

Modulation of Graft Architectures for Enhancing Hydrophobic Interaction of Biomolecules with Thermoresponsive Polymer-Grafted Surfaces

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Abstract

This paper describes the effects of graft architecture of poly(*N*-isopropylacrylamide) (PIPAAm) brush surfaces on thermoresponsive aqueous wettability changes and the temperature-dependent hydrophobic interaction of steroids in silica capillaries (I.D.: 50 μm). PIPAAm brushes were grafted onto glass substrates by surface-initiated atom transfer radical polymerization (ATRP) that is one of the living radical polymerization techniques. Increases in the graft density and chain length of PIPAAm brushes increased the hydration of polymer brushes, resulting in the increased hydrophilic properties of the surface below the transition temperature of PIPAAm at 32 °C. More hydrophobic surface properties were also observed on surfaces modified with the block copolymers of IPAAm and *n*-butyl methacrylate (BMA) than that with IPAAm homopolymer-grafted surfaces over the transition temperature. Using PBMA-*b*-PIPAAm-grafted silica capillaries, the baseline separation of steroids was successfully achieved by only changing temperature. The incorporation of hydrophobic PBMA chains in grafted PIPAAm enhanced the hydrophobic interaction with testosterone above the transition temperature. The surface modification of hydrophobicity-enhanced thermoresponsive polymers is a promising method for the preparation of thermoresponsive biointerfaces that can be effectively modulated their biomolecule and cell adsorption with the wide dynamic range of hydrophilic/hydrophobic property change across the transition temperature.

1. Introduction

Intelligent polymers having a response ability to external stimuli have attracted much attention as functional materials toward biomedical and analytical applications [1-3]. Poly(*N*-isopropylacrylamide) (PIPAAm) is one of the most remarkable stimuli-responsive polymer, exhibiting a reversible soluble/insoluble change by temperature alterations in aqueous solution at 32 °C [4]. Surface modification of PIPAAm onto solid substrate induces dramatic changes of aqueous wettabilities of the surfaces across its lower critical solution temperature (LCST). These thermoresponsive characteristics on PIPAAm-grafted surfaces are applied to switchable controls of biomolecular/cellular binding and release through mainly hydrophobic interaction, which are now widely used in microfluidic systems [5-7], cell culture substrates [8, 9], tissue engineering [10, 11], and chromatography matrices [12-15]. In order to improve the effective “on-off” interaction switching on PIPAAm-grafted surfaces, the surface wettabilities are modulated by the composition, density, and conformation of surface-grafted polymer using free radical polymerization techniques. For instance, the magnitude of hydrophilic/hydrophobic alterations on PIPAAm-modified surfaces largely depended on the mobility and density of grafted PIPAAm chains on surfaces, caused by the change of their molecular architectures [13]. Furthermore, the transition temperature is also significantly shifted from the LCST of PIPAAm by the incorporation of hydrophilic or hydrophobic monomers into random copolymers [9, 16]. The surface modification methods prepared by conventional radical polymerization in the reports described above, however, have a disadvantage in making the control of the composition, density, and conformation of polymer chains difficult [13].

Recently, our group has successfully synthesized well-defined PIPAAm-grafted surfaces by

surface-initiated atom transfer radical polymerization (ATRP) [17-20]. High-density PIPAAm brushes having controlled molecular weight are grafted onto capillary lumen [17], silica bead [18, 19], and tissue-culture polystyrene dish surfaces [20]. In temperature-dependent chromatographic analyses, dense PIPAAm-grafted silica beads were observed to give relatively longer retention time of steroids than those prepared by conventional methods. The sparse and shorter length PIPAAm brushes can hold effectively hydrophobic solutes and give a longer retention time of steroids compared to dense PIPAAm brushes at temperatures below LCST of PIPAAm [18]. This elongated retention time on sparse PIPAAm-grafted surfaces is thought to be caused by mainly hydrophobic interactions between the exposed phenethyl groups of silanes bonded on silica surfaces and steroid molecules. In addition, the polarity of interfacial layer between grafted PIPAAm brushes and silica bead surfaces may induce the hydration/dehydration alterations of PIPAAm brushes [19]. Furthermore, well-controlled ATRP facilitated the formation of dense PIPAAm brushes on the glass substrate, and their surfaces diminished fibronectin adsorption and cell adhesion [21]. These results imply that the graft architecture of PIPAAm brushes on surfaces greatly influences temperature-dependent interaction with biomolecules and cells.

In the present study, the graft architecture of PIPAAm brushes was modulated by surface-initiated ATRP technique for enhancing hydrophobic interaction with biomolecules. Hierarchical graft architecture on the surfaces can be achieved simply via sequential ATRP with other type comonomers [22-24]. Introduction of hydrophobic comonomer, *n*-butyl methacrylate (BMA), into PIPAAm sequences induce increasing the overall hydrophobicity of PIPAAm-grafted surfaces. ATRP technique can also provide the polymer brushes with changing the polymer chain length and the graft density. Therefore, thermoresponsive surface properties of grafted PIPAAm with various polymer densities, layer thicknesses,

and compositions with BMA were investigated by measuring contact angle and the elution profile of steroid molecules using PIPAAm-grafted capillaries. Thermoresponsive polymer brushes in capillaries were used to control hydrophobic interaction with steroids in laminar flow [17].

2. Experimental methods

2.1. Materials

N-Isopropylacrylamide (IPAAm) was kindly provided by Kohjin (Tokyo, Japan), and purified by recrystallization from *n*-hexane, followed by drying *in vacuo* at 25 °C. *n*-Butyl methacrylate (BMA) and *N,N*-dimethylformamide (DMF) were purchased from Wako Pure Chemical (Osaka, Japan) and purified by distillation. CuCl, CuCl₂ and 2,2'-bipyridyl (bpy) were also obtained from Wako Pure Chemicals. Tris(aminoethyl)amine (TREN) was purchased from Acros Organics (Pittsburgh, PA, USA), and tris(deimethylaminoethyl)amine (Me₆TREN) was synthesized from TREN referred to previous reports [17, 25]. 2-(*m/p*-Chloromethylphenyl)ethyltrichlorosilane was obtained from ShinEtsu Chemical Industry (Tokyo). Phenethyltrichlorosilane was purchased from Fluorochem (Glossop, UK). Cortisone and testosterone were obtained from Sigma Chemicals (St. Louis, MO). Glass coverslips (24 mm x 50 mm, average thickness 0.21 mm) were obtained from Matsunami Glass Industry (Tokyo), and fused silica capillaries (inner diameter: 50 μm) were purchased from GL Science (Tokyo). Water used in this study was purified by an ultrapure water purification system (Synthesis A10) (Millipore, Billerica, MA) unless otherwise mentioned.

2.2. Surface modification of PIPAAm by ATRP

Surface-initiated ATRP of IPAAm in water was performed according to the previously reported protocol (Fig. 1A) [17]. Briefly, the silanization of clean glass coverslips (24 mm x 50 mm, average thickness: 0.21 mm) with 2-(*m/p*-chloromethylphenyl)ethyltrichlorosilane, an ATRP initiator, was proceeded under reflux in a separable flask for 2 h at 90 °C, and the glass coverslips were baked at 110 °C for 1 h. Formations of 25, 50, and 100% initiator-immobilized surfaces were performed through the silanization of a mixed silane reagent of ATRP-initiator and phenethyltrichlorosilane with molar ratio: 25/75, 50/50, and 100/0 in feed, respectively.

Degassed solution of IPAAm in purified water (0.3 mol/L) and the silanized coverslips in separable flask were then placed into a glove box filled with N₂ gas. CuCl (1 mmol), CuCl₂ (0.1 mmol), and Me₆TREN (1.5 mmol) were completely dissolved in the degassed solution. The solution immediately added into the silanized coverslips in the separable flask, and ATRP reaction proceeded at 25 °C inside glove box for a specific time. PIPAAm-grafted glass coverslips were rinsed repeatedly with distilled water and dried for 12 h at 25 °C *in vacuo*.

PBMA-*b*-PIPAAm-grafted surfaces were prepared by ATRP of IPAAm via surfaces pre-grafted with PBMA (Fig. 1B). The preparation procedure was as follows: *n*-butyl methacrylate (BMA) monomer in *N,N*-dimethylformamide (DMF) (0.3 mol/L) was dissolved in DMF, and the solution was degassed by triplicate freeze-thaw cycles. The monomer solution in a flask and the silanized glass coverslips in a separable flask were placed into a glove box, and the inside space of the box was filled with N₂ gas by repeated vacuum and nitrogen flush. CuCl (1 mmol), CuCl₂ (0.1 mmol), and 2,2'-bipyridyl (1.2 mmol) were completely dissolved in the degassed monomer solution, and the reaction solution was then poured into the separable flask, and ATRP proceeded at 25 °C inside the glove box for 3 h. To quench ATRP, the

PBMA-grafted surfaces were immersed in DMF containing 50 mmol/L CuCl₂/bpy for 30 min under N₂ atmosphere and were then repeatedly washed with DMF to remove Cu complex [22]. Then, ATRP of IPAAm was initiated on the quenched PBMA-grafted surfaces. IPAAm (0.3 mmol) in DMF was degassed by triplicate freeze-thaw cycles, and ATRP reaction of IPAAm was proceeded at 25 °C inside the glove box for 12 h. After the reaction, PBMA-*b*-PIPAAm-grafted glass coverslips were rinsed repeatedly with DMF, and dried for 12 h at 25 °C in a vacuum oven.

Fused silica capillaries (the inner diameter: 50 µm, length: 1 m) were used as a holder for investigating the surface properties of thermoresponsive surfaces in terms of hydrophobic interactions with solute molecules. Fused silica capillaries were cleaned with 1 mol/L sodium hydroxide solution at 25 °C for 1 h, followed by extensive washing with water, and finally dried at 25 °C in a vacuum oven. Immediately after the cleaning, the reaction solution was injected into the capillary tubing by a micro-syringe pump (IC3100) (KD Scientific, Boston, MA) at a flow rate of 1 mL/h, and the luminal surfaces were reacted with ATRP-initiator silane in toluene (1, v/v%) at 90 °C for 1 day. These capillaries were repeatedly washed with toluene and finally dried at 110 °C for 20 h. For preparing PBMA-*b*-PIPAAm-grafted capillary lumen, the sequential ATRP of IPAAm and BMA was proceeded at 25 °C in N₂ atmosphere by injecting the monomer solution through the initiator-immobilized capillaries continuously using an HPLC pump (PU-980) (JASCO, Tokyo), according to the method in the previous section. PBMA-*b*-PIPAAm-grafted capillaries were then repeatedly washed with water for removing unreacted monomers and dried at 25 °C for 12 h *in vacuo*.

2.3. Surface characterization

Temperature-dependent aqueous wettabilities of the polymer-grafted surfaces were analyzed by static contact angle measurement using a contact angle meter (Type CA-X) (Kyowa Interface Science, Saitama). Sample temperature was regulated with circulating water bath (Thermo Controller 4VF) (Kyowa Interface Science) within a deviation of ± 0.1 °C. Data are expressed as the mean of three measurements with standard deviation (SD).

Morphology of PIPAAm-grafted surfaces with various densities of ATRP initiator were analyzed by an atomic force microscopy (AFM) (SPM-9500J3) (Shimadzu, Kyoto) with non-contact mode in air.

For estimating grafted polymer thickness, the surfaces were ablated by an ArF excimer laser (the wavelength: 193 nm) using a pulse width of 5 ns (L5910 IIIB) (Hamamatsu Photonics, Shizuoka) [17]. Polymer-grafted surfaces were ablated five times with 30 mJ/cm² of ArF excimer laser through an optical microscope, resulting in the ablative photodecomposition of the grafted polymer [26]. Since the ArF excimer laser-irradiated glass surface was flat and stable by AFM measurements, only polymer layers were selectively removed from the glass surface with the ablation. Partially ablated polymer-grafted surfaces were then observed by the atomic force microscopy with non-contact mode in air. The three-dimensional profile of the ablated areas on the polymer-grafted surfaces of glass coverslip gave the thickness of grafted polymer. Data are expressed as the mean of three experiments with SD.

2.4. Temperature-dependent interactions of steroid with block copolymer-grafted capillary lumens.

The property alterations of temperature-dependent hydrophilic/hydrophobic surface in thermoresponsive capillaries were analyzed by a chromatographic method. PBMA-*b*-PIPAAm-grafted capillaries were connected to an HPLC system (PU-980 and UV-875). Hydrophobic steroids, cortisone

and testosterone in water were used as makers for obtaining their chromatographic properties. Concentrations of cortisone and testosterone were 0.5 mg/mL and 0.1 mg/mL, respectively. Milli-Q water was used as the mobile phase. Thermoresponsive elution profiles of these steroids were monitored at 254 nm at a flow rate of 5 μ L/min at various temperatures using an integrator (807-IT) (JASCO). Capillary was thermostated within a deviation of ± 0.1 $^{\circ}$ C using a thermostated water bath (RC20 CS) (Lauda, Germany).

From the resulting retention times, the retention factors (k') of the steroids at various temperatures were able to be determined by the following equation:

$$k' = (t - t_0) / t_0$$

where t and t_0 are the retention times of steroids and deuterium oxide at a specific temperature, respectively. Retention time of deuterium oxide gave an estimated void volume for the capillaries [12, 14].

3. Results and Discussion

3.1. Surface wettability changes for PIPAAm-grafted surfaces with different graft densities and chain lengths.

Our laboratory has previously reported that high-density PIPAAm brush-grafted surfaces were successfully obtained by polymerizing IPAAm onto ATRP initiator-immobilized surfaces [17, 20]. Aqueous wettability of PIPAAm-grafted surfaces below the LCST increases with increasing polymerization time because of the increased hydration and molecular mobility of grafted PIPAAm chains [17]. Temperature-dependent aqueous chromatographic analyses give longer retention times of steroids

on sparsely grafted PIPAAm surfaces, compared to dense PIPAAm brushes at temperatures below the LCST of PIPAAm [18]. These results imply that the property of thermoresponsive surfaces can be modulated by the alteration of the amount and/or density of grafted PIPAAm chains. In the current study, the morphologies and wettabilities of PIPAAm-grafted coverslips with various densities and chain lengths were investigated.

Initiator-immobilized surfaces were formed with a mixture of silane agents ranging from 25 to 100% initiator molecule, 2-(*m/p*-chloromethylphenyl)ethyltrichlorosilane, in phenethyltrichlorosilane. The density of ATRP initiator on the surfaces was speculated to be proportional to the ratio of ATRP initiator and non-initiator silane in feed. PIPAAm-grafted surfaces with different ATRP initiator are abbreviated as “PI-*X*”, where *X* indicates the molar ratio of ATRP initiator silane to the total silane agents. The compositions of initiator were summarized in Table 1.

AFM images of PIPAAm-grafted surfaces prepared by ATRP with 25 and 50% initiator densities at different polymerization times were shown in the right and left columns of Fig. 2, respectively. Both initiator-immobilized surfaces were almost the same morphologies (Figs. 2A and B). Morphology of PI-0.5 was significantly smoother because of the longer polymerization time (Figs. 2E and F). With elongating the grafted PIPAAm chain lengths, the morphologies become uniform by the coverage with PIPAAm brushes. On the contrary, PI-0.25 surfaces exhibited particle-like structures with root mean square (RMS) values of 1.23 nm (Fig. 2D) and 3.55 nm (Fig. 2F). The particle-like structures became larger and more polydisperse with the longer polymerization times. Pore structures were also found in the surface regions immobilized with non-reactive phenethyltrichlorosilane. Morphological changes of polymer brush at reduced initiator densities have been reported in the previous papers [27, 28]. Jones et

al. have reported that large (30 - 100 nm diameter) and polydisperse particles are observed by AFM after ATRP with methyl methacrylate on self-assembled monolayer composed of 10% initiator [27]. Anchored PIPAAm chains onto surfaces prepared by a conventional radical polymerization are also very sparse, resulting in the formation of polydisperse particles [28]. These evidences suggested that PIPAAm chains prepared below the initiator density of 50% were sparsely grafted on the surface.

Fig. 3 shows the time course of changes in $\cos \theta$ of the PIPAAm-grafted surfaces with various densities. Aqueous wettabilities ($\cos \theta$) at 10 and 40 °C were also summarized in Table 1. Aqueous wettabilities on both silanized surfaces (PI-0.25 and -0.5) were almost identical in spite of the different initiator ratio, because phenethyltrichlorosilane is similar to 2-(*m/p*-chloromethylphenyl)ethyltrichlorosilane in chemical structure. Hydrophilicities of PIPAAm-grafted surfaces at 10 °C increased with increasing the ratio of immobilized ATRP initiator and ATRP reaction time. It has been previously reported that the increased polymerization time induces the elongation of PIPAAm chains, resulting in the enhanced hydration and higher molecular mobility of grafted PIPAAm chains at temperatures lower than 32 °C [17]. Increasing hydration with increasing the density of PIPAAm chains also increase the surface hydrophilicity. By contrast, the static contact angle of each PIPAAm-grafted surface at 40 °C was almost constant regardless of polymerization times (Fig. 3), indicating that the freely mobile ends of anchored polymer chains become highly aggregated at high temperatures due to hydrophobic interaction between dehydrated PIPAAm chains. Hydrophobicities at 40 °C were slightly increased with decreasing the density of grafted PIPAAm, because the phenethyl groups of silanes immobilized on the surface were exposed on the sparse PIPAAm-grafted surface.

3.2. Surface wettability changes in block copolymer-grafted surfaces

Although the hydrophilic properties of PIPAAm-grafted surfaces below LCST depended on their density and chain length of grafted PIPAAm, the hydrophobic properties were almost the same as that of ATRP initiator-immobilized surfaces above LCST of PIPAAm (Fig. 3). In order to enhance the total hydrophobicity on PIPAAm-grafted surfaces without the deterioration of thermosensitivity, the introduction of hydrophobic spacer between the PIPAAm moiety and the substrate was thought to be necessary. Block copolymer-grafted surfaces containing IPAAm and hydrophobic PBMA chains were then prepared via two-step ATRP from the surfaces (Fig. 1B). For comparison, the surfaces grafted with random copolymers of IPAAm and BMA (IPAAm/BMA: 97/3 molar ratio in feed) were prepared by surface-initiated ATRP in DMF (Fig. 1C). The polymer thickness of the grafted surfaces was evaluated by non-contact mode AFM observation on the excimer laser-ablated surfaces in air. Although the PBMA layer was too thin to determine the exact thickness by AFM, around 1 nm of PBMA layers was found to be grafted on the surfaces. The thicknesses of grafted PBMA-*b*-PIPAAm and P(IPAAm-*co*-BMA) on the surfaces were 16.0 ± 0.3 nm and 16.6 ± 0.9 nm, respectively. These results showed that a few percents of PBMA chains were found in the grafted PBMA-*b*-PIPAAm chains on the surface.

Thermoresponsive aqueous wettability changes on the copolymer-grafted surfaces were then investigated by measuring static contact angle (Fig. 4). Aqueous wettabilities of the copolymer-grafted surface were summarized in Table 1. PBMA-grafted surfaces exhibited more hydrophobic properties than ATRP initiator-immobilized surfaces. P(IPAAm-*co*-BMA)-grafted surfaces also showed more hydrophobic than ATRP initiator surfaces above the transition temperature. However, the wettability transition temperature of P(IPAAm-*co*-BMA)-grafted surfaces moved from that of PIPAAm-grafted

surfaces (ca. 30 °C) to 16 °C, and the wettability of the former grafted surface decreased compared with that of PIPAAm-grafted surfaces [17]. Incorporation of a small amount of hydrophobic comonomer, BMA, into PIPAAm produces a large decrease in LCST due to the decrease in overall the hydrophilicity of random copolymer [29, 30]. Moreover, BMA moieties in the grafted P(IPAAm-*co*-BMA)s enhance the aggregation of the polymer chains in water through hydrophobic interaction. The similar results have been reported in a previous paper [9]. On the other hand, the aqueous wettability of PBMA-*b*-PIPAAm-grafted surface exhibited a different character: the similar wettability transition temperature, but increased hydrophobicity above the transition temperature compared with PIPAAm-grafted surfaces. PBMA-*b*-PIPAAm-grafted surfaces had a similar transition temperature to PIPAAm-grafted surfaces regardless of the hydrophobic BMA chains at the bottom, because of the homogeneity of IPAAm units. Furthermore, the water contact angles of PBMA-*b*-PIPAAm-grafted surface above the transition temperature was found to be slightly hydrophobic ($\cos\theta = 0.3$) compared to that of IPAAm homopolymer-grafted surfaces ($\cos\theta = 0.4$) [17]. This result was due to a strong hydrophobic aggregation of PIPAAm chains induced by hydrophobic PBMA segments in the grafted chains through the strong cohesion of PIPAAm chains.

The effects of graft architectures of surface-grafted PIPAAm on aqueous wettability were summarized in Table 1. The hydrophilicities of PIPAAm-grafted surfaces at 10 °C were significantly increased with increasing their graft density and chain length. On the other hand, the decrease of graft density and incorporation of PBMA between the PIPAAm moiety and the substrate produced an increase in the hydrophobicities of the grafted surfaces at 40 °C. Therefore, the mutual controls of their graft density, chain length, and block copolymerization on PIPAAm-grafted surface were efficient for modulating the

thermoreponsive wettabilities with maintaining the phase transition temperature.

3.4. Temperature-dependent hydrophobic interaction of steroids with block copolymer-grafted capillary lumens.

Our laboratory previously demonstrated the temperature-dependent hydrophobic interactions of steroids with PIPAAm-grafted capillary luminal wall surface prepared by surface-initiated ATRP [17]. In this method, the surface properties of dynamic interaction with biomolecules can be modulated without the effects of surface geometries such as chromatographic packed column. In the current study, hydrophobicity-enhanced PBMA-*b*-PIPAAm-grafted surface by ATRP was applied to capillary lumen, and these capillaries were used as a stationary phase in chromatographic systems for regulating temperature-dependent hydrophobic interactions with steroids. Steroid hydrophobicity is estimated from conventional partition coefficients (P values) in a 1-octanol/water. The log P values of cortisone and testosterone are 1.47 and 3.32, respectively [31]. Temperature-responsive elution profiles of steroids were monitored at 254 nm with pure aqueous mobile phases.

Fig. 5 shows the capillary chromatograms of steroids at 10, 30, and 50 °C. At 10 °C, overlapped peaks were observed in PBMA-*b*-PIPAAm-grafted capillaries. As the capillary temperature increased, the retention times of both steroids increased, and the peak resolution of the steroid separation was achieved at 50 °C. The observed peaks of steroids were consistent with their log P values, indicating that the main partitioning forces of steroids were a hydrophobic interaction between steroid molecules and thermoresponsive surfaces. Fig. 6 summarizes temperature-dependent changes in the retention factors of cortisone and testosterone at various temperatures. For comparison, our previous data [17] of the

retention factors of steroids in IPAAm homopolymer-grafted capillaries prepared by ATRP is also shown in Fig. 6. The retention factors of steroids increased with increasing temperature, and the magnitude of increase in the retention factor of testosterone was clearly larger than that of cortisone in both capillaries. This result indicated that hydrophobic testosterone molecules were effectively retained on the hydrophobized thermoresponsive surfaces of capillary lumen. By comparing the characteristics of capillary surfaces, slightly increased retention factors of hydrophobic testosterone were observed on PBAM-*b*-PIPAAm-grafted surfaces than PIPAAm-grafted surfaces above the transition temperature, while those for cortisone were almost the same at all temperature ranges. From the results of aqueous contact angles in Fig. 4, the increased hydrophobicity of PBMA-*b*-PIPAAm-grafted surfaces above the transition temperature enhanced hydrophobic interaction with testosterone. Formation of strongly hydrophobic aggregation between PBMA and PIPAAm chains also gave a strong retention of testosterone on the aggregated stationary phase.

In a previous papers [12, 32], silica beads modified with random copolymers of IPAAm and BMA are prepared as a column packing material for HPLC. Enhanced hydrophobic interaction between solutes and P(IPAAm-*co*-BMA)-grafted surfaces of the stationary phases is observed even in wide range of temperatures below and above the LCST. As shown in Fig. 4, the wettability changes from hydrophilicity to hydrophobicity on P(IPAAm-*co*-BMA)-grafted surface was smaller than that on PIPAAm-grafted surfaces, because the dehydration of P(IPAAm-*co*-BMA) chains was enhanced by the introduction of BMA moieties at the vicinity of IPAAm sequences. For the useful separation of various substances on thermoresponsive stationary phase, it is desirable that thermoresponsive surface exhibits the wide dynamic range of hydrophilic/hydrophobic property change across the transition temperature such as

aqueous wettability and hydrophobic interaction with substances. From this viewpoint, PIPAAm-*b*-PBMA-grafted surfaces prepared by ATRP have a potential to give an effective separation by temperature changes through the optimization of polymer density, chain length, and hydrophobic component of block copolymer.

4. Conclusions

Graft architectures of thermoresponsive polymer chains on solid material were regulated through surface-initiated ATRP techniques, and their thermoresponsive surface properties were investigated. Density of grafted PIPAAm chains onto the surfaces was revealed to change their morphology and hydrophilic properties below the transition temperature. Surfaces grafted with PBMA-*b*-PIPAAm chains exhibited a large aqueous wettability change in response to temperature alteration and a strong hydrophobic property above its transition temperature. PBMA-*b*-PIPAAm-grafted surfaces were applied to microchannel inner lumen of silica capillaries, which was used for measuring the temperature-dependent hydrophobic interactions of steroids through chromatographic analyses. Retention times of steroids were increased with increasing temperature, and the longer retention times of testosterone were also observed compared to that on IPAAm homopolymr-grafted surfaces above LCST. This surface modification method using ATRP is simple and applicable to various geometric materials such as silica capillaries and useful for the separations of biomolecules by temperature-regulated interactions with microfluidic channels. Moreover, the surface modification of hydrophobicity-enhanced thermoresponsive polymers is a promising method for the preparation of thermoresponsive biointerfaces that can be effectively modulated their biomolecules and cell adsorption with the wide dynamic range of

hydrophilic/hydrophobic property change across the transition temperature.

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References

1. Y. Qui and K. Park, *Adv. Drug. Deliv. Rev.*, 53 (2001) 321.
2. B.A. Buchholz, W. Shi and A.E. Barron, *Electrophoresis*, 23 (2002) 1398.
3. C.D.H. Alarcón, S. Pennadam and C. Alexander, *Chem. Soc. Rev.*, 34 (2005) 276.
4. M. Heskins, J.E. Guillet and E. James, *J. Macromol. Sci. Chem.*, A2 (1968) 1441.
5. M. Ebara, J.M. Hoffman, A.S. Hoffman and P.S. Stayton, *Lab. Chip*, 6 (2006) 843.
6. D.L. Huber, R.P. Manginell, M.A. Samara, B.-I. Kim and B.C. Bunker, *Science*, 301 (2003) 352.
7. N. Idota, A. Kikuchi, J. Kobayashi, K. Sakai and T. Okano, *Adv. Mater.*, 17 (2005) 2723.
8. M. Ebara, M. Yamato, T. Aoyagi, A. Kikuchi, K. Sakai and T. Okano, *Biomacromolecules*, 5 (2004)

9. Y. Tsuda, A. Kikuchi, M. Yamato, Y. Sakurai, M. Umezu and T. Okano, *J. Biomed. Mater. Res.*, 69A (2004) 70.
10. T. Shimizu, M. Yamato, Y. Isoi, T. Akutsu, T. Setomaru, K. Abe, A. Kikuchi, M. Umezu and T. Okano, *Circ. Res.*, 90 (2004) e40.
11. K. Ohashi, T. Yokoyama, M. Yamato, H. Kuge, H. Kanehiro, M. Tsutsumi, T. Amanuma, H. Iwata, J. Yang, T. Okano and Y. Nakajima, *Nat. Med.*, 13 (2007) 880.
12. H. Kanazawa, Y. Kashiwase, K. Yamamoto, Y. Matsushima, A. Kikuchi, Y. Sakurai Y and T. Okano, *Anal. Chem.*, 69 (1997) 823.
13. A. Kikuchi and T. Okano, *Prog. Polym. Sci.*, 27 (2002) 1165.
14. J. Kobayashi, A. Kikuchi, K. Sakai and T. Okano, *Anal. Chem.*, 73 (2001) 2027.
15. K. Yoshizako, Y. Akiyama, H. Yamanaka, Y. Shinohara, Y. Hasegawa, E. Carredano, A. Kikuchi and T. Okano, *Anal. Chem.*, 74 (2002) 4160.
16. Y.G. Takei, T. Aoki, K. Sanui, N. Ogata, Y. Sakurai and T. Okano, *Macromolecules*, 27 (1995) 6163.
17. N. Idota, A. Kikuchi, J. Kobayashi, Y. Akiyama, K. Sakai and T. Okano, *Langmuir*, 22 (2006) 425.
18. K. Nagase, J. Kobayashi, A. Kikuchi, Y. Akiyama, H. Kanazawa and T. Okano, *Langmuir*, 24 (2008) 511.
19. K. Nagase, J. Kobayashi, A. Kikuchi, Y. Akiyama, M. Annaka, H. Kanazawa and T. Okano, *Langmuir*, 24 (2008) 10981.
20. A. Mizutani, A. Kikuchi, M. Yamato, H. Kanazawa and T. Okano, *Biomaterials*, 29 (2008) 2073.
21. K. Nagase, M. Watanabe, A. Kikuchi, M. Yamato and T. Okano, *Macromol. Biosci.*, 11 (2011) 400.

22. J.-B. Kim, W. Huang, M.L. Bruening and G.L. Baker, *Macromolecules*, 35 (2002) 5410.
23. J. Pyun and K. Matyjaszewski, *Chem. Mater.*, 13 (2001) 3436.
24. Q. Yu, Y. Zahng, H. Chen, F. Zhou, Z. Wu, H. Huang and J.L. Brash, *Langmuir*, 26 (2010) 8582.
25. M. Ciampolini and N. Nardii, *Inorg. Chem.*, 5 (1966) 41.
26. Y. Akiyama, A. Kikuchi, M. Yamato and T. Okano, *Langmuir*, 20 (2004) 5506.
27. D.M. Jones, A.A. Brown and W.T.S. Huck, *Langmuir*, 18 (2002) 1265.
28. N. Ishida and S. Biggs, *Langmuir*, 23 (2007) 11083.
29. H. Feil, Y.H. Bae, J. Feijen and S.W. Kim, *Macromolecules*, 26 (1993) 2496.
30. Y.G. Takei, T. Aoki, K. Sanui, N. Ogata, T. Okano and Y. Sakurai, *Bioconjug. Chem.*, 4 (1993) 341.
31. C. Hansch, A. Leo and D. Hoekman D (Eds.), *Exploring QSARs Hydrophobic, Electronic, and Steric Constants*, ACS Professional Reference Book, American Chemical Society, Washington, DC., 1995.
32. K. Nagase, M. Kumazaki, H. Kanazawa, J. Kobayashi, A. Kikuchi, Y. Akiyama, M. Annaka and T. Okano, *ACS Appl. Mater. Interfaces*, 2 (2010) 1247.

Figure captions

Fig. 1 Scheme for the synthesis of (A) PIPAAm, (B) PBMA-*b*-PIPAAm, and (C) P(IPAAm-*co*-BMA)-grafted surfaces using the surface-initiated ATRP technique.

Fig. 2 Morphologies of PI-0.5 (A, C, and E in the left column) and PI-0.25 (B, D, and F in the right column) surfaces analyzed by AFM. Polymerization times are 0 h (A and B in the top row), 1 h (C and D in the middle row), and 6 h (E and F in the bottom row). The numbers at the bottom right of each picture indicate RMS values.

Fig. 3 Effect of polymer densities and polymerization times on the surface wettability of PIPAAm-grafted surfaces. The closed and open marks show that the experiments were done at 10 and 40 °C, respectively. The diamonds, squares, and triangles represent the surfaces modified with PIPAAm having the densities of 100, 50, and 25%, respectively.

Fig. 4 Temperature-dependent wettabilities ($\cos \theta$) values of IPAAm copolymer-grafted surfaces prepared by ATRP. The open circle represents the PBMA-grafted surfaces; the open triangle, ATRP initiator-immobilized surfaces; the open diamond, PIPAAm-grafted surfaces; the closed diamond, P(BMA-*co*-IPAAm)-grafted surfaces; the closed square, PBMA-*b*-PIPAAm-grafted surfaces.

Fig. 5 Chromatograms of steroids through PBMA-*b*-PIPAAm-grafted capillaries (the inner diameter: 50 μm , the length: 1 m) at various temperatures. The peaks 1 and 2 show cortisone and testosterone,

respectively. The mobile phase is Milli-Q water. The sample volume: 80 nL. Flow rate: 5 μ L/min.

Detector: 254 nm.

Fig. 6 Temperature-dependent retention factors of steroids on PIPAAm-grafted surfaces and PBMA-*b*-PIPAAm-grafted surfaces. The data of PIPAAm-grafted surface (the close circles and squares) were taken from Ref. [17]. The data of the open marks are obtained in this study. The circles and squares represent cortisone and testosterone, respectively.

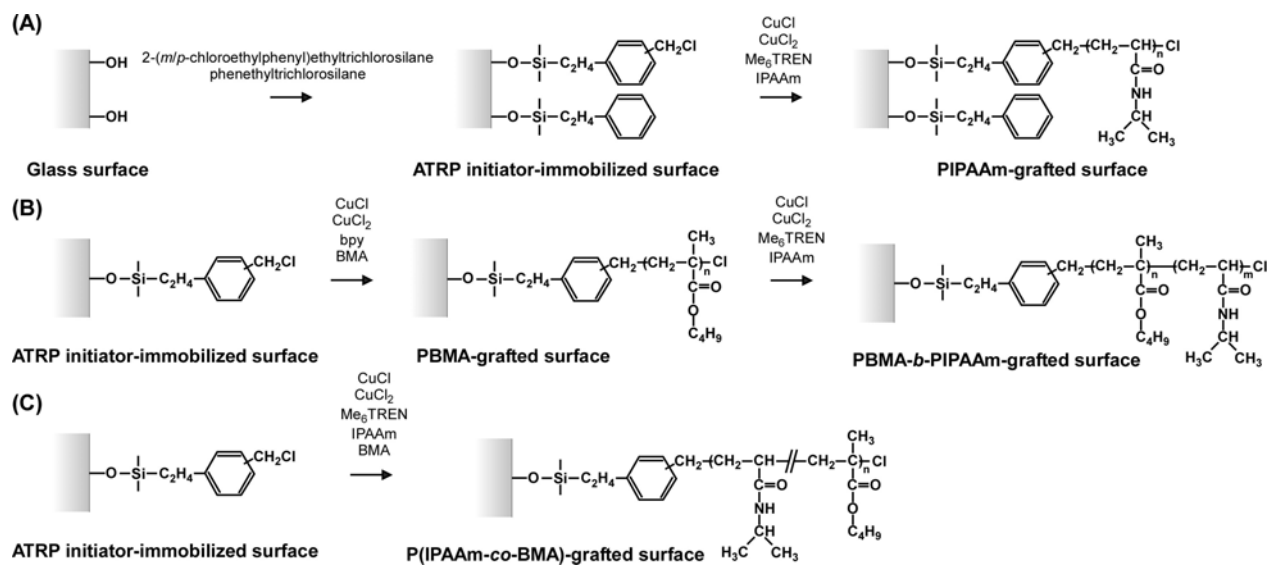


Fig. 1, Idota et al.

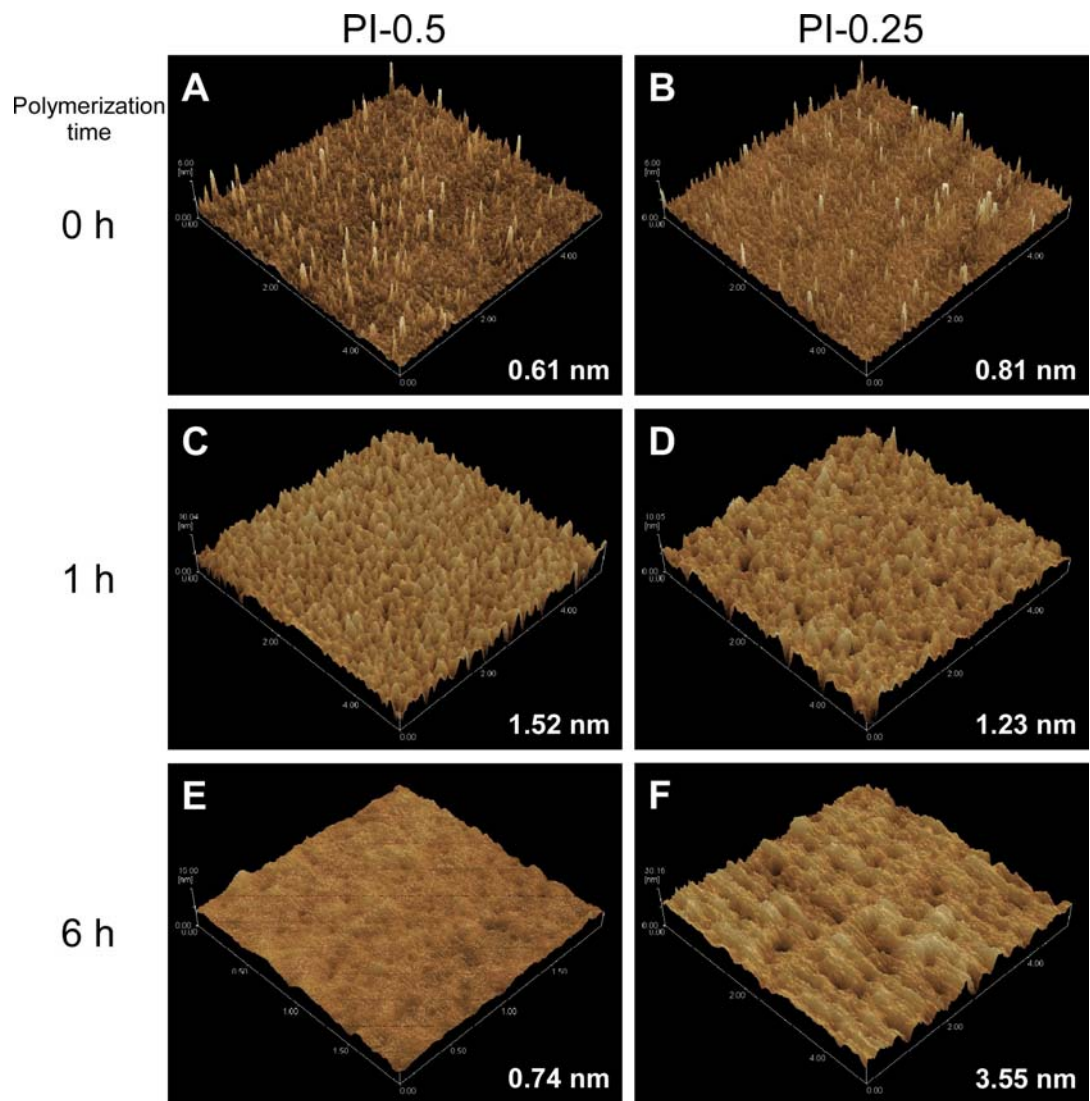


Fig. 2, Idota et al.

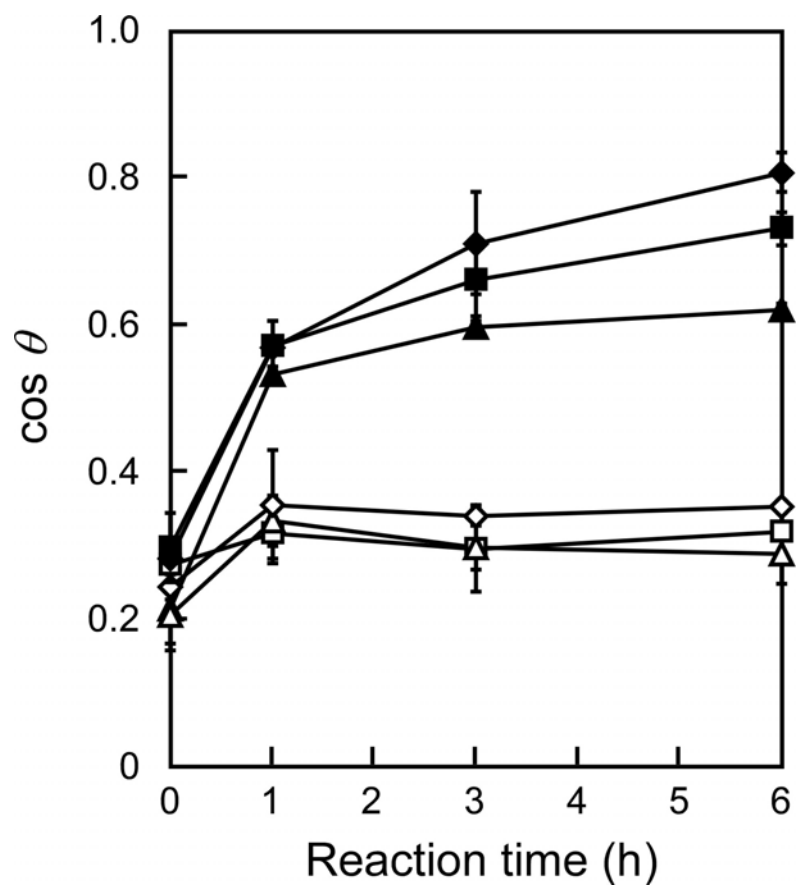


Fig. 3, Idota et al.

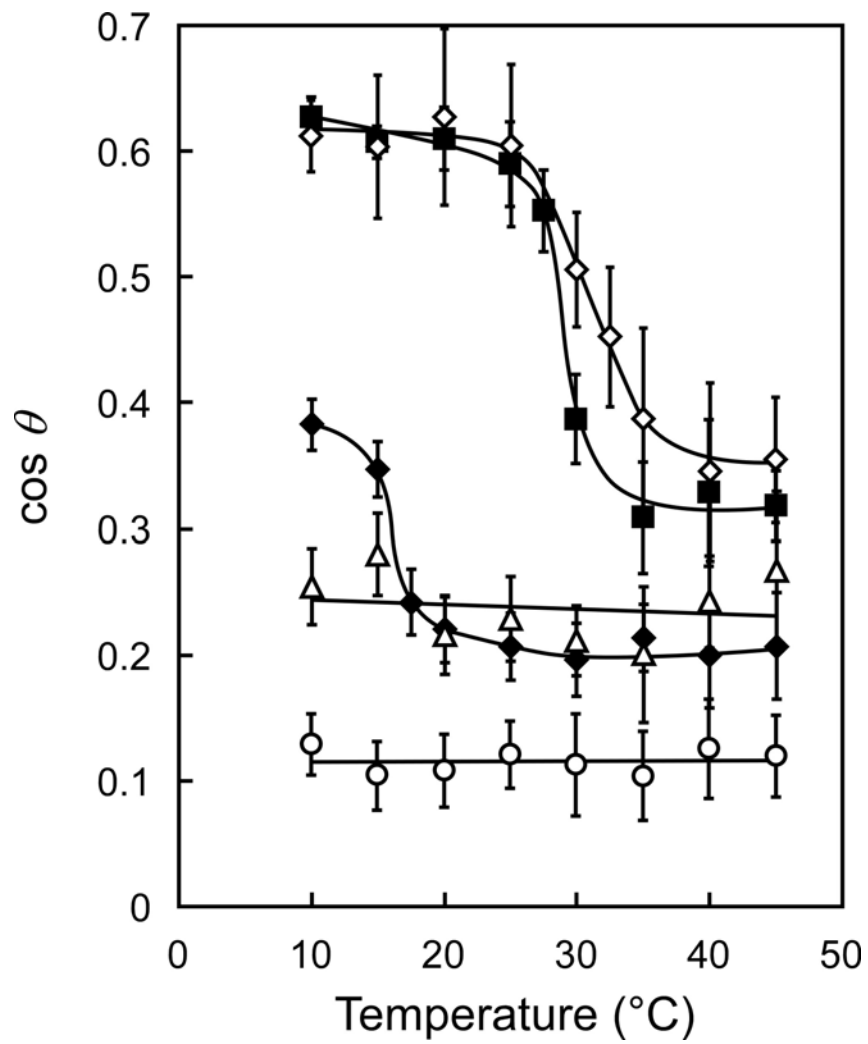


Fig. 4, Idota et al.

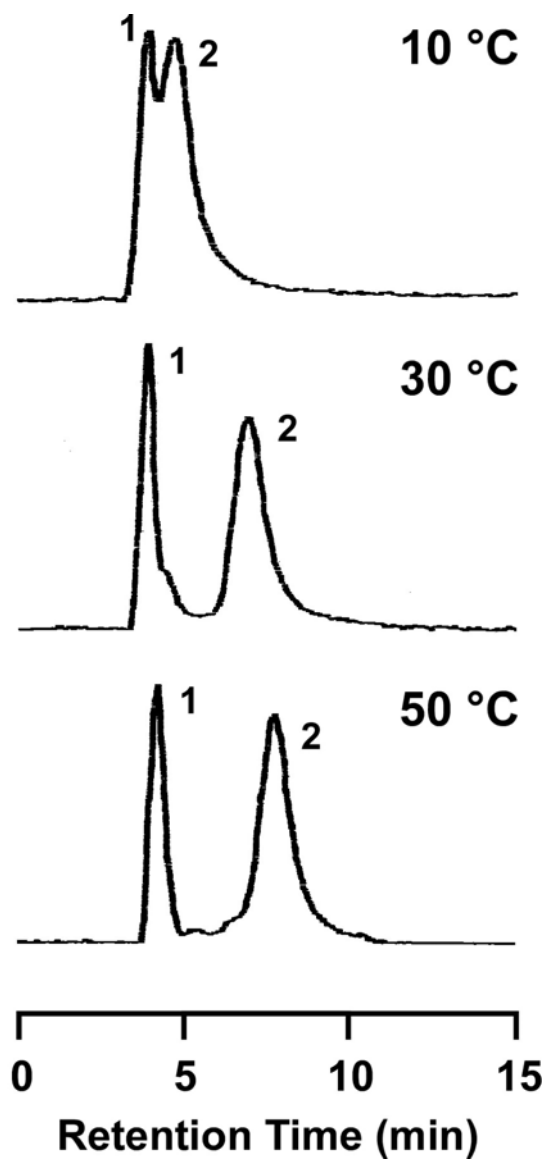


Fig. 5, Idota et al.

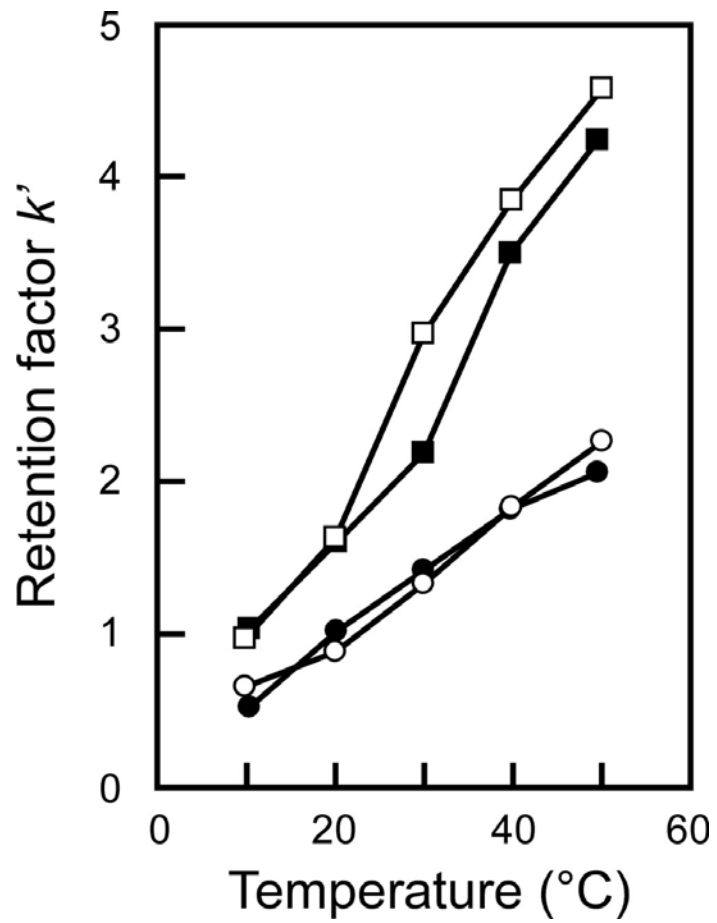


Fig. 6, Idota et al.

Table 1. Reaction conditions and aqueous wettabilities of IPAAm homopolymer and copolymer-grafted surface with different graft densities, chain length, and sequence with BMA.

code	ATRP initiator / phenethyltrichlorosilane ^a	IPAAm / BMA ^b	time (h)	solvent	cos θ^c	
					10 °C	40 °C
PI-0.25	25 / 75	100 / 0	0	water	0.213 ± 0.047	0.204 ± 0.048
			1		0.530 ± 0.012	0.332 ± 0.034
			6		0.618 ± 0.009	0.287 ± 0.041
PI-0.5	50 / 50	100 / 0	0	water	0.295 ± 0.049	0.272 ± 0.023
			1		0.569 ± 0.035	0.314 ± 0.041
			6		0.730 ± 0.022	0.316 ± 0.042
PI-1.0	100 / 0	100 / 0	0	water	0.280 ± 0.019	0.242 ± 0.017
			1		0.568 ± 0.037	0.354 ± 0.073
			6		0.806 ± 0.026	0.351 ± 0.008
P(IPAAm-co-BMA)	100 / 0	97 / 3	15	DMF	0.383 ± 0.024	0.200 ± 0.039
PBMA	100 / 0	0 / 100	3	DMF	0.129 ± 0.021	0.125 ± 0.041
PBMA- <i>b</i> -PIPAAm	100 / 0	100 / 0	12	DMF	0.627 ± 0.013	0.318 ± 0.028

^a The value represents the molar ratio of a mixed silane reagent of ATRP-initiator and phenethyltrichlorosilane in feed. ^b the value represents the molar ratio of a mixed monomer solution of IPAAm and BMA in feed. ^c the data was determined by contact angle measurement