Role of 10-MDP in the dentin bond durability of universal adhesives in etch-&-rinse mode under different degradation conditions

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Contents

This thesis is based on the published articles listed below with additional data.

Hidari T, Takamizawa T, Imai A, Hirokane E, Ishii R, Tsujimoto A, Suzuki T, Miyazaki M (2020) Role of the functional monomer 10-methacryloyloxydecyl dihydrogen phosphate in dentin bond durability of universal adhesives in etch-&-rinse mode. Dent Mater J 39, 616-623.

Summary

A functional monomer 10-methacryloyloxydecyl dihydrogen phosphate (MDP) is a common component of most universal adhesives, which can be classified as self-etch (SE) adhesive systems, due to their being one-bottle adhesives and having similar compositions to those of single-step SE adhesives. However, universal adhesives have unique characteristics in that they can be used in etch-&-rinse (ER), selective etch, or SE mode. There are important open questions concerning the dentin bond durability of universal adhesives, as several studies found that the dentin bond performance of some universal adhesives did not decline even when phosphoric acid pre-etching was performed on dentin, unlike conventional single-step SE adhesives.

It is not clear why etching mode does not appear to influence dentin bond performance. In general, considering the chemical bonding between functional monomers and hydroxyapatite (HAp), dissolving the smear layer and the HAp on the dentin surface through phosphoric acid etching may reduce chemical interactions. Thus, given the known mechanism of MDP, it might be expected that the MDP containing adhesive little influence on SBS to dentin in ER mode. Therefore, it is important to investigate the dentin bond mechanism of universal adhesives in ER mode and the role of functional monomers when phosphoric acid pre-etching is performed on dentin. The aim of this study was to determine the dentin bond durability of two universal adhesives in ER mode, with and without MDP, under simulated *in vitro* degradation tests such as thermal cycling and long-term water storage.

The universal adhesive used was Clearfil Universal Bond Quick (CU, Kuraray Noritake Dental). An experimental adhesive made with the same ingredients as CU but excluding MDP (NM, Kuraray Noritake Dental) was also used. The phosphoric acid pre-etching agent employed was Ultra-Etch (Ultradent Products). Specimen preparation was

performed in accordance with ISO 29022. Extracted mandibular bovine incisors were used as substitutes for human teeth. The dentin bonding surfaces were polished using a water coolant and 320-grit SiC paper. Fifteen specimens were used for each test group to determine the shear bond strength (SBS) to dentin with phosphoric acid pre-etching (ER mode). In addition, CU in SE mode was also used as a comparison. Both adhesives were applied in accordance with the manufacturer's instruction for CU. A resin composite was inserted into the mold-enclosed assembly on the dentin surfaces for SBS, then light irradiated for 30 s with a visible-light curing unit.

The bonded specimens were subjected to thermal cycling (TC group) or storage in distilled water at 37°C for long term period (WS group). For the TC groups, bonded assemblies were stored in distilled water at 37°C for 24 h and were then treated with 5,000, 10,000, 20,000, or 30,000 TC between 5 and 55°C, with a dwell time of 30 s. For WS groups, bonded specimens were stored in distilled water at 37°C for 3-month, 6-month, or 1-year before the SBS tests. Baseline specimens were stored in distilled water at 37°C for 24 h before the SBS tests (baseline group). The bonded specimens were loaded to failure at 1.0 mm/min using a universal testing machine. The SBS values were calculated from the peak load at failure divided by the bonded surface area. After testing, the bonded tooth surfaces and resin composite cylinders were observed under an optical microscope at a magnification of ×10 to determine the failure mode. Representative treated dentin surfaces, resin/dentin interfaces, and de-bonded fracture sites were observed by field emission scanning electron microscopy (SEM).

Defining the baseline dentin SBS value for each tested adhesive as 100%, the SBS values under TC ranged from 92.4 to 99.6% in the CU in SE mode group, from 85.5 to 90.8% in the CU in ER mode group, and from 25.7 to 74.3% in the NM in ER mode group. When comparing CU in SE mode and ER mode, although CU in ER mode groups showed lower

mean SBS than in SE mode groups at all TC periods, there were no significant differences between them. Comparing CU in ER mode and NM in ER mode, CU showed significantly higher SBS values than NM, regardless of the TC period. CU did not show any significant differences in mean SBS between treatment groups, for either ER or SE modes. On the other hand, NM showed decreased SBS values with increased TC periods, while most of the TC groups showed significantly lower SBS values than the baseline groups.

Defining the baseline dentin SBS value for each tested adhesive as 100%, the SBS values under WS ranged from 110.9 to 113.0% in the CU in SE mode group, from 90.5 to 96.3% in the CU in ER mode group, and from 59.4 to 84.2% in the NM in ER mode group. Comparing CU in ER mode and NM in ER mode, CU had significantly higher SBS than NM, just as for the TC condition. CU did not show any significant differences in SBS between WS periods, regardless of the etching mode. However, CU in SE mode had significantly higher SBS than in ER mode, for all the WS periods. NM tended towards lower SBS with increased WS periods. The 1-year NM groups had significantly lower SBS values than the baseline groups.

For CU groups, although 10 to 20% of cohesive failure in dentin or mixed failure was observed, no significant differences were detected in failure modes between degradation conditions or periods. For NM groups, all the failure modes were adhesive failures.

From SEM observations, in the specimen treated with CU in SE mode, the smear layer was partially removed and some dentinal tubules with smear plugs were observed. On the other hand, no clear morphological differences were observed between the CU and NM specimens in ER mode. The smear layer was completely dissolved and opened dentinal tubules without smear plugs were clearly observed. For restorative/dentin interfaces, MDP containing CU showed excellent adaptation between dentin substrate and adhesive, regardless of etching mode. However, the morphological appearance of CU was notably different between SE and

ER modes. For the SE mode, the smear layer remained and formed a hybrid smear layer in which the resin monomers penetrated beyond the smear layer into intact dentin surface. In contrast, hybrid layers and formed resin tags were found in ER mode. NM showed a similar appearance to CU in ER mode. In the SEM observations of de-bonded specimens of resins after SBS testing, CU in SE and ER modes predominantly showed adhesive failure at lower magnification for the baseline groups. The higher magnification images showed similar failure features, with evidence of resin tags, cracks, and cleavages. Longer resin tags were seen for CU in the ER mode than the SE mode. NM in ER had longer and clearer resin tags than the other treated groups. For the WS treatments, although CU groups showed similar failure features to the baseline CU groups regardless of the etching mode, NM groups showed different morphological appearances when compared to each group of the baseline.

This laboratory study showed that although the ranges of SBS reductions were higher for CU in ER mode than CU in SE mode, the MDP-containing adhesive CU showed significantly higher dentin bond strength than the experimental MDP-free adhesive, irrespective of the degradation method. These results suggested that the functional monomer MDP in universal adhesives might play an important role in enhancing the dentin bond durability even when ER mode is used.

Introduction

Functional monomers in self-etch (SE) adhesives can serve various purposes, such as wetting, demineralization, and chemical interaction with tooth substances (1, 2). In particular, creating chemical bonds between mineralized tissues and functional monomers are important to secure immediate bond and enhance bond durability (3). Various types of functional monomers have been employed in different conventional two- or single-step SE adhesive systems, including phosphoric acid ester, carboxylic acid, and alcohol functional monomers (4). Among these functional monomers, 10-methacryloyloxydecyl dihydrogen phosphate (MDP) is one of the best for chemical bonding to hydroxyapatite (HAp) (5). MDP can create a calcium salt within a clinically realistic time and this chemical bonding showed stability even after ultrasonication (4). Furthermore, the formed calcium salts are hydrolytically stable, leading to long-term bond durability through self-assembled nano-layers at the MDP/HAp interface (6, 7). The benefits of MDP have already been demonstrated for bonding to enamel. Tsuchiya et al. (8) investigated the effect of MDP on the enamel bond durability of single-step SE adhesives by integrating fatigue testing and long-term water storage (WS). They concluded that although similar bond strength values were obtained for adhesives both with and without MDP in etch-&-rinse (ER) mode at 24 h WS, the MDP-containing adhesive showed significantly higher enamel bond strengths than the MDP-free adhesive after long term WS. In addition, some investigations have shown that MDP-containing adhesives can form an acid-base resistant zone, which plays a key role in the prevention of secondary caries, the sealing of restoration margins, and the promotion of restoration durability (9, 10).

MDP is a common component of most universal adhesives, which can be classified as SE adhesive systems, due to their being one-bottle adhesives and having similar compositions

to those of single-step SE adhesives. However, universal adhesives have unique characteristics in that they can be used in ER, selective etch, or SE mode (11–13). Several laboratory studies have found that the enamel and dentin bond performance of universal adhesives is similar to or better than those of conventional single-step SE adhesives, thanks to optimized adhesive composition and the adoption of MDP (13–15). There are important open questions concerning the dentin bond durability of universal adhesives, as several studies found that the dentin bond performance of some universal adhesives did not decline even when phosphoric acid pre-etching was performed on dentin, unlike conventional single-step SE adhesives (13, 15, 16).

It is not clear why etching mode does not appear to influence dentin bond performance. In general, considering the chemical bonding between functional monomers and HAp, dissolving the smear layer and the HAp on the dentin surface through phosphoric acid etching may reduce chemical interactions (17). In addition, in ER mode remnant collagen fibrils that are incompletely encapsulated by resin monomers may be vulnerable and thus more easily undergo structural deterioration due to hydrolytic degradation (18–20). Thus, given the known mechanism of MDP, it might be expected that the MDP containing adhesives little influence on shear bond strength (SBS) to dentin in ER mode. Therefore, it is important to investigate the dentin bond mechanism of universal adhesives in ER mode and the role of functional monomers when phosphoric acid pre-etching is performed on dentin.

Based on these considerations, the aim of this study was to determine the dentin bond durability of two universal adhesives in ER mode, with and without MDP, under *in vitro* degradation. Two different simulated degradation methods, thermal cycling (TC) and long-term WS, were applied before SBS testing. The null hypotheses to be tested were: (i) the functional monomer MDP would not affect the dentin bond strength after *in vitro*

degradation; and (ii) phosphoric acid pre-etching would not affect the dentin bond durability of the MDP-containing adhesive.

Materials and methods

Study materials

The materials used in this study are shown in Table 1. The universal adhesive used was Clearfil Universal Bond Quick (CU; Kuraray Noritake Dental, Tokyo, Japan). An experimental adhesive made with the same ingredients as CU but excluding MDP (NM; supplied by Kuraray Noritake Dental) was also used. The phosphoric acid pre-etching agent employed was Ultra-Etch (Ultradent Products, South Jordan, UT, USA). The resin composite used for bonding to dentin was Clearfil AP-X (Kuraray Noritake Dental). A halogen-quartz-tungsten visible light curing unit (Optilux 501, sds Kerr, Danbury, CT, USA) was used, and the light irradiance (average 600 mW/cm^2) of the curing unit was checked during the course of the experiment.

Specimen preparation

Specimen preparation was performed in accordance with ISO 29022 (21). Extracted mandibular bovine incisors stored frozen for up to 2 weeks were used as substitutes for human teeth. Approximately two-thirds of the apical root structure of each tooth was removed with a diamond-impregnated disk in a low-speed saw (IsoMet 1000 Precision Sectioning Saw, Buehler, Lake Bluff, IL, USA). The labial surfaces were ground with wet 240-grit silicon carbide (SiC) paper (Buehler) to create a flat dentin surface. Each tooth was then mounted in self-curing acrylic resin (Tray Resin II, Shofu, Kyoto, Japan) to expose the flattened area. The dentin bonding surfaces were polished using a water coolant and 320-grit SiC paper (Fuji Star Type DDC, Sankyo Rikagaku, Saitama, Japan).

Storage conditions and SBS tests

The experimental protocols for the bonding procedures are shown in Table 2. Fifteen specimens were used for each test group to determine the SBS to dentin in phosphoric acid pre-etching (ER mode). In addition, CU in SE mode was also used as a comparison. Both adhesives were applied in accordance with the manufacturer's instruction for CU. Following the bonding procedures, specimens were clamped in a Bonding Clamp (Ultradent Products) and a plastic mold of 2.38 mm internal diameter and 2.0 mm height was set in place. A condenser was used to insert the resin composite into the mold-enclosed assembly on the dentin surfaces for SBS, and the resin was then light irradiated for 30 s with the visible-light curing unit.

The bonded specimens were subjected to thermal cycling (TC group) or storage in distilled water at 37°C for long term period (WS group). For the TC groups, bonded specimens were stored in distilled water at 37°C for 24 h and were then subjected to 5,000, 10,000, 20,000, or 30,000 TC between 5 and 55°C, with a dwell time of 30 s. For WS groups, bonded specimens were stored in distilled water at 37°C for 3-month, 6-month, or 1-year before the SBS tests. The storage water, which did not contain any anti-bacterial agents and was maintained at 37°C, was changed every week during the course of each experiment. Baseline specimens were stored in distilled water at 37°C for 24 h before the SBS tests (baseline group).

The SBS was measured using a Test Base Clamp (Ultradent Products) and a Crosshead Assembly (Ultradent Products) as described by ISO 29022 (21). The bonded specimens were loaded to failure at 1.0 mm/min using a universal testing machine (Type 5500R, Instron, Norwood, MA, USA). The SBS values (in MPa) were calculated from the peak load at failure divided by the bonded surface area. After testing, the bonded tooth

surfaces and resin composite cylinders were observed under an optical microscope (SZH-131, Olympus, Tokyo, Japan) at a magnification of ×10 to determine the failure mode.

Scanning electron microscopy (SEM) observations

Representative treated dentin surfaces, resin/dentin interfaces, and de-bonded fracture sites were observed by field emission SEM (ERA8800FE, Elionix, Tokyo, Japan). Dentin surfaces were first treated in accordance with the experimental protocol for bonding procedures, then rinsed with acetone and water three times. For ultrastructural morphological observations of the restorative-dentin interfaces to determine the penetration of the adhesives, bonded specimens stored in 37ºC distilled water for 24 h were embedded in epoxy resin (Epon 812, Nissin EM, Tokyo, Japan) and then longitudinally sectioned using the low-speed saw (IsoMet 1000). The sectioned surfaces were polished to a high gloss with a sequence of SiC papers (Buehler) followed by diamond pastes down to 0.25 μm particle size (DP-Paste, Struers, Ballerup, Denmark). Treated surfaces and de-bonded fracture sites were prepared directly for SEM observations. All SEM specimens were dehydrated in ascending grades of *tert*-butyl alcohol (50% for 2 h, 75% for 2 h, 95% for 2 h, and 100% for 24 h), and then transferred to a freeze dryer (Model ID-3, Elionix) for 1 h. The resin/dentin interfaces of the specimens were subjected to argon-ion beam etching (EIS-200ER, Elionix) for 40 s. Finally, all SEM specimens were coated with a thin film of gold in a vacuum evaporator (Quick Coater, Type SC-701, Sanyu Electron, Tokyo, Japan). Observations were performed at an operating voltage of 10 kV.

Statistical analysis

The data for each group were tested for homogeneity of variance (Bartlett's test) and normal distribution (Kolmogorov-Smirnov test). To analyze the effect of factors on bond strength, two-way analysis of variance (ANOVA) and subsequent Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$) were used separately for analysis of the full data

sets for TC and WS groups (factors were as follows: 1) adhesive with or without MDP and 2) storage period), excluding the data for CU in SE mode. One-way ANOVA followed by Tukey's HSD test ($\alpha = 0.05$) was used to make comparisons within subsets of the data, as described later. The statistical analysis was performed using the statistical software (SigmaPlot ver. 11.0, SPSS, Chicago, IL, USA).

Results

Comparison of SBS after thermal cycle treatment

Results for the SBS under TC conditions are shown in Table 3. The two-way ANOVA revealed that the factor of MDP significantly influenced dentin SBS values ($p < 0.001$), but the number of thermal cycles did not influence them significantly ($p = 0.07$). The interaction between factors was not significant ($p = 0.998$). Defining the baseline dentin SBS value for each tested adhesive as 100%, the SBS values under TC ranged from 92.4 to 99.6% in the CU in SE mode group, from 85.5 to 90.8% in the CU in ER mode group, and from 25.7 to 74.3% in the NM in ER mode group (Table 3). When comparing CU in SE mode and ER mode, although CU in ER mode groups showed lower mean SBS than in SE mode groups at all TC periods, there were no significant differences between them. Comparing CU in ER mode and NM in ER mode, CU showed significantly higher SBS values than NM, regardless of the TC period. CU did not show any significant differences in mean SBS between treatment groups, for either ER or SE modes. On the other hand, NM showed decreased SBS values with increased TC periods, while most of the TC groups showed significantly lower SBS values than the baseline groups.

Comparison of SBS after WS treatment

The results for the SBS under WS conditions are shown in Table 4. Two-way ANOVA revealed that the both factors MDP ($p < 0.001$) and WS periods ($p < 0.001$) significantly influenced dentin SBS values. However, interaction between factors was not significant ($p = 0.61$). Defining the baseline dentin SBS value for each tested adhesive as 100%, the SBS values under WS ranged from 110.9 to 113.0% in the CU in SE mode group, from 90.5 to 96.3% in the CU in ER mode group, and from 59.4 to 84.2% in the NM in ER mode group (Table 4). Comparing CU in ER mode and NM in ER mode, CU had significantly higher SBS than NM, just as for the TC condition. CU did not show any significant differences in SBS between WS periods, regardless of the etching mode. However, CU in SE mode had significantly higher SBS than in ER mode, for all the WS periods. NM tended towards lower SBS with increased WS periods. The 1-year NM groups had significantly lower SBS values than the baseline groups.

Failure mode analysis of de-bonded specimens

A comparison of the frequencies of different failure modes between CU groups and NM groups under different degradation conditions is shown in Table 5. For CU groups, although 10 to 20% of cohesive failure in dentin or mixed failure was observed, no significant differences were detected in failure modes between degradation conditions or storage periods. For NM groups, all the failure modes were adhesive failures.

SEM observations

Representative SEM images of surfaces treated with the two adhesives in different etching modes are shown in Figs. 1–3. In the specimens treated with CU in SE mode, the smear layer was partially removed, and some dentinal tubules with smear plugs were observed (Fig. 1). On the other hand, no clear morphological differences were observed between the CU

and NM specimens in ER mode; the smear layer was completely dissolved and opened dentinal tubules without smear plugs were clearly observed (Figs. 2, 3).

Representative SEM images of restorative/dentin interfaces are shown in Figs. 4–6. MDP-containing CU showed excellent adaptation between dentin substrate and adhesive, regardless of etching mode (Figs. 4, 5). However, the morphological appearance of CU was notably different between SE and ER modes. For the SE mode, the smear layer remained and formed a hybrid smear layer in which the resin monomers penetrated beyond the smear layer into intact dentin surface (Fig. 4). In contrast, a hybrid layer of 1 to 2 μm and resin tags were found in ER mode (Fig. 5). NM showed a similar appearance to CU in ER mode (Fig. 6).

Representative SEM observations of de-bonded specimens of resins after SBS testing are shown in Fig. 7. For the baseline groups (Figs. 7A–7C), CU in SE and ER modes predominantly showed adhesive failure at lower magnification (Figs. 7Aa, 7Ba). The higher magnification images showed similar failure features, with evidence of resin tags, cracks, and cleavages (Figs. 7Ab, 7Bb). Longer resin tags were seen for CU in the ER mode than the SE mode. NM in ER had longer and clearer resin tags than the other treated groups (Fig. 7Cb). For the WS treatments (Fig. 7D–7F), although CU groups (Figs. 7D, 7E) showed similar failure features to the baseline CU groups regardless of the etching mode, NM groups (Fig. 7F) showed different morphological appearances when compared to each group of the baseline. In particular, NM at 1-year WS showed flatter detached surfaces and fewer resin tags than the baseline of NM. SEM images of the TC treatment groups were similar to those of the WS groups.

Discussion

For universal adhesives, it may be advantageous to select the optimal etching mode in accordance with cavity configuration, size, depth, and ratio of enamel and dentin in the cavity

(12, 13, 15, 22). However, little information is available about the role of functional monomers in the dentin bond durability of universal adhesives in ER mode. Therefore, an experimental adhesive made with the same ingredients as a commercial universal adhesive was used, but without MDP. The results of this study showed that the MDP-containing adhesive CU had significantly higher SBS than the experimental MDP-free adhesive NM, regardless of the etching mode or degradation method. When comparing the CU groups and NM group at 24 h, the CU groups showed bond strengths almost three times higher than those of NM group, while the differences in SBS values between the CU groups and NM group increased with increased degradation period for both test conditions employed in the present study. These results support the hypothesis that the functional monomer MDP does indeed affect dentin bond durability in ER mode after *in vitro* degradation, and making it possible to reject the first null hypothesis stated earlier. In order to evaluate deterioration status or to predict the longevity of resin composite restorations, clinical studies observing restored teeth are clearly the best method (23, 24). However, it is difficult to standardize clinical studies for many reasons (23–25). Therefore, it may be beneficial to establish test that simulate the oral environment and can obtain rapid outcomes and provide a standardized way to determine bond durability among materials, thus helping to predict expected clinical effectiveness (12, 14). Therefore, observing multiple different degradation methods and integrating the outcomes may be helpful in grasping the deterioration process that might occur under intra-oral conditions (25, 26).

Although there were no significant differences in SBS between CU in SE and ER modes at any thermal cycle periods, significant differences in SBS were observed between the two etching modes at all the WS periods. The differences between these degradation conditions might be caused by their different degradation mechanisms and progression rates. In the TC treatment, degradation is accelerated by differences in thermal expansion of the

materials composing the bonded interfaces (23). The discrepancies in thermal expansion between tooth substrate and adhesive can lead to cracks and percolations at bonded interfaces due to mechanical stress (26). On the other hand, degradation under the WS treatment is accelerated not only by hydrolysis of hydrophilic resin components, but also by degradation of collagen fibrils (27–29). CU in SE mode did not show any significant differences in SBS values between treatment periods in either treatment condition. However, when looking at each test condition of SBS values as a percentage, CU in SE mode under TC ranged from 92.4 to 99.6%, and under WS from 110.9 to 113.0%. The reasons for increased SBS in CU with SE mode in WS may be related to post-polymerization strengthening of the adhesive in the early phase and retarded degradation.

Comparing the TC and WS treatments, SBS at TC 5,000 was similar to that at WS 6-month, while the SBS at TC 10,000 was similar to that at WS 1-year (26). This is consistent with previous reports (26, 30). However, different trends in CU in ER mode were found, that is, the ranges of SBS reductions were higher for the ER mode than the SE mode, regardless of the degradation method. Hence, it can be inferred that although the functional monomer MDP played an important role in enhancing dentin bond durability in both SE and ER mode, dissolving the smear layer and the HAp in the dentin surface through pre-etching might be disadvantageous for early phase enhancement of chemical bonding and long-term dentin bond durability.

In this study, the commercially available universal adhesive CU was employed as a positive control. In order to assess the role of MDP, the adhesive was applied to the dentin surface for 10 s, followed by a medium air blow for 5 s. This universal adhesive can be used without any waiting time, requiring just air-blowing on the adhesive-applied surface (31, 32). A previous study showed that while reduced application time had a negative impact on enamel bond strength in some universal adhesives, CU did not show any significant

differences in enamel and dentin SBS after WS 24 h for different application times (33, 34). However, universal adhesives induce larger changes in surface free energy with prolonged application time, suggesting increased chemical interactions (33, 34). For NM in the present study (without MDP), a demineralization effect in SE mode would not be expected given its pH of 7.5, while no chemical bonding was expected due to the lack of a functional monomer. However, NM showed measurable SBS values under all the experimental conditions. NM contains HEMA and a newly developed amide monomer. Both of these compounds are highly hydrophilic and mobile as monomers, and the amide monomer can polymerize to form a hydrophobic polymer. Thus, it might be reasonable to suppose that the monomers penetrate deeply into the dentin structure and polymerize there, giving rise to strong micro-mechanical interlocking. This hypothesis was supported by the SEM images of the debonded surfaces, which appeared to show many resin tags that had partially pulled out of the dentinal tubules in the testing process.

In contrast to CU, the SBS of NM decreased significantly with longer storage period, regardless of the degradation method. Although CU in ER mode tended to have lower SBS at longer periods of degradation, there were no significant differences observed for either treatment. However, significant differences between etching modes for CU in the WS treatments were observed, suggesting that the second null hypothesis (that phosphoric acid pre-etching would not affect the dentin bonding durability of the MDP-containing adhesive) should be rejected. From the SEM observations of de-bonded specimens, although the baseline NM in ER showed longer and clearer resin tags than the other treated groups (Fig. 7Cb), flatter detached surfaces and fewer resin tags than the baseline were observed at 1-year WS (Fig. 7Fb). On the other hand, CU in ER mode at 1-year WS showed a similar failure appearance to that at 24 h baseline CU in ER, with evidence of resin tags, cracks, and

cleavages. The difference in SEM images between CU and NM in ER may also indicate the chemical bonding ability of MDP.

NM had sufficient ability to penetrate into demineralized dentin without creating a chemical bond to HAp, resulting in simple failure patterns. Therefore, in the case of CU in ER mode, it can be speculated that HEMA and the newly developed amide monomer might have the ability to transport sufficient resin monomers, and that the functional monomer MDP penetrated deeply into demineralized dentin and could form chemical bonds with HAp. A previous study explained the role of MDP in enhancing bond durability by observing that the MDP treated surface becomes hydrophobic because the methacrylate group of MDP is directed away from the surface, while the long carbon spacer group may cause neighboring MDP molecules to align parallel to each other as they form the water-insoluble MDP-Ca salt (35). Although the degradation process is different for TC and WS, in both cases hydrolytic degradation occurs in the vicinity of the interface between dentin and adhesive. Therefore, the water-resistant hydrophobic layer formed by MDP might contribute to bond durability (24, 36). The present study indicates that MDP plays an important role in enhancing the dentin bond durability of universal adhesive in ER mode. In particular, even when dentin surface was etched by phosphoric acid, the MDP containing adhesive CU could sustain bond strength regardless of the degradation method.

Conclusions

Within the limitations of this *in vitro* study, although the ranges of SBS reductions were higher for CU in ER mode than CU in SE mode, the MDP-containing adhesive tested in this study showed significantly higher dentin bond strength than the experimental MDP-free adhesive, irrespective of the degradation method. These suggested that the functional

monomer MDP in universal adhesives might play an important role in enhancing the dentin bond durability even when ER mode is used.

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Table 1 Materials used in this study

Code	Adhesive (Lot No.)	Main Components	pH	Manufacturer
CU	Clearfil Universal Bond Quick (4483016)	bis-GMA, MDP, HEMA, hydrophilic amide monomer, colloidal silica, ethanol, water, NaF, CQ, silane coupling agent, chemical polymerization accelerator	2.3 (0.03)*	Kuraray Noritake Dental, Tokyo, Japan
NM	Experimental Adhesive (451192)	bis-GMA, HEMA, hydrophilic amide monomer, colloidal silica, ethanol, water, NaF, CQ, silane coupling agent, chemical polymerization accelerator	7.5 (0.04)*	Kuraray Noritake Dental
	Pre-Etching Agent			
	Ultra-Etch (G017)	35% phosphoric acid		Ultradent Products, South Jordan, UT, USA

Resin composite		
Clearfil AP-X (9B0035)	bis-GMA, TEGDMA, silanated barium glass filler, silanated silica filler, silanated colloidal silica, catalysts, accelerators, CQ, pigments, others Filler load: 83.5 wt%	Kuraray Noritake Dental

bis-GMA: 2,2-bis[4-(2-hydroxy-3- methacryloyloxypropoxy) phenyl) propane, MDP: 10-methacryloyloxydecyl dihydrogen phosphate, HEMA: 2-hydroxyethyl methacrylate, CQ: *dl*-camphorquinone, TEGDMA: triethyleneglycol dimethacrylate.
* pH measurement was conducted five times for each adhesive using a compact pH meter (LAQUAtwin-pH-33, Horiba, Tokyo, Japan).

Table 2 Application protocol for pre-etching and self-etching adhesives

Method code	**Pre-etching protocol**
SE	Phosphoric acid etching was not performed.
ER	Dentin surface was phosphoric acid conditioned for 15 s. Conditioned surface was rinsed with water for 15 s (three-way dental syringe) and air-dried.
Adhesive	**Adhesive application protocol**
CU and NM	Adhesive was applied to the air-dried tooth surface for 10 s, followed by medium air pressure for 5 s. Adhesive was light-cured for 10 s.

Table 3 Influence of thermal cycling on SBS (MPa, SD)

	Baseline	**TC 5,000**	**TC 10,000**	**TC 20,000**	**TC 30,000**
CU-SE	33.0 (3.8)^{aA} [100%]	30.5 (3.5)^{aA} [92.4%]	32.9 (6.1)^{aA} [99.6%]	30.9 (6.1)^{aA} [93.6%]	31.2 (5.1)^{aA} [94.5%]
CU-ER	32.5 (5.4)^{aA} [100%]	29.5 (3.5)^{aA} [90.8%]	28.9 (4.9)^{aA} [88.9%]	29.0 (4.8)^{aA} [89.2%]	27.8 (4.2)^{aA} [85.5%]
NM-ER	10.1 (1.5)^{bA} [100%]	7.5 (2.8)^{bAB} [74.3%]	6.4 (1.5)^{bB} [63.4%]	5.2 (2.9)^{bBC} [51.5%]	2.6 (1.9)^{bC} [25.7%]

Same lower case letter in vertical column indicates no difference at a 5% significance level.
Same upper case letter in horizontal rows indicates no difference at a 5% significance level.
SBS, shear bond strength; SD, standard deviation; CU, Clearfil Universal Bond Quick; SE: self-etching mode; ER: etch & rinse mode; NM, MDP-free experimental adhesive based on CU.

Table 4 Influence of long-term water storage on SBS (MPa, SD)

	Baseline	**3-month**	**6-month**	**1-year**
CU-SE	33.0 (3.8)^{aA} [100%]	37.1 (5.0)^{aA} [112.4%]	36.6 (6.0)^{aA} [110.9%]	37.3 (5.3)^{aA} [113.0%]
CU-ER	32.5 (5.4)^{aA} [100%]	31.3 (5.9)^{bA} [96.3%]	30.7 (5.3)^{bA} [94.5%]	29.4 (4.9)^{bA} [90.5%]
NM-ER	10.1 (1.5)^{bA} [100%]	8.5 (3.5)^{bAB} [84.2%]	7.8 (1.3)^{cAB} [77.2%]	6.0 (1.7)^{cB} [59.4%]

Same lower case letter in vertical column indicates no difference at a 5% significance level.
Same upper case letter in horizontal rows indicates no difference at a 5% significance level.
SBS, shear bond strength; SD, standard deviation; CU, Clearfil Universal Bond Quick; SE: self-etching mode; ER: etch & rinse mode; NM, MDP-free experimental adhesive based on CU

Table 5 Failure mode analysis of de-bonded specimens

	Baseline	TC				WS		
	24 h	**TC 5,000**	**TC 10,000**	**TC 20,000**	**TC 30,000**	**3-month**	**6-month**	**1-year**
CU-SE	[80/0/10/10]	[80/0/10/10]	[70/0/10/20]	[80/0/10/10]	[80/0/0/20]	[80/0/20/0]	[80/0/10/10]	[90/0/0/10]
CU-ER	[70/0/20/10]	[80/0/10/10]	[70/0/10/20]	[80/0/10/10]	[80/0/10/10]	[70/0/20/10]	[80/0/10/10]	[90/0/0/10]
NM-ER	[100/0/0/0]	[100/0/0/0]	[100/0/0/0]	[100/0/0/0]	[100/0/0/0]	[100/0/0/0]	[100/0/0/0]	[100/0/0/0]

Failure mode is presented as the percentages of each failure mode (adhesive failure/cohesive failure in resin composite/cohesive failure in dentin/mixed failure).

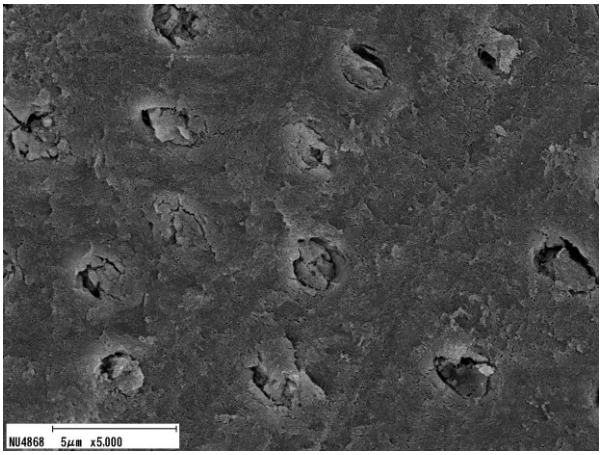

Fig. 1

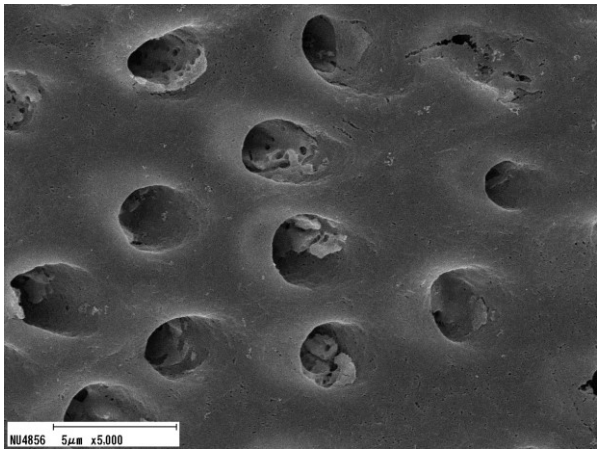

Fig. 2

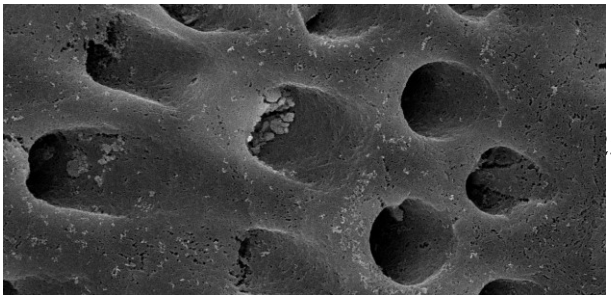

Fig. 3

Fig. 1 Representative SEM images of treated dentin surfaces. CU in SE mode at magnifications of 5,000×.

Fig. 2 Representative SEM images of treated dentin surfaces. CU in ER mode at magnifications of 5,000×.

Fig. 3 Representative SEM images of treated dentin surfaces. NM in ER mode at magnifications of 5,000×.

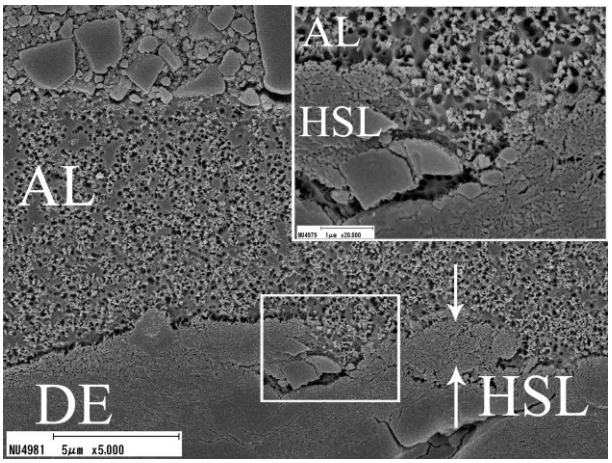

Fig. 4

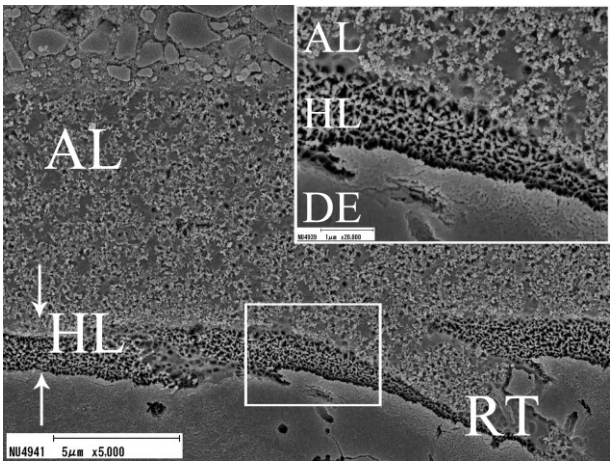

Fig. 5

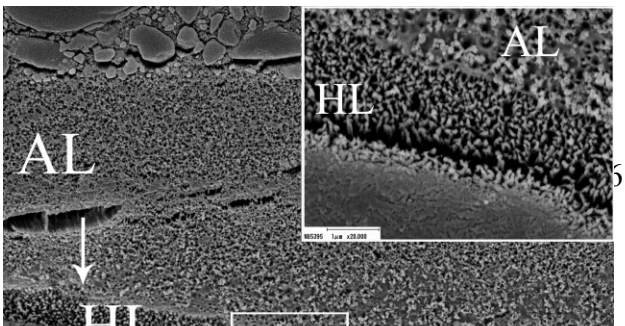

Fig. 6

Fig. 4 Representative SEM images of resin-dentin interfaces: CU in SE mode at magnifications of 5,000× and 20,000×. CU: MDP-containing universal adhesive (Clearfil Universal Bond Quick), SE: self-etching mode, AL: adhesive layer, DE: dentin, HSL: hybrid smear layer.

Fig. 5 Representative SEM images of resin-dentin interfaces: CU in ER mode at magnifications of 5,000× and 20,000×. CU: MDP-containing universal adhesive (Clearfil Universal Bond Quick), ER: etch-&-rinse mode. AL: adhesive layer, DE: dentin, RT: resin tag, HL: hybrid layer.

Fig. 6 Representative SEM images of resin-dentin interfaces: NM in ER mode at magnifications of 5,000× and 20,000×. NM: experimental MDP-free universal adhesive, ER: etch-&-rinse mode. AL: adhesive layer, DE: dentin, HL: hybrid layer.

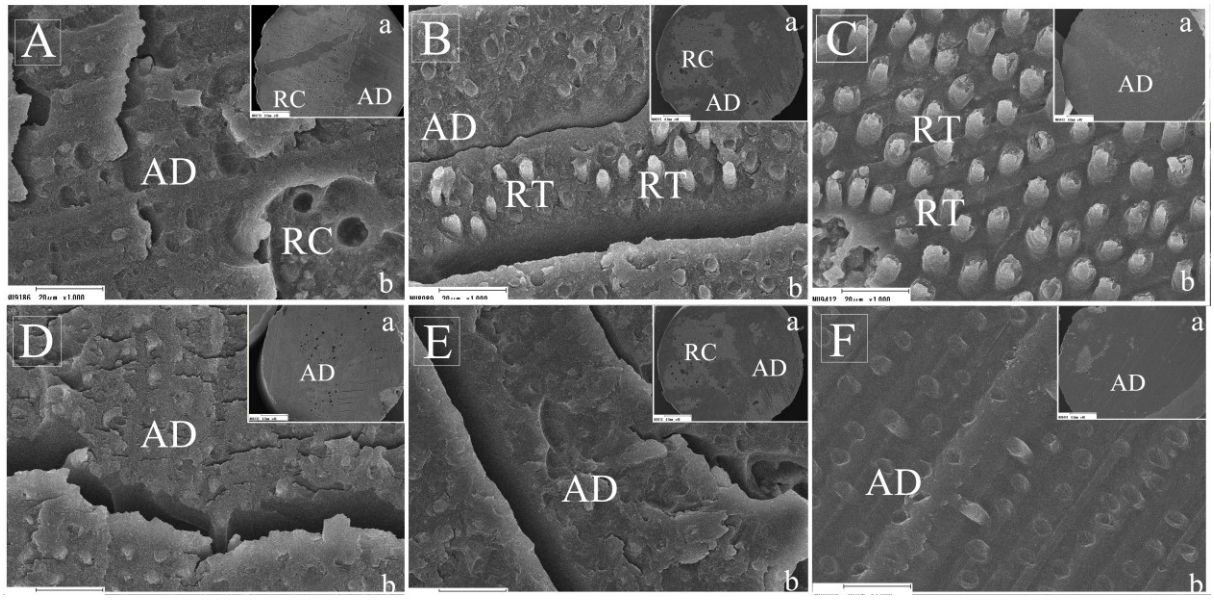

Fig. 7

Fig. 7 Representative SEM images of the de-bonded specimens. A: CU in SE mode at WS 24 h, (a) 40× and (b) 1,000×. B: CU in ER mode at WS 24 h, (a) 40× and (b) 1,000×. C: NM in ER mode at WS 24 h, (a) 40× and (b) 1,000×. D: CU in SE mode at WS 1-year, (a) 40× and (b) 1,000×. E: CU in ER mode at WS 1-year, (a) 40× and (b) 1,000×. F: NM in ER mode at WS 1-year, (a) 40× and (b) 1,000×. CU: MDP-containing universal adhesive (Clearfil Universal Bond Quick), NM: experimental MDP-free universal adhesive, SE: self-etching mode, ER: etch-&-rinse mode, RT: resin tag, RC: resin composite, AD: adhesive.