Novel Mucoadhesive Polymer: Synthesis and Mucoadhesion of Poly[acrylic acid-*co*-poly(ethylene glycol) monomethylether monomethacrylate-*co*-dimethylaminoethyl methacrylate]

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ABSTRACT: This study was to design a mucoadhesive based on the biological and physicochemical properties of the buccal mucosa to achieve optimal mucoadhesion in the aqueous buccal environment. Since the buccal surface is negatively charged, a series of novel mucoadhesive poly[acrylic acid-*co*-poly(ethylene glycol) monomethylether monomethacrylate-*co*-dimethylaminoethyl methacrylate] [poly(AA-PEGMM-DMEMA)] were synthesized by incorporating the cationic monomer DMEMA into poly(AA-PEGMM) to enhance the interactions between the mucohadhesive polymer and the buccal mucosa. The compositions of poly(AA-PEGMM-DMEMA) were varied by changing the content of DMEMA from 0 to 4.8 mol % while keeping the mole ratio of AA to PEGMM at a constant 9 : 1. It was found that the force of mucoadhesion of poly(AA-PEGMM-DMEMA) increased initially, as DMEMA content increased, and reached the maximum at 1% of DMEMA. Further increasing the content of DMEMA decreased the mucoadhesion. The polymers with 0.5 to 2.9% DMEMA appeared to have maximum mucoadhesion after prehydration for 5 min. An ATR–FTIR spectroscopy study revealed that intrapolymer interactions and intersurface interactions played opposite roles in the mucoadhesion performance of the polymers. Optimal mucoadhesion can be achieved by balancing these two interactions. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 94: 2431–2437, 2004

Key words: adhesion; biopolymers; hydrophilic polymers; cationic monomer; buccal drug delivery

INTRODUCTION

Buccal mucoadhesion, which generally refers to the binding formed between the surfaces of an adhesive material and the buccal mucosa, is crucial for retaining a transbuccal drug delivery system at the site of application for a desired period of time. The aqueous environment in the oral cavity drove the search for buccal mucoadhesives to polymers possessing a certain degree of hydrophilic properties. Poly(acrylic acid) (PAA), a hydrophilic polymer, has been found to be a good mucoadhesive. However, the mucoadhesion and mechanical strength of PAA are not optimal for transbuccal drug delivery systems. Based on the measured water contact angle of the buccal mucosa, $57.5 \pm 4.3°$,[1] the surface of buccal mucosa is relatively hydrophobic. The buccal mucoadhesion of PAA can be improved by introducing a small amount of relatively hydrophobic components into PAA to enhance the hydrophobic interaction between the polymer and the buccal mucosa while maintaining the hydrophilic properties of the polymer. The copolymer of acrylic acid and poly(ethylene glycol) monomethylether monomethacrylate [P(AA-*co*-PEGMM)] was designed and synthesized by Shojaei and Li[1] in our laboratory. The mucoadhesion of the copolymer with monomer ratio of AA to PEGMM 87 : 13 was increased significantly compared to that of PAA. The hydrophobicity in this copolymer, resulting from methylene groups in PEGMM, promotes van der Waal interactions, while the oxygen in the poly(ethylene glycol), serving as a proton acceptor, increases hydrogen bonding. The inter- and intramolecular hydrogen bonds improve both adhesion and cohesion of the mucoadhesive.

Since the buccal surface is negatively charged,[2] it was hypothesized that better mucoadhesion could be achieved by introducing a cationic monomer into the mucoadhesive to provide additional interactions between the mucoadhesive and the buccal surface. In this study, a cationic monomer, 2-(*N*,*N*-dimethylamino)ethyl methacrylate (DMEMA), was used to synthesize a series of novel mucoadhesive poly[acrylic acid-*co*-poly(ethylene glycol) monomethylether mono-

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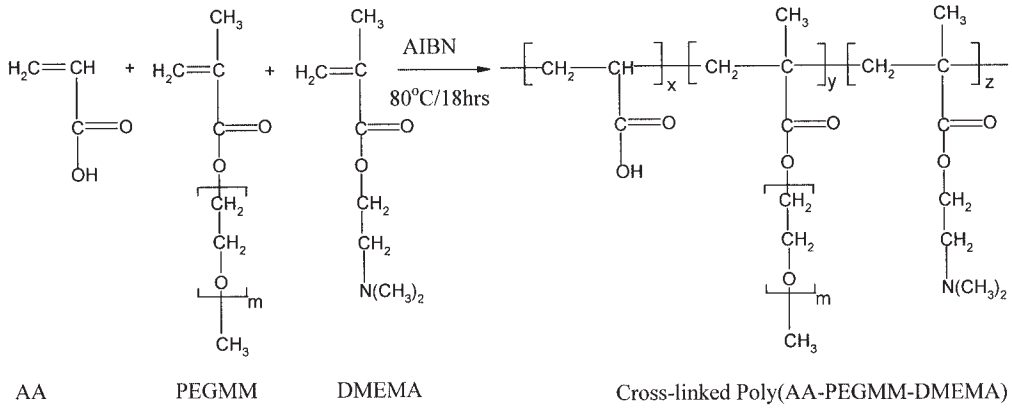

Figure 1 Synthesis of mucoadhesive polymer poly(AA-PEGMM-DMEMA).

methacrylate-*co*-dimethylaminoethyl methacrylate] [poly(AA-PEGMM-DMEMA]. In addition, the force of mucoadhesion of the polymer was determined by a tensile testing method and the mechanism of mucoadhesion was investigated from the molecular interaction point of view.

MATERIALS

Chemicals

Acrylic acid (AA) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Poly(ethylene glycol) monomethylether monomethacrylate (PEGMM) (molecular weight of PEG unit: 200 Dalton), 2-(*N*,*N*-Dimethylamino)ethyl methacrylate (DMEMA), Dehibit 100 ion exchange resin, and ethylene glycol dimethacrylate (EGDMA) were purchased from Polysciences, Inc. (Warrington, PA). 2–2′-Azobisisobutyronitrile (AIBN) was purchased from Janssen Chemical (Belgium). Sialic acid (98% min.) and Mucin (Type I-S from bovine submaxillary glands) were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals were used as received.

Polymer synthesis

AA, PEGMM, and DMEMA were dehibited by Dehibit 100 ion exchange resin for 24 h prior to polymerization. The compositions of poly(AA-PEGMM-DMEMA) were varied by changing the content of DMEMA from 0 to 4.8 mol % while keeping the mole ratio of AA to PEGMM at a constant 9 : 1. The monomer solution with the initiator, AIBN (monomer to initiator ratio = 1,000 : 1), was purged with nitrogen and then degassed by a vacuum pump. The degassed solution was filled into a mold that was constructed with two glass plates and a silicone rod as the spacer. The polymerization was carried out in an oven at 80°C for 18 h (Fig. 1). The resulting film was washed with deionized water for 48 h. The same method was used to prepare poly(acrylic acid) (PAA) crosslinked with 0.3 wt % EGDMA. The synthesized polymers from different batches were used for the following studies.

Determination of glass transition temperature

The glass transition temperature (T_g) of poly(AA-PEGMM-DMEMA) was determined by using a differential scanning calorimeter (DSC-50, Shimadzu) and a Shimadzu thermal analyzer (TA-50) data system. The measurements were conducted at a constant heating rate of 10°C/min from –20 to 120°C for the dry polymers and from −60 to 60°C for the polymers prehydrated in deionized water for 24 h. The measurements were conducted in triplicate.

Hydration study

Polymer film discs with 0.6 cm in diameter were prepared and placed in 25 mL scintillation vials. About 15 mL of normal saline was used as the swelling medium. The temperature was maintained at 37 ± 1°C by a water bath. The weight and diameter of the discs were measured and recorded after predetermined intervals. The measurements were carried out in triplicate. The percentage of hydration was calculated using the following equation:

$$H = \frac{W_{\text{Hydrated}} - W_{\text{Dry}}}{W_{\text{Hydrated}}} \times 100 \qquad (1)$$

where W_{Dry} and W_{Hydrated} are the weight of the polymer disc before and after hydration, respectively.

Tissue preparation

Porcine buccal tissues were obtained immediately after the pigs were slaughtered (Long Ranch, Manteca, CA) and were stored in normal saline at 4°C. Buccal mucosa was separated from underlying tissue by sur-

gical scissors. The mucosa was used within 2 h after slaughter. To measure the force of mucoadhesion, a piece of buccal mucosa (1.5 × 5 cm) was secured onto a plastic holder stage and fresh buccal mucosa was used in each measurement. The buccal mucosa was maintained at 37 ± 1°C during the study.

Force of mucoadhesion measurement

The mucoadhesive force measurement system, as reported in our previous studies,[1] consists of a sample holder, a load cell (GS-500, Transducer Techniques, Temecula, CA), an analog/digital (A/D) converter (Model 500A, Keithley Metrabyte, Taunton, MA), and a personal computer. Analog signals generated by the load cell were converted to digital signals by the A/D converter and were acquired by the computer. The data were recorded and analyzed by using EasyLX software (Keithley Metrabyte). The mucoadhesive force measurement system was calibrated by standard weights (Permas®, Fisher Scientific Co.). During the measurement, one side of the polymer film disc was affixed onto the glass slide (Arthur H. Thomas Co., Philadelphia, PA) by superglue (Super Duper, ITW Devcon, Danvers, MA). The discs were hydrated in the normal saline at 37 ± 1°C for a predetermined period of time and the diameters of the discs were measured. The hydrated disc was then placed on the porcine buccal surface and an external force of 50 g (including the weight of sample holder and the glass slide) was applied. The contact was maintained for 1 min. The sample holder was then raised at a constant speed of 0.3 mm/s driven by a precision motor. The maximum detachment force, which was required to separate the polymer from the buccal mucosa, was recorded as the force of mucoadhesion. The detached buccal tissues and the polymers were examined under a microscope for any possible cohesive failure. The measurements were carried out in triplicate.

ATR–FTIR spectroscopic study

Infrared spectra were obtained by using a Nicolet Impact 400 spectrometer (Nicolet Instruments, Madison, WI) with a horizontal Attenuated Total Reflectance (ATR) kit (Spectra Tech, Inc., Stamford, CT). Polymer films were prehydrated in deionized water for 3 h before being placed onto a zinc selenide ATR crystal. To investigate the interactions between the mucus and the DMEMA component in the polymer, the prehydrated polymer film was immersed in 0.01*M* sialic acid or 0.1% (wt/vol) mucin solution for 5 min. The film was then rinsed with deionized water for 5 min to remove any sialic acid or mucin on the surface of the film. Intimate contact between the surfaces of the ATR crystal and the polymer film was obtained by applying a Minigrip (Spectra Tech, Inc.). Spectra were acquired and analyzed by a personal computer with Omnic IR software (Version 1.20, Nicolet Instruments). The absorption spectra of water, sialic acid, and mucin solutions were subtracted as the background. The interactions between DMEMA monomer and sialic acid were studied by using a mixed solution containing 75% (vol/vol) DMEMA and 25% (vol/vol) 0.01*M* sialic acid. The experiments were carried out in triplicate.

RESULTS AND DISCUSSION

The role of macromonomer PEGMM in the acrylic polymer has been characterized by Shojaei in our laboratory.[1] The presence of PEG units would yield chain branching and result in crosslinking through radical polymerization by chain transfer.[3] Therefore, the polymer in this study is a slightly crosslinked polymer, which will not be dissolved in the water and other solvents. This study is focused on the role of DMEMA in the mucoadhesion performance of this novel poly(AA-PEGMM-DMEMA). Introducing DMEMA into poly(AA-PEGMM-DMEMA) would affect the crosslinking of the polymer resulting from the interactions between AA and DMEMA within the polymer. Crosslinking would restrict the movements of polymer chain segments, which could be revealed by measuring the T_g of the polymer. The measured T_g of poly(AA-PEGMM-DMEMA) decreased from 35.4 ± 0.7 to 34.3 ± 0.8°C as DMEMA content increased from 0 to 2.9%, showing no significant difference (ANOVA, $P > 0.05$) in T_g over the studied range of DMEMA content. Since T_g reflects the mobility of chain segments in a polymer, a low value of T_g indicates a high mobility of chain segments, which would facilitate interpenetration and entanglement of the polymer to the buccal mucosa in early stages of mucoadhesion.[4] Although poly(AA-PEGMM-DMEMA) without hydration appears glassy at room temperature, a rubbery state of the polymer could be achieved at body temperature. In addition, water can serve as a plasticizer[5] and significantly reduce the T_g of the polymer. The T_g of hydrated poly(AA-PEGMM-DMEMA) decreased to the range of –26.1 ± 0.5 to –26.9 ± 0.6°C as DMEMA content was varied from 0 to 2.9%.

The hydration rate and extent of a polymer is mainly controlled by the rate and extent of water penetration and the relaxation of polymer segments. The pH of the hydration media could greatly affect the hydration of the polymers with ionic components. Since poly(AA-PEGMM-DMEMA) was designed for the application on the buccal surface, the hydration and mucoadhesion studies of this polymer were conducted in normal saline with pH mimicking the physiological conditions. The hydration profiles of the poly(AA-PEGMM-DMEMA) and poly(acrylic acid) are shown in Figure 2. PAA has the fastest hydration

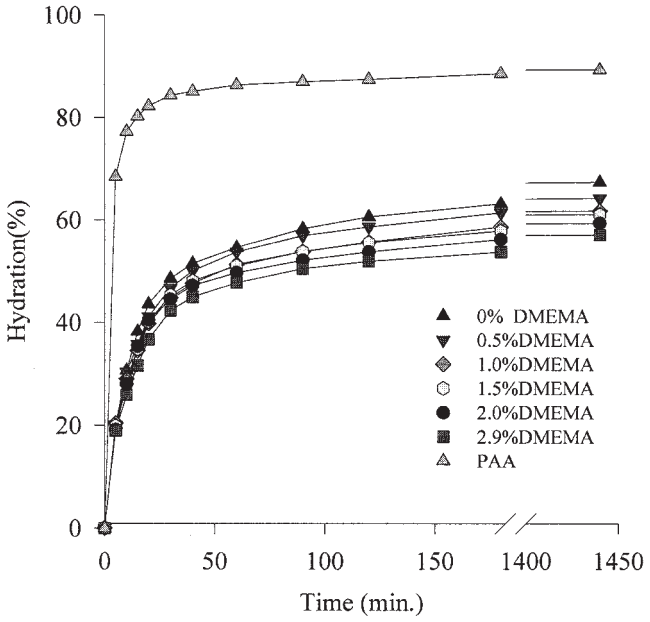

Figure 2 Hydration kinetics of poly(AA-PEGMM-DMEMA) and poly(acrylic acid) in normal saline. Each data point represents the mean ($n = 3$).

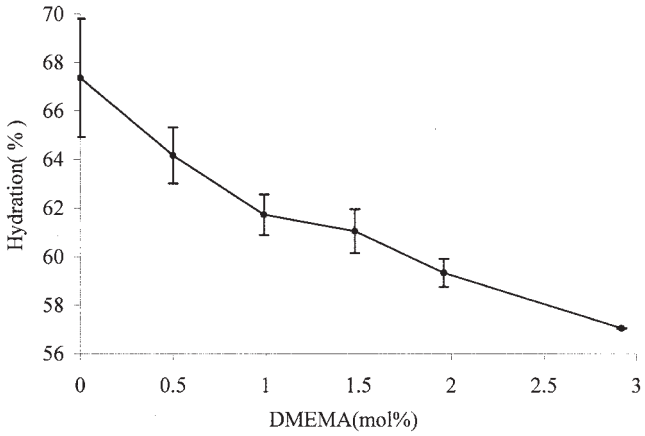

Figure 3 Effect of DMEMA on the equilibrium hydration of poly(AA-PEGMM-DMEMA) in normal saline. Each data point represents the mean and each error bar represents the standard deviation ($n = 3$).

profile among all polymers studied: the hydration of PAA increased from 0 to 68% of the equilibrium hydration within 5 min and reached equilibrium after 3 h. In contrast, poly(AA-PEGMM-DMEMA) had only about 30% of equilibrium hydration after 5 min. It took about 16 h for poly(AA-PEGMM-DMEMA) to reach the hydration equilibrium. This difference could be attributed to the complex formed between the very hydrophilic AA and the less hydrophilic PEG repeat unit, ethylene glycol (EG), within poly(AA-PEGMM-DMEMA).[1,6] The hydration of poly(AA-PEGMM-DMEMA) decreased significantly (ANNOVA, $P < 0.05$) as the DMEMA content in the polymer increased (as shown in Fig. 3). This decreased hydration could result from the increased interaction between the amino group in DMEMA and the carboxylic group in AA, leading to the increase of crosslinking in the polymer.

Various *in vitro* techniques have been developed to evaluate the bioadhesion of polymers. These techniques include tensile testing,[1,7–10] shear stress testing,[11] a fluorescent probe method,[12] flow through technique,[13] colloidal gold staining,[10,14,15] rheological examination,[16–21] ultracentrifugation,[22,23] and atomic force microscopy.[24] Currently there is no standard testing method to measure bioadhesion. Many factors can affect the results of *in vitro* bioadhesion assessment. These factors include the method of measurement, the surface nature of biological tissue used, the means of applying stress to the adhesive joint,[8] the contact time of the two substrates, the speed to remove the bioadhesive material from the biological tissue, and the external contact force applied.[9] In this study, tensile testing, which measures all interactions involved in the mucoadhesion, was used to determine the mucoadhesion of poly(AA-PEGMM-DMEMA). In addition, an ATR–FTIR study was conducted to reveal the interactions between the mucoadhesive and the substrate from the molecular perspective.

The mucoadhesion performance of poly(AA-PEGMM-DMEMA) with 5-min hydration before measurement is shown in Figure 4. Initially, the force of mucoadhesion increased as the DMEMA content increased and reached a maximum at 1% DMEMA. Further increasing the content of DMEMA caused the mucoadhesion of the polymer to decrease. Compared to poly(AA-PEGMM), the mucoadhesion of poly(AA-PEGMM-DMEMA) was significantly (ANOVA, P

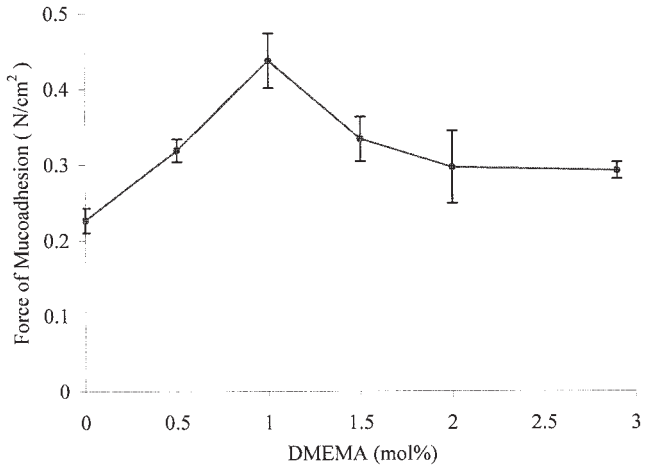

Figure 4 Force of mucoadhesion of poly(AA-PEGMM-DMEMA) measured after 5 min hydration before measurement. Each data point represents the mean and each error bar represents the standard deviation ($n = 3$).

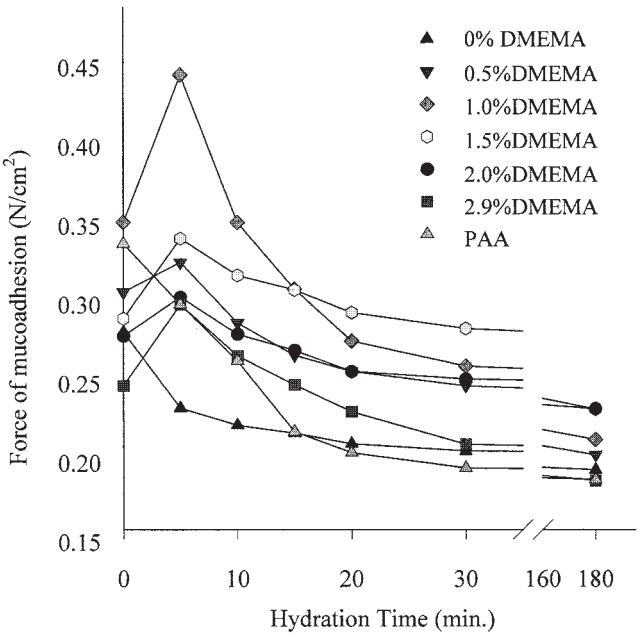

Figure 5 Force of mucoadhesion of poly(AA-PEGMM-DMEMA) and poly(acrylic acid) measured in normal saline at different hydration time. Each data point represents the mean ($n = 3$).

< 0.05) enhanced by introducing 0.5, 1, and 1.5% DMEMA in the polymer. The mucoadhesion of poly(AA-PEGMM-DMEMA) with 1% DMEMA was 0.44 ± 0.04 N/cm^2, significantly ($P < 0.05$) higher than that of crosslinked PAA, 0.29 ± 0.02 N/cm^2.

The relationships between mucoadhesion and hydration time of these polymers are plotted in Figure 5. PAA and poly(AA-PEGMM) showed the maximum mucoadhesion without hydration, and the mucoadhesion of both polymers decreased as the hydration increased. The maxima for mucoadhesion of poly(AA-PEGMM-DMEMA) with DMEMA content from 0.5 to 2.9% were observed after 5 min of hydration. The mucoadhesion then declined as the hydration increased. The mucoadhesion of hydrated poly(AA-PEGMM-DMEMA) with 1 and 1.5% DMEMA was significantly (ANOVA, $P < 0.05$) higher than that of hydrated poly(AA-PEGMM) and PAA over the range of the hydration studied. The polymer with 2.9% DMEMA did not show mucoadhesion improvement at all hydration levels compared to poly(AA-PEGMM) and PAA. No cohesive failures within the buccal mucosa and all hydrated polymers were observed during the mucoadhesion measurement.

The mucosal surface is covered by a layer of mucus gel, which is comprised of water and mucin. In a mucoadhesive joint, mucus gel is sandwiched between mucosal epithelial cells and the mucoadhesive. Mortazavi and Smart[25] found that water movement from the mucus gel to the contacting dry or partially hydrated mucoadhesive could result in a substantial increase in the cohesive and adhesive properties of the mucus gel, which in turn would strengthen the mucoadhesive joint. Mortazavi and Smart in a separate study[26] found that restricted hydration was required to prolong mucoadhesion. Two of possible approaches to restrict the hydration of the mucoadhesive are increasing the density of crosslinking and introducing hydrophobic entities in the mucoadhesive. In this study, the polymers with low hydration showed high mucoadhesion, which could be attributed to the dehydration of mucus gel to form strong adhesive joints at low hydration. As the hydration of the polymers increased, the dehydration of mucus gel decreased. In addition, the volumes of the hydrated polymers increased greatly at high hydration levels, yielding low densities of interaction sites between the polymers and the buccal mucosa to form adhesive joints. Therefore, the mucoadhesion of the polymers decreased as the hydration increased.

An ATR–FTIR study was conducted to explore the mechanism of mucoadhesion enhancement and contributions from the positively charged monomer DMEMA in this novel mucoadhesive. The FTIR spectra of the polymers are shown in Figure 6. The absorptions at 1,715 and 1,560 cm^{-1} represent the stretching vibration of carbonyl groups and carboxylate groups in the polymer, respectively. The absorption of carboxylate group at 1,560 cm^{-1} was associated with the interaction between the amino group in DMEMA and the carboxylic group in AA.[27] The ratio of areas under the peak at 1,560 and 1,715 cm^{-1} (R) was used to study the interaction between AA and DMEMA. As the con-

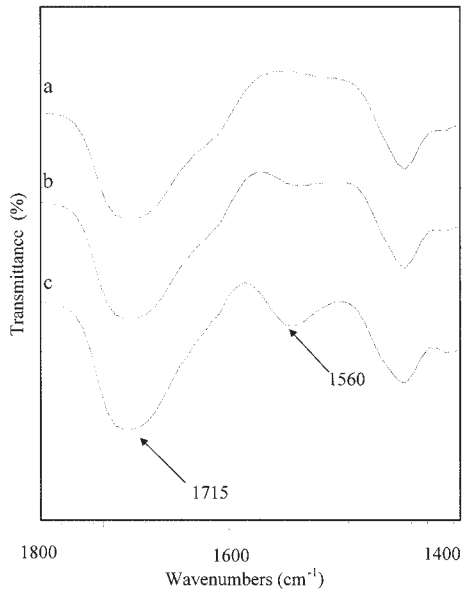

Figure 6 ATR–FTIR spectra of poly(AA-PEGMM-DMEMA): (a) 0% DMEMA, (b) 1% DMEMA, (c) 4.8% DMEMA. Water absorption spectrum was subtracted as the background in spectra a, b, and c.

TABLE I
Ratio of Areas under the Peak at 1,560 and 1,715 cm^{-1} (*R*) With and Without Treatment of Sialic acid or Mucin for Poly(AA-PEGMM-DMEMA)[a]

Treatment	DMEMA (mol %)	*R* (mean ± SD)
No treatment	0	0
	1	0.029 ± 0.002
	4.8	0.127 ± 0.005
Sialic acid	1	0.046 ± 0.003
Mucin	1	0.061 ± 0.007

[a] Mean ± SD, $n = 3$.

tent of DMEMA was increased, the absorption at 1,560 cm^{-1} increased. The value of *R* increased from 0 to 0.127 ± 0.005 when the DMEMA content was increased from 0 to 4.8% (Table I), indicating the presence of an increased interaction between AA and DMEMA. The interaction between AA and DMEMA could serve as the crosslinker in the polymer, increasing the cohesion and lowering the mobility of chain segments in the polymer. Therefore, the mucoadhesion was reduced as the content of DMEMA was increased.

Since sialic acid is positioned at the terminal ends of oligosaccharide chains in mucin and is considered as the major source of negative charges in mucus,[2] sialic acid was used to examine the interaction between the polymer and the buccal mucosa. Poly(AA-PEGMM-DMEMA) with 1% DMEMA was selected to investigate the interaction between the amino group in DMEMA and the carboxylic group in sialic acid because of its good mucoadhesion performance among the polymers studied. As shown in Figure 7, the interaction occurring between the amino group and carboxylic group in the mixed solution of DMEMA and sialic acid resulted in the absorption of carboxylate

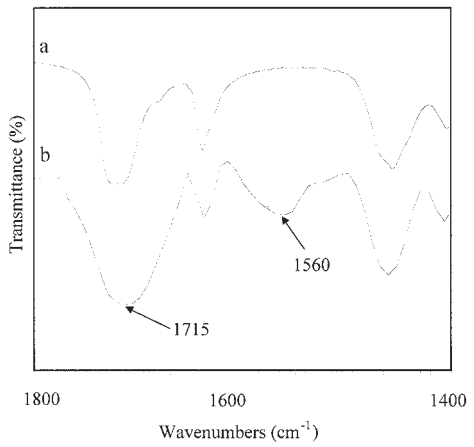

Figure 7 ATR–FTIR spectra: (a) DMEMA, (b) DMEMA and sialic acid mixed solution. Sialic acid solution absorption spectrum was subtracted as the background in spectrum b.

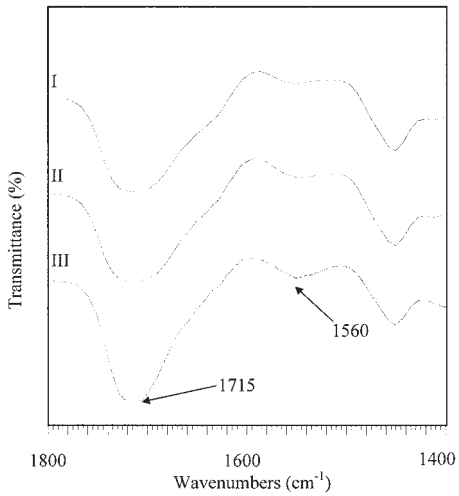

Figure 8 ATR–FTIR spectra of poly(AA-PEGMM-DMEMA): (I) 1% DMEMA without treatment; (II) 1% DMEMA after treatment with sialic acid (sialic acid solution absorption spectrum was subtracted as the background); (III) 1% DMEMA after treatment with mucin (mucin solution absorption spectrum has been subtracted as the background).

group at 1,560 cm^{-1}. When the polymer was treated with sialic acid, a further increase in absorption at 1,560 cm^{-1} [Fig. 8(II)] was observed compared to that without treatment [Fig. 8(I)]. The value of *R* increased from 0.029 ± 0.002 to 0.046 ± 0.003 (Table I). This result demonstrates the interaction between the amino group in poly(AA-PEGMM-DMEMA) and the carboxylic group in sialic acid. To further verify the existence of the interaction between poly(AA-PEGMM-DMEMA) and the buccal mucosa, mucin was utilized in this spectroscopic study. An increase in absorption at 1,560 cm^{-1} was also observed after the polymer was treated with mucin [Fig. 8(III)] and the value of *R* increased to 0.061 ± 0.007 (Table I). This result was in agreement with that obtained with sialic acid treatment.

The ATR–FTIR study revealed the existence of intrapolymer interactions within the polymer and intersurface interactions between the polymer and the buccal mucosa. While the intrapolymer interactions can increase the crosslinking within the polymer and lead to the decrease of mucoadhesion, the intersurface interactions can promote mucoadhesion of the polymer. The intrapolymer interactions and intersurface interactions played opposite roles in the mucoadhesion performance of poly(AA-PEGMM-DMEMA). When the effect of interactions between the polymer and the buccal surface outweighs that of intrapolymer interactions, high mucoadhesion is observed. However, if intrapolymer interactions are dominant, low mucoadhesion is exhibited. Optimal mucoadhesion can be achieved by balancing these two interactions.

CONCLUSION

The results of this study provide a rationale for the design of new mucoadhesives. Optimal mucoadhesion can be achieved by designing tailor-made mucoadhesive according to the biological and physicochemical properties of the buccal mucosa. The design, synthesis, and characterization of the novel mucoadhesive poly(AA-PEGMM-DMEMA) is an example of using this rationale. It was demonstrated that the interactions between the positively charged DMEMA in the mucoadhesive and the negatively charged mucosal surface can enhance the mucoadhesion of PAA-based mucoadhesives. The analysis of interactions within the polymer and between the polymer and the substrate surface revealed that an ideal mucoadhesive should have a balance between intrapolymer interactions and intersurface interactions to afford both cohesive properties and adhesive characteristics to the mucoadhesives.

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