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Micropattern Immobilization of a pH-Sensitive Polymer

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Introduction

Some polymer systems undergo reversible phase transitions in response to external stimuli such as temperature, solvent composition, pH, ionization of the network, applied electric field, and photoirradiation.[1−6] These stimulation-responsive polymers and the polymeric networks have been extensively investigated for signal manipulator or artificial muscle.[7,8] On the other hand, micropatterning is useful to control the spatial arrangement of small molecules and macromolecules on the surface of a substrate and has a wide range of potential applications in molecular electronics, micromachining, and biosensing.[9−25] Combination of the stimulation-responsive polymers and the microprocessing procedure is interesting for fabrication of intelligent materials or micromachine.

In the present investigation, pH-sensitive poly(acrylic acid) was micropattern-immobilized on a polystyrene film by the method reported by Matsuda and Sugawara[26] and the pH response of the composite polymer was observed in an aqueous solution by an atomic force microscope (AFM), which is very useful for *in situ* observation.[27−30]

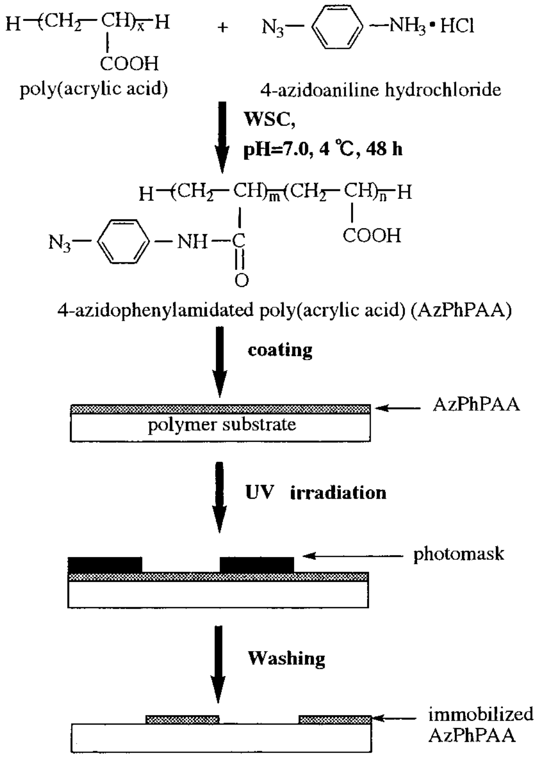

Figure 1. Schematic illustration of micropattern immobilization of azidophenyl-derivatized PAA on a polystyrene film.

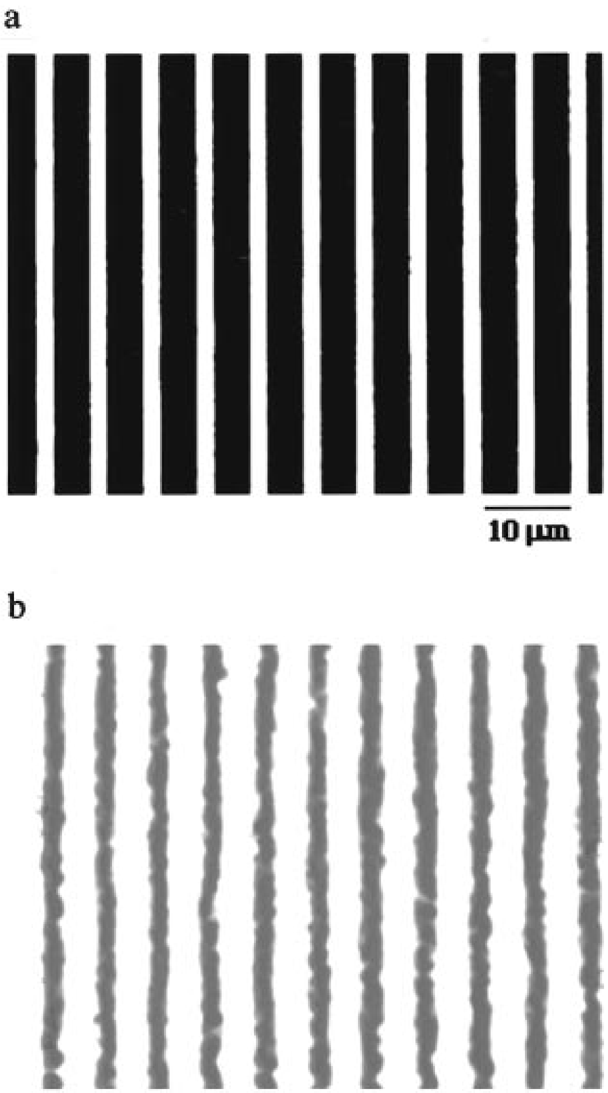

Figure 2. Optical micrographs of photomask (a) and micropattern-immobilized AzPhPAA stained with brilliant green (b).

Materials and Methods

Materials. Poly(acrylic acid) (PAA, MW = 450 000) was purchased from Aldrich Chemical Co. (Milwaukee, WI). 4-Azidoaniline hydrochloride and 1-ethyl-3-(3-(dimethylamino)-propyl)carbodiimide hydrochloride (water-soluble carbodiimide, WSC) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Micropattern Immobilization of Poly(acrylic acid). The preparation method is illustrated in Figure 1. Poly(acrylic acid) was coupled with 4-azidoaniline to prepare azidophenylamidated poly(acrylic acid) (AzPhPAA). An aqueous solution of AzPhPAA was eluted on a polystyrene film. The AzPhPAA-coated film was photoirradiated through a photolithographic mask.

The azidophenyl-derivatized poly(acrylic acid) was synthesized as follows. Poly(acrylic acid) (MW = 450 000, 1.0 mmol on monomer unit), 4-azidoaniline (0.2 mmol), and WSC (6.0 mmol) were dissolved in deionized water (110 mL). The pH of the solution was adjusted to 7.0 by adding NaOH and HCl. The solution was stirred at 4 °C for 48 h. After concentration under reduced pressure, the reaction mixture was dialyzed against distilled water through a seamless cellulose tube (cutoff molecular weight, 12 000) until the absence of azidoaniline in the washing solution was confirmed by ultraviolet spectroscopy. The dialyzed polymer (AzPhPAA) was freeze-

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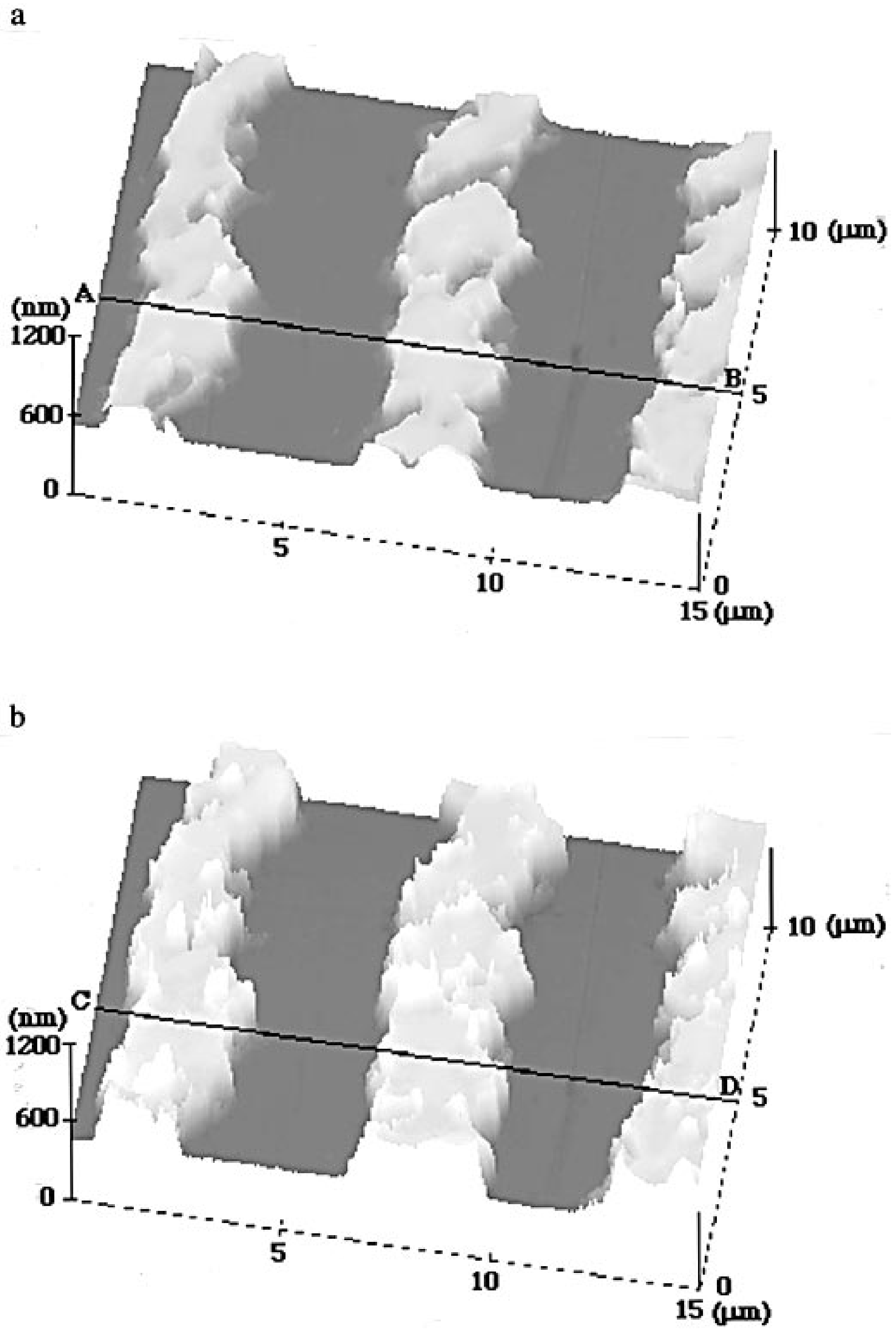

Figure 3. Atomic force micrographs of azidophenyl-derivatized poly(acrylic acid) (AzPhPAA), micropattern-immobilized on a polystyrene film, at pH 2 (a) and pH 10 (b). The cross sectional plots along the A−B or the C−D direction are shown in Figure 4.

dried to obtain a white solid. The content of azidophenyl groups in the polymer was determined by ^{1}H-NMR from the peak intensities of the azidophenyl protons at 7 ppm and those of the methylene protons of the polymer main chain at 1.3 ppm.

Photoimmobilization of the polymer was performed as follows. An aqueous solution of AzPhPAA (500 μg/mL, 0.1 mL) was dropped on a square polystyrene film (2 cm × 2 cm, thickness; 1 mm) and air-dried at room temperature for 24 h. A thin transparent layer of the polymer was formed on the polystyrene film. A photomask made of quartz plate deposited with chrome was produced by Nippon Filcon Co. Ltd. (Osaka, Japan). The photomask was attached on the AzPhPAA-covered polystyrene film and the photomask-attached film was irradiated with an ultraviolet lamp (Koala, 100 W) at the distance of 5 cm for 10 s. The treated film was washed with distilled water until the absence of AzPhPAA in the washing solution was confirmed by ultraviolet spectroscopy.

Observation by Optical Microscope. The AzPhPAA-immobilized polystyrene film was immersed in an aqueous solution containing brilliant green (pH = 7.0, 1 mg/mL) for 5 min. After washing with distilled water, the stained film was observed using an optical microscope (Olympus Co., Tokyo, Japan).

Observation by AFM. A Nanoscope IIIa (Digital Instrumemt Co.) atomic force microscope, equipped with a fluid cell containing an aqueous solution, was utilized to record the

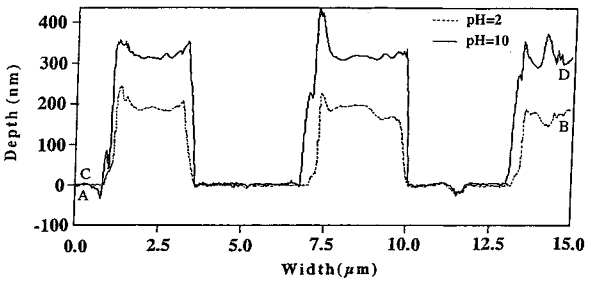

Figure 4. Cross sectional plots showing the depths and widths of the micropattern-immobilized AzPhPAA along the A-B or the C-D direction of the sample (Figure 3).

images. The AzPhPAA-immobilized polystyrene film was immersed in an aqueous solution, whose pH was adjusted to 10.0 with NaOH or to 2.0 with HCl. For the repulsive mode, commercial Si_3N_4 cantilevers (Olympus Co.) with a nominal force constant of 0.06 N/m were used.

Results and Discussion

Fifteen percent of the carboxylic groups in the poly(acrylic acid) were used for coupling with azidoaniline. The coupling percentage was less than that in the feed (20%). The content of azidophenyl groups in the polymer can be controlled by the feed concentation and affects the property of immobilized polymer. It is considered that the high content would induce a decrease of the magnitude of change in volume and that the low content would induce a reduction of the amount of immobilized polymers. Therefore, in the present investigation, the poly(acrylic acid) in which the medium percentage of carboxyl groups coupled with azidoaniline was used for micropattern immobilization.

Figure 2 shows the optical micrographs of photomask and photoimmobilized azidophenyl-derivatized poly(acrylic acid). The azidophenyl groups in the irradiated areas were photolyzed to generate highly reactive nitrene, which spontaneously formed intra- and intermolecularly covalent bonds with neighboring hydrocarbons on a polystyrene film surface. Since the carboxyl groups of poly(acrylic acid) were negatively charged, the immobilized polymer was stained with a cationic dye, brilliant green. The stained regions were the same as the blank regions of the photomask used.

Figure 3 shows an *in situ* AFM image of the pattern-immobilized AzPhPAA in an aqueous solution. It was found that micropattern-immobilized AzPhPAA swelled at pH 10.0 and deswelled at pH 2.0. Figure 4 shows the cross sectional pattern of the AFM image. The height of the photoimmobilized AzPhPAA changed in response to pH, showing different degrees of swelling at different pH values. The cross sectional area at pH 10 was 1.92 times as much as that at pH 2.0. The pH response was reversible. The swelling and deswelling of immobilized AzPhPAA can be interpreted as the result of ionization and deionization of the carboxyl groups in the immobilized AzPhPAA network. At pH 10, the carboxyl groups of the immobilized AzPhPAA network became ionized and the network swelled due to the repulsive interaction between negatively charged carboxylate groups. When the pH of the solution was lowered, the ionized carboxylate groups were deionized and the hydrogen bonds between the carboxylic groups were formed, resulting in contraction of the polymer network.

We have previously reported micropattern-immobilization of a thermoresponsive polymer, poly(*N*-isopropylacrylamide).[31] The micropattern was observed by a phase-contrast microscope only at temperatures above the lower critical solution temperature (LCST) and not below the LCST, because the polymer was hydrophobic above LCST to yield a different refractive index from the substrate polymer. The present investigation on the AFM image of the micropattern-immobilized pH-sensitive polymer showed that AFM is a powerful tool to investigate an in situ morphological change of materials. Extension and shrinking of poly(acrylic acid) or poly(methacrylic acid) grafted on a porous membrane have also been reported previously.[27,28] Microfabrication of stimulation-responsive polymers is essential for the development of a micromachine, and AFM observation should be combined to evaluate the action of the micromachine.

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