Improved long-term bonding performance of an experimental all-in-one adhesive

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The aim of this study was to determine the performance of an experimental all-in-one adhesive. The adhesive, named MTB-200 (Kuraray Medical), contained components to enhance both bond strength and hydrophobicity. The performance of the adhesive was compared with that of CLEARFIL TRI-S BOND (Kuraray Medical) and BeautiBond (SHOFU) using micro-tensile bond strength test and ultramicroscopic observations. The study revealed that the new adhesive had the highest tensile strength value among the three adhesives over time, although transmission electron microscopic images showed the phenomenon of filler de-bonding in the adhesive resin layer. In spite of modification in the experimental adhesive, the adhesive was suspected to degrade bond performance. However, revision of the composition of adhesives would be one of the solutions to enhance durability of interface.

Keywords: Aging, Bond degradation, Dentin adhesive, Filler de-bonding, Micro-tensile bond strength

INTRODUCTION

Direct composite restoration that relies on an adhesive system had been used in daily practice under the concept of minimal intervention dentistry^{1,2)}. However, since an *in vivo* study revealed degradation of resin-dentin bonding, aging of the interface has been focused on³⁾. Many researchers reported infiltration into the adhesive layer. Fluid movement through potential leakage pathways within the hybrid layer was called nanoleakage and water-treeing⁴⁻⁶⁾. Filler de-bonding in ultra-morphology shows hydrolysis of the filler-matrix coupling⁷⁾. On the other hand, potential internal enzymes, kinds of matrix metalloproteinase, contribute to the breakdown of collagen matrices⁸⁾. Those organic or inorganic decompositions are reasons for degradation of resin-dentin bonding.

In the days when two-step adhesives were the main stream of dentin adhesives, many researchers thought that clinical adhesive failure occurred due to the multiple steps involved in adhesion⁹⁾. Efforts have therefore been made to reduce the number of steps. It has traditionally been explained that there are three important actions to the superficial dentin, etching, priming and bonding, in order for hybridization between dentin and resin to occur. Either “etch-and-rinse” or “self-etch” has been a strategy of two-step types of adhesive¹⁰⁾. Dentin adhesive systems that include all 3 actions in a one-step protocol have also become available¹¹⁾. However, one-step adhesives still have problems. One-step type is not to exceed multiple-step adhesives¹²⁾, and morphologic observation has revealed that HEMA-free adhesives give rise to phase separation^{13,14)} and that HEMA-containing adhesives cause hydrolysis overtime⁷⁾.

Although an adequate amount of HEMA improves the bond strength of a one-step adhesive¹⁵⁾, both incomplete polymerization and existence of water are suspected to be reasons for the degradation of adhering

with HEMA-containing adhesive⁷⁾, and the degradation are caused by ingredients of manufacture rather than manipulation errors¹⁶⁾.

A new adhesive includes a functional additive, sodium fluoride, is thought to prevent caries. The new adhesive contains hydrophilic dimethacrylate (a cross-linking monomer), a new photo-initiator for enhanced curing and a new hydrophobic methacrylate for reduced water absorption.

In this study, we investigated the new adhesive using the traditional method for dentin adhesion. The null hypothesis was that micro-tensile bond strength of the new adhesive was not significantly different from those of contemporary single-bottle type dentin adhesives.

MATERIALS AND METHODS

Materials used

Twelve human third molars were used in this study, and every four teeth were randomly assigned to each adhesive system. The protocol of this study was approved by the Ethics Committee of Hokkaido University (admission #2010-2). Three kinds of adhesives used in this study were shown in Table 1. The three adhesives included two commercial all-in-one self-etch adhesives, CLEARFIL TRI-S BOND (TriS: Kuraray Medical, Okayama, Japan), BeautiBond (BB: SHOFU, Kyoto, Japan) and an experimental adhesive, MTB-200 (MTB: Kuraray Medical, Okayama, Japan).

Protocol of tooth specimen preparation

1. Teeth

Extracted caries-free human third molars were stored in 0.5% Chloramine-T solution at 4 degrees Celsius for preventing bacterial growth for no longer than 3 months before they were used in this study.

Table 1 Adhesives used

Name	Composition
Batch No.	
Manufacturer	Application protocol
MTB-200	MDP, Bis-GMA, HEMA, Colloidal silica, Ethanol, Water, dl-Camphorquinone, Photo initiator, Hydrophilic aliphatic dimethacrylate, Hydrophobic aliphatic methacrylate, Photo polymerization accelerator, Chemical polymerization accelerator, Sodium fluoride, Others
[Lot: 90911]	
Kuraray Medical Inc., Okayama, Japan	<ol style="list-style-type: none"> 1. Apply bond to the cavity wall for 10 s 2. Dry with mild air for more than 5 s —Dry the entire cavity wall sufficiently until the bond does not move —Use a vacuum aspirator to prevent the bond from scattering 3. Light-cure for 10 s —Halogen light for 10 s or LED light for 5 s
CLEARFIL TRI-S BOND	MDP, Bis-GMA, HEMA, Colloidal silica, Ethanol, Water, dl-Camphorquinone, Initiators, Accelerators, Others
[Lot: 00144A]	
Kuraray Medical Inc., Okayama, Japan	<ol style="list-style-type: none"> 1. Apply bond to the cavity wall for 20 s 2. Dry with mild air for more than 5 s —Dry the entire cavity wall sufficiently until the bond does not move —Use a vacuum aspirator to prevent the bond from scattering 3. Light-cure for 10 s —Halogen light for 10 s or LED light for 5 s
BeautiBond	Bis-GMA, 4-MET, TEGDMA, 6MHPAc, Acetone, Pure water, Others
[Lot: 120815]	
SHOFU INC., Kyoto, Japan	<ol style="list-style-type: none"> 1. Apply bond and leave for 10 s 2. Air-dry —blow gently without scattering for 3 s and then blow more strongly 3. Light-cure —Halogen light for 10 s or LED light for 5 s

4-MET: 4-methacryloxyethyl trimellitic acid, 6MHPAc: 6-methacryloyloxyhexyl phosphonoacetate, Bis-GMA: bis-phenol-A-diglycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate, MDP: 10-methacryloxydecyl dihydrogen phosphate, TEGDMA: triethyleneglycol dimethacrylate.

2. Bonding procedure

Occlusal enamel was ground with a model trimmer (Model Trimmer; Morita, Tokyo, Japan) to expose dentin surface. They were finished polishing by #600-SiC paper for 60 s under running water to produce a flat dentin surface with a smear layer. Dentin surfaces were clinically dried by air flowing before adhesives were applied. After applying and irradiating for each adhesive, three or four layers of the resin composite, CLEARFIL AP-X A3 (Kuraray Medical, Okayama, Japan), were incrementally made on the surface to a height of 4 mm. Each layer was light-cured for 20 s. The bonded specimens were stored for 24 h in pure water at 37 degrees Celsius.

After storage in water for 24 h, teeth were fixed with a cyanoacrylate adhesive (Model Repair II Blue; Dentsply-Sankin, Otahara, Japan) on a platform and cut vertically into 1×1 mm “beamed square” sticks using a low-speed diamond saw (Isomet; Buehler, Lake Bluff, IL, USA) under water cooling, according to the non-

trimming technique.

3. Stress test with a PCR thermal cycler

In this study, specimens were stressed with a PCR thermal cycler¹⁷⁾. There were two protocols: no thermal stress (non-TC group) and stress with a thermal cycle of 20,000 times (TC20k group) by a programmed PCR thermal cycler (Mastercycler; Eppendorf, Germany).

Up to ten beams could be quarried out from each tooth. Half of the beams from a tooth were provided for the non-TC protocol, and the remaining specimens were provided for the TC20k protocol. Every two or three beams were put into a 5 microliter tube with an adequate volume of distilled water, and the tubes were set on the PCR thermal cycle machine. Thermal cycling was carried out with the programmed protocol at temperatures of 5 and 55 degrees Celsius for 30 s each. The computed thermal cycle program was run for approximately 8 weeks.

Micro-tensile bond strength test

Specimens were promptly employed micro-tensile bond strength test. Specimens for the non-TC group were tested immediately after cutting, and specimens for the TC20k group were tested after completion of the thermal cycle stress.

Then each stick was serially numbered and was measured in 0.1 mm order with a digital caliper (Absolute Digimatic; Mitutoyo Corp., Kawasaki, Japan). A specimen was mounted in the Ciucchi's jig¹⁸⁾ using cyanoacrylate adhesive. Each specimen was loaded in tension until failure at a crosshead speed of 1 mm/min in a universal testing machine (EZ test; Shimadzu, Kyoto, Japan). Load at failure was recorded in newtons and divided by the bonding surface area in square mm in order to calculate the bond strength in MPa. A total of twenty-eight or twenty-nine specimens (from four teeth) were used for each group.

After the tensile test, dentin side specimens were stored in a dry condition and the failure surfaces of specimens were sputter-coated with Pt-Pd for 120 s at an accelerating voltage of 0.4 kV (E-1030 Ion Sputter; HITACHI, Tokyo, Japan) and then observed using a field-emission scanning electron microscope (FE-SEM: S-4000; HITACHI, Tokyo, Japan) at an accelerating voltage of 10 kV. The mode of failure was determined with microscope at a magnification of $\times 80$ and recorded as 'cohesive failure in dentin', 'cohesive failure in resin', 'interfacial failure between adhesive and dentin', 'interfacial failure between bond and composite' or 'mixed failure'.

Statistical analysis of micro-tensile bond strength

The data obtained were expressed as MPa and statistically analyzed with one-way ANOVA and Duncan's multiple comparison test at the 5% level of significance. SPSS Statistics ver.17.0.0 (IBM Japan, Tokyo, Japan) was used for the statistical analysis.

TEM observation of cross-sectional dentin specimen after de-bonding

After observation of fractured specimens with FE-SEM, the specimens were observed with a transmission electron microscope (TEM) to analyze the resin-dentin interface. Specimen preparation for TEM followed for the standard procedures for ultramorphologic TEM examination of biological tissues. The specimens were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 1 min with the solution being changed three times. The specimens were then dehydrated in ascending grades of ethanol, 50%, 75%, 95% and 100%, for 10 min each, with two changes of every new solution. Then, the specimens were immersed in 1 by 1 absolute ethanol and epoxy resin (Poly/Bed 812 kit, Polyscience Inc., PA) for 8 h with rotation at 4 rpm (Taab Rotator type N, Taab Laboratories Eqt., Aldermaston, England), before removing the specimens and placing them in 100% epoxy embedding resin in new bottles for rotation for 3–4 h. The resin-infiltrated specimens were embedded in a silicone rubber mold filled with 100% epoxy resin.

Table 2 Micro-tensile bond strength to dentin in MPa

	MTB-200	CLEARFIL TRI-S BOND	BeautiBond
non-TC	72.5 (17.2) ^A <i>n</i> =28	64.8 (14.2) ^{A,B} <i>n</i> =28	31.0 (17.1) ^C <i>n</i> =28
20,000 thermal cycles	69.7 (19.3) ^A <i>n</i> =29	58.7 (12.9) ^B <i>n</i> =29	26.9 (14.8) ^C <i>n</i> =29

Mean (SD); *n*=total number of specimens.

No specimen failed before testing.

Values with the same superscript letter are not significantly different ($p>0.05$).

The epoxy resin blocks in the mold were polymerized in an oven at 60 degrees Celsius for more than 48 h.

In order to observe the fracture area, TEM specimens were sliced parallel to the long axis of teeth using a diamond knife (DiATOME, Bienne, Switzerland) and in an ultramicrotome (Ultracut, Leica, Vienna, Austria) to a thickness of about 90–100 nm and then examined by a transmission electron microscope (JEM-1400; JEOL, Tokyo, Japan) at an accelerating voltage of 80 kV.

RESULTS

Micro-tensile bond strength

Mean micro-tensile bond strength, standard deviation and total number of specimens per group are summarized in Table 2. In the condition of 20,000 thermal cycles, MTB showed a significantly higher value than that of TriS. BB, HEMA-free adhesive, showed a lower value than those of MTB and TriS, HEMA-containing adhesives. In the non-TC group, there was no significant difference between the tensile bond strength of the HEMA-containing adhesives ($p>0.05$). There was no pre-testing failure occurred in any of the specimens.

FE-SEM observation and failure mode classification

Typical images of the de-bonded surfaces with FE-SEM are shown in Fig. 1. Table 3 shows the failure modes of the specimens according to bonding agent, number of thermal cycles and fracture type. The trends of fracture mode were different between BB and the others. Almost all of the MTB and TriS specimens showed cohesive failure in adhesive resin. On the other hand, all of the BB specimens showed mixed failure.

TEM observation of cross-sectional dentin specimens after tensile tests

Typical images of the resin dentin-interface produced by each adhesive are shown in Fig. 2. Irrespective of thermal cycles, in MTB, TriS and BB, the fracture surface indicated by the Pt-Pb ion spatter coating appeared above the adhesive resin layer. Nano-fillers were originally contained in both MTB and TriS. It was observed that those fillers scattered all over the

Fig. 1 Field-emission scanning electron micrographs of de-bonded samples show their fractured surfaces. MTB-200 samples of both non-TC and TC20k show that fractures occur in an adhesive resin. CLEARFIL TRI-S BOND samples of both conditions also show cohesive failure in adhesive resin layer. De-bonded samples which BeautiBond has been applied show characteristic appearances. The appearance indicates an uneven resin effect to dentin or an uncompleted infiltration into the affected dentin. Abbreviations: AR, adhesive resin; D, dentin

Table 3 Failure mode

	Cohesive failure in dentin	Cohesive failure in adhesive resin	Interfacial failure between adhesive and dentin	Interfacial failure between bond and composite	Mixed failure
MTB-200 non-TC	0.0%	91.3%	0.0%	0.0%	8.7%
MTB-200 TC20k	0.0%	91.7%	0.0%	0.0%	8.3%
CLEARFIL TRI-S BOND non-TC	0.0%	92.6%	0.0%	0.0%	7.4%
CLEARFIL TRI-S BOND TC20k	0.0%	81.5%	0.0%	0.0%	18.5%
BeautiBond non-TC	0.0%	0.0%	0.0%	0.0%	100.0%
BeautiBond TC20k	0.0%	0.0%	0.0%	0.0%	100.0%

Fig. 2 Transmission electron micrographs of fractured micro-tensile bond strength samples show the dentin sides of fractured samples.

All samples are non-de-mineralized and non-stained. All of the TEM samples were sliced to approximately 90–100 nm in thickness.

(a) and (b) show a specimen prepared with MTB-200 and stored for one day. In (a), the adhesive layer includes widely distributing granules. The black line with sputtering ion indicates the fractured surface. A middle level transparency band exists between the unaffected dentin and adhesive layer. (b), which is a high magnification image of (a), shows nano-fillers distributing among the adhesive layer and hydroxyapatite crystals in the hybrid layer.

(c) and (d) show a specimen prepared with MTB-200 and stored with a PCR thermal cycler for 20,000 times. In (c), the image shows a plain adhesive layer without any granules. (d), which is a high magnification image of (c), shows a simple clear adhesive layer, but hydroxyapatite crystals can be seen.

(e) and (f) show a specimen prepared with CLEARFIL TRI-S BOND and stored for one day. In (e), the adhesive layer includes granules as in the image in (a). The unaffected dentin and mid-transparency band are shown. (f), which is a high magnification image of (e), shows nano-fillers distributing all around the adhesive layer and the existence of hydroxyapatite crystals in hybrid layer.

(g) and (h) show a specimen prepared with CLEARFIL TRI-S BOND and stored with a PCR thermal cycler for 20,000 times. In (g), the image shows a plain adhesive layer with no granules. (h), which is a high magnification image of (g), shows a simple adhesive layer, but hydroxyapatite crystals can be seen.

(i) and (j) show a specimen prepared with BeautiBond and stored for one day. In (i), the adhesive layer contains nothing. The black sputtering line is smoother than another bond. (j), which is a high magnification image of (i), shows a hybrid layer containing hydroxyapatite crystals beside of adhesive layer.

(k) and (l) show a specimen prepared with BeautiBond and stored with a PCR thermal cycler for 20,000 times. In (k), the image shows a plain adhesive layer. (l), which is a high magnification image of (l), shows a hybrid layer containing hydroxyapatite crystals.

Abbreviations: AR, adhesive resin; Ep, epoxy resin; Hy, hybrid layer; Ud, unaffected dentin.

remaining adhesive resin layer (MTB and TriS, non-TC). However, the TC20k group specimens of MTB and TriS did not show any fillers. In other words, the difference between images for the non-TC group and TC20k group was the appearance of filler. On the other hand, hydroxyapatite crystals remained scattered throughout the hybrid layer.

DISCUSSION

Water storage is a common technique for examining the long-term durability of a resin-dentin interface⁷. In addition, thermal cycle test shortens time passing for aging¹⁷. A programmed PCR thermal cycling process can regulate temperature and terms automatically.

A micro-tensile bond strength test can objectively express bonding performance. Since the proposal of this method for assessing adhesives¹⁹, it has been used worldwide. Human teeth specimens for tensile testing generally have some modified forms such as “hourglass shape^{3,20}”, “beamed square¹⁹” and “cylinder form”. In the present study, the form of specimen used was a “1×1 mm squared beam”. The form is relatively less sacrifice tooth structure than trimming type²¹. The most important benefit of the “beamed square” modification is that the beams can be divided into multiple condition groups since many specimens can be obtained from a single tooth²¹: specimens for initial bonding performance and others for long-term. The second important benefit of the “beamed square” modification is size. Small diameter of specimen accelerate aging model in a relatively short period of time²¹. A PCR thermal cycler provides not only a regulation of thermal cycle process but also acceleration for aging¹⁷.

MTB specimens in the TC20k group showed a significantly higher bonding value than that of TriS specimens in the same condition. Therefore, the null hypothesis that micro-tensile bond strength of the new adhesive is not significantly different from those of contemporary single-bottle type dentin adhesives was rejected. The concept both a new hydrophobic component and an improvement of polymerization would be effective for enhancing bonding performance. On the other hand, the stress condition of 20,000 thermal cycles for approximate 2 months did not diminish bond strength in the same adhesive, although many researchers reported that the bonding value of a dentin adhesive system would decrease in the long term. Therefore, the results of the micro-tensile bond strength tests did not indicate bonding degradation in the present study. However, in spite of the similar components in MTB and TriS, the results of micro-tensile bond strength test were differing between the two adhesives.

Failure modes of BB showed mixed failure which included both cohesive failures in adhesive resin and interfacial failure at the resin-dentin boundary in all specimens. On the other hands, almost of fracture formations of MTB and TriS were cohesive failure in adhesive resin and others were mixed failure. A trend of correlation between high bond strengths and rate

of cohesive failure in this study agreed that cohesive failure was thought to be associated with higher bond strengths^{22,23}.

Fluorine plays important roles in dentistry including prevention of caries and enhancement of tooth substrates. Glass ionomer cement is one of restoration materials that contain fluoride for the two purposes²⁴. Regarding, the new experimental adhesive evaluated in the present study, hydrophobic methacrylate monomer contains a fluorocarbon chain. That is, fluorocarbon-containing polymers have a low surface energy, which are highly hydrophobic²⁵. Organo-fluorine-rich resins can therefore provide inert polymers that excellently display hydrophobicity and resistance to softening by a wide range of chemicals²⁶.

FE-SEM and TEM observations are widely used for analyzing phenomena at the interface between an adhesive resin and tooth substrate. There have been many studies using electron microscopic observations to determine the mechanisms of bonding degradation²⁷⁻²⁹ since the phenomenon of nanoleakage was described for the first time in 1995^{4,30}. Bonding degradation *in vitro* is a result of affection related to water molecules.

After the adhering step, the adhesive layer would absorb water. These residual water molecules in the adhesive layer cause activation of enzymes and/or de-bonding of bonded-fillers. Those small collapses might cause a decrease in mechanical tensile bond strength. TEM images of filler de-bonding strongly suggest that polymer hydrolysis occurred due to water⁷.

Unfortunately, TEM images also support the possibility of hydrolytic reaction because the phenomenon of filler de-bonding was clearly shown. A difference in the thermal stress conditions might strongly correlate to the appearance of filler remaining or de-bonding in TEM images. That is, aging with the thermal cycle might lead to hydrolysis between fillers and adhesive resin⁷. Concerning each adhesive, MTB showed a trend to be degraded in the long term, although the adhesive was improved comparing to TriS in the situation of TC20k. Clinically, discoloration appeared more rapidly than retention loss as an adhesive failure³¹. MTB cannot escape from those water affections, despite the addition of hydrophobic molecules. Therefore, it is an unpredicted that hydrophobic molecule additives are effective for improving adhesive degradation. However, MTB would be more durable than TriS, because it shows better bonding strength after the thermal cycle test. Result showed that filler de-bonding appeared without reduction of bond strength. This suggested a difference of chemical resolution between resin-filler bond and polymer of resin matrix. Matrix resin polymer mainly would have resisted tensile strength force in this study.

Although HEMA improves the bond strength of a one-step self-etch adhesive¹⁵, the concept of HEMA-free is expected to escape from the influence of HEMA related to water. However, phase separation is often pointed out as a disadvantage of the HEMA-free type. On the other hand, HEMA brings solvents back into solution¹³. Droplets in adhesive layer are

morphologically observed in the HEMA-free and often exist on the dentin side of the adhesive layer^{13,14}. The absence of a resin substance might cause a decrease in mechanical tensile bond strength. Figure 1 shows a fracture surface of a de-bonded specimen to which BeautiBond was applied and was stored in distilled water for 24 h. An FE-SEM image of the fracture surface of the specimen bonded with BeautiBond exhibited a characteristic appearance of residual resin. The picture aroused the result of phase separation due to both biasing distribution of ingredients in adhesive droplets and non-uniform interactions with the dentin surface.

Direct resin composite restoration is one of the most widespread practical treatments. The prognosis strongly depends on the dentin adhesive system³². Therefore, the durability of bonds between adhesive resins and dentin is important for the stability of bonded restoration¹⁵. Despite great progress in adhesive systems, the resin-dentin interface is concerned to be the sensitive portion of conservative dentistry. But the challenge with function to cover already weak point that is hydrophilic rather than bond strength in present study would be one of direction to improve the credibility of interface between dentin and adhesive resin in future.

CONCLUSIONS

1. A new experimental all-in-one adhesive exhibited better bond strength than other commercially available adhesives used in this study.
2. Filler de-bonding occurred in the new adhesive overtime.

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