Photoscissable Hydrogel Synthesis via Rapid Photopolymerization of Novel PEG-Based Polymers in the Absence of Photoinitiators[⊥]

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Abstract: Branched polyethylene glycol (four arms, MW = 15 000) having a cinnamylidene acetyl moiety as a pendant group was synthesized by an esterification reaction between polyethylene glycol and cinnamylidene acetyl chloride. The photosensitive polymer was irradiated with a 450 W medium pressure Hg lamp (λ > 300 nm) from 5 min to 3 h to produce polyethylene glycol hydrogels. These gels were swollen in water and showed characteristic properties of a hydrogel. The degree of swelling was controlled by the content of cinnamylidene acetyl moiety in the polymer and the time of ultraviolet irradiation. A reduced degree of substitution resulted in increased swellability of the synthesized hydrogel. The photoscission of the gel, which was monitored by its UV spectrum, was performed by irradiating the hydrogel with a 150 W Xenon lamp at 254 nm using a bandpass filter. The biocompatibility of the synthesized gel was also determined. The antithrombogenic behavior (99.6% reduction in platelet deposition) of the synthesized b-PEG-CA hydrogel was demonstrated by measuring platelet adhesion onto coverslips which had been coated with PMMA with a second coating film of b-PEG-CA hydrogel.

Introduction

Hydrogels, due to characteristic properties such as swellability in water, hydrophilicity, biocompatibility, and lack of toxicity, have been utilized in a wide range of biological and medical applications.[1−6] Although conventional chemical cross-linking has been extensively used as a hydrogel preparation method, relatively little work has been reported on the preparation of biocompatible hydrogels via photopolymerization of water soluble polymers.[1−7] Current photoinduced systems for hydrogel preparation include the following: (1) free radical polymerization initiated by long wave ultraviolet light or visible light[5,6] of acrylate groups attached to water soluble polymers and (2) photodimerization of photosensitive groups such as cinnamate, stilbazolium, or coumarin which are added as terminal functional groups to hydrophilic polymers.[3,4,7] Although all of the previous work demonstrated photopolymerization, most systems require the presence of initiators and/or photosensitizers. Also, the photoreversibility of all previously reported systems is very limited.[3,7] Facile photoreversibility allows for several potential applications. For example, one can envision designing a controlled release hydrogel system, where the trigger for release is light. In this paper, we describe the photosynthesis, photoscission, and preliminary blood biocompatibility of a novel nonionic hydrogel, polyethylene glycol-cinnamylidene acetate (b-PEG-CA).

The cinnamylidene acetyl group is a member of the cinnamate family and undergoes a 2 + 2 cycloaddition reaction when it is irradiated with the appropriate wavelength of UV light. Cross-links are formed by photoaddition between an excited (*) cinnamylidene acetyl group of one chain with a ground state cinnamylidene acetyl group belonging to another chain,[8−15] as described by the orbital theory of Woodward and Hoffman. We have chosen the cinnamylidene acetyl group as a photocross-linkable group because it exhibits a high photosensitivity, even to visible light, and particularly efficient photoreversibility.[8−12] Although, the cinnamylidene acetyl functionality has been used extensively in printing and photoresist applications,[14] it has not been investigated in biomedical water-soluble systems.

Materials and Methods

Materials. "Four-armed" polyethylene glycol (b-PEG, MW = 15 000) was purchased from Shearwater Polymer, Inc. All other materials were purchased from Aldrich (St. Louis, MO) and used as received.

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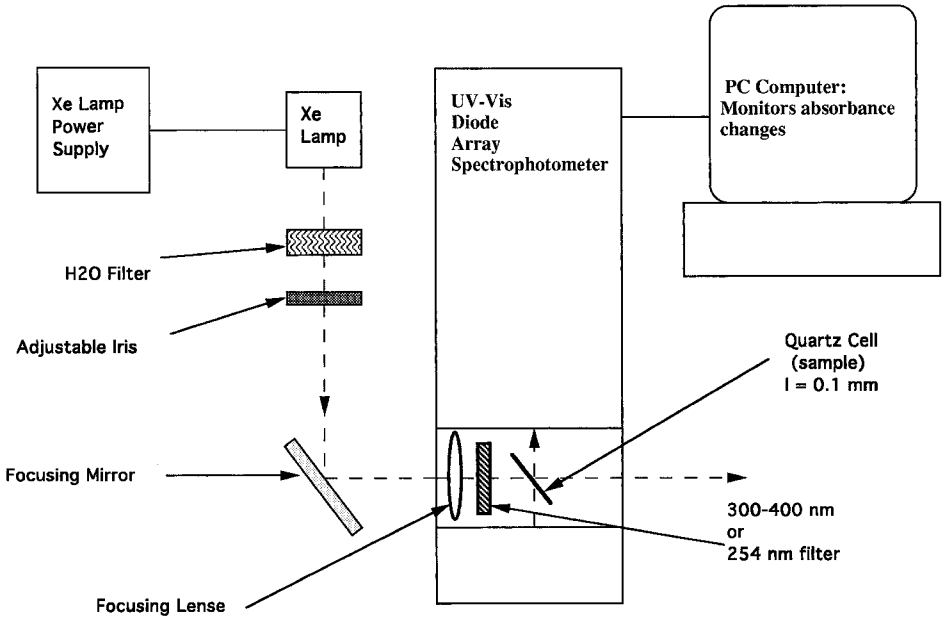

Figure 1. A diagramatic representation of the UV photolytic system.

Synthesis of Cinnamylidene Acetic Acid. Cinnamylidene acetic acid was synthesized according to the method described in U.S. patent 3 257 664.[16] Typically, 104 g of malonic acid (1 mol), 126 mL of *trans*-cinnamaldehyde (1 mol), and 90 mL of pyridine were mixed and heated mildly ($T = 40-45$ °C) for 20 min. Piperidine (0.25 mL) was then added, and the mixture was allowed to stand at room temperature for 26 h. The reaction mixture was then refluxed at 115 °C for 18 hours. The reaction mixture was allowed to cool down to room temperature, and was then poured into cold, dilute (5 w/v%) hydrochloric acid. The resulting yellowish solid cake was washed repeatedly with deionized water, and the wet product was recrystallized from ethyl alcohol to give cinnamylidene acetic acid (65 g, 40% reaction yield). The purity of cinnamylidene acetic acid was determined by ^{1}H-NMR and UV spectroscopy [^{1}H-NMR ($CDCl_3$-TMS) $\delta = 6.1$ ppm (d, ═CH-CO_2-), $\delta = 6.9$ ppm (q, ═CH-CH═), $\delta = 7.5$ ppm (m, C_6H_5-CH═)]. The −COOH proton could not be detected on the NMR spectrum. However, the product of the esterification reaction between cinnamylidene acetic chloride and polyethylene glycol establishes its existence. In addition, the melting point of the cinnamylidene acetic acid as obtained by differential scanning calorimetry was in agreement ($T_m = 167$ °C) with previous measurements:[16] UV (in dichloromethane) $\lambda_{max} = 313$ nm, $\epsilon = 37\,390$ L mol^{-1} cm^{-1}; $\lambda_{min} = 251$ nm, $\epsilon = 1985$ L mol^{-1} cm^{-1}.

Synthesis of Cinnamylidene Acetyl Chloride. Cinnamylidene acetyl chloride[17] was prepared by stirring 17 g of cinnamylidene acetic acid (0.1 mol) with 30 mL thionyl chloride (0.4 mol) in 300 mL of petroleum ether for 12 h at room temperature. The reaction mixture was then refluxed for 4 hours at 40−50 °C. Excess thionyl chloride and the petroleum ether were subsequently removed under reduced pressure to recover the product (80% yield). The melting point of the synthesized acetyl chloride was found to be 45−46 °C: ^{1}H-NMR ($CDCL_3$-TMS) $\delta = 6.2$ ppm (d, ═CH-CO_2-), $\delta = 6.9$ ppm (q, ═CH-CH═), $\delta = 7.5$ ppm (m, C_6H_5-CH═); UV (in dichloromethane) $\lambda_{max} = 333$ nm, $\lambda_{min} = 256$ nm.

Synthesis of Branched-PEG-Cinnamylidene Acetate. A typical procedure for the synthesis of the photosensitive PEG is described below. b-PEG (1.5 g, four-armed, MW = 15 000) was placed in a dry, 250-mL, three-necked flask, dissolved in 80 mL of THF (HPLC grade), and purged with N_2 (99% purity) for 15 min. Cinnamylidene acetyl chloride (1.65 g) which had previously been dissolved in 10 mL of THF (HPLC grade) was then transferred dropwise, through a septum, into the reaction flask. The reaction mixture was stirred continuously in the dark for 17 h at room temperature. Finally, the mixture was refluxed for 6 h at 45−50 °C. The resulting product was isolated by first removing the solvent by vacuum distillation, then precipitating it three times in diethyl ether, and, finally, washing it three times with acetone to remove any unreacted cinnamylidene acetyl chloride (CAC). The modified PEG (1.05 g, 70% yield) was collected, and the product was placed in a vacuum oven to dry overnight at room temperature: ^{1}H-NMR (D_2O-TMS) $\delta = 3.71$ ppm (t, -(CH_2CH_2O)$_n$-), $\delta = 6.13$ ppm (d, ═CH-CO_2-), $\delta = 7.14$ ppm (d, ═CH-CH═), $\delta = 7.5$ ppm (m, C_6H_5-CH═); UV (in dichloromethane) $\lambda_{max} = 313$ nm, $\lambda_{min} = 254$ nm.

Photocross-Linking. Typically, cinnamylidene acetate-modified polyethylene glycol was dissolved in dichloromethane and then cast over a glass slide at room temperature under air. The film was irradiated with a 450 W medium pressure UV lamp (Hanovia, Newark, NJ). The lamp was placed in a water-jacketed well, 10 cm above the coated slide, and the polymer film was irradiated for up to 3 h. A Pyrex filter was used to cut off the wavelengths below 300 nm. After irradiation was completed, the film was washed with deionized water to remove any unreacted polymer and then was dried under vacuum. In separate experiments, the photocross-linking reaction was monitored by studying the change in absorbance at $\lambda_{max} = 313$ nm of the modified b-PEG monomers dissolved in deionized water (0.002 mol/L). A UV spectrophotometer equipped with a 150 W Xenon lamp was used to measure such absorbance changes (see Figure 1).

Determination of Swelling Properties. Photosensitive b-PEG samples with varying degrees of modification were cast on glass slides from a dichloromethane solution. The polymer films were irradiated with a 450 W medium pressure Hg lamp for 75 min. Following irradiation, the polymer films were washed with deionized water to remove any unreacted polymer and then dried at room temperature under air for 2 days. The dried gels were weighed and then soaked in deionized water for 30 min. The swollen gels were removed from solution, dried of any excess of fluid with a tissue, and weighed. The degree of swelling was determined as follows

$$DS = (W_{wet} - W_{dry})/W_{dry}$$

where W_{dry} is the weight of the dry gel and W_{wet} is the weight of the swollen gel.

The degree of swelling was also determined with respect to irradiation time. b-PEG-CA (0.48 g, 69% degree of modification) was dissolved in 2 mL of dichloromethane. From the resulting solution four films were cast on 24 × 50 mm cover slips. The samples were irradiated with a 450 W lamp for 1, 2, 3, and 4 h, respectively. The

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irradiated polymer films were washed repeatedly with deionized water and soaked in water for 6 h, and, finally, the resulting hydrogels were washed with water to remove any impurities and unreacted polymer. From the purified gels, small pieces were cut and placed on 22 × 22 mm cover slips which were dried in air overnight. These samples were used for determining the degree of swelling with respect to irradiation time as given by the equation described above.

Photoscission of PEG-CA Hydrogels. The photoscission of the cross-linked PEG-cinnamylidene acetate (b-PEG-CA) was monitored using a diode array UV spectrophotometer equipped with a 150 W Xenon lamp. The acquisition of spectra during photolysis (Figure 1) required 1 s per spectrum. A small drop of a b-PEG-CA in deionized water (0.002 moles/L) was placed between two quartz plates (0.1 mm cell thickness). During photolysis (300−400 nm) the photodimerization reaction was monitored for 10 min by taking UV spectra every 2 min. At the 10th minute the filter was replaced with a 254 nm bandpass filter (Andover, Salem, NH), and the photoscission reaction was monitored for the next 4 min. At the end of the fourth minute the original 300−400 nm bandpass filter was placed back in front of the UV source, and the forward reaction (photopolymerization) was observed once again for 10 min. Finally, the 254 nm bandpass filter was placed back in front of the UV light source, and the reverse reaction was studied for a further 2 min. Photoinduced changes in the UV absorption spectrum of the b-PEG-CA were measured between 240 and 360 nm.

Fluorescein-isothiocyanate dextran (Sigma Chemical, St. Louis, MO) (0.04 g, MW = 145 000) was mixed with a 1 mL solution of b-PEG-CA (0.157 g, 69% degree of modification) in water, and the solution was poured into a Pyrex dish. The polymer solution was irradiated with a 450 W Hg lamp for 2.5 h. The resulting photocross-linked polymer was washed with deionized water to remove any uncross-linked polymer and air-dried for 2 days. The dry gel was allowed to equilibrate in 40 mL of water for 26 h. A small piece of the purified gel was irradiated with a 450 W Hg lamp at 254 nm in increments of 10 min. Following each irradiation the gel was placed in 10 mL of fresh deionized water and allowed to equilibrate for 30 min. The water solutions after each irradiation were collected, and the dextran release from the gel matrix was measured by fluorescence spectroscopy at an emission wavelength of 520 nm. The excitation wavelength for the fluorescein-isothiocyanate dextran was 495 nm. A Perkin Elmer luminescence spectrometer LS50B, equipped with a Xenon lamp, was used for the fluorescence measurements.

Blood Biocompatibility of the b-PEG-CA Hydrogels. Resistance to platelet deposition is an important characteristic for blood-contacting biomaterials, and, although not entirely sufficient, such resistance is usually considered necessary for extended blood-contacting applications.[18,19] The blood biocompatibility of the b-PEG-CA hydrogels was determined by measuring the adhesion of fluorescently labeled platelets onto polymer coated coverslips Poly(methyl methacrylate) (PMMA) was spin cast onto cleaned glass coverslips to serve as a surface which supported platelet adhesion. Half of the PMMA coated coverslips were further spin cast with b-PEG-CA monomer and photopolymerized with a 450 W Hg lamp for 75 min. Following photopolymerization the cover slips were washed with distilled water to remove any unreacted monomers. The PMMA coverslips not coated with b-PEG-CA were exposed to UV light for 75 min, and then washed with distilled water so as to serve as a control for platelet adhesion studies.

Platelets in whole human blood were labeled with 10 μM quinacrine dihydrochloride (mepacrine, Sigma Chemical, St. Louis, MO) to allow visualization and quantification of adhesion using epifluorescence video microscopy and digital image processing. The technique for quantifying platelet adhesion was similar to that described previously.[20] Briefly, blood was obtained from healthy, nonmedicated donors and was drawn from the antecubital vein into 4 U/mL of heparin (as an anticoagulant) and mepacrine (for labeling). Coated coverslips were perfused with blood at a controlled, physiologic wall shear rate of 300 s^{-1} using a flow chamber (Figure 2) connected to a syringe pump (Harvard Apparatus, South Natick, MA). The syringe pump operated in the withdrawal mode, pulling blood across the coverslip surface and displacing the phosphate buffered saline which was used to prime the system. The coverslip flow system was placed on a Zeiss Axiovert inverted stage microscope, and images were recorded at 1000× magnification from 15 randomly selected locations on the coverslip

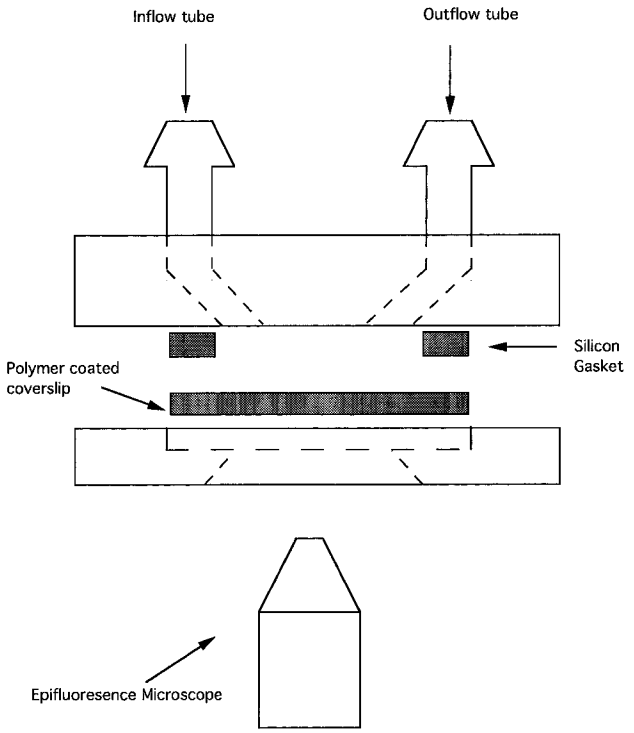

Figure 2. A diagramatic representation of the blood perfusion chamber.

following 7 min of blood perfusion. Images were recorded onto super VHS videotape and digitized using a Hammamatsu CCD camera and image intensifier. BDS imaging software (Pittsburgh, PA) allowed integration of 24 consecutive digital frames to cancel the effect of platelets flowing past in the background. For each integrated image (15 images per coverslip) the threshold of pixel intensity at which platelet adhesion occurred was determined.

Results and Discussion

Synthesis of the Photosensitive b-PEG-CA. The photosensitive polymer was prepared by an esterification reaction between b-PEG (four arms) and cinnamylidene acetyl chloride. Unmodified b-PEG in dichloromethane shows no significant absorbance from 240 to 360 nm and therefore the characteristic absorbance peak of the b-PEG-CA at 313 nm is indicative of the cinnamylidene acetate moiety. The UV absorption spectrum of the photosensitive b-PEG resembles the UV spectrum of poly-(vinyl cinnamylideneacetate) compounds which have been investigated and characterized by Tanaka et al.[8−12] The UV absorbance spectra of cinnamylidene acetic acid (CAA) and cinnamylidene acetyl chloride (CAC) in dichloromethane were also determined. The absorbance maximum and minimum for CAC (λ_{max} = 333 nm, λ_{min} = 256 nm) are at higher wavelengths than those for CAA (λ_{max} = 313 nm, λ_{min} = 251 nm). Since the absorbance maximum and minimum of CAA were similar to those for the modified b-PEG, CAA was used as a model compound for calculation of the extinction coefficient of b-PEG-CA (λ_{max} = 313 nm, ϵ = 37 390 L mol^{-1} cm^{-1}).

Assuming that the reactivity of each arm is independent of the status of the other arms, we can use the binomial distribution to estimate the functionality of the esterification product. For example, for a PEG molecule with four arms and an average degree of esterification of 70% (measured by UV), the resulting product consists of 0.8% PEG with no cinnamylidene acetyl group attached, 7.6% with one group attached, 26.5% with two groups, 41.2% with three groups, and 24% with four groups attached (Figure 3).

The appearance of characteristic peaks at δ = 7.5, δ = 7.14, and δ = 6.13 ppm in the ^{1}H-NMR spectra of the cinnamylidene acetate-modified b-PEG (69% degree of substitution by UV)

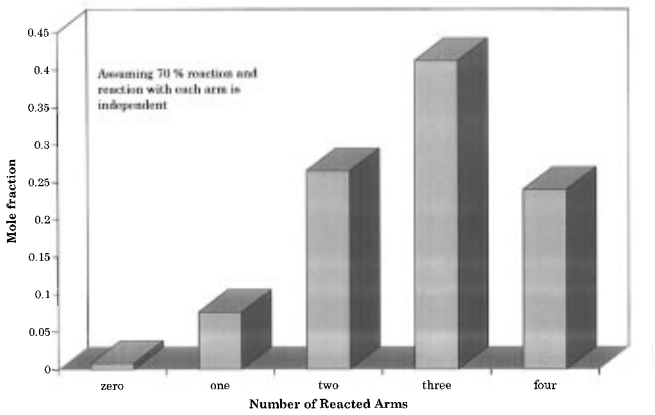

Figure 3. The fraction of b-PEG esterification product that has zero to four cinnamylidene acetyl groups for a net reaction yield of 70%, assuming that the reactivity of each arm is independent of each other.

demonstrate the presence of cinnamylidene acetyl moiety on the b-PEG molecule. The degree of substitution of photosensitive groups can also be calculated from the ratio of the areas under the ^{1}H-NMR peaks of the cinnamylidene acetate (δ = 7.5, δ = 6.9, and δ = 6.1 ppm) and polyethylene glycol protons (δ = 3.71 ppm). Both UV and ^{1}H-NMR methods give similar degree of modification of the PEG molecule (69% by UV and 71% by ^{1}H-NMR).

Photogelation of Cinnamylidene Acetate-Modified b-PEG. Photopolymerization of b-PEG-CA as summarized in Figure 4 was performed in a film cast from a dichloromethane solution on a cover slip. Figure 5 shows changes in the absorption spectra for b-PEG-CA (λ = 313 nm) during increasing time of exposure to light. Spectral changes are indirect measurements of the disappearance of the double bond adjacent to the carbonyl group of the cinnamylidene acetate moiety and, at the same time, the formation of a cyclobutane ring. The sharp isosbestic point at 265 nm indicates that a single reaction is proceeding in the system, namely, the disappearance of the double bond of the cinnamylidene acetate moiety and the formation of cross-links via cyclobutane formation. As mentioned earlier, the cinnamylidene acetyl moiety undergoes a 2 + 2 cycloaddition reaction,[8−12,14] which, in solution, transforms *trans*-cinnamylidene acetyl groups to *cis*-cinnamylidene acetyl groups upon exposure to light. The *cis*-form undergoes photodimerization via cyclobutane ring formation.[8−12] As shown in Figure 5, an 87% yield is accomplished in less than 10 min, in the complete absence of photoinitiators. Photogelation of photosensitive PEGs which were cast onto glass slides occurred as fast as 5 min. We define the gel point as the point at which b-PEG-CA monomers upon irradiation with UV light formed an insoluble three-dimensional network in water. Figure 6a is a photograph of a dry b-PEG-CA hydrogel which has been irradiated for 2 h, washed with deionized water, and dried in vacuum for 6 h. In Figure 6b the same hydrogel has been swollen in water for 10 min. Its degree of swelling was found to be DS = 16. Further irradiation gave more rigid gels with reduced swellability. No gelation occurred in the absence of the cinnamylidene acetyl group of the polymer chain, that is, in the case of a nonmodified b-PEG. Mixtures of equal amounts of cinnamylidene acetic acid and b-PEG in deionized water were cast on a glass coverslip and irradiated with a 450 W UV lamp for over 3 h; no gelation was observed. These results indicate that intermolecular cross-linking by 2 + 2 cycloaddition of the double bonds of the cinnamylidene acetate moieties in the modified b-PEG caused the formation of gels.

Table 1. Photogelation Reaction with Respect to Degree of Modification[a]

no. of expts	av deg of substitution[b]	deg of swelling (DS)
1	0	N/A
4	25%	58 ± 4
4	64%	35 ± 5

[a] The degree of swelling was determined using the equation ($W_{wet} - W_{dry}$)/W_{dry}. A 450 W Hg lamp was used to photopolymerize the PEG monomers for 75 min. Samples were cast on glass slides from dichloromethane solutions of 8.8 × 10^{-4} mol/L. [b] The degree of substitution was determined using the calibration equation for b-PEG-CA in dichloromethane.

Swelling Properties in Water. We hypothesized that the degree of swelling in water could be controlled by the initial degree of substitution of the PEG molecule and by the time of light exposure. As the degree of substitution of the hydroxyl groups of the PEG molecule increased, the resulting gels became more rigid, and the amount of water that they absorbed decreased. Table 1 demonstrates the change in the degree of swelling of b-PEG-CA hydrogel as the degree of modification is altered. Specifically, as the degree of substitution is increased the degree of swelling is decreased.

As the time of irradiation increases the gels also become more rigid, and their retention of water is decreased. In Table 2, the degree of swelling of the PEG gels (69% degree of substitution) are shown as a function of time for a 4 h period. It is important to note that even after 4 hours of irradiation, the PEG gels can still retain a large amount of water (13 times the original dry weight).

"Degelation" of b-PEG-CA Hydrogels. As mentioned above, only a few photoinduced hydrogel systems have exhibited any degree of reversibility.[3,7] The photoreversibility of b-PEG-CA was monitored by studying changes in UV absorbance (69%

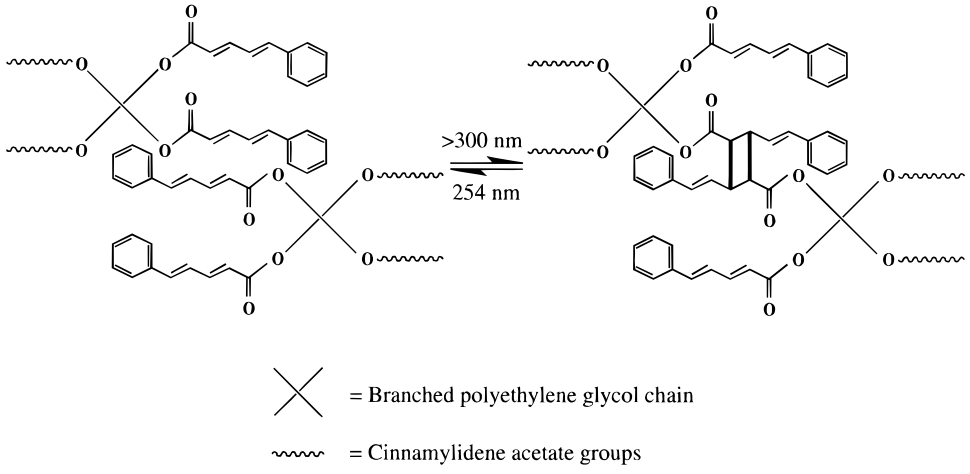

Figure 4. Photopolymerization-photoscission of b-PEG-CA in the presence of UV light.

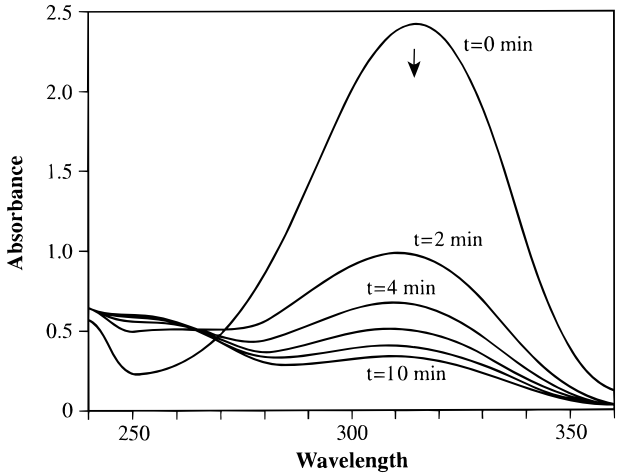

Figure 5. Change in absorption spectra of b-PEG-CA in water (2 mM) during irradiation (10 min) with a 150 W Xenon arc lamp.

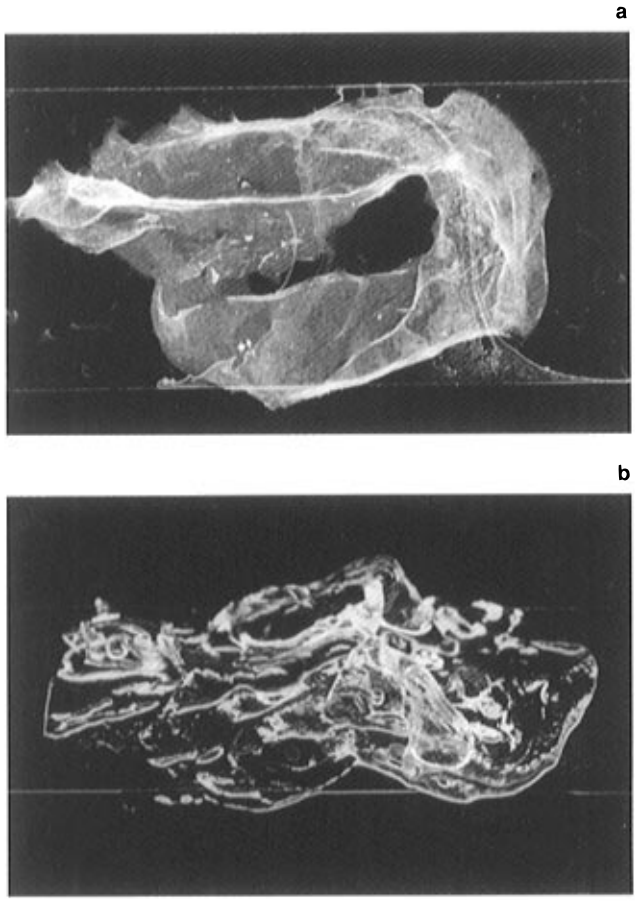

Figure 6. (a and b) The effect of water on dry photopolymerized b-PEG-CA (a) to give a swollen hydrogel (b). The DS for this gel is 16.

Table 2. Irradiation Time vs Degree of Swelling[a,b]

time (h)	deg of swelling (DS ± std dev)	no. of expts
1	22 ± 2	2
2	16 ± 1	3
3	14 ± 1	3
4	13 ± 1	3

[a] The degree of swelling was determined by using the equation $(W_{wet} - W_{dry})/W_{wet}$. [b] The degree of modification of the photosensitive PEG monomers is 69% as measured by UV. These monomers were irradiated with a 450 W Hg lamp for 1, 2, 3, and 4 h.

degree of functionalization) in water at 313 nm. Figure 7 shows two full cycles of the photoreversibility that b-PEG-CA exhibits in water when it is exposed to proper wavelength UV light.

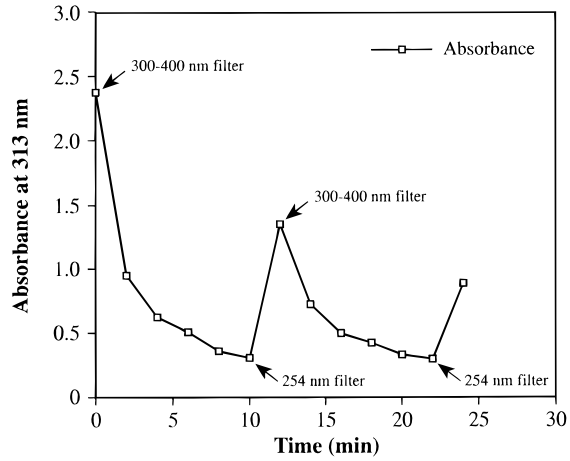

Figure 7. The effect of light on absorption at 313 nm for b-PEG-CA. A drop of 2 mM solution of b-PEG-CA monomers in water was placed in a quartz cell (0.1 mm thick) and irradiated with a 150 W Xenon lamp using the filters indicated on the figure.

For the first 10 min of irradiation, in which the forward reaction proceeds, the absorbance peak at 313 nm was decreased from 2.375 to 0.3 (87% change). After 10 min the 300−400 nm bandpass filter was replaced by a 254 ± 10 nm filter, and the reverse reaction was allowed to proceed for 2 min. During that time, the absorbance peak at 313 nm changed from 0.3 to 1.35 (a 51% recovery). Next, the 300−400 nm filter was replaced again by the 254 nm filter, and the forward reaction was observed. The absorbance peak at 313 nm decreased from 1.35 to 0.3 (77.7% change) over 10 min. Finally, the 254 nm bandpass filter was placed in front of the light source again, and the photoscission was observed. The absorbance at 313 nm increased from 0.3 up to 0.9 in the final 4 min of irradiation (29% recovery).

The extent of photoreversibility can only be estimated since a mixture of *cis* and *trans* photocleaved b-PEG-CA molecules are likely to have been produced from the irradiation at 254 nm. As a result, since the extinction coefficient of the *cis*-b-PEG-CA molecules is lower than the extinction coefficient of the corresponding *trans* molecule,[12] a lower absorbance was observed, and, consequently, a smaller extent of photoreversibility of the b-PEG-CA hydrogels was estimated. In other words, our method inherently underestimates the degree of photoscission and provides a minimum value. We have successfully converted gels with low cross-linking density to sols with just 10 s of irradiation at 254 nm.

The photoscission of the cross-linked b-PEG-CA molecules in water appears to be a much faster reaction than the forward photodimerization reaction. The highest absorbance change during the photoscission reaction is achieved after only 2 min of light exposure at 254 nm. After the 2 min of irradiation, a small decrease of the absorbance of the b-PEG-CA is observed, either due to some photodecomposition (higher energy at shorter wavelengths) or due to leakage of the 254 nm bandpass filter, which would result in some photodimerization effect on the uncross-linked b-PEG-CA molecules.

The effect of a photosensitizer (Erythrosin) was also investigated. Erythrosin is a photosensitizer which absorbs strongly between 400 and 500 nm. When b-PEG-CA solution was mixed with erythrosin and irradiated with a 150 W Xenon lamp, the mixture followed the same photochemical behavior at 313 nm as in the absence of the photosensitizer. We believe that in the presence of photosensitizers, we can photosynthesize a hydrogel using visible light as irradiation source.

We have demonstrated the photoscission of b-PEG-CA in water, when exposed to an appropriate UV light source.

Previous studies on the photoreversibility of photoinduced hydrogel systems have failed to demonstrate efficient photoreversibility.[3] We note, that in our studies of photoscission we have used a Xenon lamp equipped with a bandpass filter. The intensity at 254 nm is very low compared to the intensity at that wavelength of a mercury lamp with the same power. A low pressure Hg lamp would significantly speed the photoscission of cross-linked PEG-CA and increase the photoreversibility of our hydrogel system.

As mentioned earlier, cinnamylidene acetyl dimer molecules are cleaved by irradiation of light at wavelength at $\lambda \sim 254$ nm to regenerate cinnamylidene acetyl groups. b-PEG-CA gel fixed with dextran-FITC was subjected to photoscission by the irradiation of UV light from a 450 W Hg lamp at 254 nm. The PEG gel before irradiation appeared quite firm, with a light reddish color due to the Dextran's dye. Its degree of swelling was determined to be 20. The hydrogel was then subjected to UV light at 254 nm for 30 min. The "de-gelation" of the PEG hydrogel was measured by determining the release of dextran into a fixed volume of deionized water by means of fluorescence spectrophotometry. The intensity of fluorescence at 520 nm of the water solution due to dextran release out of the hydrogel matrix versus the irradiation time was determined. The release of dextran-FITC out of the hydrogel at zero time (no UV exposure) served as a control (relative intensity at 520 nm = 160). As the irradiation time at 254 nm is increased, the intensity of fluorescence in the water solution also increases indicating that dextran is diffusing out of the hydrogel at a faster rate due to reduced cross-link density caused by the photoirradiation at 254 nm. After 30 min of exposure at 254 nm, the fluorescence intensity at 520 nm increased to 558 and the hydrogel lost most of its integrity and appeared loose and colorless.

Blood Biocompatibility of the b-PEG-CA Hydrogels. A study of platelet adhesion to the coated PMMA surfaces as obtained by epifluorescence microscopy during human whole blood perfusion over the polymer surfaces was performed. Two PMMA coated and two b-PEG-CA coated PMMA glass coverslides were perfused, and platelet adhesion was quantified. b-PEG-CA coating of the PMMA surface reduced platelet adhesion by 99.6% compared to PMMA coating. This highly significant ($p < 0.001$) difference between the b-PEG-CA treated slides and the PMMA slides demonstrates that the b-PEG-CA coating is effective in preventing at least short term platelet deposition. Figure 8 (parts a and b) represents images of blood flow over PMMA and PMMA coated with b-PEG-CA hydrogel. These images show that platelets adhere readily to the PMMA surface. In contrast, platelets flow over the b-PEG-CA-coated PMMA surface without depositing on it. The significantly lower platelet adhesion that the b-PEG-CA hydrogel exhibits is consistent with previous reports of the blood biocompatibility of PEG[1,2,5] and indicates that the photocross-linking of the branched PEG preserves this polymer's blood biocompatibility. Further, these preliminary biocompatibility studies appear to be quite promising for future utilization of b-PEG-CA hydrogels for blood contacting applications.

Hydrogel Stability. Hydrogels, prepared from b-PEG-CA monomers with degree of substitution of 64% and 25% respectively, were stored at room temperature over a 2 month period. All of the samples retained their hydrophilic nature and water swellability, indicating that (1) the ester bonds of the b-PEG-CA are stable under these conditions, and (2) the intermolecular cross-links formed by 2 + 2 cycloaddition are also stable enough to maintain the integrity of the hydrogel for long periods of time. When the pH of the hydrogel environment is increased, the hydrogels begin to decompose. For example, when a hydrogel, synthesized by photoirradiation (2 h) of a b-PEG-CA solution cast on a cover glass (69% degree of substitution), was placed in a 5 mL NaOH solution (10 w/v%), the gel decomposed within seconds. The resulting product was soluble in water, and when it was irradiated for 1.5 h with a 450 W Hg lamp, no gel was formed. We conclude, therefore, that under neutral pH conditions the ester bonds between the PEG molecule and the cinnamylidene acetyl group remain stable, but as the environment becomes more basic, there is a hydrolysis of the ester bonds which results in the irreversible decomposition of the hydrogels.

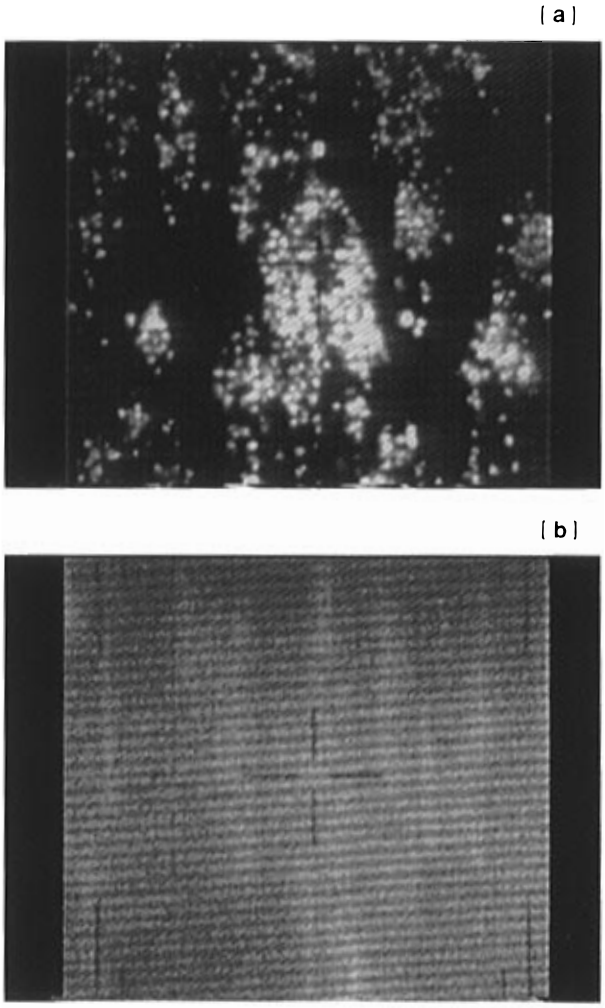

Figure 8. (a and b) Images of platelet deposition on PMMA versus PEG coated PMMA surfaces after 7 min of blood perfusion. For details see text.

Conclusion

In this study we have demonstrated the synthesis of novel, nonionic hydrogels via photopolymerization of water soluble polyethylene glycol-based molecules. The rather short irradiation times to achieve gelation in the absence of initiators (5 min), the antithrombogenic behavior that the b-PEG-CA hydrogels exhibit, and the efficient photoscission of b-PEG-CA in water make the proposed hydrogel system particularly interesting. We are currently assessing the utility of PEG-cinnamylidene acetate based hydrogels in a variety of controlled release, industrial and biomedical applications.

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