Photodependent Release from Poly(vinyl alcohol)/Epoxypropoxy Coumarin Hydrogels

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ABSTRACT: To obtain a photoresponsive hydrogel, poly(vinyl alcohol) (PVA) having coumarin residue as a pending group was crosslinked by photodimerizing the coumarin pendants. PVA having coumarin pendants was prepared by reacting the polymer with epoxypropoxy coumarin (EPC) in a strong alkali condition with EPC/PVA molar ratio of 200/1. According to the 1H NMR spectrum of PVA-EPC conjugate, the molar ratio of EPC residue to PVA was about 4.3/1, indicating that EPC was attached to PVA every 444 repeating units of vinyl alcohol. An aqueous solution of PVA-EPC (5%, w/v) became a semi-solid hydrogel by the irradiation of a light ($\lambda = 365$ nm; 400 W) for 1 hr. The dimerization degree of EPC residues of the hydrogel decreased and increased in a periodical manner under the cyclic irradiation between two UV lights ($\lambda = 365$ nm and $\lambda = 254$ nm). The release of 5(6)-carboxyfluorescein (CF) from PVA-EPC hydrogel was significantly enhanced by the 5 min-irradiation of $\lambda = 254$ nm (6 W), possibly due to the photodedimerization of EPC residues of the hydrogel. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 4339–4345, 2012

Key words: coumarin; hydrogel; poly(vinyl alcohol); photocrosslinking; photodependent release

INTRODUCTION

A few kinds of photoresponsive vehicles, which control the release of their contents in response to a light irradiation, have been developed, based on the photochemical property of coumarins.[1] Upon the irradiation of a light of which wavelength is longer than 310 nm, coumarins are dimerized to form cyclobutane bridges. In addition, the dimers are converted to monomers under the irradiation of a light of which wavelength is shorter than 260 nm.[2,3] Photoresponsive polymeric micelles were prepared by dispersing amphiphilic block copolymers having the hydrophilic block of poly(ethylene oxide) (PEO) and the hydrophobic block of coumarin methacrylate in a polar solvent (e.g., mixture solvent of tetrahydrofuran and dichloromethane) and by irradiating a light of $\lambda > 310$ nm to the dispersion.[4] The polymeric amphiphile was self-assembled into micelles, and the coumarins in the hydrophobic core of micelles were crosslinked by photodimerization. The release of a hydrophobic compound loaded in the cores was promoted under the irradiation of a light of $\lambda < 260$ nm. The photo cleavage of the dimmers loosens the cores, leading to an enhanced release. A photoresponsive nanogel was prepared using a block copolymer, which is composed of hydrophilic blocks and thermo-sensitive blocks containing coumarins.[5] Upon heating up the aqueous solution of the copolymer to a temperature above the lower critical solution temperature (LCST) of the thermo-sensitive block, micelles were formed due to the hydrophobic interaction of the thermo-sensitive blocks. When coumarins in the micelle core were photodimerized and then the micelle solution was cooled down to a temperature below LCST, the micelles became nanogels. In terms of the size change, the nanogel was sensitive to a UV light irradiation. Since the photoreaction of coumarins is reversible, the crosslinking density of the nanogel decreases under a light of $\lambda < 260$ nm. As a result, the size and the swelling degree increased, resulting in an enhanced release. Based on the same principle, a photoresponsive vesicle was prepared using another block copolymer composed of hydrophilic blocks and thermo-sensitive blocks containing coumarins.[6,7] The only photoresponsive hydrogel developed until now is a hydrogel prepared by UV irradiation of coumarin-poly(ethylene glycol) (PEG) conjugate.[8] Since one molecule of the conjugate has limited number of coumarin residues, it may be hard to control the crosslinking density of the coumarin–PEG hydrogel. However, the number of coumarin residue in poly (vinyl alcohol) (PVA)-coumarin conjugate can be easily controlled by varying the ratio of coumarin to

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PVA and extending the conjugation reaction time, so the crosslinking density of the PVA hydrogel can readily be controlled. In addition, no photoresponsive releases have been investigated using hydrogels. PVA, a water-soluble polymer, is a promising biomaterial due to its nontoxic and biocompatible properties. In addition, PVA has been widely used to prepare the hydrogel because of its good mechanical strength.[9] Chemically crosslinked PVA hydrogels have been prepared under harsh condition (e.g., use of initiators, a strong alkali condition). Accordingly, there are significant problems for their biomedical applications in drug delivery and tissue engineering. However, since photosensitive PVA hydrogels in this work is prepared only by UV irradiation, they may avoid the toxicity caused by excipients used for the preparation of the chemically crosslinked hydrogels. In this study, photoresponsive hydrogels were prepared by photocrosslinking PVA having coumarin residues as pendants. PVA having coumarin residues was prepared by reacting the polymer with epoxypropoxy coumarin (EPC) in a strong alkali condition. The hydrogel was prepared by photodimerizing EPC residues of the polymers using a light of $\lambda = 365$ nm. Change in the photodimerization degree of the hydrogel was investigated under an alternating irradiation between $\lambda = 254$ nm and $\lambda = 365$ nm. The releases from PVA-EPC hydrogel untreated by a UV light and from the hydrogel subjected to 5 min-irradiation of $\lambda = 254$ nm (6 W) were investigated using 5(6)-carboxyfluorescein (CF) as a fluorescence dye.

EXPERIMENTAL

Materials

7-Hydroxycoumarin (M.W. 162.14), poly(vinyl alcohol) (PVA, M.W. 70,000–100,000), epichlorohydrin (M.W. 92.53), KOH, and 5(6)-carboxyfluorescein (CF, M.W. 376.32) were purchased from Sigma (St. Louis, MO). All other reagents were in analytical grade.

Preparation of EPC

EPC was prepared following a method described in a previous report.[10] 7-Hydroxycoumarin (3.24 g; 20 mmol) was dissolved in ethanol (100 mL) and then an aqueous solution of KOH (25%, w/v) in distilled water was added to the solution. After the solution was stirred at room temperature for 30 min, epichlorohydrin (20 mL) was added to the solution. The mixture was heated up to 95–100°C, and the reaction was done for 2.5 hr with a reflux. Then, the solvent of the reaction mixture was completely evaporated at 40°C in a rotary evaporator under a reduced pressure to obtain dry residue. The dry residue was put in distilled water/chloroform (80/100 mL) contained in a 250 mL-separation funnel, and the mixture was hand-shaken for 5 min. Then, it was stood for 2 hr at room temperature for the partition to water and oil phase. The oil phase was separated from the water phase, and it was brought to contact with fresh distilled water (80 mL) for 2 hr to extract out impurities from the oil phase. And the solvent of the oil phase was evaporated to obtain dry residue. For further purification, the dry residue was dissolved in warm ethanol and it was recrystallized by standing the solution at room temperature. The white solid was obtained by a filtration using a filter paper (Whatman, No. 2).

Spectroscopy of EPC

The FTIR spectra of 7-hydroxycoumarin (a) and EPC (b) were taken on a Fourier Transformed Infrared spectrophotometer (FTIR, FT-3000, MX, Excalibur, in the Central Laboratory Center of Kangwon National University). EPC was dissolved in $CDCl_3$ and the ^1H NMR spectrum was obtained on a Bruker Avance 600 spectrometer (Karlsruhe, Germany, in the Central Laboratory Center of Kangwon National University). To set up the calibration curve of EPC, it was dissolved in DMSO : water (1 : 1, v/v) so that the concentration was 0.01, 0.005, 0.0025, 0.00125, and 0.000625 mg/mL. In addition, the absorbance of the solution was determined at 327 nm.

Preparation of PVA-EPC conjugates

PVA (0.5 g) was dissolved in 15 mL of distilled water (80°C) and it was cooled down to 30°C. Then, 5 mL of NaOH solution (20%, w/v) in distilled water was dropped carefully to the solution. In parallel, EPC was dissolved in 3 mL of dimethyl sulfoxide (DMSO) and the EPC solution was added to the PVA solution in a dropwise manner. The conjugation reaction was done for 48 hr at 30°C. To remove unreacted EPC and impurities, the reaction mixture was extensively dialyzed against distilled water using a dialysis membrane (MWCO 3500–5000, Spectra/Por®). The product was freeze-dried for further use.

^1H NMR spectrum of PVA-EPC

PVA-EPC conjugate was dissolved in D_2O and the ^1H NMR spectrum was obtained on a Bruker Avance 600 spectrometer (Karlsruhe, Germany, in the Central Laboratory Center of Kangwon National University).

Preparation of hydrogels

PVA-EPC conjugate was dissolved in phosphate buffer contained in a 10 mL-vial so that the

concentration was 5% (w/v). Then, the solution was subjected for 1 hr to the irradiation of a light of $\lambda = 365$ nm, generated by a lamp (400 W, HPA 400/30SD, Philips). As a control PVA solution (5%, w/v) was prepared and it was treated under the same irradiation condition. To load 5(6)-carboxyfluorescein (CF) in PVA-EPC hydrogels, 2 mL of CF solution (1 mg/mL) was layered over the hydrogel, and it was stood at room temperature under a dark condition for 24 hr. Unloaded CF solution was removed and then the surface of hydrogel was washed with distilled water.

Characterization of hydrogels

Ten milliliters-vials containing PVA-EPC solution, which had been subjected for 1 hr to the irradiation of a light of $\lambda = 365$ nm, and PVA-EPC hydrogel were tilted, and the photographs of the tilted vials were taken. The dry texture of PVA-EPC hydrogel, along with the dry texture of phototreated PVA solution was observed on scanning electron microscope (SEM, Jeol JSM-840A, in the Central Laboratory Center of Kangwon National University). The hydrogels and the PVA solution were freeze-dried, crosssectioned using a blade (No. 10 surgical blade, FEATHER), mounted on metal stubs with double-sided tape, and sputtered with gold.[11] The loading percentage of CF in the hydrogel was defined as the percent of the amount of loaded dye versus the total amount of dye used. The amount of dye loaded was determined by subtracting the amount of unloaded dye from the total amount of dye used. CF was quantified on a fluorescence spectrophotometer (F-2500, HITACHI, Tokyo, Japan) at 517 nm with excitation wavelength of 492 nm.[12]

Dimerization degree of free EPC and PVA-EPC hydrogel under cyclic irradiations

EPC was dissolved in DMSO, and the solution was diluted with distilled water so that the concentration was 0.01 mg/mL. Cyclic dimerization and dedimerization of EPC were investigated by alternating irradiations between two-wavelengths. The EPC solution was subjected to the irradiation of $\lambda = 365$ nm for 10 min and the irradiation of $\lambda = 254$ nm for 5 min in a cyclic manner.

To investigate the cyclic dimerization and dedimerization of EPC residues of PVA-EPC hydrogel, the hydrogel was prepared as described in the section of "preparation of hydrogels" except that it was prepared in a 3 mL-cuvette for a UV spectrophotometer (6505 UV–vis. Spectrophotometer, Jenway, U.K.). Cyclic dimerization and dedimerization of EPC residue was investigated under the same irradiation condition as used in observing the cyclic dimerization and dedimerization of free EPC. The degree of dimerization was determined as follows.[13]

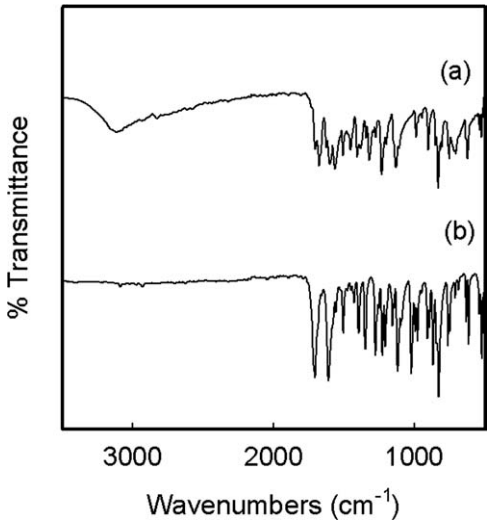

Figure 1 FTIR spectra of 7-hydroxycoumarin (a) and EPC (b).

$$\text{Dimerization } (\%) = (1 - A_t/A_o) \times 100 \qquad (1)$$

where, A_o is the absorbance of EPC at 327 nm before irradiating a UV light, and A_t is the absorbance after irradiating a UV light for a certain period.

Photodependent releases of 5(6)-carboxyfluorescein from PVA-EPC hydrogel

The CF-loaded PVA-EPC hydrogel (2.42 g), prepared in the previous section, was subjected to the irradiation of $\lambda = 254$ nm for 5 min. Then, 5 mL of phosphate buffer (pH 7.4; 30 m*M*) was layered over the phototreated hydrogel. In parallel, the same amount of phosphate buffer was put on the CF-loaded PVA-EPC hydrogel, which did not undergo the irradiation of $\lambda = 254$ nm. The two-phase systems were stood at room temperature for CF release. At the predetermined time intervals, 0.1 mL of release medium was taken to determine the amount of CF released out of the hydrogel, and the same amount of fresh phosphate buffer was put to the release medium to compensate for reduction in the volume. CF was quantified on a fluorescence spectrophotometer (F-2500, HITACHI, Tokyo, Japan) at 517 nm with excitation wavelength of 492 nm. The percentage release was defined as the percent of released mount versus total loaded amount.

RESULTS AND DISCUSSION

Spectroscopy of EPC

Figure 1 shows the FTIR spectra of 7-hydroxycoumarin and EPC. In the spectrum of 7-hydroxycoumarin

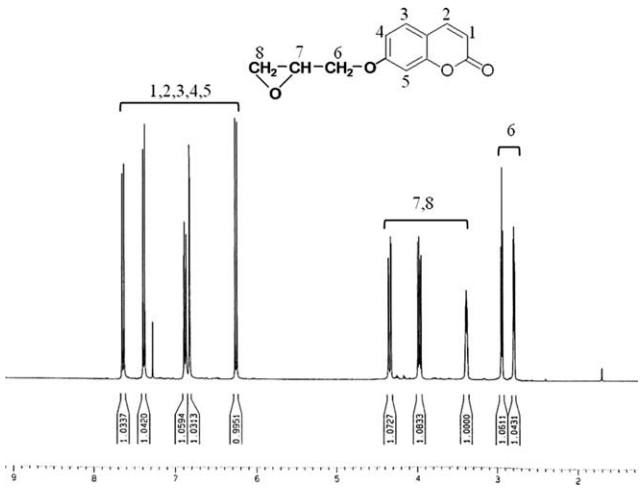

Figure 2 ^{1}H NMR spectrum of EPC.

[Fig. 1(a)], the signal of —OH stretching was found as a broad peak centered at 3116.1 cm^{-1}, C=O was found at 1703.5 cm^{-1}, C=C was found in the range of 1454–1600.5 cm^{-1}, and C—O was found at 1130.6 cm^{-1}. In the spectrum of EPC [Fig. 1(b)], the signal of —OH stretching of 7-hydroxycoumarin disappeared, indicating that 7-hydroxycoumarin participated in the reaction with epichlorohydrin. The signal of C=O was found at 1706.8 cm^{-1}, C=C was found in the range of 1506.9–1611.2 cm^{-1}, and C—O in aromatic ring was found at 1119.7 cm^{-1}.

Figure 2 shows ^{1}H NMR spectrum of EPC. The protons of aromatic rings were found at 6.25, 6.8, 6.9, 7.4, and 7.65 ppm, the protons of expoxy ring were found at 3.4, 4.0, and 4.35 ppm, and the protons of —CH_2O were observed at 2.8 and 2.95 ppm. The total area of the aromatic proton signals was 5.16, and the total area of epoxypropoxy proton signals was 2.15. Since the area ratio was almost 1 : 1, the purity of EPC is believed to be close to 100%.

A calibration curve of EPC was $Y = 61.3X + 0.0093$ (R^2=0.9998), where Y is the absorbance of EPC solution at 327 nm, and X is the concentration of the solution (DMSO/water) in mg/mL.

^{1}H NMR spectrum of PVA-EPC conjugates

Figure 3 shows the ^{1}H NMR spectrum of PVA-EPC dissolved in D_2O. The protons signals of aromatic ring of EPC residue were found at 6.2, 6.9, 7.5, and 7.9 ppm. The —CH_2— of PVA were found at 1.5 ppm. By comparing the total area of the aromatic protons signals to that of the —CH_2— signal, the molar ratio of EPC residue to PVA was calculated to 4.3/1, indicating that EPC was attached to PVA every 444 repeating units of vinyl alcohol.

Characterization of hydrogels

Figure 4 shows the tilted vials containing photo-treated PVA solution (a) and PVA-EPC hydrogel (b). PVA solution remained a fluid even after the irradiation of λ = 365 nm (400 W) for 1 hr. However, PVA-EPC solution became a hydrogel upon the irradiation. PVA-EPC is believed to be crosslinked due to the photodimerization of EPC residues, because coumarins are dimerized under the irradiation of $\lambda >$ 310 nm.[14] Figure 5 shows the SEM photos of freeze-

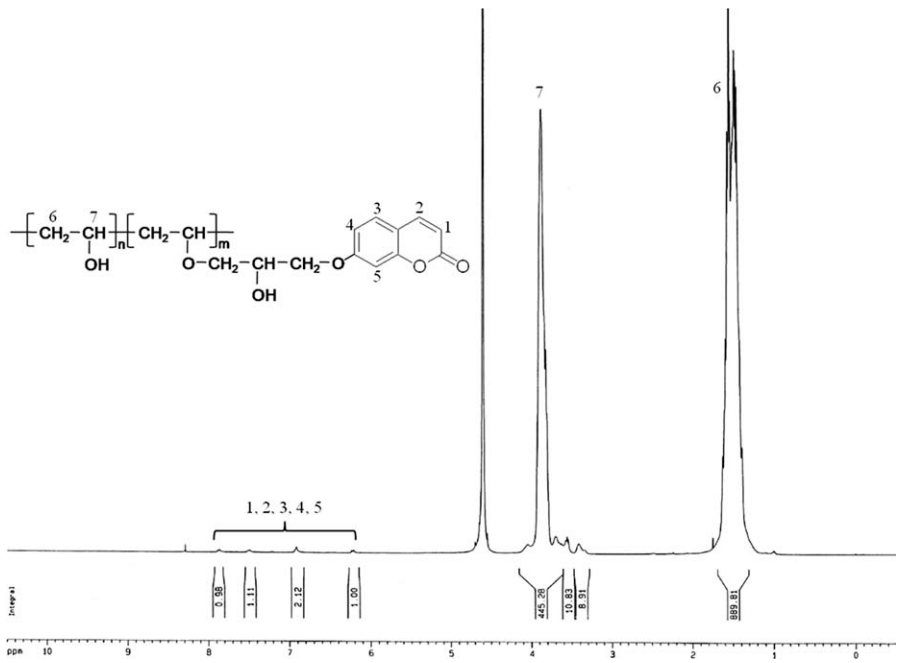

Figure 3 ^{1}H NMR spectrum of PVA-EPC conjugates.

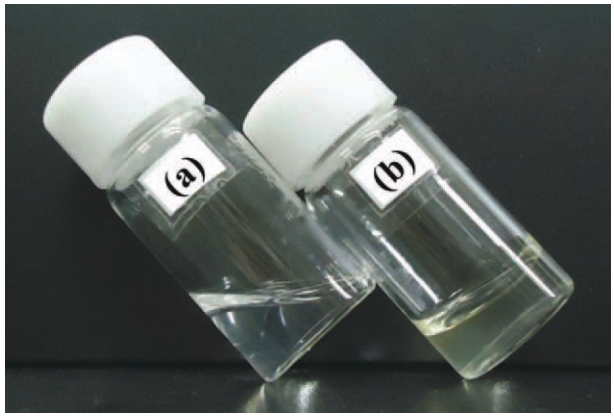

Figure 4 Tilted vials containing phototreated PVA solution (a) and PVA-EPC hydrogel (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

dried PVA solution (a) and PVA-EPC hydrogel (b). The image could hardly show the real structure of wet hydrogel because the structure could be distorted during a freezing-dry process. The macro pores is an artifact formed during the drying process, so it is hardly thought to be the mesh of hydrogel. According to the ^{1}H NMR spectrum of PVA-EPC, one EPC residue was calculated to attach to PVA every 444 repeating units of vinyl alcohol. Assuming that all coumarin residues were photodimerized, the distance between two adjacent cross-linking points (e.g., mesh size) will be about 2000 Å.

Dimerization degree of free EPC and PVA-EPC hydrogel under UV irradiation

Figure 6 shows the cyclic dimerization and dedimerization of EPC. The dimerization degree increased up to 59.9% upon 10 min irradiation of $\lambda = 365$ nm. The dimerization can account for the formation of PVA-EPC hydrogel shown in Figure 4. The dimerization degree decreased to 50.2% upon 5 min irradiation of $\lambda = 254$ nm. Coumarin and its derivatives are photodimerized under a light of $\lambda > 310$ nm to form cyclobutane ring, and the dimerization is reversible by the irradiation of $\lambda < 260$ nm.[15] Further cyclic irradiation of 365 and 254 nm resulted in a periodic increase and decrease in the dimerization degree, indicating that the dimerization is reversible.[16,17] Upon irradiation with 254 nm light to coumarin dimmers, not only photocleavage but also photodimerization occurs.[18] Accordingly, cyclic dimerization of EPC is lack of reversibility because of the photodimerization effect of 254 nm light.

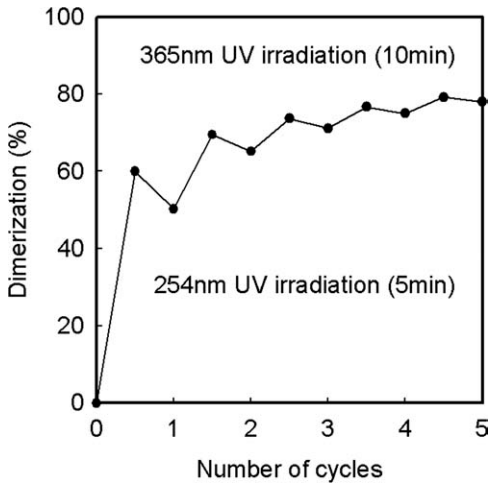

Figure 6 Dimerization and dedimerization of free EPC under the alternating irradiation, the irradiation of a UV lights of $\lambda = 365$ nm (400 W) for 10 min and then a UV lights of $\lambda = 254$ nm (6 W) for 5 min.

Figure 7 shows the cyclic dimerization and dedimerization of EPC residue of PVA-EPC hydrogel. The dimerization degree of the PVA-EPC hydrogel was about 73%, and the value decreased to about 63% upon 5 min-irradiation of $\lambda = 254$ nm and subsequently it increased to about 78% upon 10 min-irradiation of $\lambda = 365$ nm. A cyclic increase and decrease in the dimerization degree was obtained by a further cyclic irradiation.

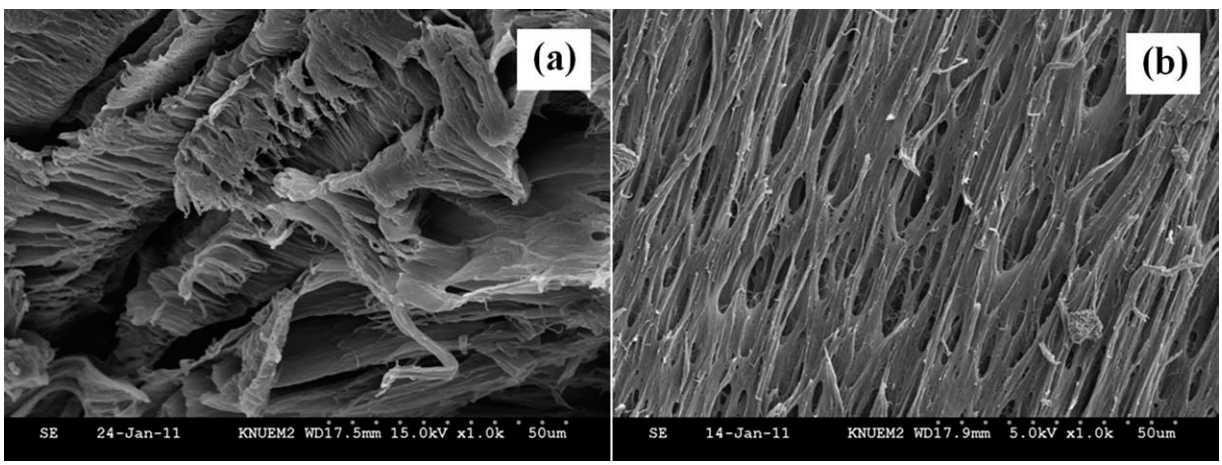

Figure 5 SEM photos of freeze-dried PVA solution (a) and PVA-EPC hydrogel (b).

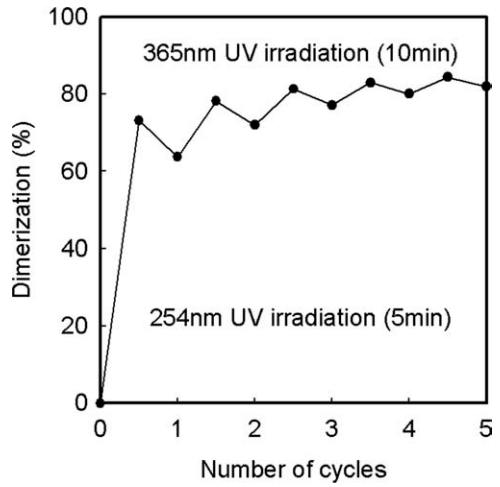

Figure 7 Dimerization and dedimerization of EPC residue of PVA-EPC hydrogel under the alternating irradiation, the irradiation of a UV lights of $\lambda = 365$ nm (400 W) for 10 min and then a UV lights of $\lambda = 254$ nm (6 W) for 5 min.

Even though the dimerization degree decreased upon the irradiation of light having the shorter wavelength, there was no transition from gel to sol throughout all the cycles. The hydrogel is a semi-solid but it imbibes a lot of water and has flexible hydrophilic segments composed of vinyl alcohol repeating units. Accordingly, the microviscosity of the hydrogel could be low enough to allow for the free movement of EPC residues, leading to the dimerization and the dedimerization even within the hydrogel.

Photodependent releases of 5(6)-carboxyfluorescein from PVA-EPC hydrogel

Figure 8 shows the releases of CF from PVA-EPC hydrogel untreated by a UV light and the hydrogel subjected to 5 min-irradiation of $\lambda = 254$ nm (6 W). The degree of release increased in a saturation manner with time. The reservoir type of vehicles are known to follow a zero-order release (the rate of release is constant with respect to time) because the solute concentration in the reservoir can remain constant for a long time, whereas the matrix type of vehicles was reported to exhibit a first-order release (the rate of release decreases with time) since the solute within the matrix is easy to be exhausted.[19] The loading percentage of CF was about 66%, and it corresponds to 0.55 mg CF/1 g hydrogel. The degree of release in 24 hr was about 53.5% for a photountreated hydrogel and about 70% for a phototreated hydrogel. The dimer of EPC residue in hydrogels was dedimerized by the irradiation of $\lambda = 254$ nm (Fig. 7). So the crosslinking density of photo ($\lambda = 254$ nm)-treated hydrogel will be lower than that of photountreated hydrogel, accounting for the higher release from the phototreated hydrogel. Figure 9 shows the dedimerization of PVA-EPC hydrogel, of which dimerization percentage was about 82%, under the irradiation of $\lambda = 254$ nm. The dimerization decreased with time in a saturation manner and a photostationary state was reached in 5 min. The photoreaction can take a place in the opposite direction under the extended irradiation of $\lambda < 260$ nm, resulting a photostationary state.[20] Accordingly, the further irradiation of $\lambda = 254$ nm would hardly have increased the degree of release.

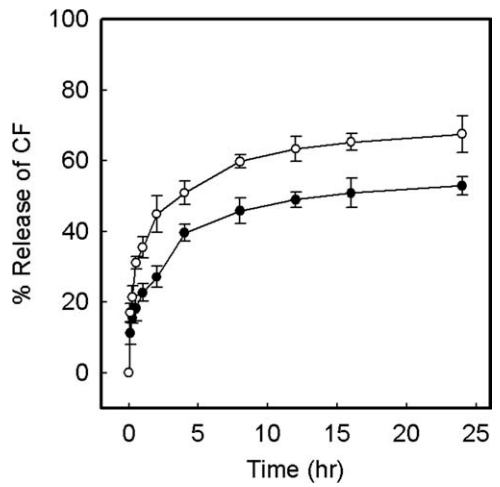

Figure 8 Releases of CF from PVA-EPC hydrogel untreated by a UV light (●) and the hydrogel subjected to 5 min-irradiation of $\lambda = 254$ nm (6 W) (○).

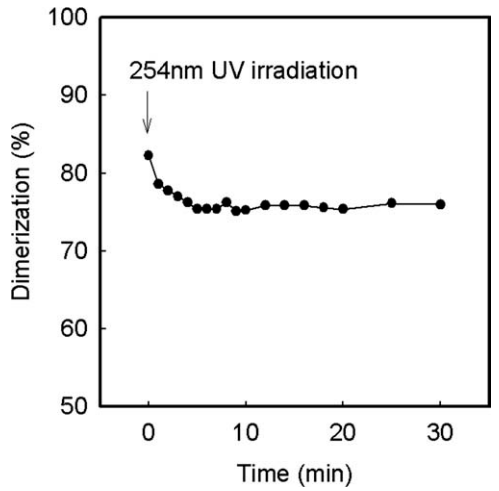

Figure 9 Dedimerization of EPC residue of PVA-EPC hydrogel under the irradiation of $\lambda = 254$ nm (6 W).

CONCLUSION

Photoresponsive hydrogel was prepared by photocrosslinking PVA-EPC conjugate. Using ^{1}H NMR spectrum of PVA-EPC conjugate, the molar ratio of EPC/PVA was calculated to be about 4.3/1. Upon the 1 hr irradiation of UV light of $\lambda = 365$ nm

(400 W), the PVA-EPC solution became a semi solid hydrogel. The dimerization degree of EPC residues in the hydrogel exhibited a cyclic increase/decrease under the alternating irradiation, the irradiation of a UV lights of $\lambda = 365$ nm (400 W) for 10 min and then a UV lights of $\lambda = 254$ nm (6 W) for 5 min. The release degree of PVA-EPC hydrogel subjected to 5 min-irradiation of $\lambda = 254$ nm (6 W), about 70% in 24 hr, was higher than that of the hydrogel untreated by a UV light, about 53.5%. Photodedimerization of EPC residues could be responsible for the enhanced release. The hydrogel prepared in this work could be used as a vehicle which releases a drug in response to a UV light irradiation. The photoresponsive PVA hydrogel would be used in the photo anticancer therapy.

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