1 h	24 h
Dentin (n=15)	18,047 (3823)	19,430 (3806) (p=1.000)	20,032 (5348) (p=1.000)	20,265 (3567) (p=0.425)	20,396 (1288) (p=0.736)	21,135 (2713) (p=0.624)
MB (n=15)	3,772 (148) ^a	3,755 (171) ^a (p=1.000)	3,862 (135) ^{a,b} (p=0.072)	3,907 (173) ^b (p=0.004)	4,210 (179) ^c (p<0.001)	4,606 (165) ^d (p<0.001)
SE (n=15)	4,193 (153) ^a	4,209 (205) ^{a,b} (p=1.000)	4,264 (130) ^{a,b} (p=0.661)	4,300 (134) ^{a,b,c} (p=0.084)	4,433 (152) ^{b,c} (p=0.005)	4,756 (207) ^d (p<0.001)

(*) Comparisons are valid between different testing points of each material. Different superscript letters indicate statistically significant differences (Bonferroni's test, $p<0.05$).

Table 4 Mean weight (standard deviations) of dentin beams (with and without bonding) and bonding resin discs (*)

Material	Mean weight (SD) in mg after removal from storage medium at					
	5 min (Baseline)	10 min	15 min	20 min	1 h	24 h
MB/Dentin beam (n=15)	16.76 (1.13) ^a	16.67 (1.15) ^b (p=0.002)	16.62 (1.17) ^{b,c} (p<0.001)	16.59 (1.19) ^c (p<0.001)	16.53 (1.17) ^{c,d} (p<0.001)	16.49 (1.18) ^d (p<0.001)
SE/Dentin beam (n=15)	15.77 (1.35) ^a	15.67 (1.36) ^b (p=0.002)	15.63 (1.35) ^{b,c} (p=0.001)	15.61 (1.36) ^{c,d} (p=0.001)	15.58 (1.37) ^{c,d,e} (p<0.001)	15.53 (1.34) ^e (p<0.001)
Dentin beam (n=15)	10.33 (0.47) ^a	10.25 (0.46) ^b (p<0.001)	10.21 (0.45) ^{b,c} (p<0.001)	10.20 (0.46) ^c (p<0.001)	10.19 (0.45) ^c (p<0.001)	10.05 (0.53) ^c (p=0.001)
MB disc (n=15)	146.93 (14.94) ^a	146.78 (14.93) ^b (p<0.001)	146.69 (14.91) ^c (p<0.001)	146.54 (14.9) ^d (p<0.001)	146.31 (14.91) ^e (p<0.001)	145.13 (14.84) ^d (p<0.001)
SE disc (n=15)	133.03 (17.04) ^a	132.85 (17.03) ^b (p<0.001)	132.80 (17.04) ^b (p<0.001)	132.72 (17.02) ^c (p<0.001)	132.49 (17.01) ^d (p<0.001)	131.39 (17.03) ^e (p<0.001)

(*) Comparisons are valid between different testing points of each material. Different superscript letters indicate statistically significant differences (Bonferroni's test, $p<0.05$).

significant differences between mean E values of MB ($F=101.865$; $p<0.001$) and SE ($F=36.243$; $p<0.001$). MB's E values became significantly different at 20 min and SE's at 1 h ($p<0.05$; Table 3). However, in the case of dentin, the differences between the E ($F=1.583$; $p>0.05$) values were not significant.

Weight-loss of dentin beams and adhesive resin discs

Gradual dehydration caused significant differences between mean weight values of bonded dentin beams (MB/Dentin: $F=41.202$; $p<0.001$ and SE/Dentin: $F=34.148$; $p<0.001$), dentin-only beams ($F=20.945$; $p<0.001$), MB discs ($F=548.364$; $p<0.001$) and SE discs ($F=794.253$;

$p<0.001$) obtained at the tested time points. For all types of weighed materials, the differences became significant at 10 min from the baseline ($p<0.05$; Table 4).

DISCUSSION

In this study, dehydration significantly affected the μ TBS of the tested adhesives to dentin ($p<0.001$). Therefore, our first null hypothesis has been rejected. For MB the significant change came early at 10 min, however, for SE it became apparent at 15 min ($p<0.05$; Fig. 1). Moreover, the bond strength of SE was higher than MB in all the tested conditions, albeit only significant at 5 min (MB5min

vs. SE5m, $p < 0.05$); therefore, the second null hypothesis was also rejected. SE is claimed to be the new improved version of MB. SE's new photo-initiator generates more free radicals during curing leading to higher monomer conversion rates and stronger bonds. In a recent study, Sato *et al.*¹⁴ also observed SE's superiority over MB in terms of μ TBS to dentin, elastic modulus, degree of conversion and lesser water sorption. It is likely that SE's higher degree of conversion and lesser water sorption keeps it less affected by dehydration, and accordingly, its bond strength values are less affected than MB.

Regardless of the type of bond strength test employed, the fractured surface usually exhibits a mixed mode of cohesive and adhesive failure²⁴. However, in this study, cohesive dentin failures prevailed over mixed failures in all the groups, except MB5m (Fig. 2). Dentin's water content increases its ability to absorb and recover energy when subjected to loading and thus promote improved durability¹⁸. Although, the tensile strength of dentin ranges from 52 to 104 MPa^{17,25–27}, dehydration increases dentin's H and makes it more brittle¹⁶, leading to its failure at a much lower stress. Our weight-loss study also suggested that both bonded and dentin-only beams started losing significant weight due to dehydration from 10 min (Table 4). These might explain why MB5m (also having the lowest mean μ TBS, 56.1 ± 7.6 MPa) had shown predominantly mixed failures and how dehydration led to a predominance of cohesive failures in dentin in other groups. Moreover, cohesive failures in composite resin were also observed in the dehydrated groups. Sato and his co researchers also reported a predominance of cohesive failures in dentin and composite resin with the same adhesives at 24 h water storage¹⁴. On the basis of these observations, we presume that, at high μ TBS values, dentin beams bonded with MB and SE show a predominance of cohesive failures which is more common in dentin. The failures in composite resin might be the combined effects of harder dentin as well as harder and stiffer adhesive resins.

In the current study, the effects of dehydration on the μ TBS of adhesives and their failure pattern were further endorsed by its effects on their H and E values. Our results indicated that gradual dehydration significantly affected the adhesive resins' mean H and E ($p < 0.001$) values at the tested dehydration points. These observations rejected the third null hypothesis. The reported H values of MB range from 154 to 275 MPa and E from 4 to 4.68 GPa^{22,28}. Our results are also in line with these studies (Tables 2 and 3). Previous reports suggested that due to increased hydrophilicity, self-etch adhesive resins absorb more water. This leads to plasticization of polymers and thus decreases the mechanical properties²². On the contrary, loss of water due to dehydration leads to increased stiffness of the polymers²⁹. Our results also showed that, from initial lower values due to water sorption, both adhesive resins' H values increased gradually due to dehydration and became significant at 10 and 15 min for MB and SE respectively ($p < 0.05$; Tables 2 and 3). Tagami *et al.* reported that hardness of an adhesive resin is directly

related to its bond strength²¹. Our results also showed that, increasing hardness values of adhesives due to gradual dehydration contributed to significantly increased bond strength values (Table 2 and Fig. 1; $p < 0.05$). Similar gradually increasing trend of values was also observed in case of adhesive resins' E; where MB's values were affected earlier (at 20 min) than SE (at 1 h; $p < 0.05$).

Marshall *et al.*³⁰ reported that nanohardness of hydrated intertubular dentin (ITD) lie between 0.15–0.51 GPa. However, according to another report, H values increased to 0.6–0.7 GPa when samples were tested in completely dry conditions³¹. Upon drying, the collagenous matrix of dentin collapses compressing the loose extrafibrillar mineral. This increases the rigidity of dentin, leading to higher surface hardness³². Our results also showed a gradual increase in dentin's H values, which became significant at 24 h (Table 2; $p < 0.05$) and remained within the reported ranges. Furthermore, the E values of ITD range from 17.7 to 21.1 GPa³⁰. Our E values are also within this range (Table 3).

In our study, the weight-loss experiment with bonded dentin beams indicated that dehydration can occur during μ TBS tests. Subsequent separate tests with dentin-only beams and adhesive resin discs further confirmed loss of water from both the components as they dehydrate. Therefore, to avoid dehydration, teeth should be immersed in aqueous media or be covered with wet tissue papers from the point of extraction to the end of the μ TBS test³³. The interval between the removal of the bonded dentin beams from the storage medium and the actual bond strength test by a universal tester can affect the test results and these effects can be further augmented by external factors like temperature and humidity²⁴. The small sized specimens (1 mm² or less) used in the μ TBS tests can dehydrate substantially when: time is allowed for adequate curing of the glue (specimen not covered with moist paper) and specimens are fixed to multiple jigs at the same time and let aside to be tested individually at a later time without accounting for the dehydration that might occur while the assembled jigs rest on the bench. The results of this mishandling can be of twofold: (1) unrealistic increase of μ TBS values owing to increased H and E of the adhesive resin, and (2) predominance of cohesive failures in dentin owing to increased brittleness.

CONCLUSION

This *in vitro* study reports previously unreported information about the mishandling of the μ TBS test method. We observed that dehydration affects the mechanical properties of adhesive resin and dentin leading to misleading bonding results. Therefore, dehydration of the test specimens should be prevented and the room condition as well as the length of interval between the specimen removal from the storage medium and the actual bond strength test should be reported.

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