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Temperature-responsive glass coverslips with an ultrathin poly(*N*-isopropylacrylamide) layer

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Abstract

A temperature-responsive cross-linked polymer gel was covalently grafted onto glass coverslips by electron beam irradiation. The grafted thickness and amount of polymer as well as the surface wettability increased with the initial monomer concentration. When the monomer concentration was 5 wt.%, the grafted polymer density was 0.84 μg cm^{-2}, and cells adhered and spread on the surface at 37 °C, but detached at 20 °C. In contrast, when the monomer concentration was 35 wt.%, the polymer density was 1.28 μg cm^{-2}, and the surfaces were cell repellent even at 37 °C. These results show a remarkable contrast to those obtained from temperature-responsive polymer-grafted tissue culture polystyrene dishes, since various types of cells showed temperature-dependent cell adhesion/detachment when the grafted density was around 2 μg cm^{-2} on these surfaces. We discuss the possible molecular mechanisms underlying this discrepancy.

Keywords: Poly(*N*-isopropylacrylamide) (PIPAAm); Electron beam; Cell adhesion; Cell detachment

1. Introduction

The temperature-responsive polymer, poly(*N*-isopropylacrylamide) (PIPAAm), possesses a lower critical solution temperature (LCST) of 32 °C in water [1]. Below the LCST, PIPAAm is fully hydrated and soluble in aqueous solution, but collapses extensively and becomes insoluble in the solution above the LCST. This unique feature of PIPAAm has been exploited for various biomedical applications [2–14], as has the use of PIPAAm's hydrophobic interactions with amino acids and proteins [15,16]. In our laboratory, chromatographic separation of a variety of bioactive molecules using the aqueous mobile phase could be achieved with temperature changes [5,6]. We have also utilized PIPAAm grafting into glass capillaries to make microfluidic valves [8]. In addition, by grafting polymer gel onto cell culture dishes using electron beam (EB) irradiation, temperature-dependent cell adhesion/detachment control could be achieved, with the development of temperature-responsive culture surfaces for tissue engineering and regenerative medicine applications [4,9–13]. In comparison to general polymerization methods, such as atom transfer radical polymerization [17] and chemical coupling [5,6], EB grafting techniques present several advantages. Using EB irradiation, it is relatively easy to graft polymer gel onto material surfaces, since no special initiators or catalysts are required and the polymerization reaction is carried out in an open system. Using EB irradiation, mass production of temperature-responsive culture dishes can be easily achieved. However, the thickness of the grafted polymer is a key factor in the control of cell adhesion/detachment in response to temperature. When PIPAAm is grafted onto tissue culture polystyrene (TCPS) dishes, a density of approximately 1.4 μg cm^{-2} (ca. 20 nm in thickness) is optimal for the

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temperature-responsive cell adhesion/detachment control of various types of cells [18]. The lower grafted density fails to enable cell detachment below the LCST. On the other hand, if the grafted density is higher than 1.4 g cm^{-2}, cells do not adhere on these surfaces even at 37 °C. The cell-repellent properties of PIPAAm grafted at higher densities can be explained by the interactions of the grafted polymer chains. In the PIPAAm-grafted layers, the polymer chains are dehydrated as they get progressively closer to the vicinity of hydrophobic TCPS, due to the restricted molecular motion of the grafted chains at the interfaces with the hydrophobic TCPS surfaces [18]. This hydrophobic property of the TCPS surfaces is also likely to promote the dehydration of the PIPAAm. At 37 °C, this effect promotes dehydration of the PIPAAm-grafted chains at the outermost surfaces, allowing for cell adhesion on the PIPAAm-grafted surfaces. When the graft thickness is increased, such progressive dehydration of the PIPAAm chains is weakened at the outermost portions of the PIPAAm chains and the surfaces fail to support cell adhesion [18]. In the present study, to investigate whether such phenomena as the thickness dependency of cell adhesion/detachment properties is observed for other substrate, we employed glass coverslips modified with 3-methacryloxypropyltrimethoxysilane (MPTMS) as the surfaces for PIPAAm-grafting. Temperature-dependent surface property changes of PIPAAm-grafted coverslips (PIPAAm-CSs) were examined in comparison to those of PIPAAm-grafted TCPS dishes (PIPAAm-TCPS).

2. Materials and methods

2.1. Reagents

N-Isopropylacrylamide (IPAAm) was kindly provided by Kohjin (Tokyo, Japan). The following materials were purchased from the respective companies: 3-methacryloxypropyltrimethoxysilane (MPTMS) from Sin-Etsu Chemical (Tokyo, Japan); 2-propanol from Kanto Chemical (Tokyo, Japan); methanol and toluene from Wako Pure Chemical Industries (Osaka, Japan); Dulbecco's modified Eagle's medium (DMEM) and Dulbecco's modified phosphate-buffered saline (PBS) from Sigma (St. Louis, MI); streptomycin and penicillin from GIBCO BRL (Gaithersburg, MD); fetal bovine serum (FBS) from Moregate Biotech (Australia); and bovine carotid artery endothelial cells (ECs) from Health Science Research Sources Bank (JCBR0099, Osaka, Japan).

2.2. Preparation of PIPAAm-grafted glass coverslip surfaces by electron beam irradiation

Glass coverslips (24 × 50 mm, 0.2 mm in thickness, from Matsunami Glass Inc., Osaka, Japan) were cleaned by oxygen plasma treatment and placed in a 500 ml separable flask with 3 ml of MPTMS. The coupling reaction of vaporized MPTMS with the coverslip surfaces (MPTMS-CS) was performed under N_2 gas at 70 °C for 3 h, as reported previously [19]. The coverslips were rinsed with toluene, methanol and distilled water, then dried for 3 h at 160 °C. IPAAm monomer in 2-propanol solution (to give a final monomer concentration of 5–50 wt.%) was spread onto silanized glass surfaces. The surfaces were then subjected to irradiation with 0.25 MGy electron beam (EB) using an area beam electron processing system (Nisshin High Voltage, Kyoto, Japan) and rinsed with cold distilled water to remove nongrafted IPAAm, PIPAAm and PIPAAm gel.

2.3. Characterization of poly(*N*-isopropylacrylamide)-grafted glass coverslips

The amount of the PIPAAm grafted onto the coverslips was determined by attenuated total reflection-fourier transform infrared spectroscopy (ATR-FTIR) (Spectrum One, Perkin Elmer Japan Co., Ltd., Kanagawa, Japan). As the base substrate was glass, a strong adsorption arising from Si–O was observed at 1000 cm^{-1} [20]. Adsorption of amide carbonyl derived from PIPAAm appeared in the region of 1650 cm^{-1}. The peak intensity ratio of I_{1650}/I_{1000} was used to determine the amount of PIPAAm grafted onto surfaces using a calibration curve prepared for a known amount of PIPAAm cast on MPTMS surfaces. The equation determined from the calibration curve was $y = 0.0147x - 0.0005$ where y is the grafted density of PIPAAm (μg cm^{-2}) and x is the peak intensity ratio of I_{1650}/I_{1000} ($R^2 = 0.989$). Samples were abbreviated as 0.84PIPAAm-CS (PIPAAm graft density = 0.84 μg cm^{-2}), 0.86PIPAAm-CS (0.86 μg cm^{-2}), 0.89PIPAAm-CS (0.89 μg cm^{-2}), 1.00PIPAAm-CS (1.00 μg cm^{-2}), 1.28PIPAAm-CS (1.28 μg cm^{-2}), 1.41PIPAAm-CS (1.41 μg cm^{-2}) and 1.49PIPAAm-CS (1.49 μg cm^{-2}). PIPAAm-TCPS were fabricated as reported previously [18], and abbreviated as 1.40PIPAAm-TCPS (1.40 μg cm^{-2}). PIPAAm-grafted surfaces were also examined by X-ray photoelectron spectroscopy (XPS) (JPS-9010TR, JOEL, Tokyo, Japan). Survey spectra were acquired at a take-off angle of 10–90° and surface elemental compositions were calculated using integrated peak areas. Contact angles of PIPAAm-CSs, MPTMS-CS and TCPS surfaces were determined by the captive bubble technique in Milli-Q water at 37 and 20 °C with a FACE contact angle meter (image processing type CA-X, Kyowa Interface Science, Saitama, Japan). Three samples were used for the determination, and each sample surface was measured at more than three points. Contact angles and the difference between those at 37 and 20 °C were represented as mean value and standard deviation. In order to evaluate the thickness of the grafted PIPAAm, the grafted PIPAAm was completely ablated to re-expose the glass surface with ArF excimer laser (wavelength = 193 nm, pulse width = 5 ns, laser fluence = 50 mJ cm^{-2}, no. of laser shots = 5) by passing a laser pulse through an optical microscope with a square photomask (L5910 IIIB, Ham-