Development of Bonding System for Resin Core Construction by MωA Adhesive

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We designed self-etching primers consisting of a series of four N-methacryloyl-ω-amino acids, MωA, of different methylene chain numbers for resin core construction. The interacted amount of MωA’s carboxylic acid with root or crown dentin apatite was determined, and its effects on dentin bond durability examined.

The addition of both dentin particles to the MωA solutions caused the carbonyl carbon peak of carboxylic acid in MωA to shift to a lower field, chiefly because of an acid-base interaction between carboxylic acid and calcium. Then, as the pKa value of MωA’s carboxylic acid increased, the amount of carboxylic acid that interacted with calcium decreased. In terms of dentin bonding durability, the four tested MωA adhesives provided noticeably higher bond strengths of resin to root or crown dentin than ED Primer II. Therefore, from the perspective of restoring pulless teeth with minimal intervention, carboxylated MωA adhesives seemed to be very useful as functional monomers for self-etching primers.

Key words: Self-etching primer, N-methacryloyl-ω-acid, Dentin bond durability

INTRODUCTION

Dual-cure composite resins have been developed for use in resin core constructions1,2. This technique is very useful because it minimizes the removal of sound tooth structure by air turbine. In addition, dual-cure composite resins provide good color matching and possess mechanical properties similar to human teeth.

To reinforce pulless teeth with the use of core resin, it is necessary to bond the core resin to root and/or crown dentin by means of acid etching. To avoid over-etching of the dentin, self-etching adhesive systems are utilized since they limit the decalcification of dentin apatite as compared with phosphoric acid etching3. It should be mentioned that as the thickness in the demineralized collagenous layer increases, so does the probability of the formation of a demineralized dentin zone — where bonding resin monomer has not diffused into and permeated the demineralized collagenous layer4,5. Consequently, the presence of a demineralized dentin zone could affect the durability of the dentin bond6–8.

With a Superbond bonding system, Masuda8 examined how the conditioning time of root and crown dentins affected resin bond strength. The optimum conditioning time, which provided the maximum bond strength, differed between root and crown dentins. This difference was caused by differences in hydroxyapatite content and in the thickness of dentinal tubules between root and crown dentins.

Previously, we synthesized methacrylamide monomer derivatives, N-methacryloyl-ω-aminoalkyl acids (MωA), for the development of MωA self-etching adhesives for resin restorations. These monomers were employed because the amide portion in the MωA structure is hydrolytically more stable than the ester portion in the methacrylester monomer9. It was found that although the amount of decalcified dentin apatite by MωA’s carboxylic acid was limited, the MωA adhesives provided noticeably high resin-dentin bond strengths, exceeding 20 MPa10.

In this study, with a view to developing a total conditioning system for both root and crown dentins, we designed four MωA self-etching adhesives for resin core construction. The interacted amount of MωA’s carboxylic acid with root or crown dentin apatite was determined. Likewise, the shear bond strength of resin to both dentins — which were conditioned by MωA adhesives — was measured before and after thermocycling. The effect of the interacted amount of MωA’s carboxylic acid on dentin bond durability was then discussed.

MATERIALS AND METHODS

Materials

A series of four MωA derivatives of different methylene chain numbers were synthesized by condensing methacrylic chloride to the amine of ω-alkylamino acids, as shown in Fig. 1. The numbers of methylene chain, as a spacer group, were I: N-

A bovine tooth was cross-sectioned by a low-speed diamond cutter under water cooling to separate the root and crown. After removing the pulp and cementum, root dentin was cut by using an air turbine with a diamond bur under water cooling. Root dentin particles were then obtained by decanting the collected cooling water, rinsed with water, and air-dried at 20°C. Crown dentin particles were also prepared by using the same procedure after removing the pulp and enamel.

Reactivity of carboxylic acid in M_ωA on root and crown dentin apatites

Five mol% of each M_ωA was dissolved in 20% aqueous deuterium oxide solution. Following which, deuterium oxide was used as the nuclear magnetic resonance (NMR) solvent. Next, 60 mg of root or crown dentin particles was suspended in 600 mg of each of the four M_ωA solutions. After each suspension’s pH value was stabilized (approximately a few of minutes later), the pH value was measured. Then, with or without dentin, each suspension’s ^13C NMR spectrum was observed using an EX270 spectrometer (JEOL, Tokyo, Japan).

A 45° pulse was used for NMR observation, with accumulation and repetition times set at 800 scans and 3.8 seconds respectively. Hexamethyldisiloxane (HMDSO) was used as an external reference. With NMR peak of tetramethylsilane (TMS) as an internal reference, chemical shift was expressed in ppm and chemical shift resolution of NMR peak was 0.009 ppm. Shift differences in the carbonyl carbon peak of carboxylic acid in M_ωA derivatives were then determined, before and after the addition of root or crown dentin. This was because the chemical shift of the carbonyl carbon peak of each M_ωA’s carboxylic acid obtained from 5 mol% M_ωA aqueous solution was the same as that when hydrochloric acid was added to 5 mol% M_ωA aqueous solution. This experiment was conducted twice for each M_ωA derivative.

Measurement of shear bond strength

A bovine tooth was cross-sectioned by a low-speed diamond cutter under water cooling to separate the root and crown. After removing the pulp, the root or crown was further cross-sectioned at the center of the pulp cavity using a low-speed diamond cutter under water cooling. The cross-sectioned root or crown dentin was then molded with a self-cured pour resin (Shofu Inc., Kyoto, Japan). To obtain a fresh dentin surface where the removed pulp had existed, the molded root or crown dentin was grounded with a sequence of silicon carbide papers, 100-, 800-, and 1000-grit, under water irrigation. A ring film, with an internal diameter of 2.0 mm and a thickness of 50 μm, was mounted onto both ground surfaces to define the adhesive area of resin.

Dentin surface inside the ring was conditioned with a 5 mol% M_ωA aqueous solution for 30 seconds and then air-dried for 10 seconds. A polyethylene tube, with an internal diameter of 2.0 mm and a height of 1.5 mm, was placed onto the ring.
film. Clearfil Photo-Bond (Kuraray Medical Inc., Tokyo, Japan) was applied to the conditioned dentin surface, air-blown for five seconds, and photocured for 10 seconds by using Curing light XL3000 (3M Dental Products, MN, Germany). Clearfil DC Core Paste (Kuraray Medical Inc., Tokyo, Japan) was immediately filled into the inside of the polyethylene tube and photocured for 20 seconds. The tube and film were then removed, and the specimens were stored in water at 37°C.

After one day, the samples were thermocycled between 4 and 60°C water baths at 10,000 times in each bath. Immersion time was 60 seconds for each bath. The specimens were then positioned on a shear testing machine (TG-5KN, Minebea, Nagano, Japan). Shear bond strength of resin to the root or crown dentin was measured under a crosshead speed of 1 mm/min, both before and after thermocycling. Number of specimens for each experiment was 10. For controls, both dentins were conditioned by ED Primer II (Kuraray Medical Inc., Tokyo, Japan), and the bond strength of resin to both dentins thereby measured.

The averaged bond strength and standard deviation (SD) of bond strength were calculated for each MωA adhesive. Results were analyzed by three-way analysis of variance (ANOVA) and post-hoc Fisher’s protected LSD test. Value of statistical significance was set at 0.05 level.

**RESULTS**

**Decalcification of root and crown dentins by carboxylic acid in MωA**

Table 1 shows the chemical shifts of carbonyl carbon peak of carboxylic acid in the MωA derivatives, as well as each MωA solution’s pH values before and after the addition of root or crown dentin. The addition of both dentin particles to the MωA solutions caused the carbonyl carbon peak to shift to a lower field as well as to lead to an increase in pH value. These effects were due to an acid-base interaction between MωA’s carboxylic acid and calcium through the decalcification of root or crown dentin apatite. However, we did not observe any decreases in intensity in the 13C NMR peaks attributed to the carbons constituting the MωA structure. This finding suggested that the MωA derivatives formed a water-soluble calcium salt.

Table 2 shows the amounts of carboxylic acid in MωA derivatives that interacted with calcium. There, the amount of interacted carboxylic acid was derived by dividing the shift difference of carbonyl carbon peak (Table 1) by the shift differential of carbonyl carbon peaks attributed to the completely dissociated and undissociated carboxylic acids. Shift differentials of carbonyl carbon peaks attributed to the completely dissociated and undissociated carboxylic acids were 3.20 ppm for Gly, 4.28 ppm for β-Ala, 4.69 ppm for Bu, and 4.93 ppm for Va, respectively12. Further, Fig. 2 shows the pKa effects of MωA’s carboxylic acid on the amount of interacted carboxylic acid with calcium. There, the pKa values of carboxylic acid were 3.5 for Gly, 4.5 for β-Ala, 4.8 for Bu, and 4.9 for Va, respectively12. As the pKa value of MωA’s carboxylic acid increased from 3.5 to 4.9, the amount of interacted carboxylic acid decreased from 34% to 12% for both root and crown dentin apatites. In terms of the

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Table 1 Chemical shifts of the carbonyl carbon peak of carboxylic acid in the MωA structure and the pH values of MωA solutions, before and after addition of root or crown dentin.

<table>
<thead>
<tr>
<th>MωA solution</th>
<th>Addition of dentin</th>
<th>Root dentin</th>
<th>Crown dentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly</td>
<td>pH</td>
<td>Chemical shift (ppm)</td>
<td>pH</td>
</tr>
<tr>
<td>1.48 (0.02)</td>
<td>173.58 (0.02)</td>
<td>3.12 (0.01)</td>
<td>174.69 (0.01)</td>
</tr>
<tr>
<td>β-Ala</td>
<td>2.08 (0.01)</td>
<td>176.16 (0.04)</td>
<td>3.64 (0.01)</td>
</tr>
<tr>
<td>Bu</td>
<td>2.28 (0.02)</td>
<td>177.85 (0.01)</td>
<td>3.85 (0.01)</td>
</tr>
<tr>
<td>Va</td>
<td>2.28 (0.02)</td>
<td>178.17 (0.00)</td>
<td>4.00 (0.00)</td>
</tr>
</tbody>
</table>

( ): Difference in chemical shift obtained between the first and second experiments.

ppm from TMS (tetramethylsilane)
degree of decalcification of root and crown dentin apatites, we were not able to observe any identifiable differences.

**Effect of interacted carboxylic acid amount on bonding to root and crown dentins before and after thermocycling**

Figs. 3A and 3B show the effects of interacted carboxylic acid amount on shear bond strength of resin to root and crown dentins, respectively, before and after thermocycling. As a control, the bond strengths to both dentins conditioned by ED Primer II were also included.

Before thermocycling, the mean bond strength to root or crown dentin — which was conditioned by MωA adhesives — was independent of the interacted amount, and ranged randomly from 13.3 MPa to 15.0 MPa (root dentin) and from 13.3 to 15.3 MPa (crown dentin). As for the controls conditioned by ED Primer II, the mean bond strength to root dentin was 13.1 MPa and that to crown dentin was 12.6 MPa. Thus, before thermocycling, no significant differences in mean bond strength were observed between the MωA adhesives and ED Primer II (p > 0.05).

With thermocycling, the mean bond strengths to both root and crown dentins decreased. For the controls conditioned by ED Primer II, the mean bond strength to root dentin decreased by 4.6 MPa (significant difference, p < 0.05) and that to crown dentin decreased by 3.2 MPa (no significant difference, p > 0.05). Conversely, with MωA adhesives, the reduction in mean bond strength to both dentins was less than 2 MPa (no significant difference, p > 0.05). In other words, we did not observe a specific reduction in bond strength with decrease in interacted carboxylic acid amount, even as the methylene chain number in MωA structure increased.

**SEM views of resin-dentin interface**

SEM views of the resin-dentin interface are shown in Fig. 4. With Gly aqueous solution, which provided the highest amount of interacted carboxylic acid with calcium, thickness of the hybrid layer was approximately 0.5 μm. However, with βAla, Bu, and Va adhesives, no formation of the hybrid layer could be identified. This was probably because the amount of decalcified dentin apatite by carboxylic acid in βAla, Bu, and Va adhesives decreased in this order. If a hybrid layer were to be created at the resin-dentin interface, the layer’s thickness was most probably less than 0.5 μm. As for the controls conditioned by ED

<table>
<thead>
<tr>
<th>MωA</th>
<th>pKa</th>
<th>SD</th>
<th>Root dentin</th>
<th>Crown dentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly</td>
<td>3.5</td>
<td>3.20</td>
<td>1.11</td>
<td>1.10</td>
</tr>
<tr>
<td>βAla</td>
<td>4.5</td>
<td>4.28</td>
<td>0.74</td>
<td>0.81</td>
</tr>
<tr>
<td>Bu</td>
<td>4.8</td>
<td>4.69</td>
<td>0.63</td>
<td>0.57</td>
</tr>
<tr>
<td>Va</td>
<td>4.9</td>
<td>4.93</td>
<td>0.63</td>
<td>0.63</td>
</tr>
</tbody>
</table>

- a): pKa of MωA’s carboxylic acid.
- b): Shift differential in the chemical shift of carbonyl carbon peaks attributed to completely dissociated and undissociated carboxylic acids.
- c): Difference in chemical shift in carbonyl carbon peak of MωA’s carboxylic acid before and after addition of root or crown dentin.
- d): Interacted amount of MωA’s carboxylic acid with calcium.
  ppm from TMS (tetramethylsilane)
Primer II, the thickness of the hybrid layer was approximately 1.5 μm. From the perspective of hybrid layer creation on root and crown dentins, neither MωA adhesives nor ED Primer II caused any difference in hybrid layer creation between the two dentins.

### DISCUSSION

In this study, we designed self-etching primers consisting of four MωA derivatives for resin core construction. The amount of MωA’s carboxylic acid that interacted with root or crown dentin apatite was determined, and its effects on dentin bond durability examined.

The addition of both dentin particles to the MωA solutions caused the carbonyl carbon peak of carboxylic acid in MωA derivatives to shift to a lower field. This lower-field shift was attributed to MωA’s carboxylic acid having an acid-base interaction with calcium through the decalcification of dentin apatite. The amount of interacted carboxylic acid decreased from 34% to 12% when the pKa value of carboxylic acid in MωA species increased from 3.5 to 4.9. On this note, the pKa value of carboxylic acid in the MωA structure strongly determines the latter’s etching potential on dentin apatite\(^{10}\). However, the mean bond strength of both dentins conditioned by MωA adhesives was not thus affected, ranging from 13 MPa to 15 MPa. In other words, we did not observe any decrease in mean bond strength of resin to both dentins conditioned by MωA adhesives, even though the amount of decalcified dentin apatite by MωA adhesives decreased as the methylene chain number in the MωA structure increased.

Nishiyama et al.\(^{13}\) studied the pH effect of Gly aqueous solution on the bond strength of resin to acid-etched dentin. It was shown that the magnitude of bond strength was strongly dependent on the pH value of Gly aqueous solution. When a Gly aqueous solution, of which the pH value was above the pKa value of 3.5 for Gly’s carboxylic acid, was applied to acid-etched dentin as a dentin primer, the mean bond strength dramatically dropped from 15 MPa to 3 MPa. In the present study, the noticeably high bond strength of 13 MPa was probably due to undissociated Gly species having hydrogen bonded interactions with dentinal collagen macromolecules; as a result, the hydrogen-bonded Gly species enhanced the bonding of resin to collagen\(^{14}\). This was possible because undissociated Gly species, which was able to participate in hydrogen bonded interactions
with collagen macromolecules, was of a proportion of 65%. Furthermore, specific decreases in mean bond strength to root or crown dentin conditioned by \( M\omega A \) adhesives were not observed, although the interacted amount of carboxylic acid decreased. This obtained high bond strength was probably due to the hydrogen bonded interaction of \( M\omega A \) with collagen macromolecules becoming stronger with an increase in the methylene chain number in the \( M\omega A \) structure\(^{15,16}\).

To evaluate the degree of decalcification of root or crown dentin by \( M\omega A \)’s carboxylic acid, SEM observations of the resin-dentin interfaces were performed. The Gly adhesive, which provided the highest amount of interacted carboxylic acid with calcium, created an approximately 0.5 \( \mu m \) thick hybrid layer. However, as the methylene chain number increased in the \( M\omega A \) structure, we were unable to identify any hybrid layer formation since the layer’s thickness was less than 0.5 \( \mu m \). This was because the amount of interacted carboxylic acid in the \( M\omega A \) structure had a direct correlation with the amount of decalcified dentin apatite and the degree of thickness of demineralized collagenous layer. In contrast, ED Primer II — which contained methacryloyloxydecelphosphoric acid, MDP, as an acidic monomer — led to the creation of a 1-1.5 \( \mu m \) thick hybrid layer. This was due to the etching potential of MDP’s phosphoric acid being higher than that of \( M\omega A \)’s carboxylic acid\(^{17}\).

The application of thermocycling resulted in a decrease in mean bond strength of resin to both root and crown dentins. However, the reduction in mean bond strength to both dentins conditioned by \( M\omega A \) adhesives was less than 2 MPa. In other words, the initial bond strengths of resin to both root and crown dentins were maintained. Therefore, although the amount of decalcified dentin apatite and the thickness of hybrid layer decreased when the pKa value of \( M\omega A \)’s carboxylic acid increased, it did not result in any specific decrease in mean bond strength to both dentins. This was possible because \( M\omega A \) adhesives not only decalcified the dentin apatite, but also enhanced the bonding of resin to collagen macromolecules through the hydrogen-bonded \( M\omega A \) species. In contrast, when ED Primer II — which facilitated the formation of a 1-1.5 \( \mu m \) thick hybrid layer — was applied, a greater reduction in mean bond strength than the \( M\omega A \) adhesives was observed. This observed higher durability of the dentin bond — obtained with the application of \( M\omega A \) adhesives — was due to the absence of demineralized dentin zone. As a result, bonding defects were limited, for a demineralized dentin zone would mean incomplete permeation and diffusion of the bonding resin monomer into the demineralized collagenous layer. This was possible since the demineralized collagenous layer became thinner as the pKa value of carboxylic acid in the \( M\omega A \) structure increased. With a demineralized collagenous layer less than 0.5 \( \mu m \), \( M\omega A \) adhesives therefore provided a high-quality hybrid layer without any bonding defects. Conversely, with increasing

![Fig. 4 SEM views of resin-dentin interface, after root or crown dentin was conditioned by \( M\omega A \) adhesives or ED Primer II. (R: resin; D: dentin; Arrow shows the hybrid layer)](image)
thickness of the demineralized collagenous layer, as in the case of ED Primer II, the probability of bonding defects creation becomes higher due to incomplete permeation and diffusion of the bonding resin monomer into the demineralized collagenous network.

From the perspective of restoring pulpless teeth with minimal intervention, the Bu and Va adhesives—which yielded the lowest amount of decalcified dentin apatite—proved to be very useful as adhesives for dentin bonding in resin core construction.

CONCLUSION

The application of MoωA self-etching primers resulted in an increase in bond strength of the resin to root and crown dentins. In particular, even when thermocycling was applied, no specific reductions in bond strength to both dentins were observed. The Bu and Va adhesives provided bond strengths above 10 MPa to both root and crown dentins, even though Bu and Va yielded the lowest amount of decalcified dentin apatite.

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