Photopolymerization of phosphoric acid ester-based self-etch dental adhesives

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The objective of the study was to gain more understanding on the photopolymerization mechanism and the role of individual monomers in the polymerization behavior of a PAE-based self-etch adhesive system with the presence of HAp and water. The photopolymerization process of the model adhesive system (2MP/HEMA) was monitored by using real-time attenuated total reflectance Fourier transform infrared (ATR/FT-IR) technique. The effect of monomer ratio, HAp incorporation, and water content were investigated. The degree of conversion (DC) and the polymerization rate (PR) of the adhesives were determined to evaluate the polymerization efficacy. The results showed that the DC and PR increased consistently as the 2MP content increased from 30% to 70%, while they declined drastically as the 2MP content was further elevated to 100%. The incorporation of HAp considerably increased the DC and PR; however, the increase in water content was found to have negative influence on the photopolymerization.

Keywords: Self-etch adhesives, Hydroxyapatite, FTIR, Photo-polymerization, Degree of conversion

INTRODUCTION

Photopolymerization has been a widespread technique in the application of preventive and restorative dentistry1-4). Typically, photocurable systems consist of mixtures of two or more monomers of various viscosities in order to achieve desirable polymer properties. In the field of adhesive dentistry, photocurable compositions are mainly based on mono- or multifunctional methacrylates, and usually combine highly viscous monomers/oligomers and low viscosity monomers5-10). A system that has been extensively investigated is composed of bisphenol A-bis(glycidyl methacrylate)(Bis-GMA) and triethyleneglycol dimethacrylate(TEGDMA)7-10). The viscous, bifunctional monomer of Bis-GMA has a high molecular weight and high reactivity. After light curing, Bis-GMA can produce a cross-linked, three-dimensional resin network11), thus the role of Bis-GMA is essential for the mechanical properties of the product. TEGDMA, on the other hand, mostly serves as a reactive diluent to enhance the handling properties and conversion potential of monomer mixtures. Studies showed that any change in the monomer ratio of a photocurable mixture such as Bis-GMA/TEGDMA would impose effect on the polymerization behavior and the properties of resulting resin, not only because of the differences in reactivities of the monomers, but also due to changes in initial viscosity of the system7-10,12).

In the present study, a self-etch adhesive system of bis[2-(methacryloyloxy) ethyl phosphate (2MP)/2-hydroxyethyl methacrylate (HEMA) was employed. The combination of these two components can be found in commercial two-step self-etch dentin adhesive formulations such as Clearfil Liner Bond 2V. As one of important category of dental resins, self-etch adhesives have been developed and gained increasing popularity among dentists due to their advantage in simplifying the clinical application procedure13,14). Contemporary self-etching and priming adhesives usually incorporate specific monomer molecules which combine unsaturated polymerizable functions with carboxylic or phosphoric acid groups5,10). Therefore, the compositional design enables self-etch systems to perform etching and bonding (or priming) simultaneously. Among the self-etch primers, the phosphoric acid ester (PAE, such as 2MP) has been widely used for composite-to-tooth bonding in adhesive dentistry. In the current 2MP/HEMA system, the significant difference in viscosities between 2MP and HEMA (1470 and 6 cP, respectively, see Table 2) is similar to that of Bis-GMA/TEGDMA system. However, as a member of PAE family, 2MP differs from Bis-GMA in its high acidity arised from the phosphonic acid functional groups (see Fig. 1) which can ionize in the presence of water. The acidic 2MP might react with dental mineral such as hydroxyapatite (HAp) and form complex structures17,18), which probably leads to the alteration of the environment (such as pH value) where the photopolymerization takes place. Under a circumstance of high acidity, there is also possibility that the polymerization of the adhesives is affected by the acidic moieties, because tertiary amines within the initiating system of the adhesives might be neutralized by the acidic functional monomers16,19,20).

Despite of the significance of the polymerization behavior of the PAE self-etch adhesives, very little research has been conducted in this regard. Our recent studies have been focused on the polymerization efficacy of the 2MP/HEMA (50/50) system after interacting with dentin20) as well as HAp22). Considering that practical application of the PAE self-etch adhesives might require different monomer ratios to achieve variable acidity and viscosity, the polymerization process of 2MP/HEMA self-
etch adhesive system with varying monomer ratios was examined in this study. To mimic the real environment where dental adhesive is applied, HAp and water were incorporated into the system, and the influences of which were also evaluated. The objective of the study was to gain more understanding on the photopolymerization mechanism and the role of individual monomers in the polymerization behavior of a PAE-based self-etch adhesive system with the presence of HAp and water.

MATERIALS AND METHODS

Model self-etch adhesive preparation
The monomer mixtures used in this study were 2MP (Aldrich, Milwaukee, WI, USA) and HEMA (Acros Organics, Morris Plain, NJ, USA). They were mixed in a mass ratio (2MP:HEMA) of 30:70, 50:50, 70:30, and 100:0 together with photoinitiators. The photoinitiator system (all from Aldrich) consisted of camphorquinone (CQ) as a photoinitiator and ethyl 4-dimethylaminobenzoate (4E) as a coinitiator. The amount of CQ or 4E used in the experiment was 0.022 mmol per gram of the monomer mixture. The chemical structures of monomers and initiators used in the study are shown in Fig. 1.

Deuterium oxide (D2O, Cambridge Isotope Laboratories, Inc., Andover, MA) with concentration of 10, 20, or 30 wt % was used in case that water was necessary to make adhesive solutions. Using D2O instead of H2O was to avoid the interference of the IR absorption of H2O to that of C=C double bonds in adhesives. To investigate the effect of mineral on the photopolymerization, HAp [(Ca10(OH)2(PO4)6, particle size ~0.5–10 µm, Aldrich] powders were added to the above solutions to obtain mass fraction of 2%. The resulting solutions were shaked and sonicated to yield well-dissolved mixtures.

Real-time ATR/FT-IR measurement
The polymerization process during photocuring of the model adhesives was in-situ monitored by using an infrared spectrometer (Spectrum One, Perkin-Elmer, Waltham, MA) equipped with an ATR attachment at a resolution of 4 cm⁻¹. The adhesive/water mixtures were cast on the diamond crystal top-plate of the ATR accessory, and covered with a transparent mylar film. The ATR crystal was zinc selenide (ZnSe) with a transmission range between 650 and approximately 4,000 cm⁻¹. A time-based spectrum collector (Spectrum TimeBase, Perkin-Elmer) was used for continuous and automatic collection of spectra during the polymerization at a rate of one spectrum every 0.4–0.6 s. At this high rate and resolution, the signal-to-noise ratio of spectra was sufficient for spectral analysis. A 40-s exposure from a conventional dental light polymerization unit (Spectrum Light, Dentsply, Milford, DE) emitting 550 mW/cm² was applied after 50 spectra had been collected. The distance from the top of the Mylar film to the distal end of the light guide was kept at ~2 mm. Real-time IR spectra were continuously recorded for 300 s after the solution was cast. Three separate replications for each adhesive formulation were conducted.

Calculation of the degree of conversion (DC) and polymerization rate (PR)
The degree of conversion of photopolymerization was calculated based on the IR intensity changes of the band at 1,637 cm⁻¹ (double bond C=C) relative to an intrinsic standard band at 1,715 cm⁻¹ (carbonyl group C=O), as shown in the following equation:

\[
DC = \left( 1 - \frac{\text{Absorbance}_{1637 \text{cm}^{-1}}}{\text{Absorbance}_{1715 \text{cm}^{-1}}} \right) \times 100\%
\]

Two-point baseline and maximum peak height ratio protocol were used to measure the absorption intensity. The DC was determined using the last 20 spectra of time-resolved spectra. One DC value was an average of 60 points. The PR was determined using the maximum slope of linear region of the DC-time plots by using the least square linear fitting. The polymerization rate of each formulation was averaged using three slopes.

Viscosity and pH measurements
To determine the influence of viscosity on the polymerization, the viscosity of the 2MP/HEMA solutions were measured using a digital viscometer (DV-E, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) equipped with a small sample adapter (SSA). The SSA consisted of cylindrical sample chambers (SC4-8R and 13R) and spindles (SC4-16 and 18), which provides a defined geometry system for viscosity measurements of small sample volumes in the order of 4 to 6 mL. A rotation speed of 20 rpm was used. Precise calibration was performed before the measurements using the viscosity standards certified within the measuring range. All samples were measured.
in triplicate.

The pH values of 2MP and HEMA aqueous solutions were measured with a pH meter (Accumet Excel XL15, Fisher Scientific, Pittsburgh, PA). Prior to measuring the self-etch adhesives, the pH electrode was calibrated with the buffer solutions. Three specimens were prepared for the measurements for each solution and the pH results were averaged.

Statistical analysis
The differences of the DC, PR and pH values among varying monomer mass ratios (2MP:HEMA of 30:70, 50:50, 70:30 and 100:0) and water (D₂O) contents (10, 20, and 30 wt%) were evaluated by one-way ANOVA with Tukey’s post test. Repeated analysis of variance and multiple comparison tests using adjusted Bonferroni method were used to identify the changes of the DC, PR and pH values before and after the reaction with HAp. All statistical analyses were performed using GraphPad InStat, version 3.06, software (GraphPad Software, San Diego, CA, USA). Significance was noted at \( p < 0.05 \).

RESULTS
Representative real-time spectra during photopolymerization of the 2MP/HEMA (70% of 2MP) system with the presence of 10% water and 2% HAp are shown in Fig. 2. The figure displays that the absorbance of 1,637 cm\(^{-1}\) band decreased while that of 1,715 cm\(^{-1}\) band remained almost unchanged with polymerization time. The decrease in absorbance at 1,637 cm\(^{-1}\) indicates consumption of methacrylate C=C bonds during polymerization. The peak intensities of these two bands were integrated (based on the peak height) and the change of the band ratios profile with 1,637 cm\(^{-1}\)/1,715 cm\(^{-1}\) was monitored to calculate the DC. Based on the calculated DC and PR, the effects of the monomer composition, addition of HAp, and water were evaluated.

Effect of monomer composition
In the present PAE system, solutions with different mass ratios for 2MP and HEMA were prepared to investigate the effect of monomer composition on the photopolymerization behavior. Figure 3 shows the real-time plots of the DC as a function of 2MP content with the presence of 10% water and 2% HAp. It can be clearly discerned from the results that the 2MP/HEMA system with varying 2MP contents exhibited distinct polymerization kinetics. The kinetics differed in both the PR and DC. In Fig. 4, the detailed results of the dependence of DC and PR on the monomer composition are given. The figure indicates that the DC and PR of the system increased consistently as the 2MP content increased from 30% to 70%. However, as the 2MP content was further enhanced to 100% (that is, no HEMA in the system), the DC dropped dramatically. The present results suggested that a 2MP mass content of 70% was the most favorable composition for the photopolymerization of the 2MP/HEMA adhesive to carry out. The quantitative data concerning the effect of monomer composition are summarized in Table 1.

In order to find out the possible mechanism of the observed polymerization behavior of the 2MP/HEMA adhesives, the viscosities of the system (with different monomer compositions) were measured since viscosity generally affects diffusion process of radical photopolymerization. The results are shown in Table 2. It can be found that due to the substantial difference in viscosity between 2MP and HEMA (see Table 2), the compositional variation led to considerable viscosity change of the system. For the 2MP/HEMA mixture with 30% 2MP, the system viscosity was relatively low because the viscosity of HEMA (with low viscosity) dominated the solution. As the fraction of 2MP was increased to 50%, 70%, and finally 100%, the viscosities in the mixture solutions were significantly enhanced.
The current results indicated that both polymerization kinetics and viscosity of the 2MP/HEMA system showed similar increasing trend as 2MP content increased from 30% to 70%. When the 2MP content was furthered enhanced to 100%, however, their dependence on 2MP content was different.

**Effect of HAp**

To investigate the role of HAp in the polymerization of the PAE-based self-etch adhesives, the polymerization kinetics of the 2MP/HEMA solution with and without addition of 2% HAp was studied and compared. As shown in Fig. 4, the results disclosed that the addition of 2% HAp could universally lead to the dramatic enhancement of DC and PR of the polymerization, despite of the monomer composition and water content. For all of the compositions studied, the DC could be raised up to 91% by incorporating 2% HAp (with the presence of 10% water), in comparison with the DC of 43% for the PAE solution without HAp. The PR of the system with...
the same composition could also be enhanced from 0.9 to 2.4%/s.

In addition, the extent of HAp-induced increment in the DC and PR (the quantitative difference of the DC and PR between the samples with 0% and 2% HAp in the each plot of Fig. 4) displayed a dependence on monomer composition: it increased as the 2MP content was elevated from 30% to 70%, and decreased when 2MP content was further elevated to 100%. The above finding indicated that the HAp-induced improvement in DC and PR was more significant in the polymerization-favorable composition (i.e., 70% 2MP).

The pH values of 2MP/HEMA adhesives before and after the addition of 2% HAp (10% water content as an example) were determined in order to understand the HAp-induced improvement of polymerization (as shown in Fig. 5). The result showed that for each monomer composition, the pH value was significantly increased by incorporating 2% HAp ($p<0.05$). The difference in pH values between with and without HAp incorporation was 0.3–0.4.

**Effect of water**

Water can affect dental adhesives in many aspects before, during, and after photopolymerization. Therefore,
the role of water is very crucial for the adhesives’ performance and the success of bonded restoration. In our current study, it was found that both the DC and PR decreased as the water content increased from 10% to 30% for all the adhesive systems investigated in this study. For example, Fig. 6 provides the results of the DC and PR as a function of water content for a 2MP/HEMA system with 70% 2MP. It can be revealed that both the DC and PR exhibited a consistent decreasing trend with the elevating water content from 10 to 30%. Other systems containing different amounts of 2MP (30, 50, 100%) showed the similar dependence of polymerization on the water content.

By analyzing the data presented in Fig. 4 and Table 1, it was found that the effect of water on the polymerization also depended on other components such as the incorporation of HAp and monomer composition. For the 2MP/HEMA systems with 0% HAp, the relationships of the DC and PR as a function of the monomer composition were diverse at different water contents (Fig. 4). At 10% water content, the DC or PR varied obviously as a function of the monomer composition. At 30% water content, the DC or PR change as the monomer composition was much less significant. If the difference between the maximum and minimum values of the DC or PR among all the monomer compositions studied is defined as the measure of the variation extent of the DC or PR, it can be seen that the extent of variation as a function of 2MP content decreased as the water content increased. As an example, for 10% water content, the extent of variation in the DC was 22.2%, while the value decreased to 9.5% for 20% water content, and further decreased to 5.1% as the water content reached 30%. The variation of PR with the 2MP content showed similar water content dependence. The phenomenon was probably related to the relatively lower polymerization efficacy in the case of no HAp incorporation in the system. The data in Table 1 indicate that even for the 10% water content, the DC and PR of the current system are quite low (less than 50% and 0.9%/s for the DC and PR, respectively). As the water content was further raised to 20% and 30%, both DC and PR would be suppressed to even a lower level for all the monomer compositions. Therefore, the DC or PR variations among various monomer compositions became less significant.

In contrast, the above-mentioned water influence on the variation extent of the DC or PR was not observed in the system with the incorporation of HAp. This might be associated with the enhanced polymerization induced by HAp. As shown in Fig. 4, along with the substantial improvement in the DC and PR by adding 2% HAp, the DC or PR variations as a function of monomer composition were still detectable even at 30% water content (the variation in the DC was 32.7% in the range of 2MP contents studied).

DISCUSSION
The monomer composition effect
The observed compositional dependence of photopolymerization might imply the influence of viscosity in the monomer mixture system. In the radical photopolymerization, the termination reaction is diffusion-controlled from the beginning of the reaction\(^\text{23}\)). At a low viscosity level in the system, the chain termination by combination of two free radical chains is at a relatively high rate. However, when the growing polymer molecules with active free radical ends are surrounded in the highly viscous mixture, the rate of termination becomes preferentially suppressed relative to propagation by diffusion restrictions. The increase in initial viscosity also contributes to the enhancement of the gel effect (or the autoacceleration effect)\(^\text{23}\)). Therefore, the initial viscosity of the 2MP/HEMA system served as a main determining factor for the polymerization rate. As a result, the DC and PR increased as the 2MP content was enhanced from 30 to 70%. As the 2MP content was raised to 100% (neat 2MP), the further increase in the initial viscosity did not enhance the gel effect because the extremely high viscosity also began to influence the molecule/chain propagation, and made the diffusion control of propagation start at early reaction stages thus...
considerably hampered the polymerization\textsuperscript{29}. This in turn resulted in the dramatic decline of the DC and PR.

Moreover, the compositional dependence of photopolymerization might also indicate the influence of pH changes in the monomer mixture system. Considering the significant difference in acidity between 2MP and HEMA (as an example, the pH values of 2MP and HEMA aqueous solutions, both containing 10% water, are 0.1 and 6.0, respectively), the pH of the mixture solutions will be affected by the compositional changes of the two monomers. The higher the 2MP content in the mixture, the lower the system pH value. This trend was found in the result shown in Fig. 5. Previous research showed that the lower pH could considerably affect the initiating efficiency of amines (co-initiators, such as 4E used in the current study) thus resulted in poor polymerization\textsuperscript{16,19,20}. This pH effect might become especially prominent as the 2MP mass fraction reached a level above 70%. Therefore, along with the viscosity effect, the low pH value of the adhesive system might cause the evident drop of the DC and PR of the polymerization. However, the viscosity effect was most likely the determining factor for the reduced DC and PR in the case of 2MP contents at below 70%.

In addition to the effect on the polymerization efficacy, the pH value altered with compositional change is of importance for dental clinical practice. For a self-etch adhesive system, the capacity of de-mineralizing/etching dentin or enamel is one of major requirements for reliable bonding. Therefore, the etching ability of the adhesive serves as one of the important criteria in the comparison and evaluation of self-etch adhesive systems\textsuperscript{25}. Studies\textsuperscript{26,27} have shown that self-etch adhesives with greater acidity could produce pronounced etching patterns on enamel comparable to those acquired using phosphoric-acid etching. In this regard, a relatively low pH value is preferred in a dental application. On the other hand, laboratory and clinical evidences demonstrated the under-performance of strong/aggressive self-etch adhesives on the bonding durability and restoration longevity\textsuperscript{28-30}. Problems with the single-bottle, strongly acidic self-etch adhesives have also been disclosed regarding the reduced hydrolytic stability of the methacrylate monomers and the continuing etching in dentin tubules\textsuperscript{16,32}. All of these facts suggest a more ‘mild’ self-etch approach. In the PAE self-etch adhesive systems, since the monomer composition not only affects the resulting viscosity (related to the resulting DC), but also directly determine the acidity (related to the etching capacity) of the system, optimizing the monomer composition is especially crucial to achieve both high monomer conversion and great etching and bonding to tooth tissues.

The HAp effect

Hydroxyapatite is the major mineral component of which the dentin and enamel comprised—it accounts for more than 70 % and 90 % (by weight) of dentin and enamel, respectively. However, there has been very few work\textsuperscript{20} about the influence of HAp on the polymerization behavior of acidic PAE monomers so far. Our investigation indicated that HAp could significantly improve the polymerization of the 2MP/HEMA self-etch adhesives. This phenomenon of polymerization improvement was most likely associated to the buffering capacity of HAp to the acidic functional monomer. HAp could chemically react with and thus neutralize the acidic monomer, which induced the augmentation of the pH values of the system. As mentioned before, the tertiary amine (as a co-initiator) in the adhesives might also be neutralized by the acidic functional monomers under a circumstance of high acidity\textsuperscript{16,19,20}. However, with the buffering effect induced by HAp, the negative influence of monomer’s acidity on the initiating efficiency of 4E might have become less significant, so that more co-initiator 4E could survive the harsh acidity environment.

As a result, the incorporation of HAp contributed to the improvement of polymerization behavior (Fig. 4). Our separate study (data not shown here) has disclosed that increasing the 4E concentration from 0.022 to 0.088 mmol per gram of monomers could significantly enhance DC and PR of the 2MP/HEMA system, which clearly demonstrated the essential role of the co-initiator (4E) in polymerization of the PAE self-etch adhesives. In addition, considering the fact that demineralization and photopolymerization take place simultaneously, there is possibility that the chemical reaction of 2MP with HAp influences photopolymerization in many aspects besides the initiating system. The reaction may induce changes in viscosity, heat (depending on exothermicity or endothermicity of the reaction) and molecular interaction within the system, which would inevitably alter the environment of the photopolymerization.

Recently, the additional chemical interaction between the PAE-based self-etch adhesives and residual hydroxyapatite on dentin or enamel has gained attention\textsuperscript{17,18}. The functional monomers have been found to interact chemically with HAp intensively and stably. The finding suggested that besides the established micro-mechanical interlocking mechanism, the interaction effect is also the basis for the long-term bonding and tight marginal adaptation at the resin-dentin/enamel interfaces\textsuperscript{19}. In the current study, both the DC and PR of the polymerization of the 2MP/HEMA system have been substantially improved with the addition of HAp. Since greater polymerization efficacy has tight relationship with a better strength of bonding to tooth tissues\textsuperscript{34,35}, the present observation indicated that the HAp-induced improvement on polymerization might have been another essential factor for the superior bonding effectiveness of PAE-based self-etch adhesives to enamel/dentin, apart from the above-mentioned mechanisms.

The water effect

Water or saliva is ubiquitous in the mouth of healthy patients. It not only exists on the surface of dentin or enamel, but also in dentin tubules which often are filled by water or oral fluids. Water in self-etch adhesives is also an essential component to enable the ionization of the functional monomers, hence to make the adhesives

The HAp effect

Hydroxyapatite is the major mineral component of which the dentin and enamel comprised—it accounts for more than 70 % and 90 % (by weight) of dentin and enamel, respectively. However, there has been very few work\textsuperscript{20} about the influence of HAp on the
acidic for effective etching or demineralization\textsuperscript{26,37}. The water-based self-etch adhesives have led to some concerns regarding the depressed monomer double-bond conversion, phase separation, and reduced shelf life\textsuperscript{38-40}. For the polymerization of an adhesive, the incorporation of water would dilute the concentration of radicals and monomers. If the dilution reaches an extent that there might not be adequate free radicals for polymer chain propagation, the polymerization rate and degree of conversion would decrease. In this study, it was observed that water could inhibit polymerization of the 2MP/HEMA adhesives. Furthermore, the present results revealed that the involvement of multi factors (such as monomer composition, HAp, and water in this study) makes the polymerization behavior of self-etch adhesives even more complicated, since these factors act differently and might interfere with each other (Fig. 4 and Table 1). The comprehensive influence of these factors needs to be fully taken into account in the related studies and clinical applications. The results presented in this study will provide valuable information on the influence of these factors on the polymerization behavior of PAE-based self-etch adhesives.

CONCLUSION

In summary, the experimental data indicated that the photopolymerization of the PAE based self-etch system of 2MP/HEMA showed remarkable dependence on monomer composition. The DC and PR of the polymerization first increased and then decreased with the 2MP mass fraction ranging from 30\% to 100\%, and a 2MP content of 70\% was found to be the most favorable composition for the photopolymerization to carry out. Alteration in both viscosity and acidity induced by varying monomer composition of the system might have contributed to the above results. Other factors such as interactions with HAp and water were also revealed to have posed significant effects on the polymerization of the current self-etch adhesives.

ACKNOWLEDGMENT

This investigation was supported in part by USPHS Research Grants T32-DE07294 and R15-DE021023 from the National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892.

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