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Cell adhesion control on photoreactive phospholipid polymer surfaces

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ABSTRACT

Non-invasive and effective cell recovery from culture substrates is important for the passage and characterization of cells. In this study, a photoreactive polymer surface, which uses UV-irradiation to control substrate cell adhesion, was prepared. The photoreactive phospholipid polymer (PMB-PL) reported herein, was composed of a both 2-methacryloyloxyethyl phosphorylcholine (MPC) unit as a cytocompatible unit and methacrylate bearing a photolabile nitrobenzyl group. The PMB-PL polymer was used to coat a cell culture substrate thus affording a photoreactive surface. Surface analysis of the PMB-PL coating indicated a strong photoresponse owing to the sensitivity of the PL unit. Before light exposure, the PMB-PL surface provided cell adhesion. Following UV-irradiation, the PMB-PL coating was converted to a neutral ζ-potential and hydrophilic surface. The photoreactive surface conversion process allowed for the detachment of adhered cells from the PMB-PL surface while maintaining cell viability. This study demonstrates the promise and significance of the PMB-PL photoreactive surface as a method to control cell attachment and detachment for cell function investigation.

1. Introduction

Recently many researchers have shown interest in stimuli-responsive surfaces for cell engineering and other applications. The properties of such "smart surfaces" are effortlessly tuned using an external stimulus [1–5]. Control of cell attachment and detachment from a substrate with continued bioactivity is important for *in vitro* cell culture analysis. The stimuli-responsive surface properties that are of interest for cell engineering development include wettability, hydrophobicity, and hydrophilicity. Previously reported surfaces were responsive to electrical [6–9], temperature [10–12], pH [13–15], or light [16–18] external stimuli. Among the stimuli, light is regarded as ideal for increased spatial and temporal resolution control.

To achieve controlled cell attachment/detachment behavior under mild conditions, it is important to suppress non-specific biomolecule interactions. We have previously reported the 2-methacryloyloxyetyl phosphorylcholine (MPC) polymers that have excellent cytocompatibility due to the inhibition of non-specific biomolecule interactions [19]. These polymers have been widely applied in various fields within the life sciences, including the area of cell engineering materials [20–23]. The MPC polymers effectively suppress the typical inflammatory reaction of adhered cells [23]. Previously, a photo-functionalized MPC polymer bearing photoreactive moieties such as azidophenyl groups and photocleavable linkers were reported to prepare micropattern surfaces for cell adhesion control [24–27].

In this study, we prepared another photoreactive MPC polymer, which controls cell detachment using UV-irradiation. The 2-nitrobenzyl moiety is a typical photocleavable protective group for surface modification, which is cleaved by UV-irradiation ($\lambda = 365$ nm) using a mercury lamp [28]. Incorporating a photoreactive MPC polymer bearing a photocleavable (PL) monomer afforded the PMB-PL polymer. Upon UV-irradiation the cell adhesive molecules were converted at the PMB-PL surface and cell detachment was achieved. In this report, characterization of the PMB-PL polymer and cell attachment/detachment behavior at the surface were investigated.

2. Materials and methods

The MPC was purchased from NOF (Tokyo, Japan), which synthesized the product using the previously reported method [29]. Methacryloyl chloride was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The photolabile linker, 4-[4-(1-Hydroxyethyl)-2-methoxy-5-nitrophenoxy]butyric acid was purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA). Other organic reagents were purchased with the highest available purity and were used without further purification.

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HeLa (*Homo sapiens* epithelial cell line established from a uterine cervix carcinoma) and L929 cells (murine fibroblast cell line established from connective tissue) were purchased from Riken Cell Bank (Ibaraki, Japan). The cells were cultured in Dulbecco's Modified Eagle Medium, (DMEM Sigma, St. Louis, MO, USA) with 10% fetal bovine serum, (FBS, Invitrogen Life Technologies, Carlsbad, CA, USA).

2.1. Synthesis of the photocleavable monomer (PL)

The photocleavable monomer (PL) was synthesized under dark conditions using lightproof vials. The photolabile linker, 4-[4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy]butyric acid (1.0 mmol) was dissolved in dry dichloromethane (DCM), which had been purged with Ar gas. Both triethylamine (TEA, 3.0 mmol) and methacryloyl chloride (MC, 2.5 mmol), were dissolved in dry DCM and added dropwise to the photolabile linker solution at 0 °C. The solution was stirred overnight at room temperature (RT). The stirred solution was washed with sodium bicarbonate (5%, w/v *aq*), dilute hydrochloric acid (1%, v/v *aq*), and water. The washed solution was evaporated and the remaining liquid product was dissolved in aqueous acetone (50%, v/v *aq*). The reaction mixture was stirred overnight at RT and the liquid monomer was extracted using DCM. The DCM layer was collected, washed with dilute hydrochloric acid (1% v/v, *aq*) and water, dried over magnesium sulfate, and evaporated to yield the photocleavable methacrylate monomer referred to as PL monomer. The structure of the PL monomer was confirmed using ^{1}H NMR (300 MHz, JEOL, Japan). The ^{1}H NMR chart and FT-IR spectrum of PL monomer was shown in Figs. S1 and S2, respectively.

^{1}H NMR (300 MHz, DMSO-*d*6): δ 12.3 (br, $CH_2COO\mathbf{H}$), 7.55 (s, Aromatic-**H**), 7.10 (s, Aromatic-**H**), 6.39, 6.05 (d, d, $OC(dO)CCH_3dC\mathbf{H_2}$), 5.3 (q, Aromatic-C**H**$(CH_3)OC(dO)CCH_3dCH_2$), 4.1 (t, Aromatic-O$\mathbf{CH_2}CH_2CH_2COOH$), 3.95 (s, Aromatic-O$\mathbf{CH_3}$), 2.5 (t, Aromatic-$OCH_2CH_2C\mathbf{H_2}COOH$), 2.1 (s, $OC(dO)CHdCH_2C\mathbf{H_3}$), 1.9 (m, Aromatic-$OCH_2C\mathbf{H_2}CH_2COOH$), 1.55 (d, Aromatic-$CHC\mathbf{H_3}$).

2.2. Synthesis of photocleavable phospholipid polymer (PMB-PL)

The photocleavable phospholipid polymer (PMB-PL) was synthesized via the conventional radical polymerization method using an α,α'-azobisisobutyronitrile (AIBN) initiator. The procedure was completed in a glass tube. The MPC (0.25 mol), BMA (0.50 mol), and photocleavable monomer (0.25 mol) were dissolved in a dioxane/ethanol mixture (1:1 by vol.) at a final concentration of 0.38 M. Thereafter, AIBN (0.38 mM) was added to the solution. The solution was purged with argon gas for 10 min and the glass tube was then sealed. The sealed tube was placed in an oil bath at 60 °C for 48 h. Following polymerization, the PMB-PL was precipitated from diethyl ether/chloroform (3:2 by vol.), and the solid product was collected. The PMB-PL solid was dried overnight, under reduced pressure.

The chemical structure of PMB-PL was confirmed using ^{1}H NMR (300 MHz, JEOL, Tokyo, Japan) and FT-IR (FT-IR 615, JASCO, Tokyo, Japan) spectroscopies. The molecular weight of PMB-PL was measured using a gel-permeation chromatography (GPC) system fitted with an OHpak SB-804HQ column (Shodex®, Showa Denko KK, Tokyo, Japan).

2.3. Surface characterization of PMB-PL

The glass cover slide (18 mm × 18 mm, thickness 0.12–0.17 mm, Matsunami, Tokyo, Japan) was cleaned using ultrasonication in hexane, ethanol, and chloroform solutions at RT for 20 min; then, the slide was treated with oxygen plasma. The glass slides were immersed in a 0.5% (w/v) PMB-PL ethanol solution, and were then dried under reduced pressure. To evaluate the photoreactive property of the PMB-PL surface, a UV-irradiation instrument (Spot-cure SP7, Ushio Inc., Tokyo, Japan) equipped with a 250-W UV lamp (UXM-Q256BY, Ushio Inc., Tokyo, Japan) was used. The power density of the UV source was 80 mW/cm^2.

Surface characterization of the PMB-PL coating was analyzed using Fourier transformed-infrared reflection adsorption spectroscopy (FT-IRRAS), X-ray photoelectron spectroscopy (XPS), static contact angle, ellipsometry, and surface ζ-potential measurement.

A FTIR-500 (JASCO, Tokyo, Japan) was used for the FT-IRRAS spectra measurement. The spectra were obtained under dry conditions at a resolution of 4 cm^{-1} and a scan number of 128.

The XPS spectra were measured using an AXIS-His instrument (Shimadzu/Kratos, Kyoto, Japan) equipped with a monochromatized, Mg-focused, X-ray source. High-resolution scans of C_{1s}, N_{1s}, O_{1s}, P_{2p}, and Si_{2p} were acquired at a photoelectron take-off angle of 90°. The energies in all spectra were corrected using the C_{1s} energy calibration peak at 285 eV.

Static water contact angle measurements were conducted at RT using a CA-W automatic contact-angle meter (Kyowa Interface Science, Tokyo, Japan). The water-in-air and air-in-water systems were applied in this study. In the water-in-air system, the typical protocol involved using a constant drop volume (200 μL) of ultra-pure water onto the surface. For the air-in-water system, the surfaces were horizontally submerged in ultra-pure water. Air bubbles were positioned on the undersides of the surfaces using a syringe equipped with a U-shaped needle. The water drops and air bubbles were monitored using a charge-coupled device (CCD) camera. The captured images were analyzed using FAMAS software (Kyowa Interface Science, Tokyo, Japan) to determine the static contact angle. The contact angle was calculated as the average of more than five values taken at different positions.

The thickness of the PMB-PL was measured under dry conditions using an ellipsometer (alpha-SE®, J.A. Woollam Co., Inc., Lincoln, NE, USA) with a He–Ne laser (632.8 nm) at a 70° incident angle. The refractive indices (n_r) of the Parylene C and poly(MPC) used in the measurement were 1.63 and 1.49, respectively, and both extinction coefficients (k_e) were 0.00. All measurements were conducted under RT air conditions. Data were collected at eight different locations from each sample.

The surface ζ-potential was measured in a 10 mM NaCl solution using a measurement unit (ELS-6000, Photal, Otsuka Electronics Co. Ltd., Osaka, Japan) with an ancillary flat plate cell (10 mm × 30 mm × 60 mm) coated with poly(acrylamide) at 25 °C. Polystyrene latex particles coated with hydroxypropyl cellulose were used as the mobility-monitoring particles.

2.4. Cell attachment/detachment at the PMB-PL surface

HeLa cells were cultured in a 100-mm cell culture dish at 37 °C in 5% CO_2 atmosphere using DMEM containing 10% FBS. After the cells reached sub-confluency, the old media was aspirated; the cells were rinsed with phosphate buffered saline (PBS) and then were exposed to trypsin (1 mL) for 2 min to detach the cells from the surface. The detached cells were added to fresh DMEM, and the cell suspension was centrifuged at 1000 rpm for 3 min. After centrifuging, the supernatant was aspirated and the HeLa cells were suspended in DMEM for the following experiments.

The PMB-PL coated cover-glass surfaces were placed into each well of a 24-well-plate cell-culture dish, sterilized with ethanol, and then washed with PBS. A cell suspension (2.0×10^4 cells/mL, 2 mL) was seeded on the PMB-PL surface and incubated under 5% CO_2 at 37 °C. After incubation for 4 h, unattached cells were washed off with warm fresh medium and the attached cells were observed