Photolithographic Synthesis of Hydrogels

Guoping Chen,[†] Yukio Imanishi,[†] and Yoshihiro Ito*[,†,‡]

Graduate School of Materials Science, NAIST, Ikoma 630-0101, Japan, and PRESTO, Japan Science and Technology Corporation, Keihanna Plaza, Hikaridai 1-7, Seika-cho, Kyoto 619-0237, Japan

Received January 20, 1998
Revised Manuscript Received April 21, 1998

Polymer gels change their volume in response to external stimuli such as changes in solvent composition,[1] pH,[2] temperature,[3] electric field,[4,5] and light irradiation.[6] They have many potential uses in various fields of biotechnology,[7] medicine,[3,8,9] and robotics.[10,11] Their practical applications will be further extended if their microscopic structures can be controlled. In the present investigation, photolithography was used to synthesize thermoresponsive microgel strings and a microgel network. They rapidly and reversibly swelled and contracted by changing temperature. The microgel network formed a unique network structure at 10 °C and recovered its original structure by raising the temperature. No such structural change of a microgel has been reported, since the photolithographic technique has been used for pattern-immobilization of hydrogels or polymers onto matrixes.[12−15]

A thermoresponsive polymer, poly(*N*-isopropylacrylamide) (PNIPAAm), shows a lower critical solution temperature (LCST) at about 32 °C.[16] Its solution suddenly becomes cloudy when the temperature is raised beyond the LCST and reversibly turns to clearness when the temperature is lowered. Because of this property, PNIPAAm has attracted much interest in various fields.[3,11,17−20] In the present investigation, we used PNIPAAm to synthesize thermoresponsive microgels in some structures, which were controlled by the photolithographic method.

A thermoresponsive copolymer, poly(*N*-isopropylacrylamide-*co*-acrylic acid) (PIA), and its photoreactive derivative, azidophenyl-derivatized PIA (AzPhPIA), were synthesized as shown in Figure 1. PIA was synthesized by copolymerization of *N*-isopropylacrylamide (NIPAAm) with acrylic acid at a molar ratio of 95/5 in isobutyl alcohol (30 mL, total monomer concentration 2.0 M) containing 2,2′-azobis(isobutyronitrile) (AIBN, 5 mg) and mercaptopropionic acid (MPA, monomer/MPA = 100/4 mol/mol). The mixture was degassed, purged with nitrogen gas, sealed, and heated at 60 °C for 24 h. The resulting mixture was diluted with methanol, and the product copolymer was precipitated in diethyl ether.

Subsequently, PIA (100 mg), 4-azidoaniline (121.5 mg), and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (water-soluble carbodiimide, WSC, 129.7 mg) were dissolved in 0.1 M 2-morpholinoethanesulfonic acid (MES)-buffered solution (20 mL, pH 4.5), and the mixture was stirred at 4 °C for 24 h. The resulting solution was then centrifuged at 45 °C. The precipitate (AzPhPIA) was dissolved in cold distilled water, and the solution was centrifuged at 45 °C again. This process was repeated until the absence of azidoaniline in the supernatant was confirmed by ultraviolet spectroscopy (280 nm).

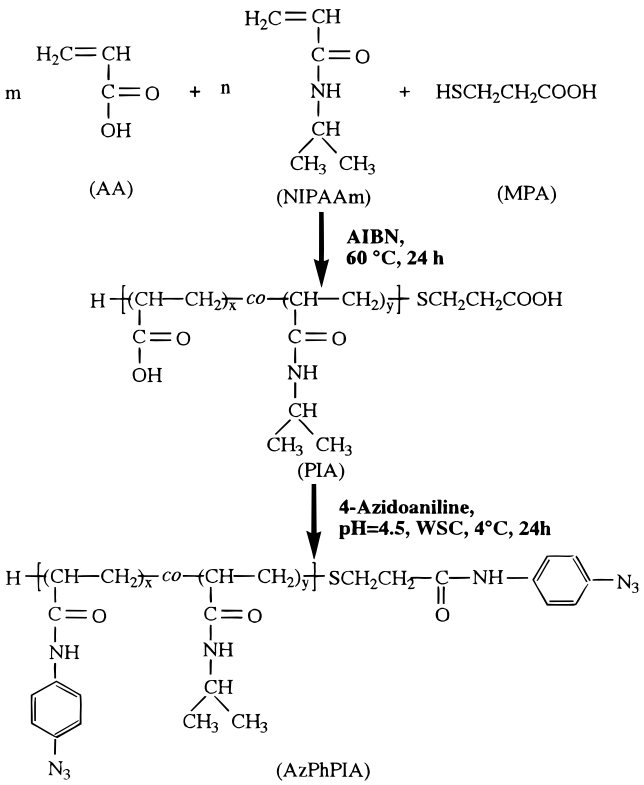

Figure 1. Synthetic scheme of poly(*N*-isopropylacrylamide-*co*-acrylic acid) (PIA) and azidophenyl-derivatized PIA (AzPhPIA).

The number-averaged molecular weight of PIA, which was calculated from the sulfur content determined from titration with barium acetate after flask combustion, was found to be 5910 ± 30. Four carboxylic groups were found in a PIA molecule by elemental analysis, and it was indicated that all of them were used for coupling with azidoaniline, from the content of azidophenyl groups in an AzPhPIA molecule determined by ultraviolet spectroscopy (280 nm). The absorbance of aqueous solution of AzPhPIA (50 mg/mL) at 280 nm was 0.44, and the molar extinction coefficient was 1141.

PIA and AzPhPIA solutions exhibited LCSTs at 38.5 and 21.5 °C, respectively.[21] The LCST increased with copolymerization of acrylic acid and decreased after the introduction of azidophenyl groups. The LCSTs depended on the hydrophilic properties of copolymers. It has been reported that random copolymerization of NIPAAm with relatively hydrophilic monomers such as acrylamide and acrylic acid resulted in the rise of LCST, while that with hydrophobic monomers such as methyl methacrylate lowered the LCST.[22−24]

Photolithographic synthesis of thermoresponsive microgels was carried out as follows. An aqueous solution of AzPhPIA (30 mg/mL, 0.1 mL) was eluted on a square slide glass (a side 15 mm) and air-dried at room temperature. The slide glass was then covered with a photomask (quartz plate deposited with chrome, Nippon Filcon, Osaka, Japan) having a prescribed pattern and irradiated for 10 s with an ultraviolet lamp (Koala, 100 W) from a distance of 5 cm (power density 70 mW/cm^2).

[†] NAIST.
[‡] PRESTO.

S0024-9297(98)00062-X CCC: $15.00
Published on Web 06/09/1998

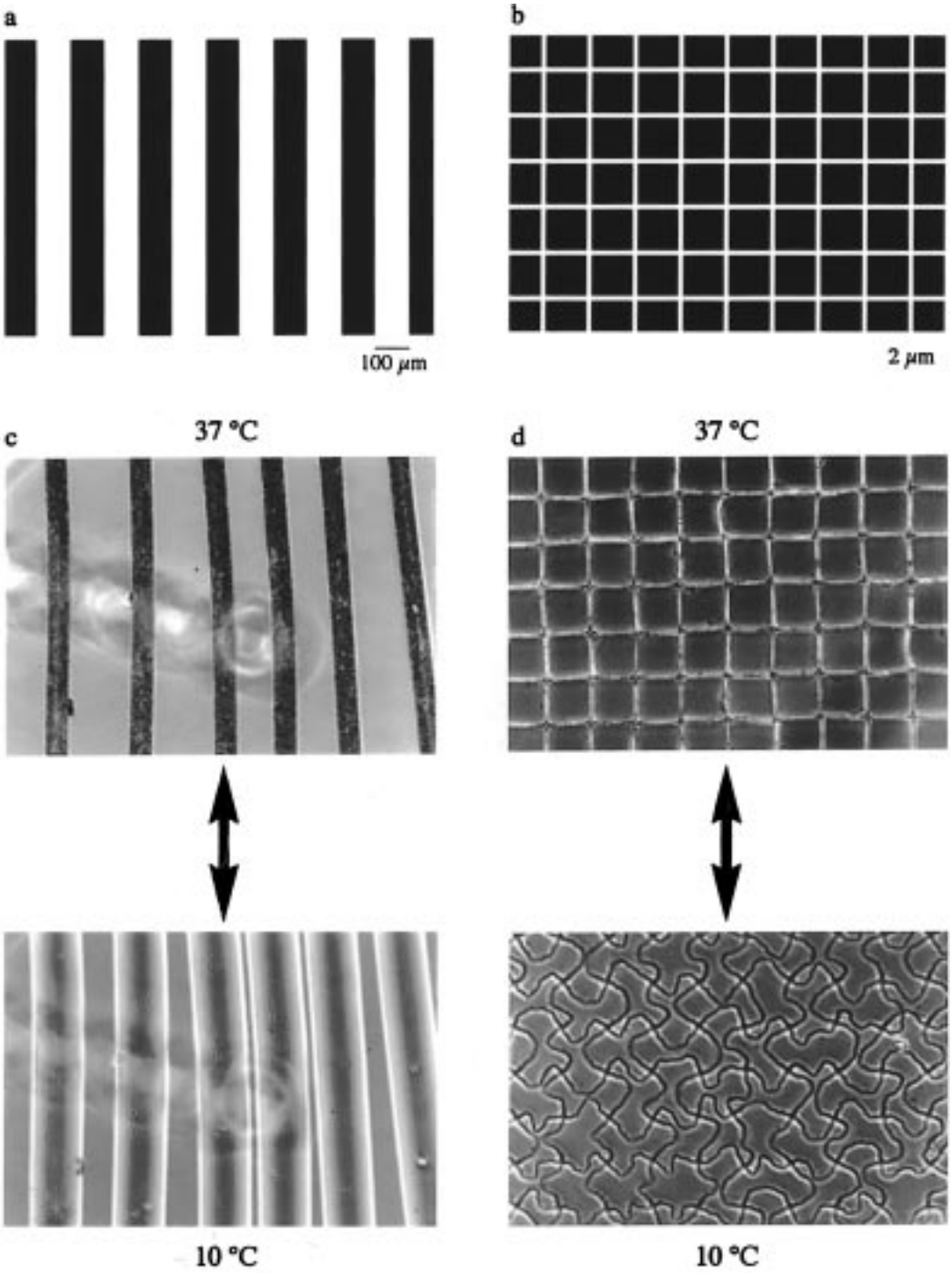

Figure 2. Phase-contrast micrographs of a photomask with 100 μm-width stripes (a) and 100 μm-width microgel strings at 37 and 10 °C (c) and a photomask having a 40 × 2-μm network structure (b) and microgel network at 37 and 10 °C (d). The double arrow represents the reversibility of the temperature-induced shape change.

Subsequently, the slide glass was immersed in and rinsed with cold distilled water (4 °C). AzPhPIA in the irradiated areas should be cross-linked. AzPhPIA in other areas should not be cross-linked and could be removed by washing with cold distilled water. With the photomask, thermoresponsive microgels of some structures were synthesized. The thickness of the microgel was about 0.1 μm.[25]

Figure 2 shows the microgel strings and microgel network. The structures of microgels were identical to the patterns of photomasks used. One end of each microgel string was attached to the glass plate by an adhesion tape. On the other hand, the surrounding of the microgel network was attached to the glass plate by adhesion tapes. When the thermoresponsive microgels were immersed in distilled water at 37 °C, they were in the contracted state. However, they rapidly swelled when the temperature was lowered. The width and length of the microgel strings increased by a factor of 2.1 when the temperature decreased from 37 to 10 °C. The swelling/contraction ratio should depend on the cross-link density,[26] although the relationship has not been investigated in the present study. The microgel network showed a unique network structure upon temperature lowering. A superficially similar "snaking" phenomenon was observed in the fine features of cross-linking negative-tone resists using nonthermoresponsive polymer.[27] When the temperature was raised, the

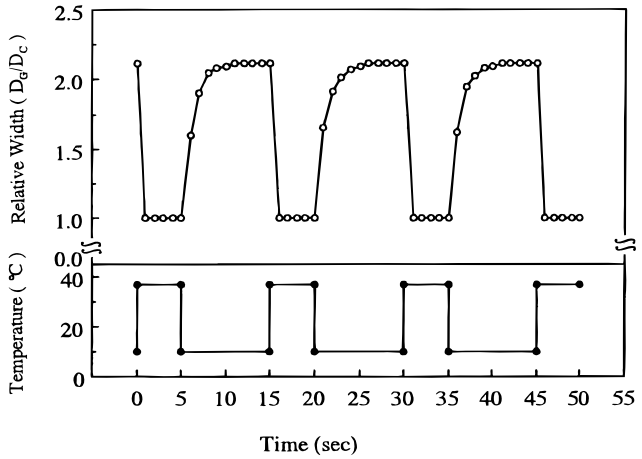

Figure 3. Swelling and contraction kinetics of the 100 μm-width microgel strings. The lower graph represents the tempeature of distilled water that was put into the container having the microgels, as described in ref 20. It does not represent the actual measured temperature.

microgels recovered their original structures. The temperature-induced structure change was repeated reversibly.

The time course of swelling and contraction of 100 μm-width microgel strings was determined by micrography[28] and is shown in Figure 3. The microgel strings swelled within 6 s when the temperature was lowered to 10 °C and contracted within 1 s when the temperature was raised to 37 °C.

Much effort has been made to improve the stimulus response of hydrogels. Okano's group synthesized rapid deswelling hydrogels by using a comb-type molecular graft structure.[18] Hu et al. have succeeded in synthesizing poly(*N*-isopropylacrylamide)−polyacrylamide interpenetrating hydrogels that have a heterogeneous or modulated structure.[11] The present investigation demonstrated that the microscopic structures of hydrogels can be controlled by photolithography according to the application purpose. Furthermore, the microgels showed rapid responsiveness to temperature change. The skilled fabrication of microgel strings and the microgel network will find wide applications for smart actuator, artificial muscle, sensor, drug delivery system, tissue engineering, etc. Photolithography provides an easy and useful means for the control of the microscopic structures of hydrogels.

Acknowledgment. We thank Dr. H. Hasegawa at Kyoto University for his valuable discussion on the measurement of microgel thickness.

References and Notes

(1) Hirokawa, Y.; Tanaka, T. *J. Chem. Phys.* **1984**, *81*, 6379.
(2) Tanaka, T.; Fillmore, D.; Sun, S.-T.; Nishio, I.; Swislow, G.; Shah, A. *Phys. Rev. Lett.* **1980**, *45*, 1636.
(3) Hoffman, A. S. *J. Controlled Release* **1987**, *6*, 297.
(4) Tanaka, T.; Nishio, I.; Sun, S.-T.; Ueno-Nishio, S. *Science* **1982**, *218*, 467.
(5) Osada, Y.; Okuzaki, H.; Hori, H. *Nature* **1992**, *355*, 242.
(6) Suzuki, A.; Tanaka, T. *Nature* **1990**, *346*, 345.
(7) Okano, T.; Yamada, N.; Sakai, H.; Sakurai, Y. *J. Biomed. Mater. Res.* **1993**, *27*, 1243.
(8) Peppas, N. A.; Langer, R. *Science* **1994**, *263*, 1715.
(9) Jeong, B.; Bae, Y. H.; Lee, D. S.; Kim, S. W. *Nature* **1997**, *388*, 860.
(10) Kajiwara, K.; Ross-Murphy, S. B. *Nature* **1992**, *355*, 208.
(11) Hu, Z.; Zhang X.; Li Y. *Science* **1995**, *269*, 525.
(12) Sugawara, T.; Matsuda, T. *Macromolecules* **1994**, *27*, 7809.
(13) Lesho, M. J.; Sheppard, N. F. *Sens. Actuators B* **1996**, *37*, 61.
(14) Ito, Y.; Chen, G.; Guan, Y.; Imanishi, Y. *Langmuir* **1997**, *13*, 3, 2756.
(15) Chen, G.; Ito, Y.; Imanishi, Y. *Macromolecules* **1997**, *30*, 7001.
(16) Heskins, M.; Guillet, J. E. *J. Macromol. Sci., Chem. A2* **1968**, 1441.
(17) Chen, G.; Hoffman, A. S. *Nature* **1995**, *373*, 49.
(18) Stayton, P. S.; Shimoboji, T.; Long, C.; Chilkoti, A.; Chen, G.; Harris, J. M.; Hoffman A. S. *Nature* **1995**, *373*, 49.
(19) Yoshida, R.; Uchida, K.; Kaneko, Y.; Sakai, K.; Kikuchi, A.; Sakurai, Y.; Okano, T. *Nature* **1995**, *374*, 240.
(20) Yoshida, R.; Takahashi, T.; Yamaguchi, T.; Ichijo, H. *Adv. Mater.* **1997**, *9*, 175.
(21) The LCST of PIA or AzPhPIA was measured as the temperature where the solution became cloudy when the temperature was raised from below the LCST to above the LCST. PIA or AzPhPIA was dissolved at a concentration of 10.0 mg/mL in 0.1 M phosphate-buffered solution (pH 7.0). The turbidity of the solution at various temperatures was monitored by the optical transmittance at 500 nm, which was recorded on a Hitachi spectrometer (Hitachi, Japan).
(22) Dong, L. C.; Hoffman, A. S. *J. Controlled Release* **1986**, *4*, 223.
(23) Park, T. G.; Hoffman, A. S. *Biotechnol. Bioeng.* **1990**, *35*, 152.
(24) Yoshida, R.; Sakai, K.; Okano, T.; Sakurai, Y. *J. Biomater. Sci., Polym. Ed.* **1994**, *5*, 585.
(25) The thickness was measured by a Zeiss laser microscope. The microgel was immobilized on a polystyrene film instead of a glass plate accroding to the same process. The immobilized microgel was freeze-dried, coated with gold layer by using an ion coater (Eiko IB-3, Tokyo, Japan), and observed by the laser microscope.
(26) Wood, J. M.; Attwood, D.; Collett, J. H. *Int. J. Pharm.* **1981**, *7*, 189.
(27) Thompson, L. F.; Willson, C. G.; Bowden, M. J. *Introduction to Microlithography*, 1994, American Chemical Society, Washinton, D.C., 1994; pp 209−210.
(28) The changing widths of the microgel strings were followed microscopically when the temperature was changed between 10 and 37 °C. The microgel strings were first equilibrated in distilled water at 10 °C, and then the water was replaced with distilled water warmed at 37 °C. After 1, 2, 3, 4, and 5 s, the micrographs were taken, and the microgel strings were soaked in cold distilled water kept at 10 °C. The widths of the microgel strings were measured from the micrographs. The swelling and contraction kinetics is defined as the change of the relative width (W_G/W_C) of the microgel strings with time. W_G represents the width of ongoing microgel strings, and W_C, the width of contracted microgel strings.

MA980062Y