Thermoresponsive Surface Prepared by Atom Transfer Radical Polymerization Directly from Poly(vinylidene fluoride) for Control of Cell Adhesion and Detachment

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ABSTRACT: Thermoresponsive surface was prepared from commercial poly(vinylidene fluoride) (PVDF) films via surface-initiated atom transfer radical polymerization. The direct initiation of the secondary fluorinated site of PVDF facilitated grafting of the *N*-isopropylacrylamide (NIPAAm) monomer. The PVDF surfaces grafted with poly(*N*-isopropylacrylamide) [P(NIPAAm)] were characterized by X-ray photoelectron spectroscopy. Kinetics study revealed that the P(NIPAAm) chain growth from the PVDF surface was consistent with a "controlled" process. The temperature-dependent swelling behavior of the surfaces in aqueous solution was studied by atomic force microscope. At 37°C [above the lower critical solution temperature (LCST, about 32°C) of NIPAAm], the seeded cells adhered and spread on the NIPAAm grafted PVDF surface. Below the LCST, the cells detached from the P(NIPAAm)-grafted PVDF surface spontaneously. The thermoresponsive surfaces are potentially useful as stimuli-responsive adhesion modifiers in the biomedical fields.
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Key words: surface-initiated ATRP; poly(*N*-isopropylacrylamide); poly(vinylidene fluoride); cell adhesion; thermoresponsive

INTRODUCTION

Smart polymers are materials that possess inherent sensing, processing, and actuating functions. These polymers can respond to external chemical or physical stimuli, such as changes in pH, ionic strength, temperature, and light or electrical potential.[1–6] Poly(*N*-isopropylacrylamide) [P(NIPAAm)] is one of the most widely studied thermosensitive polymer.[7–10] It exhibits a lower critical solution temperature (LCST) at around 32°C in aqueous solution. P(NIPAAm) chains hydrate to form an expanded structure in water when the solution temperature is below its LCST and dehydrate to form a compact structure when heated above the LCST.[11,12]

Surfaces with grafted smart polymer brushes are of particular interest because of their excellent mechanical strength and quick response to external stimulus.[13,14] Poly(vinylidene fluoride) (PVDF) has been widely studied as a membrane material because of its excellent chemical resistance, outstanding mechanical properties, and good thermal stability.[15,16] Therefore, PVDF surfaces grafted with P(NIPAAm) have become one of the most attractive research targets as smart surface.[17,18] The surface modification of polymers via molecular design is one of the most versatile approaches. Recent advances in atom transfer radical polymerization (ATRP) have provided opportunities for synthesizing polymers with well-defined macromolecular architectures on various substrates.[19,20] Thermoresponsive P(NIPAAm)-silicon hybrids have been prepared via surface-initiated ATRP for the control of cell adhesion.[21]

In this work, surface-initiated ATRP of *N*-isopropylacrylamide (NIPAAm) directly from the PVDF surfaces is reported. The direct initiation of the secondary fluorinated site of PVDF facilitated grafting of the NIPAAm monomer. The PVDF surfaces grafted with P(NIPAAm) [PVDF-*g*-P(NIPAAm)] were characterized by X-ray photoelectron spectroscopy (XPS), contact angle measurements, and atomic force microscope (AFM), respectively. The simultaneous dependence of the cell adhesion and detachment for the P(NIPAAm)-grafted PVDF surfaces on the temperature was investigated.

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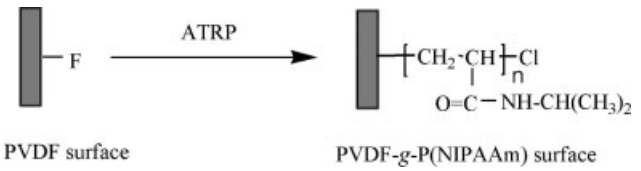

Figure 1 Schematic diagram illustrating the process of directly surface-initiated ATRP from PVDF film.

EXPERIMENTAL

Materials

PVDF films with a thickness of 0.5 mm were purchased from Goodfellow (Huntingon, England). The PVDF films were cut into rectangular strips (1 cm × 2 cm) and then washed with acetone, methanol, and double distilled water to remove the organic residues on the surface. The films were dried under reduced pressure for about 24 h and then stored in vacuum. NIPAAm, copper(I) chloride (CuCl), and copper(II) chloride ($CuCl_2$) were purchased from Aldrich Chemical. Hexamethyl tris(2-aminoethyl)-amine (Me_6Tren) was synthesized from tris(2-aminoethyl)amine according to the literature.[22] The solvents were of analytical grade and were used without further purification unless otherwise mentioned.

Surface-initiated ATRP

The reactions involved were illustrated schematically in Figure 1. PVDF comprises repeat units with secondary halogen atoms pendant that can be used as ATRP macroinitiators for the preparation of functionalized derivatives.[23] Typically, the aforementioned reactions were carried out using a [NIPAAm] : [CuCl] : [$CuCl_2$] : [Me_6Tren] molar feed ratio of 150 : 1 : 0.2 : 1.2 in 10 mL dioxane at 60°C in a flask containing the PVDF film for predetermined time, as shown in Table I. After the reaction, the P(NIPAAm)-grafted PVDF films were removed from the reaction mixture and extracted thoroughly with excess dioxane and double distilled water over 10 h to ensure the complete removal of the physically adsorbed reactants.

Cell culture on the PVDF-*g*-P(NIPAAm) surfaces

For the cell culture on the P(NIPAAm)-grafted PVDF surfaces, the samples were washed twice with phosphate-buffered saline (PBS) solution and then sterilized for about 1 h by UV irradiation, before being placed into the wells of a 12-well culture plate. 3T3 fibroblasts (ATCC, Passage 27) were seeded into the wells at a density of 1×10^4 cells/well and incubated (in 1 mL Dulbecco's modified eagle medium supplemented with 10% fetal bovine serum, 1 m*M* L-glutamine, and 100 units/mL penicillin at 37°C) for 2 days under a humidified 5% CO_2 atmosphere. After 2 days of incubation, the surfaces were washed twice in 37°C PBS solution to remove the loosely attached cells in order to study the cell adhesion on the surfaces. Fixation by 4% glutaraldehyde for 2 h and dehydration in a series of ethanol aqueous solutions (50–100%) were carried out. All the operations were carried out in 37°C incubator. After 2 days of incubation at 37°C, the samples were subjected to low-temperature treatments so as to study cell detachment from the functionalized surfaces. The samples were first transferred to an incubator equipped with a cooling unit fixed at 20°C. After a predetermined incubation time at 20°C, the samples were then transferred to 37°C incubator again, and the medium was replaced by 37°C medium, simultaneously. The same washing and fixation procedures were then carried out in 37°C incubator as described earlier for the cell adhesion studies. The surfaces with immobilized cells were imaged by an Olympus BX51M optical microscope (Olympus America). The cell number on each sample was counted on printed photographs from three or more samples and averaged.[24]

Characterization

XPS analysis was performed on a Kratos AXIS HSi spectrometer with a monochromotized Al Kα X-ray source (1486.6 eV photons). All binding energies(BEs) were referenced to the C 1s hydrocarbon peak at 284.6 eV. Surface elemental stoichiometries were determined from the sensitivity factors-corrected spectral area ratios, and were reliable within ±5%. The surface morphologies of the PVDF-*g*-P(NIPAAm) films in aqueous solutions [1 × PBS (pH 7.4)] of different temperatures were characterized on a Nanoscope AFM (Digital Instrument of Santa Barbara, CA) containing a specially designed liquid cell with temperature control. The applied voltage was between 3.0 and 4.0 V, and the drive amplitude was about 300 mV. The scan rate was 1.0 Hz. An arithmetic mean of the surface roughness (R_a) was calculated from the roughness profile determined by AFM. The thickness of P(NIPAAm) layer

TABLE I
Water Contact Angle of the PVDF-*g*-P(NIPAAm) Surfaces

Sample	ATRP time (h)	Water contact angle (±3°)
Pristine PVDF	0	93
PVDF-*g*-P(NIPAAm)1	2	85
PVDF-*g*-P(NIPAAm)2	4	78
PVDF-*g*-P(NIPAAm)3	8	70
PVDF-*g*-P(NIPAAm)4	12	62

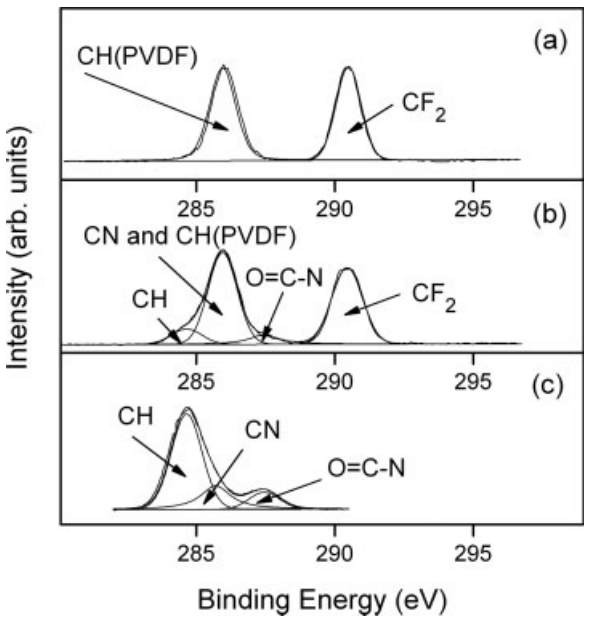

Figure 2 XPS C 1s core-level spectra of (a) the pristine PVDF surface, (b) the PVDF-*g*-P(NIPAAm)2 surface, and (c) the PVDF-*g*-P(NIPAAm)4 surface.

grafted on the PVDF substrates was determined by ellipsometry. The measurements were carried out on a variable-angle spectroscopic ellipsometer (Model 2000, J. A. Woollam, Lincoln, NE) at incident angles of 60 and 65° in the wavelength range of 370–1000 nm. For each sample, the thickness measurements were made on at least four different surface locations. Each thickness value reported was accurate to ±1 nm. Data were recorded and processed by use of the WVASE32 software package. The static water contact angles of the samples were measured on a telescopic goniometer (Rame-Hart model 10000–230). The telescope, with a magnification power of 23×, was equipped with a protractor of 1° graduation. For each angle reported, at least four measurements from different surface positions were averaged. The angle reported was reliable to ±3°.

RESULTS AND DISCUSSION

Surface-initiated ATRP of NIPAAm directly from PVDF surface

The polymerization of NIPAAm on PVDF surface was carried out by ATRP initiated at the secondary halogenated sites of PVDF. The presence of the grafted polymer on PVDF surfaces was ascertained by XPS. Figure 2 shows XPS C 1s core-level spectra of (a) the pristine PVDF surface,(b) the PVDF-*g*-P(NIPAAm)2 surface, and (c) the PVDF-*g*-P(NIPAAm)4 surface. In the case of the pristine PVDF membrane, the C 1s core-level spectrum can be curve fitted with two peak components, with BEs at 285.8 eV for the CH_2 species and at 290.5 eV for the CF_2 species.[25] The ratio for the two peak components is about 1.03, in good agreement with the chemical stoichiometry of PVDF. The C 1s core-level spectra of the PVDF-*g*-P(NIPAAm)2 surface are curve fitted with the following five chemical species. The two peak components with Bes at 285.8 eV for the CH_2 species and at 290.5 eV for the CF_2 species can be assigned to the PVDF main chains. The component with BE at 287.4 eV is assigned to the N—C=O species of the grafted NIPAAm polymer chains.[26] The component with the BE at 284.6 eV is attributed to the CH_2 species of the grafted P(NIPAAm) chain. Finally, because the CN (NIPAAm) and CH_2 (PVDF) peak components have similar BEs, they are combined and shown as a single peak component at the BE at 285.8 eV.[26] It indicates that P(NIPAAm) has been grafted on the PVDF surface via direct surface-initiated ATRP. However, the C 1s core-level spectra of PVDF-*g*-P(NIPAAm)4 surface can only be curve fitted into three peak components with BEs at about 284.6, 285.8, and 287.4 eV, attributable to the CH, CN, and O=C—N species,[26] respectively, as shown in Figure 2(c). The peak component area ratio of 3.8 : 1.1 : 1 for the three species is comparable with the theoretical ratio of 4 : 1 : 1 for the corresponding P(NIPAAm) homopolymer. The XPS results suggests that the grafted P(NIPAAm) layers is present on the PVDF surfaces with a thickness larger than the probing depth (about 7.5 nm in an organic matrix)[27,28] of the XPS technique with the increase in the ATRP time. As shown in Table I, the water contact angle of the PVDF-*g*-P(NIPAAm) surface at room temperature (25°C) decreases to about 62° with the increase in the polymerization time, compared with that of the pristine PVDF surface. However above LCST (about 40°C), the water contact angle of the PVDF-*g*-P(NIPAAm)4 surface increased to about 78°. The substantial difference in surface water contact angles below and above the LCST confirms that the grafted P(NIPAAm) directly on the PVDF surface can functionally respond to temperature changes.

The ellipsometry measurements indicate a large increase in the film thickness after the growth of the P(NIPAAm) layer on the PVDF surface. As shown in Figure 3, an approximately linear increase in the thickness of the grafted P(NIPAAm) layer on the PVDF surface with the polymerization time can be observed. These results of kinetics study indicate that the P(NIPAAm) chain growth from the PVDF surface initiated at the secondary halogenated sites of PVDF on the surface is consistent with a controlled process.

The surface morphology of the PVDF-*g*-P(NIPAAm) surface in the PBS (pH 7.4) medium is studied by AFM. The dependence of root mean

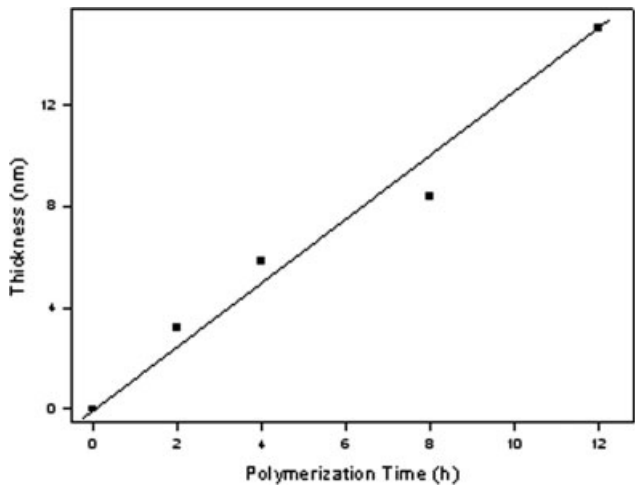

Figure 3 Dependence of the thickness of the P(NIPAAm) layer grown from the PVDF surface on the polymerization time of surface-initiated ATRP.

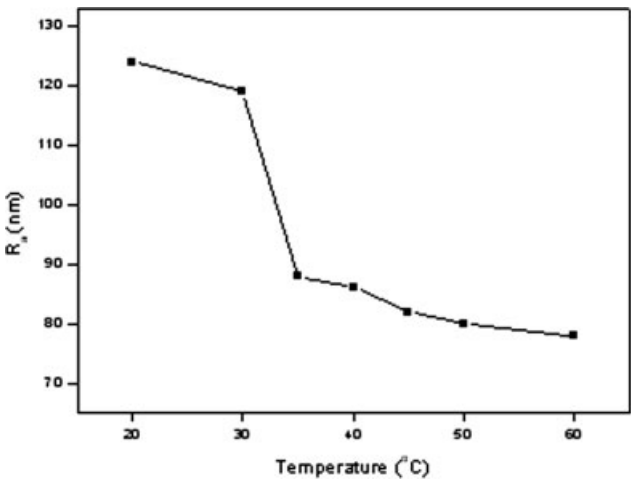

Figure 4 The dependence of R_a of the PVDF-*g*-P(NIPAAm)2 film on the temperature of the aqueous solution (in PBS, pH 7.4).

square surface roughness (R_a) (derived from the AFM images) of the PVDF-*g*-P(NIPAAm)2 film on the temperature of the aqueous solution (in PBS, pH 7.4) is shown in Figure 4. It is observed that the surface morphology of the P(NIPAAm)-grafted PVDF changes substantially with the change in solution temperature. The R_a value at 60°C is about 78 nm. However, with the decrease in temperature, the R_a value increases. At about the LCST of the NIPAAM polymer (32°C), the most drastic increase appears. When the temperature decreases to 20°C, the R_a value increases to about 124 nm. The increase in surface roughness is consistent with the change in conformation of the P(NIPAAm) chains in the aqueous solution. When the solution temperature is increased above the LCST of P(NIPAAm), P(NIPAAm) chains associate hydrophobically and form excessively compact molecular structures near the surface. With the decrease in the aqueous solution temperature, P(NIPAAm) chains become less hydrophobic and assume a more extended conformation, which leads to the observed increase in surface roughness.

Cell adhesion and detachment characteristics of the PVDF-*g*-P(NIPAAm) surfaces

P(NIPAAm) exhibits a LCST of about 32°C in an aqueous medium. On the P(NIPAAm)-grafted surfaces, cells can adhere, spread, and proliferate at 37°C. However, when the temperature decreases below the LCST of P(NIPAAm), the cultured cells detach spontaneously from the hydrophilic surfaces without enzymatic digestion.[29,30] In this work, temperature-dependent cell adhesion and detachment from the P(NIPAAm)-grafted PVDF surface is demonstrated. Cell detachment from the membrane surfaces was studied by lowering the incubation temperature. The cells can adhere and grow to some extent on the surfaces at 37°C. At 37°C, the P(NIPAAm) segments of these surfaces associate hydrophobically and collapse into globular structures, which can support cell attachment, spread, and proliferation. The aforementioned results suggest that the thermoresponsive copolymer do not restrain cell attachment at 37°C. However, significant amount of cells have detached from the P(NIPAAm)-modified silicon surfaces after the low-temperature treatment, as shown in Figure 5. When the culture temperature was lowered to 20°C, the P(NIPAAm) chains on the surface become hydrated below the LCST, producing an expanded and hydrophilic surface. This change in surface property weakens cellular adhesion and results in spontaneous cell detachment from the extended P(NIPAAm) chains. About 79, 62, and 31% of the

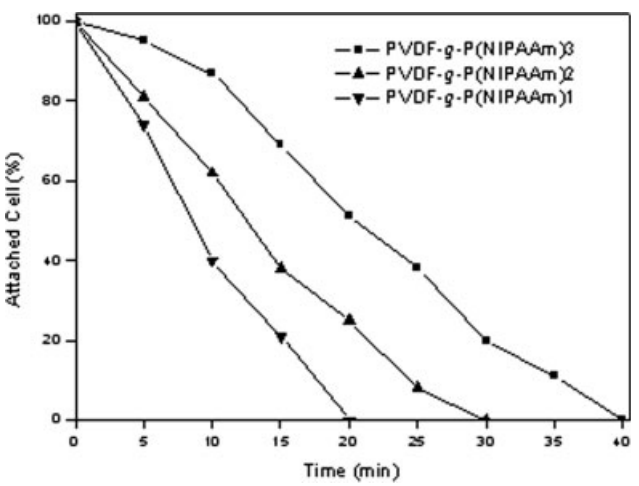

Figure 5 Time-dependent cell detachment from the surfaces of the grafted PVDF films upon reducing the culture temperature to 20°C.

adhered cells have detached from the PVDF-*g*-P(NIPAAm)1 surface, PVDF-*g*-P(NIPAAm)2 surface, and PVDF-*g*-P(NIPAAm)3 surface, respectively, within 15 min. For complete cell detachment, about 20, 30, and 40 min were required for the aforementioned surfaces. The rate of cell detachment from the PVDF-*g*-P(NIPAAm)1 surface is faster than those from the other surfaces. The phenomenon may probably be ascribed to the longer time required to hydrate the P(NIPAAm) on the surface of the PVDF film with the thicker P(NIPAAm) graft layer.

CONCLUSIONS

The approach of surface-initiated ATRP of NIPAAm directly from PVDF surface was used to prepare the PVDF-*g*-P(NIPAAm) temperaturesensitive surfaces. Kinetics study revealed an approximately linear increase in thickness of the surface graft-polymerized brushes with the polymerization time, indicating that the chain growth from the PVDF surface was consistent with a controlled process. The temperaturesensitive PVDF-*g*-P(NIPAAm) surfaces, obtained via this approach, could be applied for control of cell adhesion and detachment in biomedical microdevices.

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