Micro- and Nano-fabrication of Stimulus-responsive Polymer using Nanoimprint Lithography

Yoshiyuki Yokoyama¹,², Makiko Umezaki¹, Takahiro Kishioka³, Eiichi Tamiya⁴ and Yuzuru Takamura²

¹Toyama Industrial Technology Center, 383 Takada, Toyama, Japan
² School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi, Ishikawa, Japan
³Nissan Chemical Industries, Ltd., 635 Sasakura Fuchu-machi, Toyama, Japan
⁴Department of Applied Physics, Osaka University, 2-1 Yamadaoka, Suita, Osaka, Japan

Three-dimensionally cross-linked poly(N-isopropylacrylamide) (PNIPAAm), a thermo-responsive polymer, undergoes large volume change through reversible shrinking and swelling in response to changes in temperature. In this study, we developed a thermoresponsive resist (Bio-resist) consisting of a thermally cross-linkable PNIPAAm, thus enabling micropatterning by thermal nanoimprinting. As a result, micropatterning of Bio-resist was possible at a scale of 90 nm to 10 μm. Further, when water was dropped onto the micropattern of Bio-resist, the micropattern shrank when the substrate temperature was high (36 °C), and the micropattern swelled when the substrate temperature was low (20 °C). By utilizing the pattern deformation, a functional microchip was demonstrated that arrays, captures and releases microparticles.

Keyword : thermal nanoimprint, thermoresponsive polymer, resist, bio-chip

1. Introduction

Poly(N-isopropylacrylamide) (PNIPAAm) is a thermo-responsive polymer, the properties of which change with temperature [1]. In water at temperatures higher than 32 °C, the polymer chain is dehydrated, leading to aggregation and precipitation. At temperatures lower than 32 °C, the polymer chain is hydrated and dissolves in water [2,3]. Further, the hydrogel obtained by three-dimensionally cross-linking of the thermo-responsive polymer undergoes large changes in volume via reversible swelling and shrinking in response to changes in temperature [4]. This characteristic is expected to be useful in applications such as soft actuators (e.g., artificial muscle) and in drug delivery systems that can control the amount of drug released according to the surrounding temperature [5-9].

If a thermo-responsive polymer can be fabricated on a nanometer to micrometer scale, the material’s characteristics can be utilized in microelectromechanical systems (MEMS) devices such as micropumps, microvalves, and microtweezers, as well as biochips and micro total analysis systems (μTAS) chips. In previous research, we developed a thermoresponsive polymer that can be micropatterned under light irradiation in the same manner as photoresists for semiconductor chips [10,11]. Micropatterning is possible because the polymer is photo-cross-linkable. We named this thermo-responsive polymer “Bio-resist” because it was developed to be a photoresist for a biochip that handles cells, and because the material deforms as if it were alive, with its volume largely changing with changes in temperature.

Using Bio-resist, we fabricated a cell-array chip that can freely hold and release cells of several micrometers in diameter. On this chip, a multiple-well pattern is made of Bio-resist and each well holds only a single cell, as a consequence of the well size. When the chip
temperature is high (36 °C), Bio-resist shrinks and the inner diameter of the wells increases. Thereby, cells can be introduced into the wells or released. In contrast, when the chip temperature is low (20 °C), the Bio-resist swells and the inner diameter of the wells becomes smaller. Through this mechanism, cells can be enclosed in the wells. This clathrate-type single-cell array chip can array several hundred thousands of cells simultaneously by separating each of them. It is expected to be easily applied for single-cell application such as high-speed screening of immune cells for antibody drug development [12-14].

In this paper, we report a newly developed Bio-resist that can be patterned by thermal nanoimprinting; by using this approach, PNIPAAm can be patterned into finer shapes than in the case of Bio-resist patterned by light. Thermal nanoimprinting is a fabrication technique whereby a mold with fine convex and concave patterns is pressed against a resist film softened by heating in order to transfer the micropattern directly. This technique is now being investigated as a next-generation lithographic technique that can form nano- to micro-scale patterns with relatively inexpensive equipment [15-17]. Specifically, we developed Bio-resist that can be micropatterned by thermal nanoimprinting by making PNIPAAm thermally cross-linkable. We investigated the transfer of nano- and micro-scale patterns onto Bio-resist by thermal nanoimprinting. Further, we examined whether the obtained pattern undergoes reversible deformation with temperature change.

2. Experimental

2.1 Materials

\( N\)-isopropylacrylamide (NIPAAm), 2-hydroxyethylacrylate, dimethyl-2,2'-azobis isobutyrate (V-601), and citric acid were purchased from Wako Pure Chemical Industries. 3-(Triethoxysilyl) propylsuccinic anhydride was purchased from Gelest. Optool HD-1101Z, which was used as the mold-release agent, was purchased from Daikin Industries. Carbon black (average particle size: 120 nm), which was used as a near-IR laser absorbent, was purchased from Asahi Carbon. Fluorescent polystyrene beads (diameter: 6 μm; excitation: 488 nm; emission: 510 nm) were purchased from Cosmo Bio.

2.2 Polymer preparation

PNIPAAm copolymer, used as the base polymer of Bio-resist, was synthesized by radical copolymerization of NIPAAm and 2-hydroxyethylacrylate monomers (molar ratio: 80:20) using V-601 in THF at 70 °C for 6 h under a nitrogen atmosphere (Figure 1). After polymerization, the PNIPAAm was recovered by precipitation in n-hexane. The polymerization yield was approximately 95%.

By gel permeation chromatography (GPC) the molecular weight of the PNIPAAm copolymer was found to be ca. 20,000. \(^1\)H-NMR spectroscopy revealed the PNIPAAm to contain approximately 80 mol % NIPAAm and 20 mol % 2-hydroxyacrylate.

![Fig. 1. Synthetic scheme of PNIPAAm copolymer.](image-url)
2.3 Nanoimprinting conditions

Bio-resist films were spin-coated onto silicon or glass substrates from a solution in propylene glycol methyl ether (PGME). The resist films were pre-baked on a hot plate at 90 °C for 2 min. Thermal nanoimprinting was performed using a silicon mold with a thermal nanoimprinting apparatus (Itrix, NanoimPro Type105). The following conditions were used: load, 3 MPa; pattern transfer temperature, 120 °C; cross-linking temperature, 200 °C for 5 min; and mold removal temperature, 80 °C.

2.4 Measurements

The thermal properties of Bio-resist were measured by differential scanning calorimetry (DSC) (SII, DSC7020) and thermogravimetric analysis (TGA) (SII, TG/DTA7300). Micropatterns of Bio-resist fabricated by nanoimprinting were observed by field emission scanning electron microscopy (FE-SEM) (JEOL, JSM-6301F).

The thermal response of the Bio-resist micropatterns in water was observed under a digital optical microscope (Keyence, V170000C) with a temperature-control stage (Tokai Hit, MATS-555MRO). The thickness of the flat Bio-resist film in water was measured by confocal laser scanning microscopy (Lasertec, HD–100D). Local heating of the micropattern was performed using a near-IR laser (Micro Laser System, 784 nm continuous wave diode laser). The laser was focused by the microscope objective of a modified inverted microscope (Olympus, IX71) to a spot of approximately 10 μm in diameter. Arrayed microbeads were observed by fluorescent microscopy (Olympus, BX51).

3. Results and Discussion

3.1 Characterization of Bio-resist

As a cross-linking agent, citric acid (3 wt % relative to polymer) was added to PNIPAAm copolymer, which has a hydroxyl group that functions as a cross-linking site. The mixture was dissolved in PGME to prepare the Bio-resist solution, the chemical composition of which is shown in Figure 2. By DSC measurement, the glass transition temperature (T_g) of Bio-resist was found to be 99.6 °C. For this reason, the mold was pressed during thermal nanoimprinting at 120 °C, slightly higher than T_g.

By TGA measurement of Bio-resist, a weight decrease was observed near 180 °C. This weight decrease was attributed to the cross-linking reaction (loss of water due esterification) between PNIPAAm copolymer and the cross-linking agent. Accordingly, the three-dimensional cross-linking reaction was carried out while the mold was pressed at 200 °C, slightly higher than the temperature of the weight decrease.

![Chemical composition of Bio-resist for thermal nanoimprinting](image)

3.2 Nanoimprinting results

The thermal nanoimprinting process is shown in Figure 3. First, the Bio-resist film was formed by spin-coating onto a glass or silicon substrate surface-treated with 3-(triethoxysilyl) propylsuccinic anhydride, which is a silane-coupling agent used to ensure tight covalent bonding between the substrate surface and the Bio-resist film. This bonding prevents peeling of the Bio-resist film when the mold is withdrawn, and when micro-pattern deforms with temperature change, which is discussed later.

Next, the Bio-resist film was heated to 120 °C to soften it, and a silicon mold with a fine convex and concave pattern was pressed against the film at 3 MPa. The surface of the mold was pre-treated with the mold-release agent Optool HD-1101Z, which is a fluoropolymer [18]. Then, with the mold pressed down, the Bio-resist was heated to 200 °C to promote the three-dimensional cross-linking reaction in the Bio-resist film and the bonding between the film and the substrate surface. Finally, we cooled the substrate to 80 °C, which is lower than T_g, and withdrew the mold.
Figure 4 shows scanning electron micrographs of micropatterned Bio-resist films, fabricated by thermal nanoimprinting. These results confirm the suitable transfer of nano- and micro-patterns at scales from 90 nm to 10 µm.

3.3 Thermal response of micropatterns

The deformation of the micropatterns was observed as the temperature changed by dropping water on the various Bio-resist patterns (Fig. 5). When the substrate temperature was set to a temperature higher than 32 °C, the micropattern shrank, and the inner diameter of wells in the well pattern, as well as the space width in the line & space pattern, increased. Also, the diameter of pillars in the pillar pattern became thinner. In contrast, when the substrate temperature was lower than 32 °C, the micropattern swelled and the wells and spaces completely closed; in the pillar pattern, the diameter of the pillars increased. The wells closed in the longitudinal and latitudinal directions, in turn, due to interference between adjacent wells. The same principle, the line & space pattern naturally closed in a zigzag.

We found that this behavior is reversible and can be repeated within 10 s by changing the temperature of the substrate. Swelling and shrinking occur as water moves into and out of the Bio-resist film interior. Because the Bio-resist film was given a large specific surface area by micropatterning, water could quickly move in and out, leading to the relatively quick thermal response [19]. Moreover, the Bio-resist film did not peel off from the substrate even when the micropattern was repeatedly deformed, owing to the tight covalent bonding, mediated by the silane-coupling agent, between the substrate and film.
Even for wells as small as 500 nm in diameter, when the substrate temperature was high (36 °C) the micropattern shrank, and the shape of the well pattern could be clearly observed. In contrast, when the substrate temperature was low (20 °C), the image contrast decreased due to the swelling of the micropattern, and the pattern’s shape could not be clearly observed at the resolution of the optical microscope. However, since the well pattern re-emerged upon returning the substrate to the high temperature, it can be inferred that the thermal response is maintained even for patterns smaller than sub-μm.

We also measured the thickness of a flat Bio-resist film at high (36 °C) and low (20 °C) temperatures by confocal microscopy. At the high temperature, the thickness of the Bio-resist film was 1.2 μm due to shrinkage; at the low temperature, the film thickness was 6.2 μm due to swelling. These results show that Bio-resist underwent a 5-fold volume change when temperature was changed.

3.4 Local pattern deformation

We carried out an experiment to deform a particular part of the Bio-resist micropattern by local heating with spot irradiation of laser light [20]. A wavelength of 784 nm in the near-IR region was selected because this wavelength is not absorbed by water on the chip and does not adversely affect cells. However, since Bio-resist itself does not absorb light in near-IR region, as-prepared Bio-resist cannot be laser-heated. To overcome this, we added carbon black as a near-IR absorbent to the Bio-resist solution (0.2 wt % relative to the base polymer). In contrast to photolithography, in which patterns are formed by light, thermal nanoimprinting can make patterns on resist materials that have extremely low light transmittance. Therefore, we could obtain a suitable micropattern even in the case of the Bio-resist film containing carbon black, which is a light-shielding material.

Figure 6 shows the result of the local pattern deformation induced by spot irradiation with near-IR laser light onto a micropattern (Φ10 μm well pattern) of Bio-resist containing dispersed carbon black. When the substrate temperature was high (36 °C), the micropattern shrank and all the wells were open, whereas at low temperature (20 °C), the micropattern swelled and all the wells were closed. We could open a single well selectively within 1 s by focusing the near-IR laser to a spot of 10 μm in diameter. Laser power as low as 2 mW effectively heated the dispersed carbon black, which in turn heated the Bio-resist film and induced a volume change. The well closed within 1s when the near-IR laser irradiation was stopped, and the heat was rapidly dissipated to the surroundings.
3.4 Array of microparticles

We carried out an experiment on arraying polystyrene φ6 µm beads in a φ10 µm well pattern and a 10 µm line & space pattern; both patterns were fabricated with Bio-resist. Figure 7 shows the experimental procedure. First, we maintained the high chip temperature (36 °C) to shrink the Bio-resist. Then, while the patterns were in an open state, a suspension of beads was dropped onto the surface. The beads were allowed to naturally settle for 1 min. Next, the chip temperature was lowered to 20 °C to swell the Bio-resist and switch the patterns to a closed state. The beads were firmly held within the wells and spaces. Using low-temperature (20 °C) water, excess beads were washed from the chip. When the temperature was raised to 36 °C, the Bio-resist shrank, and the patterns returned to their open states, releasing the beads. Figure 8 shows optical micrographs of the beads held in and released from the wells. Figure 9 shows fluorescent micrographs of the fluorescent beads in a single-dot array with Bio-resist φ10 µm well patterns, and in a single-line array with Bio-resist 10 µm line & space pattern.
4. Conclusion

We developed a thermally cross-linkable thermoresponsive polymer, namely “Bio-resist” that could be micropatterned by thermal nanoimprinting. The micropattern could be reversibly deformed through the swelling and shrinking of Bio-resist. Further, we fabricated a chip that could capture and release microparticles by means of the pattern deformation of Bio-resist. This technology is expected to find applications in biochips, photonic devices, and electronic devices because various microparticles, such as cells, quantum dots, magnetic beads, can be simultaneously arrayed with high density and high precision.

Moreover, this technique is expected to be applied not only for thermoresponsive polymer but also for other polymers that respond to external stimuli such as pH, UV light, and electricity [21-23]. In the future, more advanced microdevice can be constructed if such polymers can be modified to be micropatterned similarly to Bio-resist developed in this study.

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