Formation of Thermoresponsive Surfaces by Surface-Initiated, Aqueous Atom-Transfer Radical Polymerization of *N*-Isopropylacrylamide: Application to Cell Culture†

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Stimuli-responsive surfaces, which switch their physical, chemical and biological properties in response to external stimuli, have a great potential in many technologically important areas such as nanoelectromechanical systems, bioanalysis, and biomimetics.[1] In the biotechnological fields, stimuli-responsive surfaces could also be utilized for developing surface adhesion modifiers, biochemically triggered actuators or valves, supports for cell culture, and tissue engineering.[2] For example, thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAAm) was grafted on tissue culture polystyrene (TCPS) cell culture surfaces by electron beam irradiation, and the PNIPAAm-grafted surfaces were used for "cell sheet engineering".[2a] Cells adhere, spread and proliferate at 37 °C, and at 25 °C the cultured cells are detached spontaneously from the surfaces without any enzymatic or mechanical means because of the phase transition of PNIPAAm: the lower critical solution temperature (LCST) of PNIPAAm is about 32 °C and the phase transition of PNIPAAm in water takes place over a narrow range of temperature (1-2 °C).[3] Above the LCST, PNIPAAm is hydrophobic (cell-adherent) in water due to dehydration (loss of hydrogen bonding between the isopropylamide moiety and water molecules) and subsequent aggregation of the polymer chains, while PNIPAAm is hydrophilic (cell-repellant) in water due to the hydrogen bonding below the LCST.

Formation of stimuli-responsive surfaces is usually achieved by surface modification of solid substrates,[4] and among the methods for modifying surfaces, surface-initiated polymerization, where a polymerization initiator is directly bound onto a surface and a polymer chain is grown from the surface, has intensively been investigated as a result of possibility of controlling the density and thickness of grafted polymers.[5] Huck and collaborators reported a surface-initiated, atom-transfer radical polymerization (SI-ATRP) of *N*-isopropylacrylamide (NIPAAm) and its derivatives in a water : methanol (1 : 1) solution.[6] In this communication, we report the *aqueous*, SI-ATRP of NIPAAm on gold surfaces and a preliminary result of cell culture on the PNIPAAm-grafted surfaces.

Self-assembled monolayers (SAMs)[7] of the disulfide terminating in bromoester,[8] were formed by immersing a freshly prepared gold substrate in a 1 mM ethanolic solution of the disulfide for 24 h at room temperature. The SAM-coated gold substrate was then placed in an aqueous solution of NIPAAm (0.5, 1.0, 2.0, or 4.0 M), CuBr (1 mol% relative to NIPAAm) and 2,2'-dipyridyl (2 mol% relative to NIPAAm), and the mixture was stirred for 2 h at room temperature. Grazing-angle infrared spectrum showed characteristic peaks of PNIPAAm after the aqueous SI-ATRP: 1662 cm^{-1} (amide I) and 1546 cm^{-1} (amide II). Figure 1 shows the effect of NIPAAm concentrations on the thickness of PNIPAAm films. We were able to grow about 300-nm thick PNIPAAm films in a *purely aqueous* solution, whereas a 100-nm thick PNIPAAm film was grown in the water : methanol (1 : 1) solution.[6a] The thickness of the PNIPAAm films increased as the monomer concentration was increased: 123-nm, 205-nm, 237-nm, and 270-nm thick PNIPAAm films were formed with 0.5, 1.0, 2.0, and 4.0 M NIPAAm, respectively.

Surface-grown PNIPAAm forms a polymer brush, where polymer chains are forced to stretch away from the surface to avoid overlap. Theoretical and experimental studies suggest that the properties of polymer brushes are different from those of polymers in solution and the phase transition of PNIPAAm (that is, coil-to-globule transition) proceeds *continuously* as temperature is changed.[9] The continuous phase transition of PNIPAAm brushes would make it required to scrutinize the temperature dependency of cell attachment/detachment on a surface-grown PNIPAAm film. In this respect, it is noteworthy that the detachment of L929

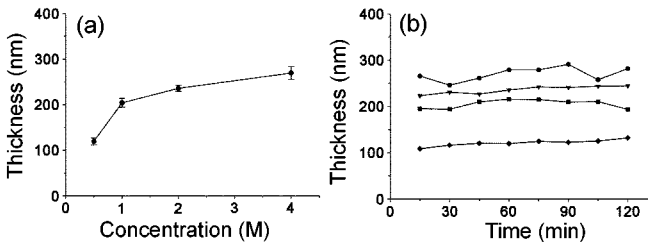

Figure 1. (a) A graph of the thickness of PNIPAAm films *vs*. NIPAAm concentrations. (b) A graph of the thickness of PNIPAAm films *vs*. polymerization time. (◆) 0.5 M; (■) 1 M; (▼) 2 M; (●) 4 M.

†This paper is dedicated to Professor Yong Hae Kim.

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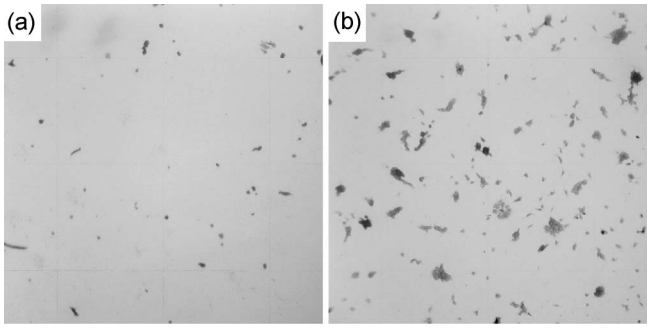

Figure 2. Optical micrographs of NIH 3T3 fibroblasts after the cultivation on a 120-nm thick PNIPAAm film (a) at 37 °C and (b) 40 °C. Cells were fixed with 10% formalin solution and stained with 1% rhodamine solution.

mouse fibroblasts occurred from a surface-grafted hydrogel of PNIPAAm-*g*-poly(ethylene glycol) when temperature was changed only by 1 °C,[10] which implies that there is a critical point in the continuous phase transition of surface-grafted PNIPAAm films for the cell detachment. We, therefore, studied cell attachment/detachment with the PNIPAAm surfaces grafted by surface-initiated polymerization, "grafting-from approach". Figure 2 shows optical micrographs of NIH 3T3 fibroblasts after the cultivation on a 120-nm thick PNIPAAm film at 37 and 40 °C. At each temperature, NIH 3T3 fibroblasts were cultivated for 24 h in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in 10% CO_2. Of interest, we observed no (or little if any) attachment of fibroblasts onto the PNIPAAm surface at 37 °C (Figure 2a), which implies that the thermoresponsive property of the surface-grown PNIPAAm film was altered in terms of cell-adherent property and the surface-grown PNIPAAm was still hydrated (cell-repellant) in water at 37 °C.[11] We presumed that increase in temperature would make the PNIPAAm brushes further collapse, which consequently made the surface cell-adherent: increasing the cultivation temperature to 40 °C led to the increased number of attached fibroblasts (Figure 2b).

In summary, we demonstrated the first example of a surface-initiated, *aqueous* atom-transfer radical polymerization of *N*-isopropylacrylamide and studied temperature-dependent bioadhesion of the formed poly(*N*-isopropylacrylamide) (PNIPAAm) films by using cell cultivation of fibroblasts. PNIPAAm-based materials have been exploited in sensors, responsive membranes, drug-delivery vehicles, anti-fouling surfaces, and tissue engineering. Facile control over physicochemical properties of PNIPAAm films by surface-initiated polymerization, therefore, would yield a useful tool for developing tunable, "smart" surfaces.

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11. Our result suggests that the phase transition of surface-grafted PNIPAAm greatly depends on grafting methods: it was reported that at 37 °C cells attached on PNIPAAm films grafted by "grafting-onto" method,[12] where a polymer was synthesized in solution and the synthesized polymer was grafted onto a surface, and by electron beam irradiation.[2a] We presume that the properties of PNIPAAm brushes are different from those of PNIPAAm grafted by other methods. Control over the thermoresponsive property of SI-ATRP-derived PNIPAAm surfaces is under investigation.
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