博 士 論 文

Does gradual dehydration affect the mechanical properties and bonding outcome of adhesives to dentin?
（乾燥処理時間は象牙質の物性と接着性能に影響を与えるか？）

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Title

Does gradual dehydration affect the mechanical properties and bonding outcome of adhesives to dentin?

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Keywords

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Abstract

Objectives

To evaluate the effects of dehydration on the Hardness (H) and Elastic Modulus (E) of adhesives and dentin, and on the Microtensile Bond Strength (μTBS) of adhesives to dentin.

Methods

Flat, mid-coronal dentin surfaces of twenty human third molars were exposed and polished with 600-grit SiC paper. They were then randomly treated with Clearfil Mega Bond (MB) or Clearfil SE Bond 2 (SE2) and built-up with composite resin. After water-storage (37° C; 24 h), μTBS of wet (SE2W and MBW; tested at 5 min after removal from the storage) and dry (SE2D and MBD; tested at 10 min) specimens were obtained by subjecting resin/dentin beams (1 mm^2) to a universal tester at a crosshead speed of 1 mm/min. Fracture modes were determined by scanning electron microscope. The changes in the H, E and weight of dehydrating dentin-only beams and adhesive discs were monitored over time. The μTBS data were analyzed by two-way ANOVA and Tukey's test. The H, E and weight-loss data were analyzed by one-way repeated measures ANOVA and Bonferroni's test ($\alpha = 0.05$).

Results

Significant differences in bond strength were observed for adhesives ($p < 0.05$) and for conditions (dry vs. wet, $p < 0.001$). Dehydration caused significant gradual changes ($p < 0.05$) in the H, E and weight-loss of adhesives and dentin. However, the changes in dentin's E were not significant ($p > 0.05$).

Significance

Gradual dehydration of μTBS testing specimens can cause significant changes in the test outcomes and should be avoided as a significant source of test variation.

1. Introduction

Among the current adhesive systems, self-etch adhesives are preferred by the clinicians[1-4] for their ease of application, less technique-sensitivity, proven clinical performance and limited or no post-operative sensitivity[5-7]. Currently, among the self-etch systems, one-step 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[8, 9]. The superior bonding efficiency of 'mild' two-step self-etch adhesive systems has been proven extensively, in *vitro* [1, 10-12] and *in vivo* [13-15]. Therefore, they are most commonly used and have been considered as the gold standard for all self-etch systems[16]. Nevertheless, the quest for further improvement of self-etch systems is constant. Consequently, 12-methacryloyloxydodecylpyridinium bromide (MDPB) was incorporated in the self-etch primer system to add antibacterial property to the arsenal[17]. Recently, in addition to the extensively employed camphorquinone (CQ)[18], a new photo-initiator has been introduced to one system. This photo-initiator significantly enhances monomer conversion rates, leading to stronger bonds[19].

Regardless of the method, bond strength testing imply 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 modulus of elasticity of the substrates that compose the joint can significantly affect the outcome of the bond strength test[20]. Dentin's mechanical properties are affected by its hydration status[21-23] and water sorption of adhesives can cause significant reduction of their properties[24]. Moreover, superior properties of the adhesives have been associated with increased

durability of the bonds[25-27]. Particularly in the case of microtensile bond strength (μTBS) testing method, the specimens are of smaller in dimensions, usually 1 mm^2 or less of cross-sectional area and approximately 6-8 mm in length. When testing μTBS, 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 beams does not affect the adhesives' microtensile bond strength (μTBS) to dentin, (2) there is no significant difference between the μTBS of the adhesives to dentin at the tested conditions and (3) dehydration does not affect the hardness (H) and elastic modulus (E) of cured adhesive resins and dentin.

2. Methods

2.1 Teeth preparation and bonding procedures for μTBS test

The composition and application instructions of the adhesives used in this study are shown in Table 1. Clearfil Mega Bond is identical to Clearfil SE Bond and is commercially available in Japan. Clearfil SE Bond 2 is the new improved version of Clearfil SE Bond.

The study was approved by the local Ethical Committee (# 2013-7). All 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. Twenty flat, occlusal dentin surfaces of third molars were exposed by using a gypsum model trimmer under water coolant and subsequently polished with 600-grit SiC paper (Sankyo-Rikagaku Co., Saitama, Japan) under running water for 60 s to produce standardized smear layers. They were then randomly divided into four groups (n = 5; see bellow in 2.2 for details of wet vs. dry groups): Clearfil SE Bond 2 Dry (SE2D), Clearfil SE Bond 2 Wet (SE2W), Clearfil Mega Bond Dry (MBD) and Clearfil Mega Bond Wet (MBW). Adhesives were applied according to the manufacturer's instruction and light cured (Optilux 401, Demetron/Kerr, Orange, CA, USA) at ≥550 mW/cm^2. Following composite resin (Clearfil AP-X, Kuraray Co, Ltd, Osaka, Japan) build-up, the specimens were stored in distilled water at 37° C for 24 h. Resin/dentin beams (cross-sectional area: 1 mm^2) were prepared by a low-speed diamond saw (IsoMet 1000, Buehler, Lake Bluff, IL, USA) and a total of 15 beams per group were randomly selected and tested.

2.2 Microtensile Bond Strength (μTBS) test

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 Ciucchi's jig with a cyanoacrylate adhesive (Model Repair II Pink, Dentsply-Sankin, Tokyo, Japan). In the wet groups (MBW and SE2W), each beam was tested 2 min after fixing to the grips of the testing device to allow adequate setting of the cyanoacrylate adhesive to prevent glue failure. During this period, a small piece of wet paper (Kimwipe S-200, Nippon paper Crecia Co., Tokyo, Japan) was used to cover the beams of the wet group to prevent dehydration. In the dry

groups (MBD and SE2D), each beam was kept on the bench for 7 min after fixing to the jig without wet paper covering. All tests were conducted at room conditions (23° C and 30% RH). Therefore, the wet group was tested at 5 min after removal from the storage medium and the beams were kept wet until tested; and the dry group was tested at 10 min after removal from the distilled water and underwent free dehydration during this period. The μTBS test was carried out at a crosshead speed of 1 mm/min in a desktop testing apparatus (EZ-S, Shimadzu Co., Kyoto, Japan) until failure occurred.

2.3 Fracture mode analysis

After μTBS test, for 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, Tokyo, Japan) 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 categories[28)]: A, Adhesive failure; CD, Cohesive failure in dentin; CC, Cohesive failure in composite; M, Mixed failure.

2.4 Specimen preparation for Hardness (H) and Elastic Modulus (E) tests

Five additional dentin discs (approximately 7 mm x 11 mm x 1.5 mm) were prepared by cutting with a low-speed diamond saw (IsoMet 1000, Buehler, Lake Bluff, IL, USA). The discs were then sequentially finished with no. 1000-, 1200-, and 2000-grit waterproof SiC paper (Sankyo-Rikagaku Co., Saitama, Japan) under running water; and polished with 6, 3, and 1 μm particle size diamond pastes (DP-Paste, Struers, Denmark) for a period of 1 min each. The discs were then further cut to prepare 1 mm^2 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, Gao Hui Mechanical and Electrical International Trade Co. Ltd., Nanjing, China; model FU-2H) with phosphate buffered saline solution (PBS; Wako Pure Chemical Ind., Ltd., Osaka, Japan) for 3 min after every finishing and polishing step. Then the beams were preserved in PBS and tested within three days after preparation.

Adhesive disks (n = 5) measuring 2.0 ± 0.3 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.

2.5 Hardness (H) and Elastic modulus (E) test

Pilot studies were done for establishing indentation procedures with material-specific settings. Individually, fifteen polished dentin beams (3/tooth) were removed from PBS, blotted of excess water (Kimwipe S-200, Nippon paper Crecia Co., Tokyo, Japan), fixed on glass slides and tested with a dynamic ultra micro hardness tester (DUH-211, Shimadzu, Japan) having a triangular pyramidal diamond indenter with a tip angle of 115° and radius 0.1 μm. Samples were tested in the range of ambient temperatures 22° C - 24° C with a maximum humidity of 30%. The dentin at the centre of each beam was targeted. If any part of the indentation mark occurred on a dentinal tubule, the data was discarded and retaken. Indentations were performed at 5 min, 10

min, 15 min, 20 min, 1 h 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 μm which corresponded to the maximum loads of 5.04 mN.

In a similar sequential manner, five discs for each adhesive were tested at 5 min, 10 min, 15 min, 20 min, 1 h and 24 h after removal from distilled water at a constant speed of 0.2926 mN/s, with a 10 s holding time at peak load. The maximum depth of indentation was 1.275 μm, which corresponded to the maximum loads of 5.04 mN. Each disc was tested three times in the same sequence. The discs were kept in distilled water for 24 h before and between the consecutive sequences of H and E test. H and dynamic E values were obtained from the default software of the testing device. At least a 10 μm distance between adjacent indentations was maintained for all materials. Poisson's ratio assumed for both dentin and adhesive resin was 0.30.

2.6 Test for weight loss of dentin beams and adhesive discs

Polished dentin beams (n = 5, 1/tooth) were removed from PBS, quickly blotted dry and placed on the stage of a digital balance (METTLER TOLEDO, AB204-S Analytical Balance). The weight loss of the specimens was monitored over time and the weight recorded after 5 min, 10 min, 15 min, 20 min, 1 h and 24 h of free, ambient dehydration. Adhesive discs (n = 5) were also removed from distilled water and weighed in the same sequence as dentin. Mean weight loss of the dentin beams and adhesive discs were calculated.

2.7 Statistical analysis

The normality of all data was tested using the Shapiro-Wilk test. The μTBS data were analyzed by two-way ANOVA to demonstrate the effects of adhesive and condition (dry vs. wet), followed by Tukey'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 19.0 for Windows (SPSS, Chicago, IL, USA).

3. Results

3.1 Microtensile bond strength

There was no pre-test failure. Two-way ANOVA indicated significant differences in bond strength for adhesives ($F = 8.97$; $p < 0.05$) and for conditions (dry vs. wet; $F = 15.63$; $p < 0.001$). The interaction between the factors was not significant ($F = 2.004$; $p > 0.05$). While dehydration significantly increased the μTBS of MB ($p < 0.05$, Table 2), the bond strength increase in case of SE2 was not significant ($p > 0.05$). SE2 showed significantly higher μTBS than MB (SE2W vs. MBW; $p < 0.05$) without dehydration.

3.2 Fracture modes

SEM observations of the debonded surfaces revealed a predominance of cohesive dentin failure in all the groups except for MBW, where the mixed failure prevailed over other failure patterns (Table 2).

3.3 Hardness (H) and Elastic modulus (E) of cured bonding resins and dentin

Our results indicated that gradual dehydration caused significant differences between MB's mean H ($F = 68.685$; $p < 0.001$) and E ($F = 4.635$; $p < 0.05$) values obtained at the tested time points. While H became significantly different at 20 min ($p < 0.05$; Table 3 and Fig 1) from the baseline (5 min), for E, the difference became significant at 24 h ($p < 0.05$; Table 4 and Fig 2). Similar trend was observed for SE2's mean H ($F = 34.856$; $p < 0.001$) and E ($F = 6.004$; $p < 0.05$) values, where H became significantly different at 1 h ($p < 0.05$; Table 3 and Fig 1) from the baseline. However, for E, the difference became significant at 24 h ($p < 0.05$; Table 4 and Fig 2).

In case of dentin, while gradual dehydration caused significant differences between mean H (F = 11.260; $p < 0.001$) values obtained at the tested time points, the differences between the E (F = 1.583; $p > 0.05$) values were not significant. For dentin, H became significantly different at 24 h ($p < 0.05$; Table 3 and Fig 1) from the baseline.

3.4 Weight loss of dentin beams and adhesive discs

Gradual dehydration caused significant differences between mean weight values of dentin beams (F = 15.807; $p < 0.001$), MB discs (F = 160.977; $p < 0.001$) and SE2 discs (F = 193.627; $p < 0.001$) obtained at the tested time points. For dentin, the difference became significant at 10 min ($p < 0.001$; Table 5 and Fig 3) from the baseline (5 min), for MB discs at 20 min ($p < 0.05$; Table 5 and Fig 3) and for SE2 discs at 10 min ($p < 0.05$; Table 5 and Fig 3).

4. Discussion

In this study, dehydration significantly affected the μTBS of adhesives to dentin ($p < 0.001$). Therefore, our first null hypothesis has been rejected. MB's μTBS was significantly increased due to dehydration (Table 2; $p < 0.05$). However, for SE2, the increase was not significant ($p > 0.05$). The bond strength of SE2 was higher than MB for both tested conditions, albeit only significant for the wet condition ($p < 0.05$); therefore, the second null hypothesis was also rejected. SE2 is claimed to be the new improved version of MB. 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 (SE2 Brochure). In a recent study, Sato et al.[19] also observed SE2's superiority over MB in terms of μTBS to dentin, modulus of elasticity, degree of conversion and lesser water sorption. It is likely that SE2's higher degree of conversion and lesser water sorption resulted in non significant effect of dehydration in the bond strength. However, these findings apply only to the dehydration time

used in this study. Longer dehydration times may have further consequences to the bond strength as suggested by the findings of H and E (see further below).

Regardless of the testing mode, the fractured surface usually exhibits a mixed mode of cohesive and adhesive fracture[29]. However, in this study, cohesive dentin failures prevailed over mixed failures in most of the groups (Table 2). Dentin is an intrinsically hydrated tissue. The water content of dentin increases its ability to absorb and recover energy when subjected to loading and thus promote improved durability[23]. Although, the tensile strength of dentin ranges from 52 to 104 MPa[22, 30- 32], gradual dehydration increases dentin's H and E (Fig 1 and 2; Table 3 and 4) and makes it more brittle[21], leading to its failure at a much lower stress. Our weight-loss study also suggested that dentin beams became significantly dehydrated at 10 min. These explain why MBW (also having the lowest mean μTBS, 56.1 ± 14.9 MPa) had shown predominantly mixed failures and how dehydration led to a predominance of cohesive failures in dentin in case of MBD. Under tensile load, stress concentrates at the interface between two dissimilar materials. Increase of E intensifies the stress[20]. Therefore, high stress concentrations in harder, brittle dentin led to its failure. Moreover, cohesive dentin failure also prevailed as the μTBS values reached in excess of 70 MPa in case SE2W and SE2D (Table 2). We presume the combined effects of stiffer dentin and harder adhesives increased the bond strength values in the dry groups (MBD and SE2D) increasing the chances of cohesive failure in the composite resin.

In the current study, the effects of dehydration on the μTBS of adhesives and their failure pattern were further endorsed by the H and E values of the adhesives and dentin. Our results indicated that gradual dehydration made significant differences between MB's mean H ($p < 0.001$) and E ($p < 0.05$), SE2's mean H ($p < 0.001$) and E ($p < 0.05$) and dentin's H ($p < 0.001$) values at the tested time points. These observations reject the third null hypothesis. The reported H values of

MB range from 154 to 275 MPa and E between 4 to 4.68 GPa[27, 33]. Our results are also in line with the previous studies (Table 3 and 4). 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 properties[27]. Our results also showed that, from initial lower values due to water sorption, adhesives' H values increased gradually due to dehydration which became significant for MB at 20 min and for SE2 at 1 h ($p < 0.05$; Table 3 and 5). Tagami et al. reported that hardness of an adhesive is directly related to its bond strength[26]. Our study also showed that, increasing hardness values of adhesives either due to gradual dehydration (Table 3; MB; 5 min vs. 10 min) and difference of composition (Table 3; MBW vs. SE2W at 5 min) contributed to significantly increased bond strength values (Table 2; $p < 0.05$). Contrary to H values, both adhesives' E values became significantly different at 24 h ($p < 0.05$). The default software of the nanoindenter calculates the elastic modulus by Oliver and Pharr method[34]. Although this method is effective for elastic-plastic materials, it tends to overestimate the elastic modulus for viscoelastic materials like polymers. Therefore, to address this issue a hold segment at the peak load is employed[33-35]. In the current study, it is likely that the 10 s holding time at peak load was not sufficient for the adhesive disc's creep recovery prior to unloading when tested at 5 min. Hence, the 5 min's elastic modulus values of both adhesives are probably overestimations with respect to their values obtained at other time points in this study.

Marshall GW et al.[36] reported that nanohardness of hydrated intertubular dentin (ITD) lie between 0.15-0.51 GPa. However, H increased to 0.6-0.7 GPa when samples were tested in completely dry conditions[37]. Upon drying, the collagenous matrix of dentin collapses compressing the loose extrafibrillar mineral. This increases the rigidity of dentin, leading to

higher surface hardness[38]. Our results also showed a gradual increase in dentin's H values, which became significant at 24 h ($p < 0.05$) and lie within the reported ranges (Table 3). Furthermore, the E values of ITD range from 17.7 to 21.1 GPa[36]. Our E values are also within this range.

In order 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 μTBS test[39]. The duration of the time elapsed 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 humidity[29]. The small sized specimens (1 mm^2 or less) used in the μTBS 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 three fold: unrealistic increase of μTBS values owing to increased H and E of the adhesives, predominance of cohesive failures in dentin owing to increased brittleness (usually not accepted as μTBS values) and increased standard deviation. The results achieved from this study are in agreement with these.

5. Conclusion

This *in vitro* study reports previously unreported information about the mishandling of the μTBS test method. We observed that dehydration affects the mechanical properties of adhesives and dentin leading to misinterpretation of bonding results of adhesive resins to dentin by increasing the μTBS values with concurrently increasing the number of cohesive dentin failures. Therefore, the bonded dentin beams should not be allowed to dehydrate during μTBS test. The results

achieved from this study might not be relevant to clinical situations. However, they may shed lights on alleviating confounding effects of dehydration induced material and substrate modifications, leading to more representative μTBS results of bonding systems in laboratory settings.

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Tables

Figures & legends

Fig. 1 – Mean hardness of dentin and adhesives at tested times. The black-filled marker points indicate the time at which the values become significantly different ($p < 0.05$) from the baseline.

Fig. 2 - Mean elastic modulus of dentin and adhesives at tested times. The black-filled marker points indicate the time at which the values become significantly different ($p < 0.05$) from the baseline.

Fig. 3 – Mean weight loss values of dentin and adhesives. The black-filled marker points indicate the time at which the values become significantly different ($p < 0.05$) from the baseline.

Conflicts of interest

None

Table 1

Adhesive (Code/Manufacturer/ Lot number)	Type	Composition	Application instructions
Clearfil Mega Bond (MB/Kuraray Noritake Dental Inc., Japan/000040)	Two-step self-etch	**Primer:** 10-MDP, HEMA, Hydrophilic aliphatic dimethacrylate, CQ, DEPT, Water **Bond:** 10-MDP, Bis-GMA, HEMA, Hydrophobic aliphatic dimethacrylate, CQ, DEPT, 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 (SE2/Kuraray Noritake Dental Inc., Japan/000013)	Two-step self-etch	**Primer:** 10-MDP, HEMA, Hydrophilic aliphatic dimethacrylate, dl-CQ, Water **Bond:** 10-MDP, Bis-GMA, HEMA, dl-CQ,Hydrophobic aliphatic dimethacrylate, 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
Bis-GMA: bisphenol-A-diglycidyl methacrylate; CQ: camphorquinone; DEPT: N, N-diethanol-p-toluidine; HEMA: 2-hydroxyethyl methacrylate; 10-MDP: 10-methacryloxydecyl dihydrogen phosphate.			

Table 2

Adhesive \ Condition		Wet	Dry
MB (n=15)	μTBS ± SD (MPa)	56.1 ± 14.9 [a]	73.9 ± 12.3 [b]
	Fracture mode (%) A/CD/CC/M	7/33/0/60	7/53/7/33
SE2 (n=15)	μTBS ± SD (MPa)	70.7 ± 13.8 [b]	79.2 ± 9.8 [b]
	Fracture mode (%) A/CD/CC/M	13/73/0/13	0/53/7/40

Different superscript letter indicates statistically significant difference (Tukey's test, $p < 0.05$). A: adhesive; CD: cohesive failure in dentin; CC: cohesive failure in composite resin; M: mixed failure.

Table 3

Material	Hardness ± SD (MPa)					
	5 min	10 min	15 min	20 min	1 h	24 h
Dentin (n=15)	403 ± 119.9 [a]	436.6 ± 92.9 [a]	443.6 ± 59.5 [a]	457.4 ± 101.3 [a]	522.2 ± 120.9 [a, b]	664 ± 140 [b]
MB (n=15)	114.6 ±18.4 [a]	128.5 ± 17.6 [a]	135.2 ± 15.8 [a, b]	136.1 ± 11.2 [b]	147.1 ± 20.4 [b]	194.8 ± 4.7 [c]
SE2 (n=15)	134.5 ± 15.2 [a]	140.9 ± 12.8 [a, b]	144.6 ± 13.2 [a, b]	145.7 ± 13.0 [a, b]	159.8 ± 22 [b]	188.6 ± 14.7 [c]

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

Table 4

Material	Elastic modulus ± SD (MPa)					
	5 min	10 min	15 min	20 min	1 h	24 h
Dentin (n=15)	18047 ± 3823	19430 ± 3806	20032 ± 5348	20265 ± 3567	20396 ± 1288	21135 ± 2713
MB (n=15)	4497 ± 837	4073.2 ± 419 [a]	3952 ± 434 [a]	3890 ± 320 [a]	4315 ± 621 [a, b]	4403 ± 185 [b]
SE2 (n=15)	4201 ± 443	4090 ± 315 [a]	4082 ± 315 [a]	4064 ± 357 [a]	4263 ± 299 [a, b]	4549 ± 282 [b]

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

Table 5

Material	Mean weight (mg) after removal from storage medium at					
	5 min	10 min	15 min	20 min	1 h	24 h
Dentin beam (n = 5)	11.46 ± 1.88 [a]	11.36 ± 1.88 [b]	11.3 ± 1.85 [b]	11.22 ± 1.74 [b]	11.04 ± 1.73 [b]	10.9 ± 1.68 [c]
MB disc (n = 5)	180.88 ± 41.64 [a]	180.62 ± 41.65 [a, b]	180.46 ± 41.62 [a, b]	180.28 ± 41.62 [b, c]	179.86 ± 41.49 [c]	177.42 ± 41.56 [d]
SE2 disc (n = 5)	186.94 ± 31.98 [a]	186.72 ± 32.0 [b]	186.56 ± 32.06 [a, b ,c]	186.42 ± 32.09 [b, c]	185.92 ± 32.02 [c]	183.58 ± 32.08 [d]

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

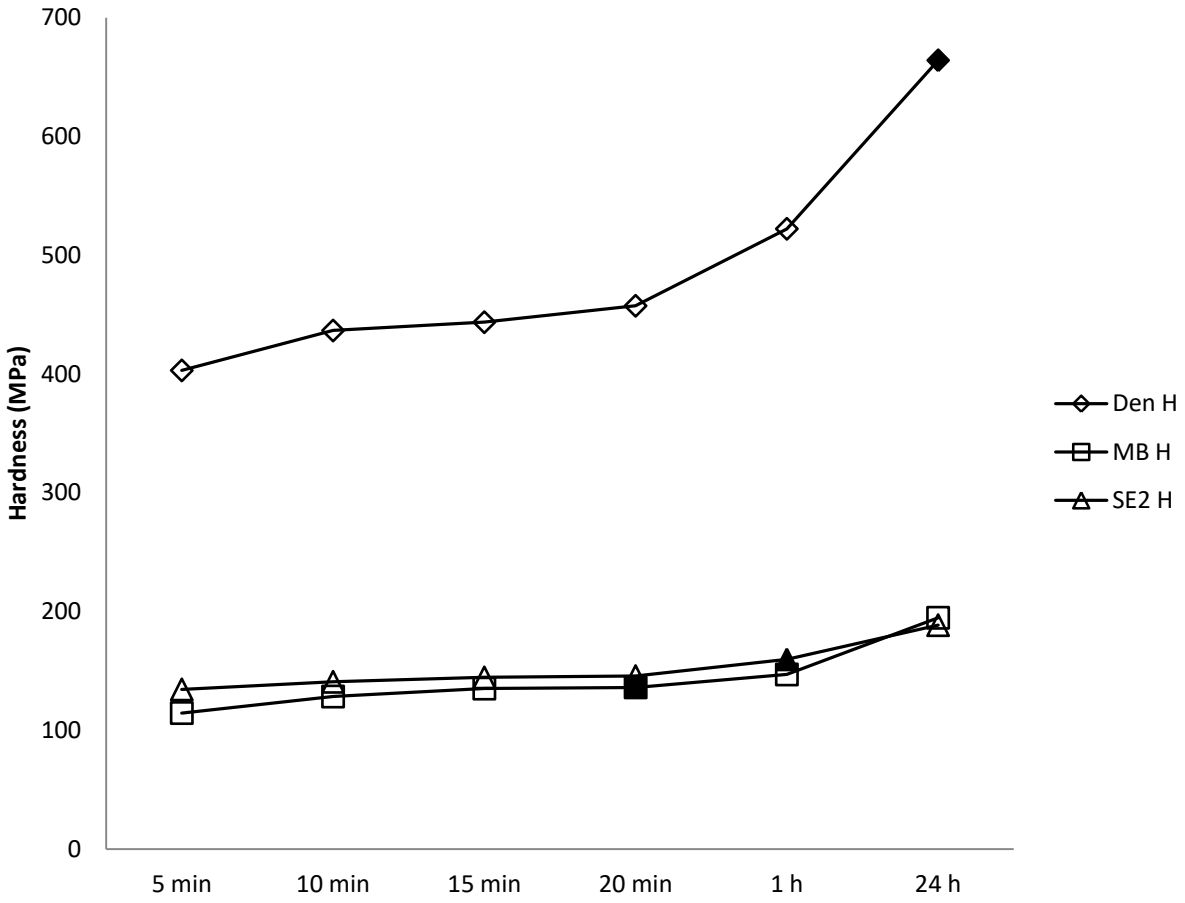
700
600
500
400
300
200
100
0
Hardness (MPa)
5 min
10 min
15 min
20 min
1 h
24 h
Den H
MB H
SE2 H

Fig. 1

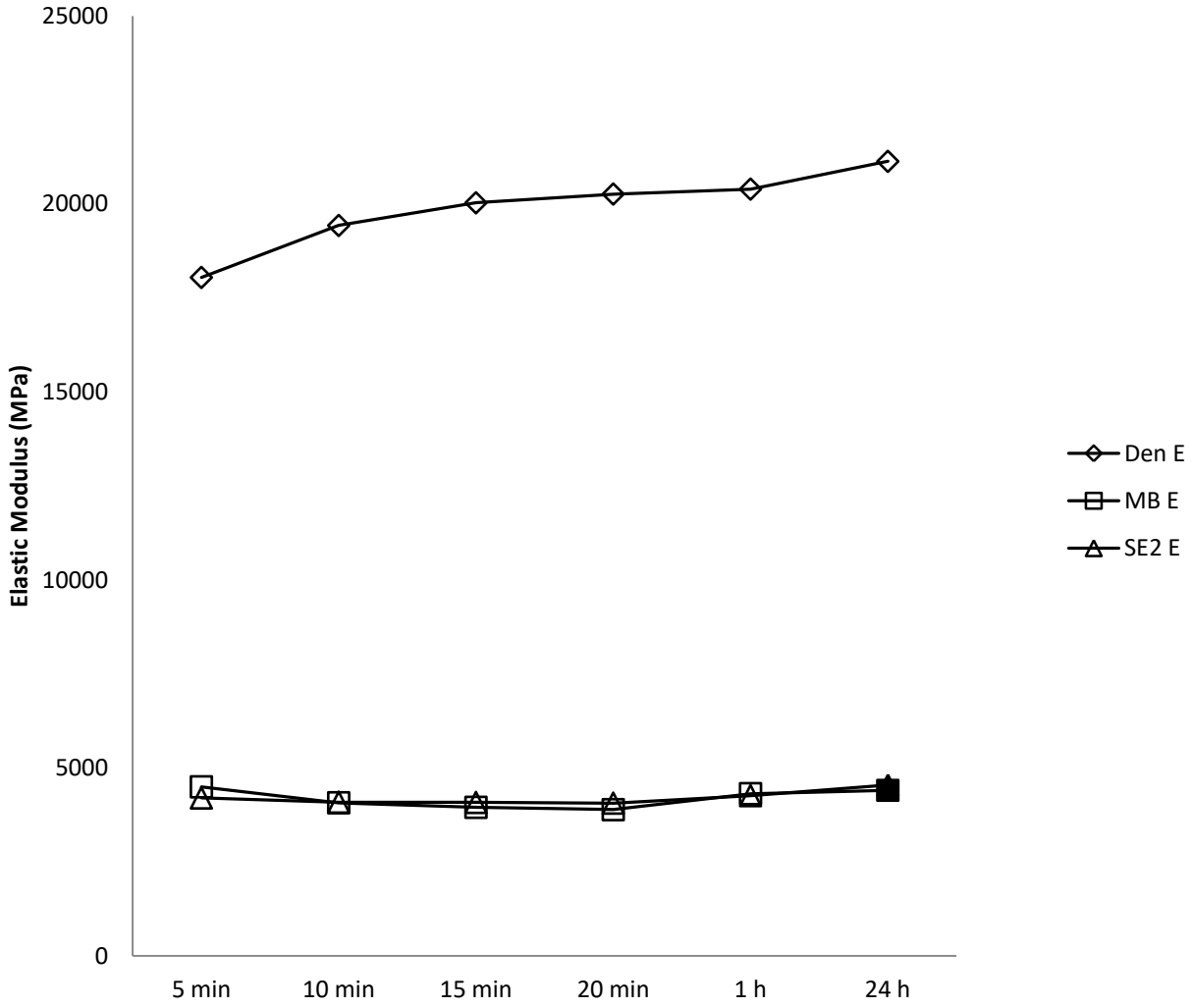
25000
20000
15000
10000
5000
0
Elastic Modulus (MPa)
5 min
10 min
15 min
20 min
1 h
24 h
Den E
MB E
SE2 E

Fig. 2

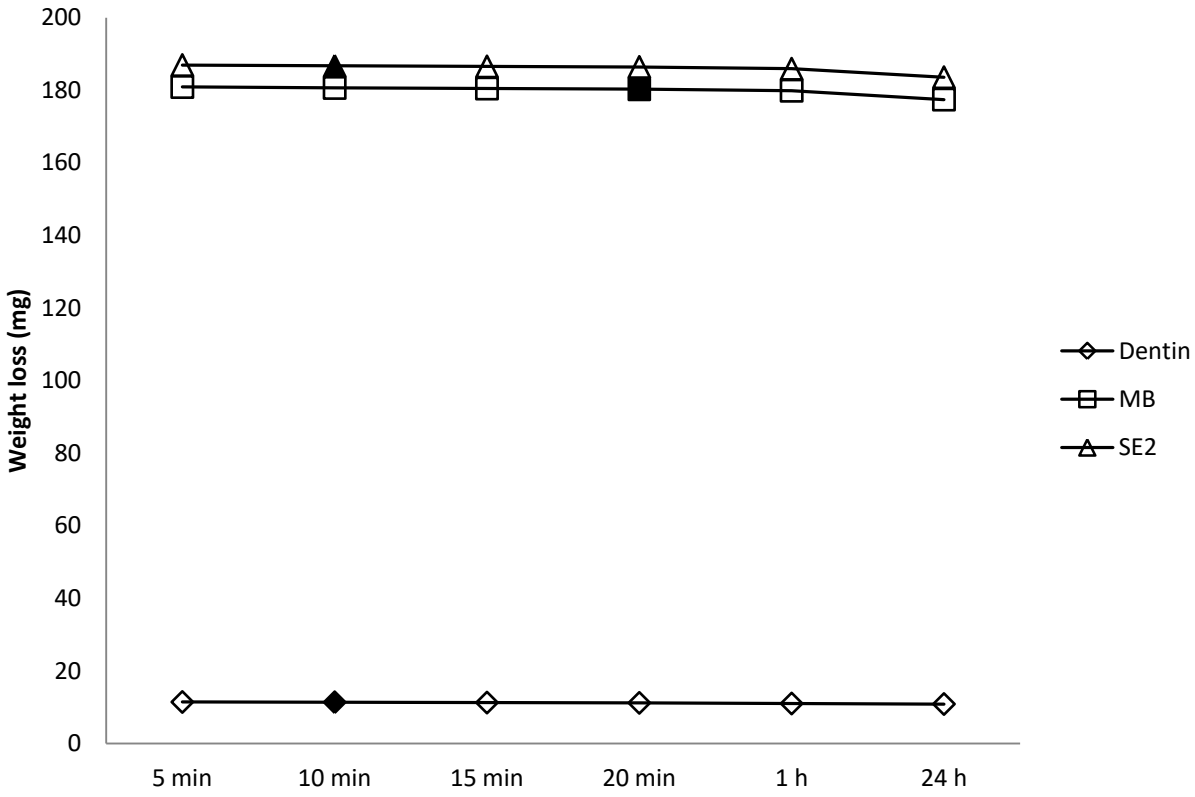
200
180
160
140
120
100
80
60
40
20
0
Weight loss (mg)
5 min
10 min
15 min
20 min
1 h
24 h
Dentin
MB
SE2

Fig. 3