Improving the durability of resin-dentin bonds with an antibacterial monomer MDPB
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The 12-methacryloxydodecylpyridinium bromide (MDPB) has been reported to act as a matrix metalloprotease (MMP) inhibitor. In this study, the effects of application of MDPB on resin-dentin bonds were evaluated. The resin-dentin bonded specimens were prepared with a commercial MDPB-containing self-etching primer or a self-etching primer without MDPB, and stored 24 h or 1 year. Surfaces were pretreated with chlorhexidine or MDPB-containing cavity disinfectant. Additionally, we compared the degradation patterns between the two self-etching adhesives and etch and rinse system. Water tree formations were observed as the typical morphological phase of the two tested self-etching adhesives for both 24 h and 1 year groups. The degradation phase of collagen network depletion was observed in the adhesive interface of the etch-and-rinse system in the 1 year group. Pretreatment with chlorhexidine did not prevent bond strength reduction after 1 year. The cavity disinfectant improved the bond durability for the self-etching adhesive.

Keywords: MDPB, Bond strength, Durability, Resin adhesive

INTRODUCTION

Agents such as fluoride or silver ions have traditionally been incorporated into materials to introduce antibacterial properties¹²; however the antibacterial activity decreases over time and may adversely affect the material’s physical properties. Cationic compounds, such as chlorhexidine or cetlylpyridinium chloride, are widely used as antibacterial agents. The antibacterial monomer, 12-methacryloxydodecylpyridinium bromide (MDPB), is a polymerizable resin monomer synthesized by combining a quaternary ammonium compound, dodecylpyridinium bromide, with a methacryloyloxy group. MDPB, as a derivative of a quaternary ammonium compound, has strong antibacterial activity against various caries-related or endodontic pathogenic bacteria before polymerization³⁴. The antibacterial component in a polymer network is immobilized by polymerization of MDPB, and the immobilized antimicrobial agent does not leach out from cured resins. Clearfil SE Protect (Kuraray Noritake Dental, Tokyo, Japan) was the first commercialized antibacterial adhesive system. Employing an MDPB-containing antibacterial, self-etching primer, the system provides antibacterial effects even for long-term clinical use. Additionally, the bond strength of the resin-dentin bonds and the curing performance of the complex of the primer and bonding resin are not adversely affected by the addition of MDPB into the primer⁵⁷. An experimental cavity disinfectant containing 5% MDPB, developed, for use in various direct and indirect restorations⁹, has been shown to effectively eradicate bacteria in dentin without causing any adverse influence on the bonding ability of resinous luting cements.

Recently, several studies have reported that MDPB can be used to improve bonding durability by inhibiting matrix metalloproteases (MMPs) and cathepsins⁸¹⁰. MMPs have attracted widespread attention because of their possible role in decreasing durability of resin-dentin bonds¹¹¹². Dentinal MMPs may slowly modify the structure of the mineralized dentin in a normal physiological process. After restoration of the tooth with resin composite, the naked collagen network in the demineralized dentin zone (hybrid layer-mineralized dentin junction) is easily digested by MMPs, causing the bond strength to decrease significantly with age¹³¹⁴. Inhibition of MMPs is thought to improve the durability of the resin-tooth bond. We speculate that application of MDPB as a pretreatment of the dentin surface or as a component of the bonding resin monomer will improve the long-term durability of resin-dentin bonds. Therefore, the purpose of this study was to evaluate the effect of MDPB monomer on the longevity of resin-dentin bonds after storage in water for 1 year, using a microtensile bond test, fractography with a scanning electron microscope (SEM), and interfacial observation with a transmission electron microscope (TEM). The null hypothesis tested was that the incorporation of MDPB in self-etching primer or the experimental cavity disinfectant does not prevent the reduction of bond strength at the resin-dentin interface after aging for 1 year.

MATERIALS AND METHODS

Test tooth
Sound human premolars were obtained under a protocol approved by the Ethics Committee of the Dentistry of Osaka University (H25-E23). The crown was cut to obtain flat dentin surfaces using a low-speed diamond...
saw (Isomet saw, Buehler, Lake Bluff, IL, USA) under water cooling. Then the smear layer thickness was standardized by polishing the dentin surface with #600 SiC paper with copious water coolant.

### Bonding procedure for self-etching adhesives

Chemical formulations and bonding procedures for self-etching adhesives and cavity disinfectants (CD) are provided in Tables 1 and 2. The sectioned teeth were randomly divided into three groups, according to the dentin surface pretreatment for Clearfil SE Bond/SEB (Kuraray Noritake Dental): 1) application of distilled water for 30 s (control); 2) application of the experimental cavity disinfectant containing 5% MDPB for 30 s; and 3) application of 0.2% chlorhexidine diacetate (CHX) solution (CHX) for 30 s. Pretreatment for Clearfil SE Protect/PR (Kuraray Noritake Dental) consisted of application of distilled water for 30 s. Excess pretreatment solution was completely removed from the surface by strong air-blasting prior to bonding. The primer or bonding agent was applied according to the manufacturer’s instructions (Table 2). After the application of the bonding resin, a 5 mm high resin composite block (Clearfil APX, Kuraray Noritake Dental) was built up incrementally, and each increment was light cured for 40 s using a light-curing unit (Pencure 2000, Morita, Kyoto, Japan). The restored teeth were then stored in deionized water and kept in an incubator at 37°C for 24 h. We checked the distilled water once after 6 months. At that time, we checked the pH of the test water with litmus paper.

### Table 1  Materials used and their chemical formulations

| Cavity disinfectants | Chemical formulation |
|----------------------|----------------------|
| Experimental disinfectant containing MDPB/CD | MDPB (5%), ethanol (80%), water (15%) |
| Chlorhexidine diacetate/CHX | Chlorhexidine diacetate (0.2 %), water (99.8%) |

| Adhesives (manufacturer) | Chemical formulation |
|--------------------------|----------------------|
| Clearfil SE Bond/SEB (Kuraray Noritake Dental, Tokyo, Japan) | Primer: MDP, HEMA, DET, hydrophilic DMA, water Bond: MDP, Bis-GMA, HEMA, hydrophilic DMA, DET, silica fillers |
| Clearfil SE Protect/PR (Kuraray Noritake Dental) | Primer: MDP, MDPB, HEMA, DET, hydrophilic DMA, water Bond: MDP, Bis-GMA, HEMA, hydrophilic DMA, DET, silica fillers, surface treated sodium fluoride crystals |
| Adper Single Bond 2/SB2 (3M ESPE, St Paul, MN, USA) | Etchant: 37% phosphoric acid Primer/bond: Bis-GMA, HEMA, ethanol, water, polyalkenoic acid copolymer, dimethacrylate, amine, silica fillers |

*Each bonding resin contained photoinitiators.

Bis-GMA: bisphenol-A-diglycidyl methacrylate, DET: diethyltriamine, DMA: dimethylamine, HEMA: 2-hydroxyethyl methacrylate, MDP: 10-methacryloyloxydecyl dihydrogen phosphate, MDPB: 12-methacryloyloxydodecylpyridium bromide.
Bonding procedure for etch-and-rinse adhesives
The sectioned dentin was conditioned with 35% phosphoric acid (Scotchbond etchant, 3M ESPE, St Paul, MN, USA) for 15 s and then rinsed with deionized water. After blot drying, the dentin surfaces were treated as follows: 1) application of distilled water for 30 s (control); 2) application of CD for 30 s; or 3) application of CHX for 30 s. Excess pretreatment solution was removed from the surface with absorbent paper prior to bonding (wet-bonding technique). The bonding agent (Adper Single Bond 2/SB2, 3M ESPE) was applied according to the manufacturer’s instructions (Table 2). The resin composite block was built up as previously described. The restored teeth were stored in deionized water in an incubator at 37°C for 24 h. We changed the distilled water once after 6 months. At that time, we checked the pH of the test water with litmus paper.

Microtensile bond test and SEM fractography
After storage in water for 24 h, resin-dentin bonded beams (adhesive area: approximately 0.9 mm²) were cut with a high-precision cutting machine using a diamond saw (Isomet saw) under water cooling. The cross-sectional area of each beam was individually measured to calculate the adhesive area using a caliper (Model 500-144, Mitutoyo, Kawasaki, Japan). Three beams from each tooth were tested 24 h after the bonding procedure. The remaining specimens (three beams) were stored in distilled water at 37°C for 1 year. For each group (24 h or 1 year after bonding), the beams were fixed to a testing device with cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin, Tochigi, Japan) and subjected to a microtensile bond test in a mechanical testing machine (EZ-S, Shimadzu, Kyoto, Japan) at a crosshead speed of 1.0 mm/min. Two-way analysis of variance (ANOVA) and Tukey tests were used to analyze the effect of type of adhesive, dentin surface pretreatment, and storage period on bond strength (MPa). The significance level was 5% for all analyses (n=18 for each group). Immediately after testing, the debonded halves of each beam were dried and stored in closed receptacles at room temperature until analysis of the fractured surface was undertaken by SEM (JSM-6390BU, JEOL, Tokyo, Japan) at 80 kV. We categorized the fractured surface from upper to lower: resin composite, bonding resin (adhesive), hybrid layer, demineralized dentin, and mineralized dentin.

Nanoleakage analysis by TEM
In each group, four 0.9-mm-thick beams (kept in water for 24 h or 1 year) were randomly selected and immersed in 50 wt% ammoniacal AgNO₃ solution (pH 9.5) in darkness for 24 h according to the protocol of nanoleakage visualization described by Tay et al. After immersion in the tracer solution, followed by rinsing in deionized water, the slabs were immersed in photo-developing solution for 8 h under a fluorescent light to reduce silver ions into metallic silver grains. Each slab was fixed with 2.5% glutaraldehyde fixative and was dehydrated in an ascending ethanol series (60 to 100%). Subsequently, the slabs were embedded in epoxy resin (EPON 812, Taab, Reading, UK), and ultra-thin sections (100 nm) were prepared using a diamond knife on a microtome (Sorvall MT-5000, Du Pont, Wilmington, DE, USA). The sections were examined using a TEM (H-7100, Hitachi, Tokyo, Japan) at 80 kV.

RESULTS

Microtensile bond test
Bond strength results are shown in Fig. 1. No statistical significantly differences were found in the 24 h bond strength of SB2, SEB and PR (p>0.05). After aging for 1 year, statistically significant reductions in bond strength were found in the group of SB2 (CHX), SEB (water), and SEB (CHX) groups (p<0.05). However, no bond strength reductions were found in the SB2 (water and CD), SEB (CD), and PR (water) groups (p>0.05).

SEM fractography
Figure 2 shows the dentin side of the fractured surfaces of self-etching adhesives. No morphological differences were observed between 24 h and 1 year specimens. Additionally, there were no morphological differences among the 24 h specimens between the different surface treatments. Figure 3 shows the dentin side of the fractured surfaces of SB2. Many droplets were observed in the fractured adhesive layer as circles (A: water, 24 h) or voids (C: CHX, 24 h). A fractured region of demineralized dentin was located at the demineralized-mineralized dentin junction (E and F: CHX, 1 year). The orifice of the side branches of the dentinal tubules...
Fig. 2 SEM images of the fractured surface of self-etching adhesives.
No morphological differences were observed in the fractured surfaces between the 24 h and 1 year specimens. The fractures were mainly localized at the border between the top of hybrid layer and the bonding resin layer. A: (SEB, water, 24 h), B: (SEB, water, 1 year), C: (SEB, CD, 1 year), D: (SEB, CHX, 1 year), E: (PR, water, 24 h), F: (PR, water, 1 year). All images were taken at the same magnification.
R: resin adhesive

was widened in the 1-year group of CHX (F). The same morphological phase (demineralized dentin zone and widened orifice of the lateral branches) was found in 1 year water storage group (not shown). However, this morphological phase could not be found in the 24 h specimens.

Fig. 3 SEM images of the fractured surface of SB2. Many droplets were observed in the fractured bonding resin layer (A and C). Fracture at the demineralized dentin was typically observed in 1 year specimens (F). The widened orifices of the lateral branches of the dentinal tubules were frequently observed in 1 year specimens (F). However, these morphological phase cannot be found in 24 h specimens. The white arrows show droplets in the fractured resin (A and C). The black arrows show the widened orifices of the lateral branches of the dentin tubules (F).
A: (water, 24 h), B: (CD, 24 h), C: (CHX, 24 h), D: (water, 1 year), E: (CHX, 1 year), F: (CHX, 1 year).
DD: demineralized dentin, R: resin adhesive

TEM images
Figure 4 shows the resin-dentin interface of silver-impregnated, unstained, and undemineralized specimens for SEB and PR. Silver deposits were observed in the hybrid layer of all test groups of self-etching adhesives. Water tree formations were observed from the dentin surface through to the adhesive layer (A, C, and F). The hybrid layer was less than 1 µm thick. Figure 5 shows the resin-dentin interfaces of silver-impregnated, unstained, and undemineralized dentin (for SB2). The hybrid layer was 3–5 µm thick. Heavy silver deposits were observed in the hybrid layer. Many water droplets caused by phase separation were observed at the bonding resin layer (A, black arrow). Figure 5F shows the hydrogel layers of carboxylic acid between the bonding resin and hybrid layers (white arrow). We did not find a clear degradation phase, even in the aged specimens.

DISCUSSION
In Fig. 1, the experimental cavity disinfectant containing MDPB (CD) effectively prevented the reduction of bond strength in SEB specimens after immersion in water for 1 year. However, it is not clear whether this effect also occurred in SB2 specimens because no bond-strength reduction was observed in the control group with the immersion in water. In addition, the PR adhesive system with MDPB-containing primer and NaF-containing adhesive prevented bond strength reduction. However, significant reduction in bond strength was found after storage for 1 year in water in the control specimens of SEB (without MDPB). Therefore, the null hypothesis that the incorporation of MDPB in the self-etching
Fig. 4 TEM images of unstained, non-demineralized sections of the resin-dentin interface of specimens with self-etching primer adhesives. The specimens were stained with ammoniacal silver nitrate solutions for nanoleakage evaluation. A: (SEB, water, 24 h), B: (SEB, CD, 24 h), C: (SEB, water, 1 year), D: (SEB, CD, 1 year), E: (PR, water, 24 h), F: (PR, water, 1 year). Water tree formations were found in all self-etching adhesive specimens. The black arrows in A and F show water trees. Silver deposits could be identified in the hybrid layer. These deposits were found even in the 24 h bonding specimens. Numerous filler particles were found in resin adhesive layer as nano-sized gray deposits. H: approximately 0.5 µm thick hybrid layer, D: demineralized dentin, R: resin adhesive primer or experimental cavity disinfectant does not prevent the reduction of bond strength at the resin-dentin interface after aging for 1 year, has been rejected for the self-etching adhesive system.

Collagen hydrolysis has been found to occur over time at the resin-dentin bonded interface in previous in vitro and in vivo studies\textsuperscript{12,16,17}. In 2004, a study attributed this collagen hydrolysis to the action of host-derived dentinal MMPs\textsuperscript{11}. In recent years, the MMP concerned with degradation has gained widespread attention in the research field of resin-dentin bonds. MMPs are a class of zinc and calcium dependent endopeptidases. MMP are trapped in dentin during the tooth development process. When the dentin is treated with various agents of adhesive resin systems, the MMPs are released and activated during the

Fig. 5 TEM images of unstained, non-demineralized sections of the resin-dentin interface of SB2 specimens. The specimens were stained with ammoniacal silver nitrate solutions for nanoleakage evaluation. In D and F, the silver deposits were predominantly located along the bottom of the hybrid layer. Water tree formations were not observed in SB2 specimens. Silver deposits were mainly found in the hybrid layer, not in the adhesive resin layer. Hydrogel droplets of carboxylic acid were found as circle shapes in the adhesive resin layer (D: white arrow) and as a layer at the top of hybrid layer (F: white arrow). A: (water, 24 h), B: (CD, 24 h), C: (water, 1 year), D and E: (CHX, 1 year), F: (CD, 1 year). The black arrow in A shows a droplet caused by phase separation. H: 3–5 µm thick hybrid layer, D: dentin, R: resin adhesive.
bonding procedure\textsuperscript{18}. A recent study measuring loss of dentin mass and using three-point flexure tests and hydroxyproline assays has shown that antibacterial monomers of dimethylaminododecyl methacrylate (DMADDM) and MDPB inhibit dentinal MMP\textsuperscript{20,19}. These monomers are cationic, while both mineralized and demineralized dentin substrates have net anionic charges. Therefore, they can bind electrostatically to dentinal collagen that may alter the configuration of the active site of MMPs so they are unable\textsuperscript{9,10} to accept collagen fibrils. However, further studies are required to clarify the detailed mechanism of the interaction between MMPs and monomers.

The self-etching systems (Clearfil SE Bond and Clearfil SE Protect) exhibited no significant morphological differences between 24 h and 1 year specimens under SEM fractography. In the 1 year SB2 specimens, a demineralized dentin zone was found in the fractured surface (Fig. 3). A hybrid layer was formed with the infiltration of the resin monomer into the dentinal collagen network. Many studies have shown that a demineralized dentin zone is formed between the hybrid layer and the mineralized dentin in etch-and-rinse adhesive systems\textsuperscript{11,12}. The demineralized dentin zone is a naked collagen layer created where the resin has failed to infiltrate into collagen network. Activation of MMPs in this demineralized dentin zone induces hydrolysis of exposed collagen fibrils over the long-term. The lateral branches of the dentinal tubules observed on the debonded surfaces in the water-1 year and CHX-1 year groups of the etch-and-rinse system might be morphological evidence of collagen hydrolysis within the demineralized dentin zone of the resin-dentin bond (Fig. 3F). Lateral branch of dentinal tubules are normally present in human dentin. However, the diameter of lateral branch is generally small in the fractured dentin or fractured hybrid layer. In our previous study, the lateral branches were enlarged on dentin surface when treated with sodium hypochlorite solution after conditioning with maleic acid, more than did the dentinal tubules\textsuperscript{12}. The peritubular dentin of lateral branches would be more susceptible to organic removing agents than that of dentinal tubules\textsuperscript{12}. It is speculated that hydrolysis increased the diameter of lateral branches of dentinal tubule in the naked collagen layer of the demineralized dentin zone\textsuperscript{19}. This degradation pattern is not directly observable in the fractured surfaces of the 24 h specimens. The lateral branches of the dentinal tubule in the naked collagen layer of the demineralized dentin zone are more easily created in etch-and-rinse adhesives than in self-etching adhesives because this zone is formed by the discrepancy between the depth of the acid etched zone and the degree of resin monomer impregnation. The acidic monomer of the self-etching system demineralizes dentin without rinsing with water after application. It is well known that demineralization of dentin and resin impregnation occur simultaneously preventing the formation of a naked collagen layer in the bonds. However, in this study, such degradation did not induce a reduction in the bond strength in SB2 specimens, even after 1 year of water storage (Fig. 1). Although many previous reports have shown a micromorphological phase of collagen hydrolysis under TEM observation\textsuperscript{11,15,18}, this degradation phase was not observed in our TEM images. It is speculated that the TEM specimens with a degraded interface, such as collagen degradation or major nanoleakage, are likely to have failed at the interface during the specimen preparation process with a microtome or the high vacuum chamber of the TEM. Hence severe defects in the bonds could not be observed by TEM examination. A study of clinical data for class V resin restorations concluded that two-step self-etching and three-step etch and rinse adhesive are preferred over one-step self-etching adhesives\textsuperscript{19}. This clinical finding can be explained in terms not only of collagen hydrolysis but also of resin hydrolysis. In this study, many defects in the adhesive resin (droplets and void formation) were observed in both 24 h and 1 year SB2 specimens. These defects are caused by phase separation between the water and resin, or the gel phase formation of polyalkenoic acid\textsuperscript{20}. A study of nanoleakage expression found that silver tracer depositions increased in size and density within the bonding resin after 1 year of water storage\textsuperscript{21}. This may be attributed to the initial water trap and subsequent nanoleakage propagation by hydrolysis within the bonding resin. However, we did not observe nanoleakage propagation of SB2 under SEM and TEM examination. Additionally, although typical water tree formations were found in the 24 h and 1 year specimens of the self-etch systems (SEB and PR), there was no extension in size and density after 1 year. Further studies on the specificity of the degradation of resin material are warranted.

Chlorhexidine is well known as an antibacterial agent, and is used for various purposes in dentistry. Since CHX is an MMP inhibitor, the surface pretreatment with CHX is thought to improve the longevity of bonds. CHX is an amphiphilic molecule that may bind the catalytic site (Ca\textsuperscript{2+} or Zn\textsuperscript{2+}) of MMPs by a cation-chelating mechanism. It inhibits MMPs at very low concentrations\textsuperscript{19}. However, adverse effects were found in the bond strength of SB2 specimens after 1 year of water storage (Fig. 1). During the bonding process, CHX remains bound to the dentin surface; therefore, this binding is responsible for the long-term efficacy as an MMP inhibitor in the bond interface\textsuperscript{22}. Several studies have shown that after pretreatment with CHX, bonding properties decreased after storage in water for 24 h storage\textsuperscript{23,24}. It has been speculated that pretreatment with CHX inhibits polymerization of the bonding resin and increases water sorption\textsuperscript{24}. This incomplete polymerization may accelerate the hydrolysis of the bond structure. Similar results for the bond test were found for the self-etching adhesive, SEB.

MDPB-containing primer is thought to inhibit the degradation of bonding after immersion in water for 1 year because no reduction in bond strength was found in PR specimens. Similar results were also reported in a previous study\textsuperscript{25}. The CHX also inhibited the MMP activity of the bonds, resulting in improved long-term...
bonding performance. However, the incorporation of CHX is considered to be an agent-releasing material, the MMP inhibition effect, and the physical properties of the resin, decreased over time. However, the antibacterial monomer MDPB is a non-agent releasing material because the immobilized MDPB does not leach out from the cured resin. This mechanism overcomes the disadvantage of agent-releasing materials. Nawareg et al. investigated the long-term durability of resin-dentin bonds to evaluate CHX-methacrylate and CHX-digluconate as surface treatments. They found that the effect of CHX-methacrylate lasted much longer than that of CHX-digluconate because CHX-methacrylate was able to copolymerize with other monomers of the adhesive resin. Because the salt of CHX used in this study is immiscible with resin monomers, the presence of CHX decreased the physical properties of the bonding resin or decreased the bond strength. Therefore, the chemical structure of MDPB or CHX-methacrylate is favored as a component of the adhesive resin system for long-term clinical use. However, it has a possibility that chemical components of 10-MDP or NaF modify the dentin surface that contributes to the overall resin-dentin bond durability. The results of one study have shown that fluoride-releasing adhesive could improve the durability of dentin bonds. Additionally, it has been reported that fluoride has the potential to prevent collagen degradation as and MMP-inhibitor. In the Clearfil SE Protect system, the primer contains MDPB and the adhesive contains NaF crystals, which can release fluoride for a long-time. Therefore, further research is required to evaluate the effect of chemical modification of dentin surface on the bonding mechanism or the durability.

CONCLUSIONS

Chlorhexidine pretreatment did not prevent bond strength reduction after 1 year. However, the experimental cavity disinfectant improves the longevity of resin-dentin bonds. There is a trend towards developing dental materials with MDPB as an MMP inhibitor.

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