Rewritable Microrelief Formation on Photoresponsive Hydrogel Layers

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In recent years, photoresponsive polymers and gels have attracted interest as functional materials and have been utilized as cell culture substrates,[1] drug delivery systems,[2] permeable membranes,[3] microvalves,[4] and microactuators.[5] Light irradiation is a useful and important means of control because it can be applied instantaneously and with high resolution, without physical contact. Several photoresponsive gels have been developed by various researchers and the volume change of these gels on the basis of the photothermal effect,[6] photochemical effect,[5,7] or photoisomerization of a comonomer[8] have been reported.

The most common material used to develop photoresponsive gels is poly(*N*-isopropylacrylamide) (pNIPAAm), which is a well-known thermoresponsive polymer.[9] In water, pNIPAAm gels undergo a volume phase transition at their lower critical solution temperature (LCST), around 34 °C, which can be substantially affected by modifying the chemical structure of the polymer network by copolymerization.[10] In our previous studies, we synthesized novel copolymers by modifying pNIPAAm with spirobenzopyran (SP). The charged state of SP is dramatically responsive to light irradiation, and the photoresponsive characteristics of the aqueous solutions of these copolymers were analyzed.[11] The advantages of SP are as follows: the photoisomerization of the SP is totally reversible, the hydrophilicity of the two isomers is significantly different, and the isomerization is induced by blue light, which is more favorable in the biological applications compared to UV light.

In the present study, we report the quick photoresponse of thin hydrogel layers composed of thermoresponsive pNIPAAm with an acrylated SP chromophore incorporated in the polymer backbone (pSPNIPAAm). Drastic photoinduced volume change of the hydrogel was examined, and the instant formation of microrelief on a thin hydrogel layer was demonstrated by means of micropatterned light irradiation. Because of the significant change in the structure and the charge of SP as a result of blue light irradiation in 0.5 mM HCl aqueous solution, selective adsorption of negatively charged latex particles onto the surface of the pSPNIPAAm gel layer was also observed.

First, we prepared a pSPNIPAAm hydrogel of initial thickness 1 mm by free radical polymerization of *N*-isopropylacrylamide (NIPAAm) and acryl ester of 1′,3′,3′-trimethyl-6-hydroxyspiro(2*H*-1-benzopyran-2,2′-indoline) (acrylated SP) with *N*,*N*-methylene-bis(acrylamide) as cross-linker in a THF/water mixture. The molar ratio of SP to NIPAAm monomer was only 0.01 in the polymer gel. Figure 1 shows the chemical structure of the cross-linked pNIPAAm hydrogel functionalized with SP. In the dark, most of the SP chromophores in the chains of the pSPNIPAAm gel in acidic aqueous solution are in a protonated open-ring form. The gel is transparent yellow in color because of the color of the chromophores in the protonated open-ring form. The positively charged, hydrophilic chromophores contribute to the swelling of the gel. Upon irradiation with blue light, the SP chromophores are isomerized to their colorless closed-ring form and effectively lose their charge.[11] Because the chromophores in this state are nonionic and basically hydrophobic, the hydration of the gel chains is reduced and shrinking of the gel results. After the light is turned off, the chromophore returns spontaneously to the protonated open-ring form, which is more stable than the closed-ring form in the dark. In our previous studies, it was confirmed that this photoisomerization greatly affects the hydration of pSPNIPAAm in a certain temperature range around 30 °C.[12]

The equilibrium size of the prepared gel discs with and without continuous blue light irradiation was measured in the temperature range 21−38 °C. The wavelength and the

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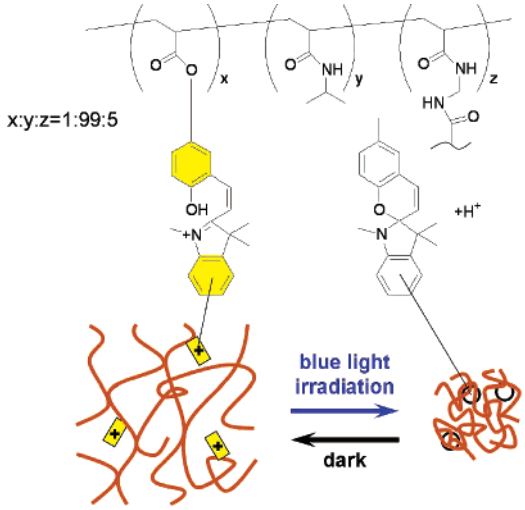

Figure 1. Chemical structure of the cross-linked pNIPAAm hydrogel functionalized with SP and a schematic illustration of the photoinduced shrinking of pSPNIPAAm hydrogel.

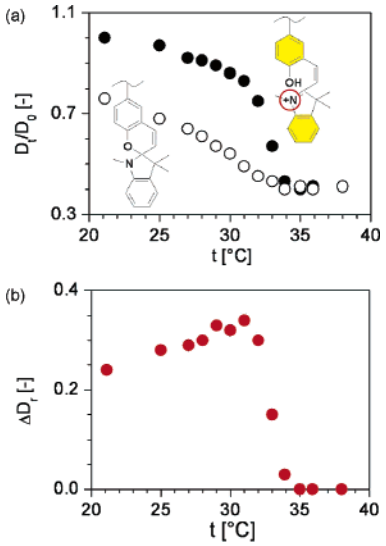

Figure 2. (a) Variation in equilibrium size ratio, D_t/D_0, as a function of temperature of (●) non-irradiated and (○) irradiated pSPNIPAAm gels in 0.5 mM HCl aqueous solution. (b) Relative size change, ΔD_r, as a function of temperature.

intensity of blue light were 436 nm and 5 mW/cm^2, respectively. Figure 2a shows the variation in equilibrium size ratio, D_t/D_0, of the non-irradiated and irradiated pSPNIPAAm gel discs as a function of temperature, where D_t and D_0 are the diameters of the discs at a given temperature and at 21 °C in the dark, respectively. In the dark, some discontinuous behavior originating from thermoinduced phase transition was observed at around 34 °C, just as is observed for ordinary pNIPAAm gels. Upon blue light irradiation, however, such a tendency was found to be less marked. Below the LCST, light irradiation resulted in considerable shrinking of the gels at every given temperature. At 21 °C under blue light irradiation, D_t/D_0 decreased from 100 to 76%. To obtain the same size change of the gel only by heating, we need to elevate the temperature by more than 10 °C. Because the intensity of the irradiation was low and the sample was well-thermoregulated throughout the entire process, photoisomerization is considered to be the dominant factor in the photoinduced shrinking observed here.

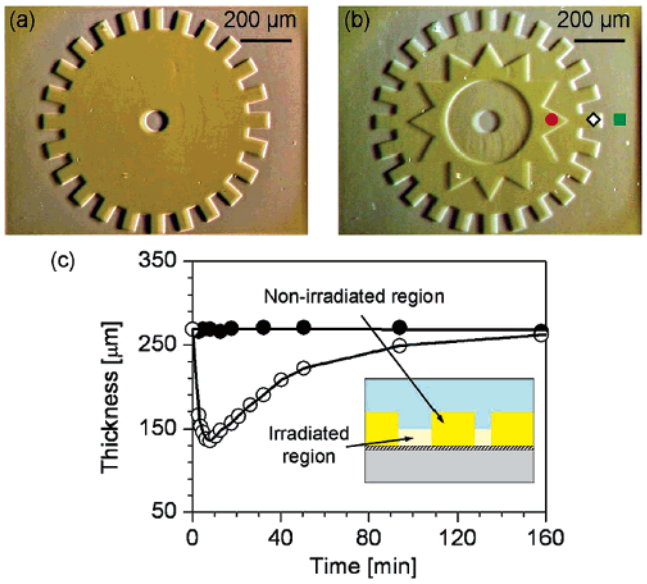

Figure 3. (a, b) Images of the pSPNIPAAm hydrogel layer just after the micropatterned light irradiation. Duration of irradiation was (●, red) 0, (◊) 1, and (■, green) 3 s. (c) Height change of the hydrogel layer in (●) non-irradiated and (○) irradiated region as a function of time after 3 s blue light irradiation.

Figure 2b shows the change in the equilibrium size (ΔD_r), which was calculated as the difference in D_t/D_0 between non-irradiated and irradiated gels, as a function of temperature. ΔD_r increased slightly with rise in temperature below 31 °C. In the temperature range from 31 to 35 °C, however, it decreased drastically with the temperature, and no photoinduced shrinking was observed at temperatures above 35 °C. Accordingly, the most pronounced photoresponse was observed just below the LCST of pSPNIPAAm hydrogel in the dark. This result suggests strongly that the photoisomerization of the chromophore leads the hydrogel to such a drastic shrinking through disturbing the sensitive hydration balance of pNIPAAm main chain. To obtain large ΔD_r stably, we carried out further experiments to investigate the photoinduced deformation at 29 °C.

To demonstrate microrelief formation, a pSPNIPAAm hydrogel layer of 270 μm thickness was prepared by in situ polymerization on a glass surface modified with 3-(trimethoxysilyl)propyl methacrylate so the gel layer was attached covalently to the surface of the flat glass plate. The blue-light-induced deformation of the pSPNIPAAm hydrogel layer in 0.5 mM HCl aq. at 29 °C was observed with an inverted microscope (IX-71, OLYMPUS) equipped with a cooled CCD camera. The micropatterned light irradiation onto the pSPNIPAAm hydrogel layer was carried out by using a PC-controllable micropattern projection system (DESM-01, ENGINEERING SYSTEM) mounted on the microscope. The wavelength and intensity of the irradiating light were 436 nm and 120 mW/cm^2, respectively. The microscopic observation indicated that 2 s irradiation provided significant change in the profile of the surface (Figure 3a). Figure 3b shows a complex microrelief with three individual levels of thickness after an additional 1 s light irradiation on a different region.

To quantitatively analyze the photoinduced change in the surface profile, we irradiated the gel layer with blue light

for 3 s only once. After the light irradiation, the thickness change of the hydrogel layer was evaluated as a function of time. The result is shown in Figure 3c. Although no significant change was observed in the non-irradiated region, the thickness of the hydrogel layer in the irradiated region decreased immediately after the 3 s irradiation by about 30% and continued to decrease to half of the original thickness in the following 8 min. The yellow color of the gel also faded in the first 3 s. From these results, it was concluded for the photoinduced shrinking of the hydrogel layer that ring closure and charge loss of the majority of chromophores were completed in 3 s of light irradiation and then shrinking of the hydrogel proceeded because of the less hydrophilic nature of the resulting polymer network.

Because the photoinduced decrease in thickness was at most ca. 130 μm, the resultant depth of the microrelief was comparable to the smallest line width of the patterns applied in this experiment. The thickness of the gel finally recovered its initial value after 3 h in the dark following light irradiation, indicating that the photoinduced shrinking process is reversible. However, the inscribed structure could be maintained for longer times by reiterating short periods of blue light irradiation. The reswelling time is considered to be determined by spontaneous protonation of the SP chromophore from the nonionic closed ring form.[11] Furthermore, we confirmed that new microrelief was formed by a second irradiation of the hydrogel layer, after it had recovered from the previous deformation.

To study the change in the electrostatic properties of the surface of the pSPNIPAAm gel layer due to the structural change of the SP chromophore, negatively charged fluorescent polystyrene latex particles (ø = 0.39 μm) were introduced on the gel surface by applying an aqueous dispersion containing 0.5 mM HCl for 3 min immediately after the microrelief formation. The irradiated pattern is shown in Figure 4a. A fixed-point observation system composed of a motorized XY-stage and a confocal laser scanning microscope (FluoView 300, OLYMPUS) were used to estimate the number of adsorbed particles that remained after replacing the latex dispersion with 0.5 mM HCl aq. A schematic illustration of the adsorption of negatively charged latex particles on the surface of the pSPNIPAAm gel layer after microrelief formation is shown in Figure 4b. Confocal microscopy yielded a quantitative estimate of the number density of adsorbed latex particles at different levels in thickness direction. It was found that many latex particles were adsorbed on the non-irradiated region (Figure 4c), whereas the number density of adsorbed latex particles on the irradiated region (Figure 4d) was estimated to be only 1/3 that of the non-irradiated region. Because the latex

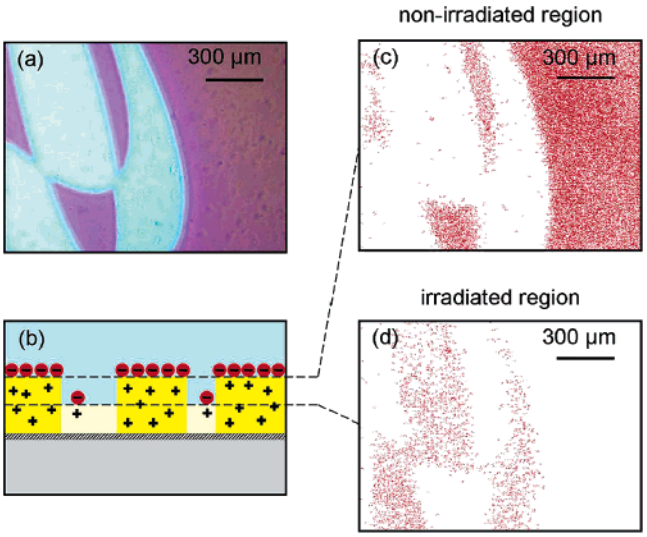

Figure 4. (a) Image of the irradiation by blue light. (b) Schematic illustration of the adsorption of negatively charged latex particles on the surface of pSPNIPAAm gel layer after micropattern formation. Negatively charged latex adsorption (c) on the non-irradiated and (d) on the irradiated region observed by confocal laser scanning microscopy.

particles are assumed to be adsorbed by virtue of the electrostatic attraction between the anionic sulfate groups on latex particles and the protonated and cationic SP, this result indicates that photoisomerization of SP by light irradiation effectively reduces the positive charge in the hydrogel layer.

In conclusion, we have confirmed that micrometer-scale microrelief with a high aspect ratio can be formed instantly on a thin hydrogel layer composed of thermoresponsive pNIPAAm and photoresponsive spirobenzopyran, just by irradiating with blue light. We also observed through the adsorption of anionic latex particles that the charge density is substantially reduced as a consequence of light irradiation. Such drastic photoresponsive properties are expected to be applicable to full command control of integrated microsystems such as microfluidic chips.

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Supporting Information Available: Details of preparations and methods (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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