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A photoresponsive soft interface reversibly controls wettability and cell adhesion by conformational changes in a spiropyran-conjugated amphiphilic block copolymer

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ABSTRACT

The functionalities of soft interfaces including cell adhesion can be enhanced by dynamic conversion of polymer properties and movement via external stimuli. Light is a superior stimulus, and various surfaces modified with photoreactive molecules have been prepared. However, in most of these studies, the surface properties are irreversibly changed due to photo-degradation, and reversible adhesion and collection of cells is not feasible. In this study, we developed a photoresponsive polymer soft interface that was able to spatiotemporally control wettability, cell adhesion, and detachment in a reversible manner. Spiropyran molecules were introduced into the hydrophobic block of an amphiphilic diblock copolymer consisting of poly(methyl methacrylate) and polyethylene glycol, and the monomer unit numbers of these components were optimized. The copolymer was immobilized on a glass substrate as a nanofilm. With alternating irradiation using UV and visible light, the surface exhibited reversible changes in hydrophobicity and hydrophilicity, and the direction of change was opposite to the polarity change in photo-isomerization of spiropyran. We also achieved photo-control of effective cell adhesion and detachment with sequential irradiation with UV and visible light. These remarkable functions could be ascribed to conformational changes triggered by photo-isomerization of spiropyran. This photoresponsive polymer soft interface may have applications as a powerful tool in biological studies by facilitating sequential changes in wettability and bioaffinity.

Statement of Significance

We developed a photoresponsive polymer soft interface, which was able to spatiotemporally control wettability and cell adhesion/detachment in a reversible manner, by introducing spiropyran into the hydrophobic block of an amphiphilic diblock copolymer. With alternating irradiation using UV and visible light, the surface exhibited unique reversible wettability changes; the direction of hydrophobicity and hydrophilicity change was opposite to the polarity change in spiropyran photo-isomerization. Light-dependent reversible control of spatiotemporal cell adhesion and detachment was also achieved with sequential UV (adhesion) and visible light irradiation (detachment). Cell detachment using noncytotoxic visible light was realized for the first time. Cell-patterning capability stably lasted for 25 days. This photoresponsive surface could be applied to fabrication of engineered tissues comprised of several cellular species.

1. Introduction

Soft interfaces, which have useful functions that can be ascribed to the structures, physicochemical properties, and movement of polymer materials, have attracted increasing interest owing to their various potential applications in biological fields. Dynamic conversion of the polymer properties and/or movement with

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external stimuli triggers induction or switching of the higher functionalities, including cell adhesion and detachment, in biotechnologies. Temperature-responsive polymer interfaces, which exhibit changes in hydrophilicity and hydrophobicity with dynamic coil-globule transitions in the polymer chains in response to external thermal stimuli, have been developed and successfully applied to noninvasive collections of cell sheets as grafts in tissue engineering and transplantation therapy [1–3]. For the use of thermal stimuli, however, spatial control is not easy, particularly when applying to small spots, such as single cells, on the substrate. Moreover, the temperature-responsive substrate does not rapidly reach thermal equilibrium. Moderate changes in ambient temperature not to cause denaturation of proteins or cytotoxicity take time as thermal stimulations.

Light is superior to various other types of stimuli with regard to certain parameters. For example, light can be focused on a micron- or submicron-sized area and affects the target in a contact-free manner. Photoreactions proceed rapidly in general. Thus, various types of surfaces have been modified with photoreactive molecules [4–18], and control of protein adsorption or cell adhesion by light stimuli has been studied. These photoreactive molecules can be classified into photocleavable and photoswitchable molecules.

(2-Nitrophenyl)alkyl ester derivatives are a typical example of a photocleavable molecule and have been introduced at the end [4,5] or in the middle [6,7] of silane-coupling reagents to be immobilized on a surface. In these studies, the surface properties have been irreversibly changed due to photocleavage of the molecules. Hence, reversible adhesion and collection of cells are not feasible, and the generated fragments may exhibit cytotoxicity. Therefore, photoswitchable molecules, which sequentially and reversibly change the properties of a surface and do not generate any byproducts [9], are preferable for various applications, e.g., *in situ* sequential cell patterning of different species to form engineered tissues in highly ordered structures and noninvasive collection of cells for regenerative therapies.

As photoswitchable molecules, azobenzene [9,10] and spiropyran have been frequently utilized for surface modifications. These molecules reversibly photo-isomerize in response to UV and visible light irradiation without releasing or incorporating any other molecule. Conjugation with cell-adhesive peptides for photocontrol of cell adhesion and detachment were also studied [11]. In particular, spiropyran forms two unique isomers-spiropyran (Sp) and merocyanine (Mc). These two isomers have dramatically different molecular structures and physicochemical properties [12–16]. The Sp isomer is a nonpolar molecule, whereas the Mc isomer has a large electric dipole moment owing to charge separation by the ring-opening reaction [17]. Due to this difference in polarity, the contact angles of the surface-immobilized Sp-containing low-molecular-weight compounds are reversibly changed with variance up to 15° by photo-isomerization [18,19]. However, cell adhesion and detachment are not altered, suggesting that the changes in surface structure and polarity during photoisomerization of individual Sp molecules are not sufficient for controlling the adsorption of extracellular matrix (ECM) proteins. Therefore, introducing a number of Sp molecules into a polymer chain to induce dynamic movement and immobilizing these macromolecules on a surface to form a functional soft interface would be a promising strategy to control cellular behaviors on the surface.

Polyethylene glycol (PEG) is a polymer material that is frequently used to modify a surface to effectively repel proteins and cells [20–24]. However, the conformation of the PEG chain cannot be controlled effectively at arbitrary times. Hence, dynamic regulation of cell adhesion and detachment is not feasible using PEG alone. An Sp-containing photoresponsive substrate anchoring PEG chains by weak interactions, e.g., van der Waals forces and hydrogen bonds, and releasing them with light-stimulus has been reported [8]. This substrate enables specific cell adhesion to light-irradiated spots; however, changes in surface properties without releasing any molecule and reversible control of cell adhesion and detachment have not been achieved.

In this paper, we report a photoresponsive polymer soft interface that was able to temporally and spatially control cell adhesion and detachment in a reversible manner. Sp molecules were introduced into the hydrophobic block of an amphiphilic diblock copolymer consisting of the hydrophobic block poly(methyl methacrylate) (PMMA) and the hydrophilic block PEG. The copolymer was immobilized on a glass substrate as a nanofilm by spin-coating. Alternative irradiation with UV and visible light modified the surface wettability in a unique manner and altered cell adhesion and detachment. Dynamic changes in the polymer conformations are also discussed to elucidate the mechanisms of these remarkable functions.

2. Materials and methods

2.1. Materials

Methyl methacrylate (MMA) was purchased from Wako Pure Chemicals (Osaka, Japan) and purified by distilling at 40 °C and 110 mmHg. Poly(ethylene glycol) monomethyl ether (monomer unit number = 45, M_w 2000; $MeOPEG_{45}$) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and was used without further purification. Spiropyran methacrylate monomer (SpMA) was synthesized as previously reported (see Supporting Information) [25]. 4-Cyanopentanoic acid dithiobenzoate (CPADB) and poly (ethylene glycol)-based chain transfer agent (PEG-CTA) were synthesized as previously described, with slight modifications (see Supporting Information) [26,27]. 4,4′-Azobis(4-cyanopentanoic acid) (ACPA) and 1,4-dioxane were purchased from Wako Pure Chemicals and were purified by recrystallization and distillation at 100 °C, respectively. Other chemicals were also purchased from Wako Pure Chemicals and were used as received. Milli-Q water (resistivity 18 MΩ cm^{-1}) was prepared with a water purification system (Millipore, Billerica, MA, USA) and was used throughout in this study. Dulbecco's modified Eagle's medium with high glucose (DMEM) and basic fibroblast growth factor (bFGF) were obtained from Wako Pure Chemicals. Dulbecco's phosphate-buffered saline and trypsin-ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma-Aldrich. Penicillin and streptomycin were obtained from Gibco Life Technologies (Grand Island, NY, USA). Fetal bovine serum (FBS) was obtained from Biowest (Nuaille, France).

2.2. Preparation of photoresponsive polymers

Amphiphilic diblock copolymers consisting of a hydrophobic PMMA block containing photoresponsive Sp molecules and a hydrophilic PEG block (P(SpMA-*co*-MMA)-*b*-PEG) were prepared by changing the monomer ratios of SpMA and MMA in RAFT polymerization, using PEG-CTA (RAFT agent) and ACPA (initiator) at the ratio of 1/0.2 (Fig. 1A). The composition of SpMA in feed was varied from 0 mol% (i.e., PMMA-*b*-PEG) to 13 mol% or 27 mol%. The total monomer concentrations of SpMA and MMA were kept constant at 1.8 M to generate similar chain lengths for the hydrophobic block. The target setpoint of the total molecular weight was approximately 8000, as determined according to pilot experiments in which the reactivities of both monomers were assessed. The average molecular weight of PEG in PEG-CTA was kept constant at M_w 2000, as described in Section 2.1.