Effects of Solvents and pH Values on the Chemical Affinity of 10-Methacryloyloxydecyl Dihydrogen Phosphate toward Hydroxyapatite

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ABSTRACT: This study aimed to investigate the effects of solvents and pH values on the chemical interaction between 10-methacryloyloxydecyl dihydrogen phosphate (MDP) and hydroxyapatite (HAp). The chemical affinity of MDP toward HAp dissolved in different solvents (E-MDP: 10 wt % MDP and 90 wt % ethanol; E-W-MDP1: 10 wt % MDP, 75 wt % ethanol, and 15 wt % water; A-W-MDP: 10 wt % MDP, 75 wt % acetone, and 15 wt % water; and E-W-MDP2: 10 wt % MDP, 45 wt % ethanol, and 45 wt % water) was investigated. The pH of E-W-MDP2 was increased from 2.04 to 5 (E-W-MDP2/5) and to 7 (E-W-MDP2/7). The reaction products were characterized by Fourier transform infrared spectroscopy, X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), thermogravimetric analysis (TGA), and nuclear magnetic resonance (NMR). XRD and NMR results revealed that no MDP-calcium salt formed in E-MDP. XRD, TGA, and XPS results indicated that MDP interacted with HAp, producing the MDP-calcium salt in all groups except E-MDP. NMR results revealed that the dicalcium salt of the MDP dimer (DCS-MD) and the MDP tripolymer (DCS-MT) and the monocalcium salt of the MDP monomer and the MDP dimer were formed in E-W-MDP1. DCS-MD and DCS-MT were also formed in E-W-MDP2 and A-W-MDP. In E-W-MDP2/5 and E-W-MDP2/7, DCS-MD was obtained. Both the solvents and pH values affect the chemical interactions between MDP and HAp and the types of reaction products formed. MDP and HAp do not form any MDP-calcium salt in pure ethanol; the structural stability of MDP-calcium salts is dependent on the solvent water content and the pH value. The ethanol/water mixture is recommended as the main solvent in an MDP-containing primer, and the ideal pH value is 2−7; if these conditions are satisfied, sufficient amounts of MDP-calcium salts with stable structures are expected to be formed, thus improving the longevity of dentin/enamel bonding.

1. INTRODUCTION

Phosphate ester monomers can form a chemical coupling between hydroxyapatite (HAp) and the resin monomer because of the phosphoric acid terminal and the vinyl terminal in their molecular structure, and phosphate ester monomers have therefore been a key functional component in enamel and dentin adhesives.1 The chemical affinity of 10-methacryloyloxydecyl dihydrogen phosphate (MDP), the most widely used phosphate ester monomer, to HAp is stronger than that of other monomers such as 4-methacryloyloxyethyl trimellitic acid and 2-methacryloyloxyethyl phenyl hydrogen phosphate.2−4 The chemical affinity of MDP to HAp is a key factor affecting the bonding performance of dental adhesives bonded to dentin or enamel.5,6

Pure MDP is a viscous colloid; so, without a solvent, its optimal dispersal on the HAp surface, necessary for the initiation of the reaction between MDP and HAp, cannot be achieved.1 Therefore, a solvent is required to facilitate optimal MDP dispersal that is necessary for P−OH dissociation and reaction with Ca.1 The dissociation of MDP is the first step in the reaction between MDP and HAp. MDP easily undergoes hydrolysis in water, giving two protons and thus exhibits a strong acid-etching ability,7,8 but water is an inefficient solvent for MDP and many other functional adhesive components, such as 2-hydroxyethyl methacrylate and 2-hydroxypropyl methacrylate;9 therefore, the adequate dissolution of these functional components cannot be achieved by using pure water as the solvent. In addition, water is considered to cause collagen degradation at the dentin bonding interface, and hydrolysis of the ester bonds in the MDP-calcium salt at the bonding interface aggravates the damage to the hybrid layer.9

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Further, because of the high boiling temperature and the low vapor pressure, the complete removal of water is difficult. Ethanol and acetone are efficient solvents for MDP and other functional components. Commercially available adhesives usually contain a mixture of ethanol-based solvents with water and/or acetone. The vapor pressure of ethanol is higher than that of water, resulting in quicker evaporation. Hydrogen bonds form between water and alcohol, resulting in the azeotropic water—alcohol mixture, so the evaporation of water—ethanol aggregates is quicker than that of pure water. The ethanol wet-bonding theory proposes that gradient ethanol can replace water in the dentin matrix and promote the permeation of the hydrophobic resin into the collagen network, thus improving the bonding strength and durability. Acetone is an alternative to ethanol as a solvent in adhesives. Its high dipole moment and relatively low dielectric constant allow the simultaneous dissolution of both polar and non-polar compounds. Therefore, acetone has been commonly used in adhesives that contain both hydrophobic and hydrophilic components. However, the vapor pressure of acetone is about four times that of ethanol, making the former more volatile; the resultant rapid evaporation causes an unavoidable reduction in the shelf life of the adhesives. In addition, acetone is flammable. Owing to safety concerns and the volatility of acetone, few commercial adhesives contain acetone as the main solvent.

The effect of the different component proportions in solvents on the chemical affinity of MDP to HAp remains unknown. In addition, regardless of the solvent used, the pH values of commercial MDP-containing self-etching primers are usually between 1.0 and 2.6. A recent study reported that the incorporation of Ca(OH)$_2$ resulted in the pH of self-etching adhesives (SEAs) increasing up to 3, thus improving the degree of conversion (DC %) of the C—C bonds of the monomer into polymeric C—C single bonds within the hybrid layer without increasing nanoleakage or affecting the bonding strength of the SEAs to dentin. A further increase in the pH to 7 suppresses the demineralization ability of MDP because HAp dissolution is not possible without an acidic environment; consequently, no calcium ions are released from HAp and thus dicalcium phosphate dihydrate is not formed. However, to the knowledge of the authors, the relationship between the chemical affinity of MDP to HAp and the pH value has not been analyzed.

In order to determine the effects of solvents and pH values on the chemical affinity of MDP to HAp, in this study, the chemical bonds between MDP and HAp and the reaction products formed using different concentrations of ethanol, acetone/water, and ethanol/water solvent mixtures with pH ranging from 2 to 7 were investigated. Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), thermogravimetric analysis (TGA), and nuclear magnetic resonance (NMR) spectroscopy were employed to characterize the chemical reaction products. The null hypotheses were that the chemical interactions and reaction products between MDP and HAp would not be affected by the solvents (i) and pH values (ii).

2. RESULTS

2.1. FTIR Analysis. Figure 1 shows the infrared spectra in the 2000—525 cm$^{-1}$ region for HAp powder samples. All groups showed absorbance peaks corresponding to the P—O stretching vibration (at 1024 and 563 cm$^{-1}$). The C==O stretching vibration (1719 cm$^{-1}$) of the methacryloxy carbonyl group was detected for the MDP-primer-treated HAp after washing with ethanol but not detected for the untreated HAp sample. No significant differences were found between the groups. The wavenumber region selected for spectral comparisons was limited to the 1750—1600 cm$^{-1}$ range, where C==O stretching vibrations were observed. The infrared spectra and the peak fitting results of those specific wavenumber domains are presented in Figure 2. Approximately 80 data points were selected from the target region.

2.2. TG Analysis. The TG curves of the six groups and untreated HAp are presented in Figure 3. Figure 4 shows thermogravimetric-derivative thermogravimetric (TG-DTG) spectra of the HAp powder samples. In the E-MDP group (10 wt % MDP and 90 wt % ethanol), a weight loss of 7% occurred very quickly up to 337 °C. In the E-W-MDP (10 wt % MDP, 75 wt % ethanol, and 15 wt % water) and A-W-MDP group (10 wt % MDP, 75 wt % acetone, and 15 wt % water), the first weight loss of 2% occurred quickly from 30 to 190 °C, while the second weight loss of 28% occurred from 200 to 360 °C. In the E-W-MDP$_2$ group (10 wt % MDP, 45 wt % ethanol, and 45 wt % water), the first weight loss of 2% occurred quickly from 30 to 190 °C, while the second weight loss of 28% occurred from 200 to 360 °C. In the E-W-MDP$_{2/5}$ group (45 wt % ethanol with 45 wt % water, pH value was increased to 5 using NaHCO$_3$) and E-W-MDP$_{7/7}$ group (45 wt % ethanol with 45 wt % water, pH value was increased to 7 using NaHCO$_3$) samples exhibited very quick weight losses of 32 and 30%, respectively, up to approximately 400 °C.

2.3. XRD Analysis. XRD patterns of MDP-primer-treated HAp and untreated HAp are shown in Figures 5 and 6. No significant difference was observed in the spectra between the E-MDP$_1$ group and the untreated HAp sample. However, the patterns of the E-W-MDP, and A-W-MDP groups showed two characteristic peaks representing the MDP-calcium salt, at 2θ =
4.76° and 2θ = 6.94°. Further, E-W-MDP1 showed three characteristic peaks at 2θ = 2.42°, 2θ = 4.92°, and 2θ = 7.46° and E-W-MDP2/3 showed three characteristic peaks at 2θ = 2.32°, 2θ = 4.64°, and 2θ = 7.00°. In addition, E-W-MDP2/7 showed three characteristic peaks at 2θ = 2.26°, 2θ = 4.70°, and 2θ = 7.00°.

2.4. XPS Analysis. The wide-scan XPS spectra of HAp powder samples treated with four experimental primers and of untreated HAp are shown in Figure 7A. The peak intensities (C 1s) of the E-W-MDP1, A-W-MDP, and E-W-MDP2 groups were significantly higher than that of the untreated HAp. Narrow-scan spectra of HAp powder samples treated with the four primers and of untreated HAp are shown in Figure 7B–F. The C 1s region of all samples, including the untreated sample, revealed a C–C peak at 284.6 eV and a C–O peak at 285.8 eV. Further, a –COO peak at 288.5 eV was detected in the untreated HAp sample (Figure 7B), and the same peaks were observed at 288.9, 288.5, 288.6, and 288.5 eV in the E-MDP (Figure 7C), E-W-MDP1 (Figure 7D), A-W-MDP (Figure 7E), and E-W-MDP2 (Figure 7F) groups, respectively. From the XPS results (O 1s, Ca 2p, P 2p, and C 1s), the Ca/P, O/Ca, and C/Ca ratios were calculated (Table 1). The Ca/P ratios of HAp powder samples treated with the four experimental primers were lower and the C/Ca and O/Ca ratios were higher than those of the untreated HAp sample. The results can be attributed to the adsorbed MDP.

2.5. NMR Analysis. By considering the results of a previous study,15,16 the 31P NMR peaks detected in each HAp powder sample are assigned as listed in Table 2. Figures 8 and 9, respectively, show the typical 31P NMR spectra and curve-fitting results of reaction products formed when the HAp powder samples were treated with the six experimental primers. The 31P NMR labeled 3, 4, 5, and 6 were assigned to the four types of MDP-calcium salts, namely, the dicalcium salt of the MDP dimer (DCS-MD), the dicalcium salt of the MDP triplymer (DCS-MT), the monocalcium salt of the MDP monomer, and the monocalcium salt of the MDP dimer, respectively. No peaks representing the MDP-calcium salt appeared in the case of E-MDP. The predominant peaks for E-W-MDP1 were peaks 3, 4, 5, and 6, while those for A-W-MDP and E-W-MDP2 were peaks 3 and 4. In addition, E-W-MDP2/5 and E-W-MDP2/7 exhibited peak 3 as the predominant peak.

3. DISCUSSION

The MDP-calcium salt formed from MDP and the free calcium ion released from demineralized dentin is the most stable and effective among the known functional monomers.17–19 However, the formation of the MDP-calcium salt is affected by many factors, including the concentration and purity of MDP in the adhesives and the actuation duration. When the concentration and purity of MDP was increased and the actuation duration was increased, a stronger nanolayer structure was detected.20–22 Each nanolayer comprises two rows of parallelly oriented MDP molecules with their methacrylate groups directed toward each other in the center and their functional hydrogen phosphate groups directed away from each other.4 Ethanol is a type of polar solvent that forms hydrogen bonds with its solutes. Ethanol is more suitable than water as a solvent for non-polar or low-polarity solutes (like MDP)
Figure 4. TG-DTG results of HAp samples, including the untreated HAp. Solvents were (A) 90 wt % ethanol, (B) 75 wt % ethanol with 15 wt % water, (C) 75 wt % acetone with 15 wt % water, (D) 45 wt % ethanol with 45 wt % water (pH = 2.04), (E) 45 wt % ethanol with 45 wt % water (pH value was increased to 5 using NaHCO₃), and (F) 45 wt % ethanol with 45 wt % water (pH value was increased to 7 using NaHCO₃).
because its dielectric constant is much lower than that of water.3 The FTIR results of the present study show that as long as ethanol is present in the solvent, regardless of the water content, the methacryloxy carbonyl peak corresponding to MDP is observed; this suggests that the dissociation of MDP in ethanol resulted in the chemical reaction with HAp, and MDP molecules are therefore adsorbed on the HAp surface. When the MDP-containing primers contain 75 wt % acetone and 15 wt % water as the solvent, the methacryloxy carbonyl peak in MDP is also observed in the reaction products, indicating that the reaction also occurs in the acetone–water mixture. Similar to ethanol, acetone and water can also form azetropic mixtures. Although fewer hydrogen bonds are formed with ketones (C=O) than with alcohols (–OH),3 MDP may also dissociate in the acetone–water mixture. The reaction pathways for MDP dissociation in vacuum and in water, ethanol, and acetone are shown in Figure 10.

In the ethanol–water solution, MDP reacts with HAp to form the MDP-calcium salt, Ca(RHPO4)2, which is stable in ethanol but metastable in water in the absence of ethanol and may dissociate to CaRPO4 and RH2PO4.23 The chemical reactions can be expressed as follows

\[
\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \rightarrow 10\text{Ca}^{2+} + \text{PO}_4^{3-} + 2\text{OH}^- \quad (1)
\]

\[
\text{PO}_4^{3-} + \text{RPO}_4\text{H}_2 \rightarrow \text{HPO}_4^{2-} + \text{RHPO}_4^- \quad (2)
\]

\[
2\text{Ca}^{2+} + \text{HPO}_4^{2-} + 2\text{RHPO}_4^- + 2\text{H}_2\text{O} \quad \downarrow + \text{Ca}^{2+} \downarrow + \text{CaRPO}_4 \downarrow
\]

The stable structure of CaRPO4 contributes to the formation of the durable nanolayer of the MDP-calcium salt in the adhesive interface, thus extending the clinical life of the restorations.24

Previous studies have reported that the amounts of CaHPO4·2H2O and Ca(RHPO4)2 deposited on HAp depend on the equilibrium of the chemical reactions 1–3, and the solubility of the deposited salts in the water–ethanol environment.23 The TGA analysis results of the present study showed that the amount of MDP deposited on HAp was affected by the water content in the experimental primers. The weight loss value of the HAp powder treated with the MDP-containing primers without water was 7%, which was higher than that of untreated HAp. This might be attributed to the pyrolysis of the reaction products.25 The observed weight loss values in the three groups of water-containing experimental primers were similar. The greatest weight loss occurred from 200 to 375 °C (the temperature corresponding to the maximum pyrolysis rate is approximately 320 °C), suggesting that the backbones of the long carbon chain of MDP collapsed. This indicates that the presence of water will increase the reaction yield. After treatment with the MDP-containing primers without water, no characteristic peak assigned to the MDP-calcium salt was visible, indicating that HAp and MDP react in the pure ethanol solvent even in the absence of water but do not form a calcium salt. The explanation given in a previous paper is that in absolute ethanol, the reaction of PO43− of HAp and P−OH of MDP results in the formation of very small amounts of the pyrophosphate groups instead of the MDP-calcium salt on the HAp surface.23 The powder samples treated with 15 wt % water and 75 wt % ethanol acetonitrile-containing primers showed two peaks in the 2θ range of 0–10°, which can be assigned to the MDP-calcium salt. The amount of the MDP-calcium salt was considered to be small, and another peak around 2θ = 2.30° was too small to be relevant for XRD characterization. With an increase in the water content to 45 wt % in primers, the HAp powder showed three characteristic peaks in the 2θ range of 0 to 10°, which represented the MDP-calcium salt, and the peak intensity was also higher than that in the case of 15 wt % water content. The results of previous studies show that a significant correlation exists between the intensity of the characteristic XRD peaks
Figure 7. (A) Wide-scan XPS spectra of HAp powder samples treated with four experimental primers and of untreated HAp. (B) Untreated HAp powder exhibited a peak at 288.5 eV corresponding to $-\text{COO}$ binding. (C) HAp treated with 10 wt % MDP and 90 wt % ethanol exhibited a peak at 288.9 eV assigned to $-\text{COO}$ binding. (D) HAp treated with 10 wt % MDP, 75 wt % ethanol, and 15 wt % water showed a peak at 288.5 eV corresponding to $-\text{COO}$ binding. (E) HAp treated with 10 wt % MDP, 75 wt % acetone, and 15 wt % water exhibited a peak at 288.6 eV representing $-\text{COO}$ binding. (F) HAp treated with 10 wt % MDP, 45 wt % ethanol, and 45 wt % water showed a peak at 288.5 eV assigned to the $-\text{COO}$ binding.
and effectiveness of the formed nanolayers.\textsuperscript{21} The higher the peak intensity is, the higher is the amount of the formed MDP-calcium salt, resulting in the improved stability of the nanolayer, bonding stability, and durability.\textsuperscript{21}

The XPS results of the present study agreed well with the TGA results. In the narrow-scan XPS spectra of the C 1s region, changes in the binding energy corresponding to each component peak among the different groups suggest that the chemical affinity of MDP to HAp is influenced by the solvent. The Ca/P ratio of primers without water is smaller than that of the primers with water, and an increase in the water content in the solvent increases the content of MDP bonded to HAp, that is, it accelerates the reaction between MDP and HAp. This may be the reason why most commercial adhesives contain water in the solution. When a certain proportion of ethanol is replaced with the same proportion of acetone, the Ca/P ratio remains almost unchanged. In the “strong” SEAs, the acidic monomers with a low pH result in a highly permeable structure, which in turn causes the hydrolysis of the hybrid layer, thereby deteriorating the dentin bonding strength and durability.\textsuperscript{13} In contrast, “mild” SEAs cannot generate sufficient micromechanical retention, which adversely affects the dentin bonding.\textsuperscript{26,27} Therefore, the content and types of the reaction products at different pH values were also investigated in this study. The FTIR spectra showed that when the pH value of the solvent was 2–7, the methacryloxy carbonyl peak of MDP was visible, indicating that in this pH range, irrespective of the primer pH, the chemical reaction between MDP and HAp occurs. The XRD results were consistent with FTIR results. The characteristic peaks representing the MDP-calcium salt did not disappear with the primer pH change, and the position and intensity of the characteristic peaks did not change significantly. With an increase in the pH values to 5 and 7, the temperature corresponding to the maximum pyrolysis rate increased to 395 and 397 °C, respectively, which may be ascribed to the high stability of MDP-calcium salts. Therefore, by controlling the pH value of the MDP-containing adhesives appropriately, new SEAs can be developed.

In this study, curve-fitting analyses were employed to determine the molecular formula of the MDP-calcium salt produced using the different MDP-containing primers. Based on the chemical shifts, intensities, intensity ratios, and intensity changes of the four simulated peaks (Figure 9), simulated peaks “5” and “6” were determined to correspond to the MDP-calcium salt in which one of the two −OH groups bonded to the phosphate group reacted with a calcium ion from HAp, while simulated peak “3” was determined to correspond to the MDP-calcium salt in which two −OH groups of the phosphate group have reacted with calcium ions; further, simulated peak “4” was determined to correspond to the MDP-calcium salt containing both the phosphate group with two −OH groups that reacted with calcium ions and two phosphate groups with one −OH group reacted with a calcium ion.\textsuperscript{15} However, curve-fitting analyses clearly showed that Ca(RHPO$_4$)$_2$ and CaRPO$_4$ were formed after treatment with 15 wt % water and 75 wt % ethanol primer; when the water content was 45 wt %, fewer types of Ca(RHPO$_4$)$_2$ were formed. It is presumed that the stability of the calcium salt structure improves with increasing water content. This is consistent with the results of a previous study that showed that MDP and HAp form CaRPO$_4$ after washing multiple times with both absolute alcohol and distilled water, as shown in chemical reaction \textsuperscript{4}.\textsuperscript{24} Interestingly, fewer types of Ca(RHPO$_4$)$_2$ were formed after treatment with the

| atomic ratio | untreated HA | E-MDP | E-W-MDP$_1$ | A-W-MDP | E-W-MDP$_2$ |
|-------------|-------------|-------|-------------|---------|-------------|
| Ca/P        | 1.88        | 1.35  | 0.79        | 0.82    | 1.00        |
| C/Ca        | 1.09        | 2.01  | 11.19       | 10.18   | 5.41        |
| O/Ca        | 2.35        | 2.62  | 5.21        | 3.09    | 3.68        |

Table 1. Atomic Ratio Based on XPS Analysis Results for HAp Treated with MDP

Table 2. Chemical Structures of Four MDP-Calcium Salts Detected after HAp Powder Reactions
primer containing 75 wt % acetone and 15 wt % water, which may be due to the limited solubility of MDP in the acetone−water mixture. The P−OH dissociation rate of MDP molecules for releasing protons in the acetone−water mixture was higher than that in the ethanol−water mixture with the same component ratio; this dissociation results in the two −OH groups of MDP ionically reacting with the calcium ion. The above results provide a potential guide for the ratio of ethanol/acetone to water in further adhesives. However, the evaporative pressure and flammability of acetone have to be taken into account. Therefore, MDP-containing adhesives generally contain ethanol, rather than acetone, as the main solvent. When the pH of the primers was increased gradually, the reaction between MDP and HAp produced CaRPO4; when the pH was 7, the MDP-calcium salt, DCS-MD, was formed. The hydrolytic stability of the MDP-calcium salt is speculated to be different under different pH values. Thus, the null hypotheses that the chemical interactions and reactions of MDP with HAp will not be affected by the solvent (i) and pH (ii) can both be rejected.

4. CONCLUSIONS
Within the limits of the present study, it may be concluded that both the solvent and pH affect the chemical interactions and reactions between MDP and HAp. There is no MDP-calcium salt formation between MDP and HAp when pure ethanol is used as the solvent. The structural stability of the MDP-calcium salt improves with the increase in the solvent water content. The type of the MDP-calcium salt formed depends on the pH value, wherein DCS-MD and DCS-MT were formed when the pH value was 2 in the aqueous ethanol solution, while single DCS-MD was formed when pH values ranged from 2 to 5 or 7.

Based on the results of the present study, the ethanol/water mixture should be the main solvent in the MDP-containing primer and the pH value should be 2−7. The stable structure of the resultant MDP-calcium salt is expected to enable the formation of durable nanolayers of the salt at the adhesive interface and thus improve the longevity of dentin/enamel bonding.

5. MATERIALS AND METHODS
5.1. Preparation of Experimental MDP-Containing Primers. Six experimental primers each containing 10 wt % MDP (DM Healthcare Products, Inc., San Diego, CA, USA) were prepared using ethanol, acetone, water, or their mixtures as solvents with different pH values. Sodium bicarbonate (NaHCO3, Macklin, Shanghai, China) was used to adjust the pH of the E-W-MDP2 to 5 (E-W-MDP2/5) or 7 (E-W-MDP2/7). The pH values of different primers were measured using an electronic pH meter (FE28, Mettler Toledo, Zurich, Switzerland). Prior to measuring the primers, the pH electrodes were calibrated with buffer solutions at pH 7.00 and pH 4.01. Three specimens were prepared for the

Figure 8. Typical 31P NMR spectra of HAp powder samples. Solvents were (A) 90 wt % ethanol, (B) 75 wt % ethanol with 15 wt % water, (C) 75 wt % acetone with 15 wt % water, (D) 45 wt % ethanol with 45 wt % water (pH = 2.04), (E) 45 wt % ethanol with 45 wt % water (pH value was increased to 5 using NaHCO3), and (F) 45 wt % ethanol with 45 wt % water (pH value was increased to 7 using NaHCO3). The arrows denote the NMR peaks assigned to the phosphorus atoms of the MDP-calcium salts. Peak α was assigned to HAp. The numbered peaks were assigned to the phosphorus atoms in the corresponding salts in Table 2.
Figure 9. Curve-fitting results corresponding to the observed $^{31}$P NMR spectra (black lines) for HAp powder samples. Solvents were (A) 90 wt % ethanol, (B) 75 wt % ethanol with 15 wt % water, (C) 75 wt % acetone with 15 wt % water, (D) 45 wt % ethanol with 45 wt % water (pH = 2.04), (E) 45 wt % ethanol with 45 wt % water (pH value was increased to 5 using NaHCO$_3$), and (F) 45 wt % ethanol with 45 wt % water (pH value was increased to 7 using NaHCO$_3$). The green lines show the simulated $\alpha$ peak for HAp. The sky blue lines are the simulated peaks 3, 4, 5, and 6 for the four MDP-calcium salts. The red line is the resulting overall synthetic spectrum.

Figure 10. Reaction pathways of MDP dissociation in vacuum, water, ethanol, and acetone.
5.2. HAp Powder Conditioning. Six 0.2 g of the HAp powder samples (Sigma Chemical Co., St. Louis, MO, USA) were treated with 2.0 g of the six MDP-containing primers for 24 h, with untreated HAp as the control. The treated HAp powder samples were washed with ethanol, centrifuged three times, and then dried in air at ambient temperature for 48 h.

5.3. FTIR Analysis. Infrared spectra were recorded between the spectral range of 2000 and 525 cm\(^{-1}\) using an FTIR spectrometer (Nicolet 6700, Thermo Scientific, Waltham, USA) with an attenuated total reflection setup at 4 \(^{\circ}\) resolution and 32 scans.

5.4. TG Analysis. TGA of HAp powder samples was conducted using a thermogravimetric analyzer (TGA-4000, PerkinElmer, Waltham, USA). The untreated HAp powder was used as the control. Approximately 5 mg of each dried powder sample was placed in a platinum crucible and heated from 28 to 700 °C at a rate of 15 °C/min.

5.5. XRD Analysis. The crystal phases of the HAp powder samples were identified by XRD (D8 ADVANCE, Bruker, Karlsruhe, Germany) at an acceleration voltage of 40 kV, a current of 200 mA, and a scanning rate of 0.02° s\(^{-1}\) for 2θ/θ scans.

5.6. XPS Analysis. The E-MDP, E-W-MDP\(_p\), A-W-MDP, and E-W-MDP\(_b\) groups were subjected to XPS analysis (Escalab 250xi, Thermo Fisher Scientific, Waltham, USA) with monochromatic A1 K\(_\alpha\) radiation, and untreated HAp was used as the control. The C 1s spectra were recorded using XPS Peak 4.1 software with the Lorentz–Gauss ratio fixed at 80%.

5.7. NMR Analysis. \(^{31}\)P NMR spectra of the reaction products were recorded (AVANCE III HD 400M, Bruker, Karlsruhe, Germany) with the chemical shift expressed as ppm, by using 85% H\(_3\)PO\(_4\) as the external reference. The spectra were analyzed using Mestrenova and OriginPro 8.0 Data Analysis and Graphing Software (OriginLab Co., Northampton, MA, USA). Dicalcium phosphate dihydrate is considered to be produced along with several types of MDP–calcium salts as byproducts.\(^{15,29–31}\) The intensity of each simulated peak used for the curve-fitting analyses of the reaction products was then determined for each experimental group.

Table 3. Composition of the Six Experimental MDP-Containing Primers (in wt %)

|        | MDP | ethanol | acetone | water | mean pH values and their S.D.s. |
|--------|-----|---------|---------|-------|----------------------------------|
| E-MDP  | 10  | 90      | 0       | 0     | 3.07 (0.13)                      |
| E-W-MDP\(_p\) | 10  | 75      | 0       | 15    | 2.68 (0.18)                      |
| A-W-MDP | 10  | 0       | 75      | 15    | 2.60 (0.08)                      |
| E-W-MDP\(_b\) | 10  | 45      | 0       | 45    | 2.04 (0.06)                      |
| E-W-MDP\(_b\)/5 | 10  | 45      | 0       | 45    | 5.00 (0.04)                      |
| E-W-MDP\(_b\)/7 | 10  | 45      | 0       | 45    | 7.00 (0.10)                      |

Notes

The authors declare no competing financial interest.

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