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Effects of Storage Temperature on the Shelf Life of One-step and Two-step Self-etch Adhesives

S Ma • K Fujita (Nakajima) • N Nishiyama

Clinical Relevance

Storage temperature affects the alteration rate of one-step adhesives for all-in-one adhesive systems and self-etching primer for two-step adhesive systems. Their shelf life is strongly dependent on the storage temperature and time period. Storage under 20°C extends their shelf life.

SUMMARY

Recently, self-etch adhesive systems, the one-step (all-in-one) and two-step adhesive systems, have become widely utilized due to their simplified application procedures and low technique sensitivity. In the current study, in order to understand the effects on shelf life of the two types of self-etch adhesives, the effects from storage temperature and time period on the alteration stage of 10-methacryloxydecyl dihydrogen phosphate (MDP)-based, a one-step adhesive (all-in-one adhesive system) and an MDP-based self-etching primer (two-step adhesive system) were examined.

Clearfil Tri-S Bond (TSB), an MDP-based one-step adhesive (all-in-one adhesive system), and Clearfil Mega Bond Primer (MBP), an MDP-based self-etching primer (two-step adhesive system), were used. Both TSB and MBP, received within two days after they were produced, were immediately stored at 8°C, 20°C or 40°C for 1, 3, 7 and 14 weeks, respectively. At the end of each storage period, ^{13}C NMR observations were performed by an EX-270 spectrometer. In addition, NMR observations of TSB and MBP were immediately performed within two days, after both were produced as a control. The effects from the storage temperature and time period on the alteration rate and stage of TSB and MBP were examined by determining the amount of hydrolyzed 2-hydroxyethyl methacrylate (HEMA) and MDP in both materials. Specimens

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bonded to dentin by using TSB or MBP in different alteration stages were prepared. The degradation stage effect of TSB or MBP on bond durability was examined by measuring the shear bond strength before and after thermocycling (20,000 times).

With increases in storage temperature and time period, the relative intensities of the NMR peak "ε" assigned to both methylene carbons in the ethylene glycol (EG) and to the NMR peak "ζ" assigned to the terminal methylene carbon bonded to the hydroxy group in the 10-hydroxydecyl dihydrogen phosphate (HDP) produced by the hydrolysis of the ester portion in HEMA or MDP, respectively, increased. The alteration stages of TSB and MBP were strongly dependent on storage temperature and time period. When TSB or MBP, stored at 40°C for 14 weeks, was applied to dentin, specific decreases in mean bond strength were observed in both adhesive systems. However, the application of thermocycling did not exhibit any specific decreases in the mean bond strength, even though the alteration stage of TSB and MBP progressed.

From the results of the current study, storage temperature and time period significantly affect the alteration rate and stage of TSB and MBP. However, TSB and MBP exhibit expectant bond strength and bond durability when both are stored below 20°C.

INTRODUCTION

Recently, self-etch adhesive systems have become widely utilized due to their simplified application procedures and low-technique sensitivity when compared to etch and rinse adhesives.[1] Self-etch adhesive systems can generally be classified as either a one-step (all-in-one) adhesive system or a two-step adhesive system, based on whether or not a bonding agent is applied.[2-3] In addition, a two-step adhesive system reportedly exhibited relatively higher bonding performance of resin to tooth than a one-step self-etch adhesive system.[4-6]

Sadr and others[7] reported that the bond strength of resin to dentin conditioned by a one-step adhesive (Clearfil Tri-S Bond, TSB) for the all-in-one adhesive system or a self-etching primer (Clearfil Mega Bond Primer, MBP, marketed as Clearfil SE Bond outside of Japan) for a two-step adhesive system that had been stored at 4°C, 23°C or 37°C decreased when their storage temperature and time period were increased. However, the effects from the alteration stages of stored TSB or MBP, as evidenced by the hydrolysis stage of the 2-hydroxyethyl methacrylate (HEMA) and 10-methacryloxydecyl dihydrogen phosphate (MDP) utilized in both materials on dentin bond durability, have not yet been thoroughly identified. The alteration stage effects of TSB and MBP are of importance since byproducts, produced by hydrolysis of the methacryloxy ester portion in HEMA[8] and MDP, may degrade the hybrid layer's quality and reduce dentin bond durability. Specifically, hydrolysis of the HEMA may degrade the quality of the hybrid layer. This is of importance, since HEMA plays a key role in the bonding process: (1) HEMA's carbonyl group in the methacryloxy ester portion forms a hydrogen-bonded interaction with the collagen fiber exposed by acid-etching, (2) hydrogen-bonded HEMA promotes the hybridization of resin to collagen fiber and 3) as a result, it enhances bonding of resin to the collagen fiber.[9-10]

In the current study, effects from storage temperature and time period on the alteration stage of TSB and MBP, as determined by the amounts of hydrolyzed HEMA and MDP utilized in these materials, were examined. In addition, the effects from the alteration stages of TSB or MBP on dentin bond durability were investigated before and after thermocycling. The shelf life of TSB and MBP was then discussed. The null hypotheses were: 1) storage temperature and time period of TSB and MBP would not affect the alteration stage and rate; 2) the alteration stage of TSB and MBP would not affect the thickness of the hybrid layer and 3) the alteration stage of TSB and MBP would not affect the initial bond strength of resin to dentin (before thermocycling) and dentin bond durability (after thermocycling).

METHODS AND MATERIALS

1. Materials

Clearfil Tri-S Bond (TSB, Kuraray Medical Inc, Osaka, Japan) was used as a one-step adhesive for the all-in-one adhesive system. Clearfil Mega Bond Primer (MBP, Kuraray Medical Inc) was used as a self-etching primer for the two-step adhesive system. TSB and MBP were received within two days after both were produced. Clearfil Mega Bond (Kuraray Medical Inc) was used as a bonding agent for the two-step adhesive system. Chemical compositions and lot numbers are shown in Table 1.

2. Methods

Determination of Quantitative Amounts of Hydrolyzed HEMA and MDP Using the ^{13}C NMR Technique

TSB and MBP received within two days after both were produced were immediately stored at 8°C (refrigerator), 20°C (thermo-stabilized room) or 40°C (incubator) for 1, 3, 7 and 14 weeks, respectively. At the end of each storage period, ^{13}C NMR observations of the stored TSB or MBP were performed by an EX-270 spectrometer (JEOL, Tokyo, Japan) so as to determine the quantitative amounts of HEMA and MDP whose methacryloxy ester portion had become hydrolyzed. The accumulation

Table 1: *Composition and Lot Numbers of the One-step and Two-step Adhesives and Bonding Agents Used in This Study*

Adhesives	pH	Components	Lot Numbers
Tri-S bond	2.7	Water, MDP, Bis-GMA, HEMA, Ethanol, Hydrophobic DMA, CQ, Silanated colloidal silica	0755AA
Mega Bond Primer	2.0	Water, MDP, HEMA, Hydrophilic DMA, CQ, DET	00087A
Bonding Agent			
Mega Bond		Bis-GMA, MDP, HEMA, Hydrophobic DMA, CQ DET, Silanated colloidal silica	01078A

Bis-GMA: bis-phenol A diglycidylmethacrylate; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate; DMA: dimethacrylate; DET: N,N-diethanol p-toluidine; CQ: camphorquinone
pH of the adhesives as provided by the manufacturer.

and repetition times were 1,000-5,000 times and nine seconds, respectively. NMR samples were prepared after dissolving 0.200 g of the stored TSB or MBP into 0.500 g of dimethylsulfoxide-d_6 (DMSO). In addition, NMR observations of TSB and MBP, received within two days after both were produced, were immediately performed as a control (storage period: 0 day).

Preparation of the Specimens for Scanning Electron Microscope (SEM) Observation and Adhesion Test

Bovine teeth frozen immediately after extraction and kept frozen for one month were unfrozen and cross-sectioned into crown and root. After removing the pulp, the facial enamel surface of the crown was ground by a 100-grit silicon carbide paper under running water as a coolant in order to obtain a flat dentin surface. The ground dentin was then molded with a self-curing-type pour resin (Shofu, Kyoto, Japan) in a brass ring mold (internal diameter = 12 mm, height = 14 mm). The molded specimens were then removed from the brass mold after the pour resin had hardened. To obtain a fresh crown dentin surface, the molded dentin surface was polished with a sequence of 100-grit, 600-grit and 1000-grit silicon carbide papers under running water. To adjust the adhesive area, 80 μm double-faced tape (Nichiban, Tokyo, Japan) with a circular hole (internal diameter = 3.2 mm) was placed onto the grounded dentin surface. The dentin surface inside of the circular hole was conditioned for 20 seconds by a TSB (storage period: 0 day, control) or a TSB stored at 8°C, 20°C or 40°C for 14 weeks. Thereafter, in order to remove the solvent and water from the applied TSB, a high-pressure air flow was applied to the conditioned dentin surface for five seconds. Visible light was then irradiated for 10 seconds by using Curing Light XL3000 (3M ESPE, Gratfenau, Germany). A distance of approximately 1 mm from the exit of the visible light to the top surface of the bonding layer of the applied TSB was maintained. A 1-mm thick silicone ring mold with a circular hole (internal diameter = 3.2 mm) was placed onto the double-faced tape. Then, a resin composite (Clearfil AP-X, Shade A3.5, Kuraray Medical Inc) was carefully filled into the silicone ring mold's circular hole and was light-irradiated for 20 seconds. After removing the silicone ring mold and double-faced tape, the bonded specimens were immersed in water at 37°C.

In addition, the grounded dentin surface inside of the 80 μm double-faced tape was conditioned for 20 seconds with an MBP (storage period: 0 day, control) or an MBP stored at 8°C, 20°C or 40°C for 14 weeks, then dried thoroughly with a mild air flow. Following application of Clearfil Mega Bond, a gentle air flow was applied to the bonding agent. Visible light was applied to Clearfil Mega Bond for 10 seconds. The resin composite was immediately applied, then light irradiated for 20 seconds. After applying the same procedures as previously described, the bonded specimens were immersed in water at 37°C. The number of bonded specimens for each experimental group was 26.

SEM Observations of the Resin-dentin Interface

After one day, the bonded specimens were cross-sectioned perpendicular to the resin-dentin interface by a low-speed diamond saw under water-cooling. Thereafter, the cross-sectioned surfaces were polished using a 0.25 μm diamond paste, then argon-ion showered by a flat milling device (E-3000, Hitachi, Tokyo, Japan) for 60 seconds. The specimens were then dehydrated by using 70, 80, 90 and 100 vol% of ethanol aqueous solutions. After immersion in tertiary-butyl alcohol for one day, the specimens were freeze-dried by using Freeze Dryer (Hitachi ES-2030, Tokyo, Japan). The specimens were mounted onto aluminum stubs, then sputter-coated with a platinum-palladium alloy. Each specimen was examined at numerous magnifications and tilt angles by a scanning electron microscope (Hitachi S-4500) at 20.0kV. The number of bonded specimens for each experimental group was two.

Measurements of Shear Bond Strength Before and After Thermocycling

After the bonded specimens were stored in water at 37°C for one day, they were divided into two experimental groups: before and after thermocycling. The shear bond strength of resin to dentin was measured

under a crosshead speed of 1 mm/minute by a conventional testing machine (TG-5KN, Minebea, Nagano, Japan). The shear bond strength was measured 12 times by using the bonded specimens for each experimental group.

For the thermocycling groups, the specimens were thermocycled between 5°C and 55°C in a water bath 20,000 times for each bath. The dwell time in the water bath was 60 seconds. The transfer time was seven seconds.

The average bond strength and standard deviation (SD) were calculated for TSB or MBP with different alteration stages. The results were analyzed by one-way analysis of variance (ANOVA) and Scheffé's multiple comparison tests. The value of statistical significance was set at the 0.05 level.

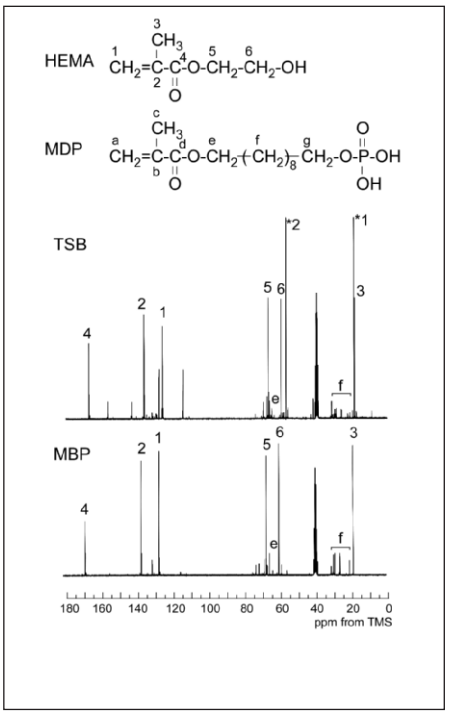

Figure 1. *The ^{13}C NMR spectra of TSB and MBP and the ^{13}C NMR peak assignments of carbons attributed to MDP and HEMA, as represented in both TSB and MBP.*
*Upper spectrum is of TSB. Lower spectrum is of MBP. Both TSB and MBP, received within two days after both were produced, were measured upon receipt (storage period: 0 day), as a control. The hydrolysis stages of HEMA and MDP utilized in TSB or MBP were 2.0 and 0% or 1.9 and 2.4%, respectively. In addition, the ^{13}C NMR spectrum clearly demonstrated that TSB was diluted by ethanol, since NMR peak "*2," assigned to methylene carbon and NMR peak "*1," assigned to methyl carbon for ethanol, were detected.*

RESULTS

1. Effects From Storage Temperature on the Alteration Rate of TSB and MBP

To understand the effects on the shelf life of TSB and MBP, ^{13}C NMR observations of the stored TSB or MBP were performed under different storage temperature and time period conditions.

Figure 1 shows the typical ^{13}C NMR spectra of TSB (upper spectrum) and MBP (lower spectrum). NMR observations of TSB and MBP were performed within two days after TSB and MBP were produced. The ^{13}C NMR peaks attributed to carbons constituting HEMA and MDP were assigned based on previous papers of the current authors.[8,11] The unassigned small ^{13}C NMR peaks detected in the ^{13}C NMR spectrum of the TSB or MBP were probably assigned to carbons of the other chemical substrates utilized in both TSB and MBP, as shown in Table 1.

To investigate the hydrolytic stabilities of the methacryloxy ester portion in HEMA and MDP utilized in TSB and MBP, the authors focused on the methylene region in the ^{13}C NMR spectrum of HEMA and MDP. Since the methacryloxy ester portion in HEMA hydrolyzed and ethylene glycol (EG) and methacrylic acid (MA) were, as a result, produced, the NMR peak "ε" assigned to both methylene carbons in the EG appeared at approximately 63.2 ppm for TSB (left side) or 63.4 ppm for MBP (right side), respectively, as shown in Figure 2. Specifically, when the storage temperature of both materials was increased to 40°C, dramatic increases in peak intensity (peak area) of the NMR peak "ε" were observed when compared to the peak intensity of NMR peaks "5" and "6" assigned to both methylene carbons in HEMA. The magnitude of the peak intensity in NMR peak "ε" detected in the methylene region of the MBP was greater than that of TSB.

Similar to HEMA, since the methacryloxy ester portion in MDP hydrolyzed and 10-hydroxydecyl dihydrogen phosphate (HDP) and MA were produced as a result, the NMR peak "ζ" assigned to the terminal methylene carbon bonded to the hydroxy group in HDP appeared at approximately 61.3 ppm in the methylene region of TSB (left side) when the storage temperature of TSB was increased from 8°C to 20°C, as shown in Figure 3. The peak intensity of NMR peak "ζ" dramatically increased when the storage temperature was increased to 40°C, thus reflecting a decrease in peak intensity of NMR peak "ε" attributed to the methylene carbon of the methacryloxy ester portion in MDP when compared to the NMR peak "γ" attributed to methylene carbon of the phosphoric ester portion in MDP. In contrast, the NMR peak "ζ" was detected at approximately 61.7 ppm in the methylene region of MBP (right side), even though MBP was stored at 8°C.

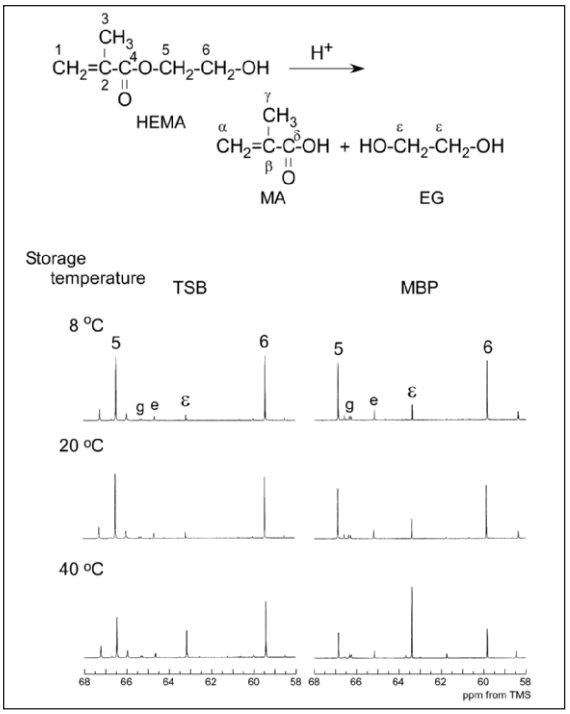

Figure 2. *The expanded ^{13}C NMR spectra of the methylene region of HEMA utilized in TSB and MBP under different storage temperature conditions, and NMR peak assignments of carbons attributed to HEMA and EG, as represented in both TSB and MBP.*
The left side NMR spectra show the TSB. The right side NMR spectra show the MBP. There, both TSB and MBP were stored at different temperatures for 14 weeks. The upper spectra were obtained from both TSB and MBP stored at 8°C. The middle spectra were obtained from both TSB and MBP stored at 20°C. The lower spectra were obtained from both TSB and MBP stored at 40°C.
The quantitative amount of HEMA, whose methacryloxy ester portion had hydrolyzed, was determined by dividing the relative peak intensity of the NMR peak "ε" (attributed to both methylene carbons in the EG) against the peak intensity of both NMR peaks "5" and "6" (attributed to both methylene carbons in HEMA).

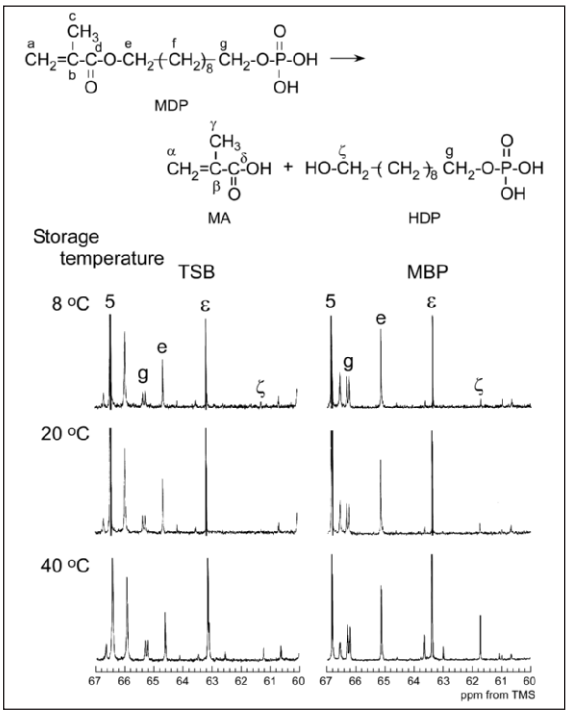

Figure 3. *The expanded ^{13}C NMR spectra of the methylene region of MDP utilized in TSB and MBP under different storage temperature conditions and NMR peak assignments of carbons attributed to MDP and HDP, as represented in both TSB and MBP.*
The left side NMR spectra show the TSB. The right side NMR spectra show the MBP. There, both TSB and MBP were stored at different temperatures for 14 weeks. The upper spectra were obtained from both TSB and MBP stored at 8°C. The middle spectra were obtained from both TSB and MBP stored at 20°C. The lower spectra were obtained from both TSB and MBP stored at 40°C.
The quantitative amount of MDP, whose methacryloxy ester portion had hydrolyzed, was determined by dividing the relative peak intensity of the NMR peak "ζ" (attributed to the terminal methylene carbon in the HDP) against the peak intensity of both NMR peaks "e" and "g" (attributed to the methylene carbons of both methacryloxy and phosphoric ester portions in MDP).

Similar to the hydrolysis behavior of MDP utilized in TSB, the NMR peak "ζ" peak intensity dramatically increased when the storage temperature was increased from 20°C to 40°C. The magnitude of the peak intensity in NMR peak "ζ" detected in MBP was greater than that of TSB.

To understand the hydrolysis behavior of HEMA and MDP utilized in TSB or MBP, the ratio of the hydrolyzed HEMA (percentage) was determined by calculating the relative peak intensity of peak "ε." There, the relative peak intensity of peak "ε" was derived by dividing the peak intensity of NMR peak "ε" by the peak intensity of NMR peaks "5" and "6." In addition, the ratio of the hydrolyzed MDP (percentage) was determined by dividing the peak intensity of NMR peak "ζ" by the peak intensity of NMR peaks "e" and "g." The effects from storage temperature and time period on the hydrolysis rate of HEMA and MDP are summarized in Figures 4 and 5, respectively.

The hydrolysis rate of HEMA and MDP utilized in TSB or MBP is strongly dependent on its storage temperature. The hydrolysis rate of HEMA was faster than that of MDP. The alteration rate of TSB and MBP obtained at a storage temperature of 8°C was almost the same as that obtained from a storage temperature of 20°C. However, when the storage temperature was increased to 40°C, the alteration rate of TSB and MBP was nearly four times faster when compared to the hydrolysis rate obtained at a storage temperature of 20°C.

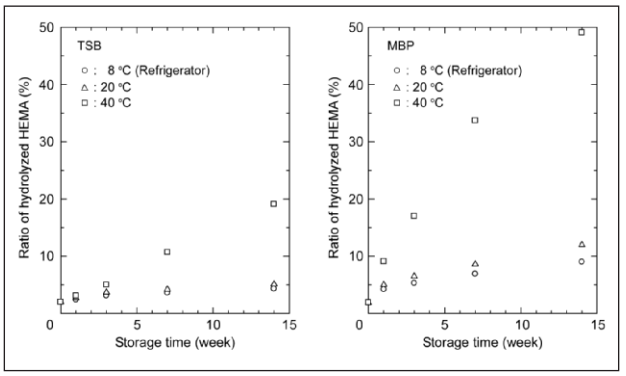

Figure 4. *The effects of storage temperature and time period on the amount of hydrolyzed HEMA utilized in TSB and MBP.*
The ratio of hydrolyzed HEMA in TSB or MBP was determined by dividing the relative peak intensity of the NMR peak "ε" (attributed to methylene carbons in the EG) against the peak intensity of both NMR peaks "5" and "6" (attributed to both methylene carbons in HEMA).

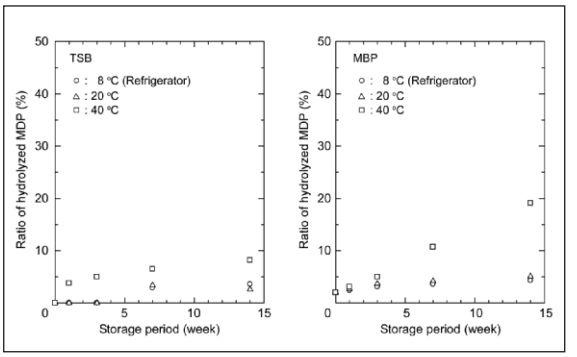

Figure 5. *The effects of storage temperature and time period on the amount of hydrolyzed MDP utilized in TSB and MBP.*
The ratio of hydrolyzed MDP in TSB or MBP was determined by dividing the relative peak intensity of the NMR peak "ζ" (attributed to terminal methylene carbon in HDP) against the peak intensity of both NMR peaks "e" and "g" (attributed to the methylene carbons of both methacryloxy and phosphoric ester portions in the MDP).

As shown in Table 2, the alteration stages of TSB were: 2.0% for HEMA and 0% for MDP (storage period: 0 day, control), 4.3% for HEMA and 3.6% for MDP (stored: 8°C, 14 weeks), 5.1% for HEMA and 2.6% for MDP (stored: 20°C, 14 weeks) or 19.1% for HEMA and 8.2% for MDP (stored: 40°C, 14 weeks). Conversely, the alteration stages of MBP were: 1.9% for HEMA and 2.4% for MDP (storage period: 0 day, control), 9.0% for HEMA and 3.7% for MDP (stored: 8°C, 14 weeks), 12.0 for HEMA and 6.0% for MDP (stored: 20°C, 14 weeks) or 49.1 for HEMA and 15.5% for MDP (stored: 40°C, 14 weeks).

2. Alteration Stage Effects of TSB and MBP on Creation and Quality of the Hybrid Layer

Figure 6 shows the effects from the alteration stages of TSB or MBP on the creation and quality of the hybrid layer. A series of four altered TSB or MBP in different alteration stages, as described above, were utilized in this experiment.

The thicknesses of the hybridized layers were 0.2-0.3 μm for TSB and 1-1.2 μm for MBP, respectively. The thickness of the hybrid layer, obtained from an alteration stage of TSB of 2.0% for HEMA and 0% for MDP or for MBP of 1.9% for HEMA and 2.4 for MDP (for both, storage period: 0 day, control), remained unchanged even though the alteration stages of TSB or MBP progressed. However, when MBP, whose alteration stages were 49.1% for HEMA and 15.5% for MDP, was applied to the dentin, a bonding defect of a void appeared within the hybrid layer. In contrast, in all SEM views of TSB, the authors of the current study did not observe the creation of any bonding defect within the hybrid layer, even though the alteration stages of TSB equaled 19.1% for HEMA and 8.2% for MDP.

3. Alteration Stage Effects of TSB and MBP on Bond Strength and Bond Durability

Effects from the alteration stages of TSB or MBP on the shear bond strengths of resin to dentin are shown in

Table 2: *Effects From the Storage Temperature and Period on the Hydrolyzed Amounts of HEMA and MDP*

Storage Period (weeks)	Hydrolyzed Amounts of HEMA						Hydrolyzed Amounts of MDP					
	TSB Temperature (°C)			MBP Temperature (°C)			TSB Temperature (°C)			MBP Temperature (°C)		
	8	20	40	8	20	40	8	20	40	8	20	40
Control	2.0	2.0	2.0	1.9	1.9	1.9	0.0	0.0	0.0	2.4	2.4	2.4
1	2.4	2.7	3.1	4.2	5.0	9.1	0.0	0.0	3.8	2.5	3.8	5.5
3	3.1	3.7	5.0	5.3	6.5	17.0	0.0	0.0	5.0	3.3	4.3	7.0
7	3.6	4.2	10.7	6.9	8.6	33.7	2.9	3.4	6.5	3.5	4.8	9.9
14	4.3	5.1	19.1	9.0	12.0	49.1	3.6	2.6	8.2	3.7	6.0	15.5

The TSB and MBP were received within two days after both materials were produced.
After receipt of both materials, NMR observations of the TSB and MBP were conducted as a control (storage period: 0 days).

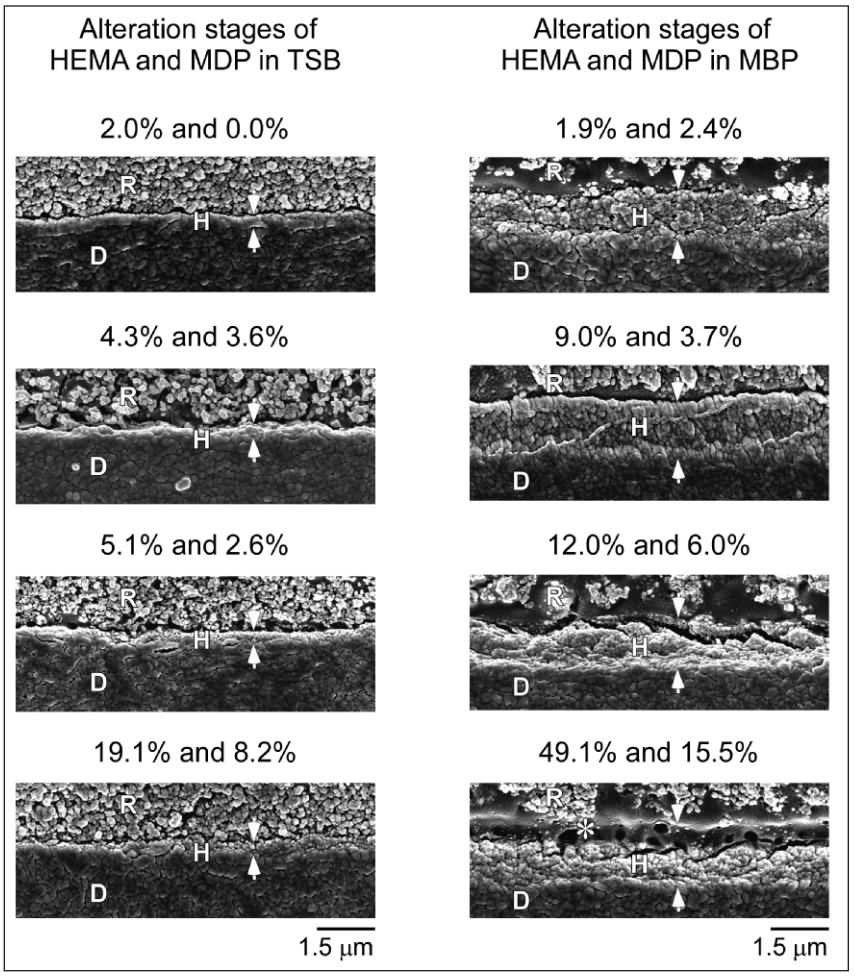

Figure 6. *SEM views of the hybrid layer at the resin-dentin interface created by TSB or MBP with different alteration stages.*
R: polymerized resin; D: dentin; H: hybrid layer.
The distance between the arrowheads demonstrates the thickness of the hybrid layer. The asterisk shows a void developed.

Table 3. A series of four altered TSB or MBP in different alteration stages, as described above, were utilized.

Before thermocycling, the mean bond strengths to dentin conditioned by TSB, alteration stages of 2.0% for HEMA and 0% for MDP or by MBP with alteration stages of 1.9% for HEMA and 2.4% for MDP, were 16.4 or 21.0 MPa (for both—storage period: 0 day, control). These initial mean bond strengths remained relatively unchanged, even though the alteration stages of TSB progressed to 5.1% for HEMA and 2.6% for MDP or MBP progression of 12.0% for HEMA and 6.0% for MDP. However, when the alteration stages of TSB equaled 19.1% for HEMA and 8.2% for MDP or for MBP of 49.1% for HEMA and 15.5% for MDP, specific decreases in mean bond strength were observed from 16.4 to 13.6 MPa for TSB and from 21.0 to 17.1 MPa for MBP. These reductions in mean bond strength were 16.6% for TSB and 18.7% for MBP, respectively.

After thermocycling, specific decreases in mean bond strength before and after thermocycling were not observed for all experimental groups (no significant difference), even though the alteration stages of TSB progressed. In contrast, when MBP was applied, the mean bond strength gradually decreased (no significant difference), with an increase in the alteration stage of MBP.

DISCUSSION

In the current study, in order to understand the effects on the shelf life of one-step adhesives for the all-in-one adhesive system and self-etching primer for the two-step adhesive system, the effects from the storage temperature and time period of TSB and MBP on their alteration rate and stage were studied. Furthermore, the effects from the alteration stage of TSB and MBP on dentin bond durability were then examined.

When TSB and MBP were stored, they became altered due to hydrolysis of the methacryloxy ester portion in HEMA and MDP utilized in TSB and MBP. The alteration rate and stage of TSB and MBP are strongly dependent on the storage temperature and time period. Therefore, the hypothesis that the storage temperature and time period of TSB and MBP would not affect the alteration stage, was rejected.

The observed faster alteration rate of MBP compared to that of TSB was due to the MDP utilized in MBP providing sufficient amounts of protonated protons to induce hydrolysis of HEMA and MDP, rather than that of TSB. This is possible, since the pH value of MBP (pH=2.0, referred from the instruction) is lower than that of TSB (pH=2.7, referred from the instruction). This higher pH value of TSB compared to MBP may be due to: 1) TSB being diluted by non-electrolytic ethanol[12] and/or 2) the water/MDP ratio utilized in TSB being lower than that in MBP.[7] This was possible, since: 1) the degree of dissociation of an acid becomes inhibited when the acid is diluted by non-electrolytic ethanol[13] and 2) the degree of dissociation of phosphoric acid in MDP became inhibited when the water/MDP ratio was decreased, since the degree of dissociation of an acid was limited by an increase in the concentration of an acid.[13]

Specifically, when the storage temperature of TSB and MBP was increased to 40°C, the alteration rate of both materials was accelerated. The observed temperature dependency of the alteration rate of TSB and MBP was probably due to the degree of dissociation of phosphoric acid in MDP becoming higher as the storage

Table 3: *Effects From the Alteration Stage of the TSB and MBP on the Shear Bond Strength, Before and After Thermocycling*

	Storage Temperature (°C)	Hydrolyzed Amounts of HEMA (%)	Hydrolyzed Amounts of MDP (%)	Shear Bond Strength (MPa)	
				Before	After
TSB	Control	2.0	0.0	16.4 (2.6) *	15.8 (2.3) *
	8	4.3	3.6	16.2 (3.3) *	16.7 (1.9) *
	20	5.1	2.6	15.1 (2.8)	15.8 (3.2) *
	40	19.1	8.2	13.6 (2.1)	13.0 (2.8)
MBP	Control	1.9	2.4	21.0 (4.1)	19.8 (3.8) *
	8	9.0	3.7	21.3 (3.9) *	19.3 (3.5) *
	20	12.0	6.0	20.1 (4.0)	18.5 (2.7) *
	40	49.1	15.5	17.1 (5.2)	15.3 (3.1)

**: significant difference (p‹0.05), One-way ANOVA (Scheffe)*
The TSB and MBP were received within two days after both materials were produced.
After receipt of the TSB and MBP, measurements of the shear bond strengths were conducted as a control (storage period: 0 days).
The both materials were stored under different storage temperatures for 14 weeks.

temperature increased. This is plausible, since the degree of dissociation of an acid accelerates as the temperature of the acidic solution rises.[13]

Reflecting a lower degree of dissociation of phosphoric acid in MDP utilized in TSB compared to MBP, TSB produced a noticeably thinner hybrid layer than that observed with MBP. The thickness of the hybrid layers remained relatively unchanged at 0.2-0.3 μm for TSB and 1-1.2 μm for MBP, even though the alteration stage of TSB progressed from 2.0% for HEMA and 0% for MDP to 19.1% for HEMA and 8.2% for MDP or it progressed for MBP from 1.9% for HEMA and 2.4% for MDP to 49.1% for HEMA and 15.5% for MDP. Therefore, the hypothesis that the alteration stage of TSB and MBP would not affect the thickness of the hybrid layer was accepted. The fact that no changes in thickness of the created hybrid layer was observed suggests that the etching efficacy of dentin by TSB and MBP remains unchanged, even though hydrolysis of MDP progresses and the concentration of HDP in TSB or MBP increases. This was possible, since phosphoric acid in HDP was able to demineralize the dentin apatite and form a water-insoluble calcium salt, since HDP contains a hydrophobic group (10 methylene carbons) similar to MDP.

However, the application of MBP, whose alteration stage was 49.1% for HEMA and 15.5% for MDP to dentin, resulted in the development of a bonding defect of a void within the hybrid layer. This bonding defect was due to an increase in water-soluble byproducts, such as EG, which has no vinyl group that is necessary for a polymerization reaction. Conversely, when TSB, whose alteration stage was 19.1% for HEMA and 8.2% for MDP, was applied, the authors of the current study did not observe any bonding defect within the hybrid layer. This was plausible, since the alteration stage of TSB is lower than of MBP.

The effects from the alteration stage of TSB or MBP on the shear bond strength of resin to dentin were investigated. Before thermocycling, the initial mean bond strengths remained relatively unchanged until the alteration stage of TSB reached 5.1% for HEMA and 2.6% for MDP and MBP reached 12.0% for HEMA and 6.0% for MDP. However, when the alteration stage of TSB equaled 19.1% for HEMA and 8.2% for MDP or for MBP of 49.1% for HEMA and 15.5% for MDP, specific reductions in mean bond strength of 16.6% for TSB and 18.7% for MBP were observed. Therefore, the hypothesis that the alteration stage of TSB and MBP would not affect the bond strength of the resin to dentin was rejected. This specific decrease in bond strength was due to the production of byproducts, such as EG, HDP and MA produced by hydrolysis of the methacryloxy ester portion in HEMA and MDP. This was plausible, since sufficient amounts of EG, HDP and MA would remain within the adhesive layer. In particular, the production of a water-soluble EG might reduce the quality of the adhesive layer, since EG has no vinyl group for co-polymerization with functional monomers utilized in TSB or Mega Bond (bonding agent).[14]

However, the degree of reduction in the mean bond strength of MBP was almost the same as that of TSB, even though the alteration stage of MBP was significantly higher by nearly twice that of TSB. This unexpected smaller reduction in mean bond strength of MBP, when compared with TSB, was due to the fact that, when Mega Bond was additionally applied to dentin conditioned by the altered MBP, the adhesive

monomers utilized in Mega Bond permeated and diffused into the demineralized collagenous layer and created a hybrid layer.

After thermocycling, specific reductions in mean bond strength were not observed, even though the alteration stage of TSB progressed from 2.0% for HEMA and 0% for MDP to 19.1 for HEMA and 8.2% for MDP. In contrast, with an increase in the alteration stages from 1.9% for HEMA and 2.4% for MDP to 49.1% for HEMA and 15.5% for MDP, the degree of reductions in mean bond strength slightly increased (no significant difference). Therefore, the hypothesis that the alteration stage would not affect the bond durability of resin to dentin was accepted for TSB but was rejected for MBP. The observed reduction in mean bond strength appeared to be greater from 5.7% to 10.5%, as the alteration stages of MBP increased. The degree of reductions in dentin bond durability would be related to a decrease in the quality of the adhesive layer. This was possible, since the adhesive layer contains sufficient amounts of byproducts that induce the development of a bonding defect.

CONCLUSIONS

As per the respective manufacturer's instructions, the shelf life of TSB and MBP is 21 months when both adhesives are stored at 2°C-8°C. However, preventing alterations in TSB and MBP was not possible, even though both materials were stored in a refrigerated condition (8°C). However, when TSB and MBP were stored below 20°C for 14 weeks, the alteration rate of TSB and MBP were delayed, and the expected bond strength and bond durability were obtained at a similar level to what was observed with a TSB and MBP that was immediately used upon receipt, two days after being produced by the manufacturer (storage period: 0 day). The current study limited the testing period to 14 weeks; however, in actual applications, a longer period would better reflect real life conditions. In future studies, the authors of the current study would like to examine strength versus time conditions that may better reflect real life conditions. It is recommended that, even though a manufacturer may list a longer shelf life, in order to obtain the expected bond strengths of the resin to the tooth, one-step and two-step self-etch adhesives should be kept refrigerated when not in use, and equally important, self-etch adhesives should be utilized as quickly as possible upon receipt, since the pertinent historical information on the self-etch adhesive's shelf life is normally not clearly known, including when the primer was produced, how long it was stored and at what temperature the primer was stored.

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