PHOTO-CLEAVABLE CROSSLINKER CAPABLE OF PREPARING PHOTODEGRADABLE HYDROGEL BY A TWO COMPONENT REACTION FOR HYDROGEL MICRO PATTERNING

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ABSTRACT

Photodegradable hydrogels have emerged as a powerful platform for studying and directing cell behavior in a spatiotemporally controlled manner. Several research groups have previously reported the formation of photodegradable hydrogels by redox-mediated radical polymerizations, Michael-type addition reactions, and orthogonal click reactions. In this work, we synthesized a photocleavable crosslinker with an ester activated by *N*-hydroxysuccinimide, and we prepared photodegradable hydrogels by means of a one-step mixing reaction of the crosslinker and a biocompatible polymer containing amino moieties (amino-terminated tetra-arm poly(ethylene glycol) or gelatin). We then micropatterned the prepared photodegradable hydrogels to fabricate microstructures.

KEYWORDS: hydrogel, crosslinker, photodegradation, micropatterning

INTRODUCTION

Micropatterned hydrogels have been used to engineer three-dimensional (3D) tissue constructs with specific microstructures. For fabrication of the microstructures, several technologies have been developed, including top-down and bottom-up approaches [1]. In particular, photocrosslinking polymers are popular materials for controlling not only hydrogel micro-structure and stiffness but also cellular behavior in and on the hydrogels. Several research groups have previously reported hydrogel micropatterning for tissue engineering with photocrosslinking polymers, including derivatives of poly(ethylene glycol) (PEG) polysaccharides and proteins (e.g., gelatin) [2]. Recently, photodegradable hydrogels have attracted significant attention for their tunable mechanical and chemical properties and their use in the creation of 3D microstructures and in biomaterials and tissue engineering research fields [3]. The physical and chemical properties of photodegradable hydrogels can be temporally and spatially controlled by irradiation with light (single- and two-photon) and photodegradation is compatible with living cells. Current methods to fabricate photodegradable hydrogels are restricted to reactions between synthetic molecules, such as Michael-type conjugations, redox reactions, and orthogonal click reactions. In addition, most of the resulting photodegradable hydrogels are composed of derivatives of PEG, which is biologically inactive; cells can neither bind to nor degrade it. Although the cell-binding sequence Arg-Gly-Asp and other peptides can be incorporated into these photodegradable hydrogels using a combination of synthetic homo- and heterofunctional crosslinkers, development of simple systems to prepare biologically functional photodegradable hydrogels can be expected to facilitate widespread use of this technology.

THEORY

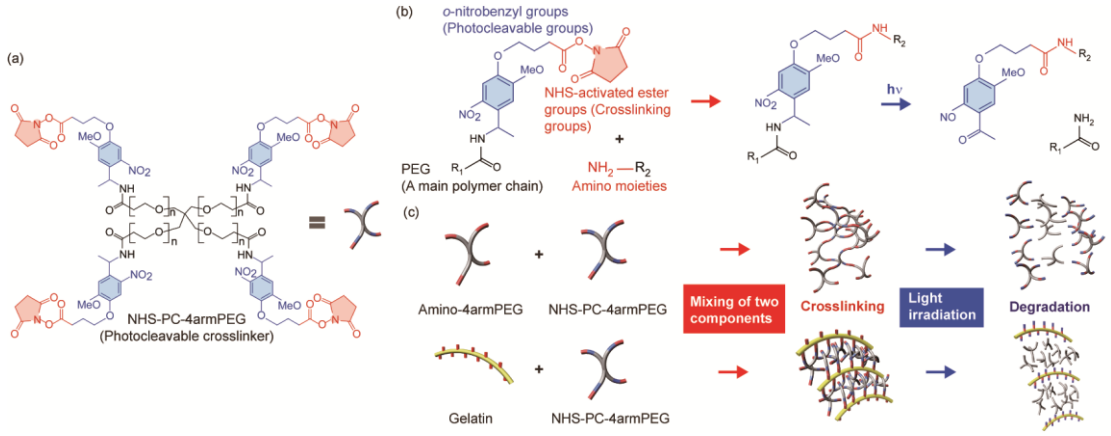

Figure 1. Schematic diagram of hydrogel formation by crosslink-ing amine-containing polymers and subse-quent photodegradation by irradiation with light. (a) NHS-PC-4armPEG, composed of a PEG main polymer chain, photocleavable o-nitrobenzyl groups, and NHS-activated-ester crosslinking groups. (b) Crosslinking of NHS-PC-4armPEG with primary amines and subsequent photo-cleavage. (c) Schematic of the overall process.

One class of hydrogel preparation methods of particular interest is the multicomponent mixing reaction, in which two (or more) molecular-scale components react with each other and gelation occurs spontaneously after mixing. Such systems are highly tunable because either component can be easily modified to change the hydrogel performance or to introduce additional functionality. In this work, we synthesized an activated-ester-type photocleavable crosslinker, which

formed photodegradable hydrogels by means of a one-step, two-component mixing reaction with a biocompatible polymer containing amino moieties (amino-terminated tetra-arm PEG (amino-4armPEG) or gelatin).

EXPERIMENTAL

We synthesized an *N*-hydroxysuccinimide (NHS)–terminated photocleavable tetra-arm PEG (NHS-PC-4armPEG; Mw = 12,062) crosslinker composed of the following functional groups (Figure 1). We studied the photocleavage of NHS-PC-4armPEG upon light exposure by measuring absorption spectra and ^{1}H NMR spectra before and after cleavage. For measurement of absorption spectra, a 0.01% w/v crosslinker solution in distilled water was pipetted into a quartz cell and exposed to light (365 nm, 30 mW/cm^2) from a UV light source. A prepolymer solution containing either 10 mM amino-4armPEG (M_w = 9,617) or 5% w/v gelatin was prepared in a 1:1 mixture of DPBS and 0.3 M HEPES buffer (pH 7). A solution of the synthesized NHS-PC-4armPEG crosslinker (10 mM, 12.1% w/v) was prepared in 10 mM phthalate acid buffer (pH 4) with 140 mM NaCl. The prepolymer and crosslinker solutions were mixed at a 1:1 v/v ratio. Immediately after the two components were mixed, 10–30 µL of the mixture was transferred to an amino-coated glass slide. The slide was covered with a cover slip, which was separated from the glass slide by PET films or cover slips as spacers to control the hydrogel thickness. The mixtures were then incubated at 37 °C for 30 min to form photodegradable hydrogels. A photomask was placed on the cover slip on the photodegradable hydrogel. Subsequently, the hydrogel was exposed to light from the UV light source through the photomask. To develop the photodegraded region in the hydrogel, the sample was immersed in DPBS solution at 37 °C for 24 h. For evaluation of the degradation depth of the micropatterned hydrogels, 3 µm fluorescent microparticles were deposited on the micropatterned surface. Three-dimensional images were obtained with a laser scanning confocal microscope, and the degradation depth of the micropattern was measured.

RESULTS AND DISCUSSION

An aqueous solution of the crosslinker was slightly yellow and optically clear, but upon irradiation with light (365 nm, 30 mW/cm^2), the solution changed color. Therefore, we evaluated cleavage of the crosslinker by means of absorption spectroscopy (Figure 2a). A light exposure of 0.3 J/cm^2 induced cleavage of the o-nitrobenzyl groups, as evidenced by an increase in the absorbance at 390 nm. We also used ^{1}H NMR spectroscopy to estimate the molar ratio of uncleaved to cleaved crosslinker after irradiation. The amount of uncleaved crosslinker gradually decreased as the light exposure increased (Figure 2b). To form a photodegradable hydrogel from amino-4armPEG or gelatin, we allowed a 10 mM solution of the NHS-PC-4armPEG crosslinker in 10 mM phthalate acid buffer (pH 4) with 140 mM NaCl to react with a prepolymer solution containing 10 mM amino-4armPEG or 5.0 % gelatin in a 1:1 v/v mixture of DPBS and 0.3 M HEPES (pH 7). The NHS moiety is stable under acidic conditions but reacts quickly with primary amines at neutral pH; the NHS moiety was stable in the crosslinker solution for a few hours, and hydrogel formation took place within 30 min after mixing. Homogeneous photodegradable hydrogels were obtained from both amino-4armPEG and gelatin. The rate of the crosslinking reaction with the amine-containing polymers could be controlled by changing the pH of the reaction buffer. At pH 8, the reaction rate was too quick to handle the solutions at the concentration range described above; at pH 6, the reaction was slow, and hydrogel formation required more than three hours.

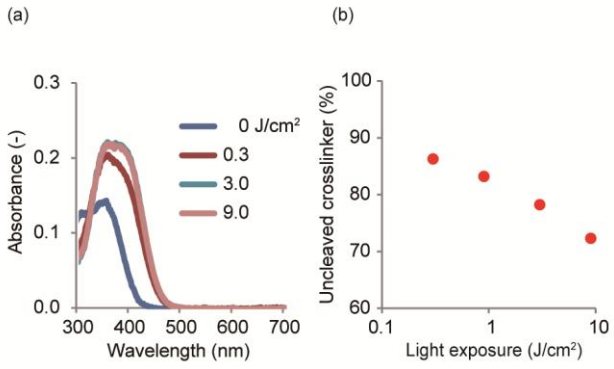

Figure 2. Effect of irradiation with light (365 nm, 30 mW/cm^2) on the photocleavable crosslinker. (a) Absorption spectra of 0.01% aqueous NHS-PC-4armPEG before and after irradiation. (b) Light exposure–dependence of molar ratio of uncleaved to cleaved NHS-PC-4armPEG (1.25%) after irradiation in deuterated water.

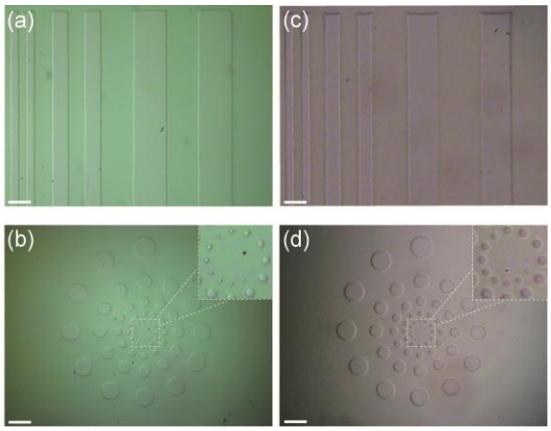

Figure 3. Microscope images of micropatterned photodegradable hydrogels prepared with (a, c) amino-4armPEG and (b, d) gelatin. Irradiation of the hydrogel through the photomask induced degra-dation of exposed regions of the hydrogel and created (a, b) lines or (c, d) circles. Scale bars = 500 µm.

To assess hydrogel micropatterning, we irradiated the hydrogels with micropatterned light (365 nm, 30 mW/cm^2) by using photomasks. After irradiation, the color in the irradiated region changed slightly, as was observed for the photocleavable crosslinker solution. To erode the degraded polymer in the exposed region of the hydrogels, we immersed

them in DPBS for a day in an incubator at 37°C with 5% CO_2. Micropatterns corresponding to the photomask pattern were created by means of the erosion process (Figure 3). The time required for erosion depended on the thickness of the hydrogel, the concentrations of the crosslinker and the amine-containing polymer, and the light exposure. Both lines and circles could be precisely created, and the patterning resolution ranged from 20 to 500 μm. Unexposed regions remained intact. The resolution of the micropatterning do not differed substantially between the hydrogel prepared with amino-4armPEG and that prepared with gelatin.

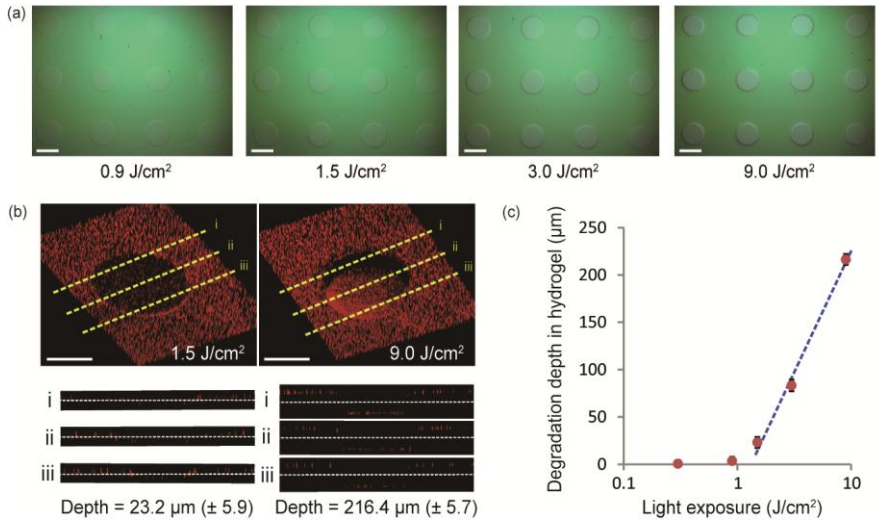

Figure 4. (a) 2D micrographs and (b) 3D confocal micrographs of micropatterned hydrogels prepared with amino-4armPEG. (c) Effect of light exposure on degradation depth. Error bars indicate standard deviations for four samples. Scale bars = 500 μm.

We evaluated the effect of the light exposure on the degradation depth in the micropatterned hydrogels prepared with amino-4armPEG. The visibility of the micropatterns corresponding to the photomasks increased with increasing light exposure (Figure 4a). To evaluate the degradation depth, we characterized the surface profile and morphology of the micropatterned hydrogels by confocal laser scanning microscopy (Figure 4b) and measured the degradation depth in the x-z plane (Figure 4c). We did not observe significant degradation at light exposures of <1.2 J/cm^2, although the absorbance and NMR spectra indicated the crosslinker was cleaved at light exposures of <1.2 J/cm^2. Under these conditions, photo-cleavage of the crosslinker induced a decrease in crosslinking density, but photocleavage was insufficient for complete degradation. We did observe hydrogel degradation after irradiation at light exposures of >1.2 J/cm^2, and the degradation depth increased linearly with the log of the exposure. This linear relationship can be explained by the Beer–Lambert law, in which the log of the transmitted intensity is proportional to the depth from the sample surface. On the basis of this correlation, the degradation depth of photodegradable hydrogels can be controlled simply by varying the light exposure.

CONCLUSION

In conclusion, we synthesized a photocleavable crosslinker that has the ability to form a photodegradable hydrogel upon reaction with amino-4armPEG or gelatin. We demonstrated that these hydrogels could be micropatterned by irradiation with light through photomasks. Our new photocleavable crosslinker is a potentially versatile and convenient material for preparing photodegradable hydrogels with biocompatible polymers containing amino moieties. The degradation depth of the micropatterns could be varied by altering the light exposure. The approach presented herein is potentially useful for manipulating cells on and within the hydrogels to form engineered tissue constructs, owing to its versatility in chemical composition and the simplicity of the hydrogel preparation procedure.

ACKNOWLEDGEMENTS

This research was supported by KAKENHI (24106512).

REFERENCES

[1] P. Zorlutuna, N. Annabi, G. Camci-Unal, M. Nikkhah, J.M. Cha, J.W. Nichol, A. Manbachi, H. Bae, S. Chen, A. Khademhosseini, *Adv. Mater.*, vol 24, pp. 1782-1804 (2012)

[2] J.W. Nichol, S.T. Koshy, H. Bae, C.M. Hwang, S. Yamanlar, A. Khademhosseini, *Biomaterials* vol 31, pp. 5536-4400 (2010)

[3] C.A. DeForest, K.S. Anseth, *Nature Chem.* vol 3, pp. 925-931 (2011)

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