The control of cell adhesion and detachment on thin films of thermoresponsive poly[(N-isopropylacrylamide)-r-((3-(methacryloylamino)propyl)-dimethyl(3-sulfopropyl)ammonium hydroxide)]

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\textbf{ABSTRACT}

This paper describes the formation of poly(N-isopropylacrylamide) (PNIPAAm) and poly[(N-isopropylacrylamide)-r-((3-(methacryloylamino)propyl)-dimethyl(3-sulfopropyl)ammonium hydroxide)] (P[NIPAAm-r-MPDSAH]) films on a glass surface via surface-initiated, atom transfer radical polymerization as a cell-culture platform. The films of PNIPAAm with various thicknesses and of P[NIPAAm-r-MPDSAH] with various ratios of NIPAAm and MPDSAH are formed to investigate the behaviors of cell adhesion and detachment. In the case of the PNIPAAm-grafted glass surfaces, the optimal film thickness, achieving the effective control of both cell adhesion and detachment, is estimated to be 11–13 nm for NIH 3T3 fibroblast cells. The adhesion and detachment behaviors of NIH 3T3 fibroblast cells are further tuned by incorporating the hydrophilic and non-biofouling MPDSAH moiety into the PNIPAAm system. The cell adhesion and detachment are controlled best, when the ratio of NIPAAm and MPDSAH is 75:1 (NIPAAm:MPDSAH).

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\textbf{1. Introduction}

Stimuli-responsive polymers have drawn immense interest, as they could provide a variety of applications for biomedical uses. These polymers undergo large physicochemical changes in response to small environmental changes in temperature, electric or magnetic fields, mechanical stress, pH, ionic factors, chemical agents, etc. [1–3]. The interfacial interactions of stimuli-responsive polymers with external stimuli are especially useful in biotechnological applications, because the interactions are similar to the common processes of biopolymers in living organisms. Among various kinds of stimuli-responsive polymers, thermoresponsive poly(N-isopropylacrylamide) (PNIPAAm) is one of the most intensively studied polymers. PNIPAAm exhibits the reversible phase transition in aqueous solutions with its low critical solution temperature (LCST) of 32 °C. This inherent thermoresponsive property of PNIPAAm has extensively been used in numerous biomedical applications, such as drug delivery systems [4–7], microfluidics [8], and chromatography [9–11]. In addition, PNIPAAm-coated plates were fabricated as an innovative cell-culture substrate that enabled reversible adhesion and detachment of cells with a simple control of temperature [12–18]. At temperatures above the LCST (e.g., 37 °C), the PNIPAAm chains are of the compact and collapsed conformation, making the polymeric surface hydrophobic; cells are shown to easily attach and proliferate on the hydrophobic PNIPAAm-coated substrates. When temperature is lower than the LCST, the PNIPAAm-coated surface is hydrophilic because of the hydrogen bonding between PNIPAAm chains and water, inducing cell detachment from the surface.

Although thermoresponsive PNIPAAm-coated surfaces have been applied to various fields of biotechnology and biomedical sciences, recent advances in materials science impose progresses in the fabrication of more sophisticated systems that possess advanced surface properties. Especially, previous studies showed that the adhesion and detachment of cells on the PNIPAAm-coated surfaces were critically determined by several factors, such as grafting density and thickness of PNIPAAm films as well as cell types [16,19–22]. The demand for advanced systems that control the cell adhesion and detachment more facilely has motivated the use of block copolymers of PNIPAAm with other functional monomers [15,20,23–27]. The incorporation of 2-carboxyisopropylacrylamide [15] or poly(ethylene glycol) (PEG) [20,23,24] into PNIPAAm was shown to lead to more rapid cell detachment during the temperature transition than PNIPAAm itself.
Poly((3-(methacryloylamino)propyl)-dimethyl(3-sulfopropyl)-ammonium hydroxide) (PMPDSAH) was introduced as one of the zwiterionic polymers that exhibited non-biofouling property [28]. Because of the zwiterionic moieties along the polymer chains, the PMPDSAH-coated surfaces were neutral and hydrophilic, effectively minimizing the nonspecific adsorption of proteins. The PMPDSAH-coated surface was found to exhibit the enhanced non-biofouling property even compared with PEG [28,29]. Based on these properties, we reasoned that the incorporation of PMPDSAH into the PNiPAAm films would enable us to finely tune the behaviors of cell adhesion and detachment in a controlled manner. In this work, we investigated how molar ratios of PNiPAAm:PMPDSAH in the P(NiPAAm-r-MPDSAH) films affected the cell adhesion and detachment on glass substrates in the aim of developing advanced cell-culture systems.

2. Materials and methods

2.1. Materials

Glass slides (76 mm × 26 mm × 1 mm, 90° ground edges) were obtained from Paul Marienfeld GmbH & Co. KG. Silicon wafers (boron-doped p-type, 100) orientation, and thickness of 500–250 μm) were purchased from ICM Engineering (Bulgaria). Copper (I) bromide (CuBr, 99.99%, Aldrich), 2,2'-bipyridyl (bpy, >99%, Aldrich), (3-(methacryloylamino)propyl)-dimethyl(3-sulfopropyl)ammonium hydroxide, inner buffer solutions (PBS, Sigma) and antibiotics–antimycotic solution (100 unit/mL penicillin, and 100 μg/mL streptomycin) were purchased from Gibco. Ultrapure water (18.3 MΩ cm) was used for all the experiments.

2.2. Synthesis of the polymerization initiator

The polymerization initiator, (3-(2-bromo-2-methylpropionyloxy)propyl)trichlorosilane, was synthesized by following the procedure reported in the literature [30,31]. At first, allyl alcohol (2.13 mL, 31.3 mmol) and triethylamine (3.87 mL, 31.3 mmol) were mixed and stirred in 20 mL of dry dichloromethane on ice bath. 2-Bromoisobutyryl bromide (4.36 mL, 31.3 mmol) were mixed and stirred in 20 mL of dry dichloromethane on ice bath. Then, 10.5 mL of dry triethylamine in 30 mL of dry toluene. After formation of the SAMs, unreacted trichlorosilane was removed under vacuum. The remaining yellow 118.52, 131.41, 171.32 (C), 10.5 Hz, 1H), 5.86–5.96 (m, 1H);13C NMR (100 MHz, CDCl3, δ): 171.54 (C=O).

The obtained allyl 2-bromo-2-methylpropanoate was reacted with trichlorosilane to produce a silane initiator. To a stirred solution of allyl 2-bromo-2-methylpropanoate (4.35 g, 21.0 mmol) in trichlorosilane (excess, 30 mL) under dry argon, dropwisely added was a solution of H2PtCl6 in a 1:1 mixture of ethanol and water. The mixture was stirred at room temperature for 24 h. The reaction mixture was transferred to a separative funnel followed by addition of 0.1 M solution of HCl. The organic phase was separated, dried over MgSO4, and filtered. The solvent was removed under reduced pressure, and the colorless oily residue was purified by flash column chromatography (hexane:ethyl acetate = 9:1 (v/v)) to give 4.35 g (yield, 67.1%) of the ester.1H NMR (400 MHz, CDCl3, δ): 1.92 (s, 6H), 4.65 (d, J = 5.5 Hz, 2H), 5.25 (dd, J1 = 10.5 Hz, J2 = 0.9 Hz, 1H), 5.36 (dd, J1 = 17.1 Hz, J2 = 0.9 Hz, 1H), 5.86–5.96 (m, 1H);13C NMR (100 MHz, CDCl3, δ): 30.78, 55.64, 66.35, 118.52, 131.41, 171.54 (C=O).

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2.5. Surface-initiated, atom transfer radical polymerization of P(NiPAAm-r-MPDSAH)

A mixture of NiPAAm and MPDSAH was used for the formation of random copolymers of NiPAAm and MPDSAH at the surfaces. NiPAAm, MPDSAH, CuBr (5 mol% relative to the sum of NiPAAm and MPDSAH), and bpy (12 mol% relative to the sum of NiPAAm and MPDSAH) were added to a Schlenk flask, and the mixture was degassed under vacuum and purged with dry argon. The prepared monomer solution was transferred to the Schlenk flask containing the initiator-coated glass substrate by using a syringe, and the polymerization was carried out at room temperature. To attain the concentration of the monomer solution was set to be 0.05 M, and the polymerization time was 2 min for all the component ratios of NiPAAm and MPDSAH. After the polymerization, the P(NiPAAm-r-MPDSAH)-coated glass substrate was taken out, rinsed with water, methanol, and absolute ethanol, and dried under a stream of argon. All the procedures for the formation of the SAMs and PNiPAAm-r-MPDSAH films were similarly applied to the Si/SiO2 substrates for the ellipsometric measurement of the film thickness.

2.6. Cell-culture

NH 3T3 fibroblast cells were maintained in continuous growth in cell culture media (DMEM supplemented with 10% FBS and 1% antibiotic solution at 37 °C in a humidified atmosphere containing 5% CO2 on tissue culture T25 polystyrene flasks. Cells were removed from the flask surfaces by washing twice with 2 mL of DPBS, followed by incubation in 0.5 mL of 0.25% trypsin/EDTA for detachment. The detached cells were resuspended in 2 mL of cell-culture media and the number of cells was counted with hemocytometer. On each PNiPAAm or P(NiPAAm-r-MPDSAH)-grafted surface, placed in a petri dish (60 × 15 mm), cells were seeded (5 × 104 cells/cm2 dispersed in 6 mL cell-culture media) and incubated at 37 °C in a humidified atmosphere of 5% CO2 on tissue culture T25 polystyrene flasks. Cells were removed from the flask surfaces by washing twice with 2 mL of DPBS, followed by incubation in 0.5 mL of 0.25% trypsin/EDTA for detachment. The detached cells were resuspended in 2 mL of cell-culture media and the number of cells was counted with hemocytometer. On each PNiPAAm or P(NiPAAm-r-MPDSAH)-grafted surface, placed in a petri dish (60 × 15 mm), cells were seeded (5 × 104 cells/cm2 dispersed in 6 mL cell-culture media) and incubated at 37 °C in a humidified atmosphere of 5% CO2. After 24-h incubation, the behaviors of cell adhesion were studied at 37 °C with an optical microscope. The substrates were then taken out from 37 °C cell-culture media and placed in the media that had been cooled to 25 °C. The cell behaviors were studied with an optical microscope immediately.

2.7. Characterization

The thickness of the SAMs and polymer films was measured with a Gaertner L116s ellipsometer (Gaertner Scientific Corporation, IL) equipped with a He–Ne laser (632.8 nm) at a 70° angle of incidence. A refractive index of 1.46 was used for all the samples. More than five different points on each sample were measured, and the average values were reported in this paper. Contact angle measurements were performed using Phoenix 300 apparatus (Surface Electro Optics Co. Ltd, Korea) equipped with a video camera. The static contact angles of water drops of ~5 μL were measured at five different locations on each sample, and average values were reported in this paper. The X-ray photoelectron spectroscopy (XPS) study was performed with a VG-Scientific ESCALAB 250 spectrometer (UK) with a monochromatized Al Kα X-ray
polymerization time were varied to generate PNIPAAm films with different thicknesses. The polymerization times were 2, 5, 30, and 60 min for the 0.05-M NIPAAm solution, and 10, 30, and 60 min for the 0.1-M NIPAAm solution. The same set of the reaction conditions was applied to the Si/SiO₂ surfaces (silicon wafers) for the determination of ellipsometric thickness; it was practically difficult to measure the polymer thickness on glass, and we supposed that the thickness of the polymer films formed on glass would be identical or at least similar to that on the Si/SiO₂ surfaces under the same polymerization conditions. The ellipsometric thicknesses were measured to be 3.3, 4.4, 11.6, and 13.8 nm, when the polymerization times were varied with the 0.05-M NIPAAm solution. The 13.0-, 16.6-, and 19.1-nm-thick PNIPAAm films were obtained after 10-, 30-, and 60-min reactions with the 0.1-M NIPAAm solution, respectively (Table 1). The 19.1-nm-thick PNIPAAm-coated substrate was characterized as a representative before and after SI-ATRP by X-ray photoelectron spectroscopy (XPS). In the XPS spectra, the characteristic peaks of the polymerization initiator were observed at 533.6 (O1s), 288.8 (C1s), and 73.42 (Br3d) eV before SI-ATRP (Fig. 2a), and an additional peak of N1s appeared at 401.3 eV after SI-ATRP (Fig. 2b).

3.2. Cell adhesion and detachment behaviors on PNIPAAm-grafted surfaces

Recent studies on PNIPAAm-coated cell-culture substrates showed that the grafting amount of PNIPAAm on the surface had a significant influence on the behaviors of cell adhesion and detachment [16,17,21]. There have been three previous reports, including ours, that used SI-ATRP for forming PNIPAAm films as a cell-culture platform: the solid surfaces were gold [19], Si(100) [20], and polystyrene [21]. In the present work, we formed the PNIPAAm films with various thicknesses on a glass substrate and investigated effects of the thickness of the PNIPAAm films on the water contact angle and behaviors of cell adhesion and detachment. Especially, we sophisticatedly adjusted polymerization conditions to obtain 20-nm-thick PNIPAAm films at maximum, because the PNIPAAm films with the thickness of below 20 nm were shown to be operational for reversible adhesion and detachment of cells [17], and the thick PNIPAAm films (>100 nm) were resistant to cell attachment at 37°C or even higher [19]. In the case of ultrathin PNIPAAm films (<20 nm), the mobility of PNIPAAm brushes was thought to be highly restricted, because of a close contact with the substrate. When the thickness of PNIPAAm films was greater than 20 nm, PNIPAAm brushes could be stretched away from the substrate surface relatively well and display enhanced chain mobility. Therefore, as the films became thicker, they tended to be more hydrophilic (or less hydrophobic) at 37°C, due to the enhanced interaction with water molecules. At 25°C, both ultrathin and thick PNIPAAm films were hydrophilic because of hydration; however, when the PNIPAAm layer was too thin (<5 nm in our case), the hydration was insufficient to make the surface hydrophilic at 25°C.

Thickness-dependent behaviors of cell adhesion and detachment were investigated first with the 3.3-nm-thick PNIPAAm film. NIH 3T3 fibroblast cells adhered well on the surface and proliferated to confluency at 37°C (left image in Fig. 3a). However, we observed only a partial cell detachment, when temperature was lowered to 25°C (right image in Fig. 3a). In this case, the grafting amount of PNIPAAm was thought to be too small to show effective hydrophobic and hydrophilic switching required for cell detachment. When the thickness was increased to 4.4 nm, the detachment behavior was still similar to that of the 3.3-nm-thick film: the detachment of the cells was observable, but most cells remained on the surface (Fig. 3b).

The PNIPAAm polymer films were formed on glass substrates with several different thicknesses by varying the conditions of surface-initiated, atom transfer radical polymerization (SI-ATRP) [32,33]. PNIPAAm has been grafted onto cell-culture substrates by various techniques, such as electron beam-irradiated polymerization, plasma deposition, and UV irradiation [34–38]. These methods, however, suffer from batch-to-batch variability, and ATRP has recently been adopted for the thickness-controlled formation of dense PNIPAAm films on cell-culture substrates [19–22]. Fig. 1a shows a general procedure of SI-ATRP for the formation of PNIPAAm films on a glass substrate. The SAMs of the polymerization initiator, 3-(2-bromo-2-methyl)propionyloxy)propyltrichlorosilane, were formed on a glass substrate, and the monomer solution, containing the monomer (PNIPAAm), a catalyst (CuBr) and a ligand (2,2’-bipyridyl), was then added to the initiator-coated glass substrate. The concentration of the monomer solution and the

![Fig. 1. Schematic description for the formation of (a) PNIPAAm and (b) P(NIPAAm-r-MPDSAH) films on glass.](image-url)
The controlled adhesion and detachment of cells were achieved with the 11.6-nm-thick PNIPAAm film (Fig. 3c). On this sample, the cells attached and proliferated well at 37 °C, and were detached completely from the surface at 25 °C. We also observed the same controlled behaviors of cell adhesion and detachment for the 13.0- and 13.8-nm-thick films (Data not shown). In other words, the PNIPAAm film layers with the thickness of around 11–13 nm offered an optimized hydrophobic surface at 37 °C. The hydrophilicity of the surfaces increased as the thickness of the PNIPAAm films reached 16.6 nm, and therefore the cells could not attach well throughout the surface at both 37 °C and 25 °C (Table 1).

Our results were in a qualitative agreement with the previous reports. Okano and co-workers studied the adhesion and detachment behaviors of bovine endothelial cells (ECs) with 15.5- and 29.3-nm-thick PNIPAAm films that were grafted onto tissue culture polystyrene (TCPs) by electron beam irradiation [17], and reversible adhesion and detachment of ECs were observed on the 15.5-nm-thick film, but no cell adhesion and proliferation were observed on the 29.3-nm-thick film. In addition, Okano and co-workers also reported the formation of PNIPAAm films on TCPs by SI-ATRP, where poly(4-vinylbenzyl chloride) was spin-casted and used as an initiator of SI-ATRP [21]. In the report, they showed that the adhesion of ECs decreased as the thickness increased. Based on the results, they concluded that the hydrophilicity of the surfaces increased both at 37 and 20 °C as the thickness increased, and the increased hydrophilicity of the thick films prevented cells from being adhered onto the surfaces at 37 °C. Our results showed the similar trend in terms of cell adhesion: the cell adhesion decreased as the film thickness increased (Fig. 3, left column). Our previous report – thin PNIPAAm films were generated on gold by SI-ATRP, and the 120-nm-thick films did not induce cell adhesion – was also consistent with the present results [19]. The absolute value of the films thickness was, however, different between present and previous cases in present case, fibroblasts did not proliferate at the thickness above about 16 nm. Nonetheless, it is noteworthy that only about 3-nm difference (13.8 vs. 16.6 nm) made the surfaces act differently in the aspect of cell adherence in our system.

Regarding the cell detachment, the ultrathin 1.8-nm-thick PNIPAAm film on polystyrene, formed by SI-ATRP, was reported to exhibit a cell-detaching property at 20 °C [21]. However, thin films (3.3 and 4.4 nm in thickness) did not show cell-detaching property in our system. Moreover, PNIPAAm films on Si(100) did show an opposite trend in the aspect of cell adhesion [20]: the adhesion of fibroblasts increased as the thickness increased. The detailed mechanism of cell adhesion and detachment remains to be seen, but our results, along with the previous reports, indicated that the control of cell adhesion and detachment was a complicated process, determined interweavely by many factors, such as substrate types, grafting methods, surface density of polymers, polymer thickness, and cell types.

To investigate the possible relationship between the surface morphology and cell adhesion/detachment behaviors, the surface morphology of Brush-11.6 was imaged at both 25 and 37 °C by the solution-phase atomic force microscopy (AFM). The surface was, however, found to be fairly smooth at both temperatures with a slightly increased roughness at 25 °C (see the Supporting information for the AFM micrographs; Fig. S1).
3.3. Water contact angles

Although detailed mechanisms on cell adherence and detachment on PNIPAAm films remain to be seen, hydrophilicity/phobicity of surfaces seems to play an important role in the control. In this respect, we investigated the possible correlation between the water contact angles and the behaviors of cell adherence/detachment for thin PNIPAAm films (<20 nm in thickness) (Table 1).

Table 1 shows that the water contact angle became smaller – i.e., the apparent hydrophilicity of the films increased – at both temperatures, as the film thickness increased. As described in the previous reports [21,22], the molecular motion of the grafted
PNIPAAm chains became larger with the increase of the chain lengths, facilitating the hydration of the polymer brushes in their outer regions. Therefore, the surfaces became more hydrophilic both at 25 and 37 °C, as the thickness of PNIPAAm layer was increased.

In our work, thickness-dependent contact angles also gave an inkling of some correlations between the contact angles and the cell behaviors. The set of the PNIPAAm films in Table 1 were further divided into three groups based on the cell behaviors: adherence at 37 °C and adherence at 25 °C (AA); adherence at 37 °C and no adherence (i.e., detachment) at 25 °C (AD); no adherence at 37 °C and no adherence at 25 °C (DD). In the AA case (Brush-3.3 and 4.4), the static water contact angles were measured to be around 70° at 37 °C, and decreased slightly to around 67°–69° as temperature was lowered to 25 °C. When the film thickness was 11.6 nm (only 7.2-nm increase from Brush-4.4), the contact angle at 25 °C was measured to be 61.8° (for Brush-11.6), which was a greatly decreased value from the value for Brush-4.4 (66.7°). The decrease in the contact angle (i.e., the increase in hydrophilicity) could explain the observed cell detachment. The same trend was observed in the other AD cases (Brush-13.0 and 13.8). The contact angles at 25 °C were below 60°, while those at 37 °C were about 64°. The AA and AD cases indicated that cell adherence was favorable at the contact angle of about 64° and higher, and cell detachment at the contact angle of about 62° and lower. This correlation implies that the contact angles might be used as a ruler of cell adherence and detachment on PNIPAAm films. The contact angles of Brush-16.6 and 19.1 (the DD case) were measured to be lower than 62° (57°–61°) at both 37 and 25 °C; the cells failed to adhere onto the surfaces at both temperatures. The previous report also supports this suggestion: cell detachment was observed on PNIPAAm-grafted S(100) surface at the contact angles of 58°–61° [20].

3.4. Surface-initiated, atom transfer radical polymerization of P(NIPAAm-r-MPDSAH)

The studies on pure PNIPAAm films on glass in the previous sections indicated that the PNIPAAm films with the thickness of around 3–4 nm, providing more hydrophobic surfaces compared with the PNIPAAm films with the thickness of around 11–19 nm, offered the best environments for cell adhesion at 37 °C (see the left column of Fig. 3). In other words, cells preferred (ultra)thin PNIPAAm films for their adherence at 37 °C. However, under these conditions, the adhered cells were, unfortunately, not detached from the surfaces, presumably because the thin polymer films were not hydrophilic (strictly speaking, non-biofouling) enough for cell detachment at 25 °C. The main object of this work was to develop a method for finely tuning cell adhesion and detachment properties of PNIPAAm films by incorporating functional monomers, and we thought that the thickness of 3–4 nm would be a legitimate range for further studies in our system.

To give additional cell-detaching property to the system, we incorporated the hydrophilic and non-biofouling MPDSAH into the PNIPAAm films as a form of the random copolymer, P(NIPAAm-r-MPDSAH). The procedure for the formation of P(NIPAAm-r-MPDSAH) films is described in Fig. 1b. After formation of the SAMs of the polymerization initiator on glass, the SI-ATRP of P(NIPAAm-r-MPDSAH) was carried out with a mixture of PNIPAAm and MPDSAH as the monomers. The mole ratio of NIPAAm and MPDSAH was varied to 300:1, 200:1, 100:1, 75:1, 50:1, and 25:1. The other reaction conditions were set to be the same as those for the formation of the 3.3-nm-thick PNIPAAm film (monomer concentration: 0.05 M; polymerization time: 2 min). The same set of reaction conditions was applied to the Si/SiO2 surfaces for the determination of ellipsometric thickness. The ellipsometric thicknesses were measured to be 3.6, 2.7, 3.4, 3.2, 3.1, and 3.3 nm for P(NIPAAm-r-MPDSAH) films formed on Si/SiO2 with the different mole ratios of NIPAAm and MPDSAH (from 300:1 to 25:1) (Table 2). The resulting films were designated as Brush-300:1, Brush-200:1, Brush-100:1, Brush-75:1, Brush-50:1, and Brush-25:1, respectively. The thickness-dependent thermoresponsiveness and cell adhesion/detachment ability of thicker P(NIPAAm-r-MPDSAH) films were not examined in this study, because the surface properties would reach the states that were inadequate for cell adhesion/detachment control: enhanced chain mobility in the thicker films, leading to more hydration of P(NIPAAm-r-MPDSAH) brushes, was insufficient to prevent cell adhesion at both 37 and 25 °C. As a representative, the XPS spectrum of Brush-25:1 showed an S 2p peak at 168.6 eV (Fig. 2c), confirming the successful formation of P(NIPAAm-r-MPDSAH) on glass.

3.5. Cell adhesion and detachment behaviors on P(NIPAAm-r-MPDSAH)-grafted surfaces

The MPDSAH moiety clearly contributed to the control over cell adhesion and detachment, especially cell hydrophobicity (and/or non-biofouling property) (Fig. 4). In the cases of Brush-300:1 and Brush-200:1, the cell behaviors were the same as those for the thin PNIPAAm films: the cells were finely adhered and proliferated at 37 °C, and mostly remained on the surfaces at 25 °C (Fig. 4a and b). We thought that the small fraction of the added MPDSAH was not sufficient enough to yield hydrophilicity at 25 °C. When we increased the mole ratio to 100:1, we observed an enhanced detachment of cells at 25 °C (Fig. 4c). The cell behaviors were quite comparable with those on the 4.4-nm-thick PNIPAAm film only, indicating the effect of the MPDSAH moiety. However, the hydrophilicity of the surface at 25 °C seemed to be still insufficient to generate a clear detachment of cell from the surface. The controlled adhesion and detachment were achieved in the case of Brush-75:1 (Fig. 4d). The cells were finely adhered on the surface and proliferated to confluency at 37 °C, and completely detached from the surface when the temperature was lowered to 25 °C. When the relative ratio of MPDSAH was increased further to 50:1 and 25:1, the non-biofouling property of MPDSAH surpassed the thermoresponsive character of PNIPAAm: the cells were not adhered at 37 °C (Fig. 4e and f).

The contribution of the MPDSAH moiety to control over the cell detachment of surfaces was confirmed. In the case of ultrathin PNIPAAm films (3–4 nm in thickness), the surfaces were relatively hydrophobic at 37 °C (contact angles > ~69°, Table 1) because of the inherent, thermoresponsive character of PNIPAAm. However, their surface property could not be switched to show enough hydrophilicity for cell detachment at 25 °C (contact angles > ~67°, Table 1), presumably because the grafting of PNIPAAm was insufficient to have enough interactions with water molecules. Therefore, the cells adhered at 37 °C could not be detached well from the surface at 25 °C (Fig. 3a and b). On the other hand, in the case of P(NIPAAm-r-MPDSAH), the lack of hydrophilicity could be supplemented, as the

### Table 2

<table>
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<tr>
<th>Sample</th>
<th>Mole ratio of NIPAAm and MPDSAH (NIPAAm/MPDSAH)</th>
<th>Thickness (nm)</th>
<th>Contact angle</th>
<th>37 °C</th>
<th>25 °C</th>
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<tr>
<td>Brush-300:1</td>
<td>300:1</td>
<td>3.6 ± 0.2</td>
<td>71.1 ± 0.8°</td>
<td>69.8 ± 0.4°</td>
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<tr>
<td>Brush-200:1</td>
<td>200:1</td>
<td>2.7 ± 0.2</td>
<td>70.8 ± 1.9°</td>
<td>69.0 ± 1.3°</td>
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<tr>
<td>Brush-100:1</td>
<td>100:1</td>
<td>3.4 ± 0.1</td>
<td>69.6 ± 2.1°</td>
<td>69.1 ± 1.4°</td>
<td></td>
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<tr>
<td>Brush-75:1</td>
<td>75:1</td>
<td>3.2 ± 0.1</td>
<td>69.0 ± 2.2°</td>
<td>66.0 ± 1.2°</td>
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<tr>
<td>Brush-50:1</td>
<td>50:1</td>
<td>3.1 ± 0.3</td>
<td>62.1 ± 0.6°</td>
<td>61.6 ± 0.3°</td>
<td></td>
</tr>
<tr>
<td>Brush-25:1</td>
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<td>3.3 ± 0.1</td>
<td>62.2 ± 0.5°</td>
<td>59.9 ± 0.8°</td>
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</table>
Fig. 4. Optical micrographs of NIH 3T3 fibroblasts on P(NIPAAm-r-MPDSAH) films with different mole ratios of NIPAAm and MPDSAH. The ratios (NIPAAm:MPDSAH) were (a) 300:1, (b) 200:1, (c) 100:1, (d) 75:1, (e) 50:1, and (f) 25:1.
4. Conclusions

To further investigate the role of the MPDSAH moiety in the controlled detachment of cells, we measured the water contact angles (Table 2). As a reference, the water contact angle of the MPDSAH films was reported to be about 5° when the thickness of film was 32 nm [28]. The contribution of MPDSAH to the water wettability of the surface was insignificant, when its relative ratios were low (Brush:300:1, 200:1, and 100:1): the water contact angles of these AA cases were around 69–70° at both temperatures. As the relative amount of the incorporated MPDSAH increased (Brush:50:1 and 25:1), the surface became much more hydrophilic than the PNIPAAm films with the same thickness. The contact angles of around 62° at 37 °C manifested the DD cases. The obtained DD cases implied that the cell adhesiveness could be tuned by the incorporation of MPDSAH; the 3-nm-thick film was either cell-adherent (Fig. 3a) or cell-repellent (Fig. 4e and f).

The full-fledged control over cell adherence and detachment (the AD case) was achieved with the 75-to-1 ratio of PNIPAAm and MPDSAH (Brush:75:1). The surface was thermoresponsive, changing the water contact angles from 69° at 37 °C to 66° at 25 °C. This change in the contact angles was enough to achieve the controlled detachment of fibroblasts from the surface at 25 °C (right image of Fig. 4e). It seems that the non-biofouling property of MPDSAH also took a role in the control of the cell detachment along with its hydrophilicity. The contact angle of Brush:75:1 at 25 °C was measured to be 66°. The value of 66° was an indicator for the cell adherence onto the PNIPAAm films: in the case of PNIPAAm films, surfaces with the contact angle of 64–72° were favorable for cell adhesion, and cells were completely detached from surfaces when the contact angle was below 62°. In contrast, cell detachment was observed for a P(NIPAAm−r-MPDSAH) film with the contact angle of 66°, while cells were finely adhered on the surface with the contact angle of around 69°. These results indicated that the critical value of contact angles for cell detachment depended upon the chemical compositions of the films, and that the value of 66° would be used as the adherence indicator for PNIPAAm films. The observed phenomena could be explained by the supplemented non-biofouling effect from the MPDSAH moiety, on top of the hydration effect of PNIPAAm polymer brushes, when the temperature was lowered to 25 °C. Taken together, all the results indicate that it would be possible to finely tune the behaviors of cell adherence and detachment by incorporating MPDSAH into the PNIPAAm films in a designed manner.

4. Conclusions

In this paper, we prepared the films of poly(N-isopropylacrylamide) (PNIPAAm) and poly[(N-isopropylacrylamide)-r-(3-(methacryloylamino)propyl)-dimethyl(3-sulfopropyl)ammonium hydroxide)](P(NIPAAm−r-MPDSAH)) on glass surfaces via surface-initiated, atom transfer radical polymerization, and investigated the effects of polymer thicknesses and chemical compositions on the control over the adhesion and detachment behaviors of NIH 3T3 fibroblast cells. Based on the results, we could draw several conclusions. (1) The cell behaviors on surfaces – attachment, adherence, proliferation, detachment, etc. – were sophisticatedly (and tightly) controlled by many factors: a subtle change in the thicknesses or chemical compositions drastically changed the cell behaviors on surfaces. (2) The contact angle measurements suggested the possible correlation between the contact angles and the behaviors of cell adhesion and detachment. (3) Of most importance, the cell behaviors on surfaces were controlled or finely tuned by the incorporation of MPDSAH into the PNIPAAm films: the MPDSAH moiety converted the cell adherence of surfaces to the cell-repellency by its non-biofouling and hydrophilic properties. With the optimized mole ratio of NIPAAm and MPDSAH, we achieved the advanced control over the cell behaviors: cells were more favorably adhered onto the surface, and the adhered cells were detached facilely from the surfaces by lowering the temperature. The (P(NIPAAm−r-MPDSAH) films with the thickness of around 3–4 nm and 75:1 mole ratio of NIPAAm and MPDSAH provided the best environments for both cell adhesion at 37 °C and cell detachment at 25 °C. We believe that our approach demonstrated in this work would be beneficial in the design of advanced cell-culture systems that utilize copolymers of NIPAAm and other functional monomers.

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Appendix

Figures with essential colour discrimination. Figs 3 and 4 in this article are difficult to interpret in black and white. The full colour version can be found in the on-line version, at doi:10.1016/j.biomaterials.2009.07.016.

Appendix. Supporting information

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.biomaterials.2009.07.016.

References


