Preparation of Photolabile and Cytocompatible Polymer Surface to Control Cell Adhesion and Detachment

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In this study cell attachment and detachment on the surface was selectively controlled using a photoreaction. We prepared a photolabile and cytocompatible polymer surface to control on cell attachment and detachment. As a polymer, poly[(2-methacryloyloxyethyl-phosphorylcholine(MPC)-co-n-butylmethacrylate(BMA)-co-photocleavable-monomer(PL)](PMB-PL) was synthesized. Photocleavable linker with active ester catches suitable for biomacromolecules, therefore cells bind for a culturing on a glass surface. The irradiation with UV light removes the cell adhesive molecules and biofouling surface is remained. Thus, it will be able to detach attached cells with photolirradiation on the surface. The photochemical activity of PMB-PL was examined by absorbance spectra. Features of before and after photoirradiated PMB-PL surface were characterized by Fourier transformed infrared reflection adsorption spectroscopy, X-ray photoelectron spectroscopy, and static contact angle measurement. Also, photo-induced detachment of cells on the surface was examined. As a result, 91% of seeded 4×10⁴ cells were attached onto PMB-PL surface and 67% of attached cells were detached by photochemical reaction. We expect expansion of this tool can be applied for various field, e.g., single cell analysis system, cell and tissue engineering.

Key words: cytocompatible surface, photocleavable polymer, single cell, cell adhesion, photocleavage

1. INTRODUCTION

The value of research and development on molecular analysis in single living cell has increased tremendously over the past decade. Since the advent of proteomics and genomics, researchers often perform bulk techniques because they are simple, available, and well established [1]. Biologists worked on millions of cells and thereby they reported on population averages rather than their distribution and missed rare but important events. However, each cell is different from the others and cell heterogeneity is well known in bacteria and eukaryotic cells [2-4]. When using primary and rare cells (e.g., stem cells, progenitor cells) there are normally fewer cells available and unfortunately, those cells display a distribution of heterogeneous behaviors. Therefore, we could not apply the conventional high-throughput population-based protocols in this system. Additionally, the population based average protocols are often misleading. An example of how bulk experiment on protein levels among cells could be misleading has been reported [5]. To determine how heterogeneously different cell populations are and to understand every individual living cell behaviors in same environment, a number of new techniques has been developed for single cell analysis in last few years [6-9]. An evolution of single cell analysis technologies has provided advantages of looking at dynamic study on single living cell by temporally and spatially, cell-cell and cell-surface interactions, and physical signals for environmental stimuli.

Single cell analysis has been applied in various fields under the development of numerous microdevices used for studies based on well-, trap-, pattern-, and droplet based structures. One of those common technologies for single cell is a microwell plate device, which has designed in numerous different ways. In these microwell studies, polydimethylsiloxane, glass, silicon surfaces were prepared [10, 11]. Preparation of surface is key technology for developing all of those microdevices. A highly controlled and cytocompatible surface is vital to the functionality and survival of the single cell analysis technology.

In this study we demonstrate a new approach to fabricate a novel surface can regulate cell attachment and detachment selectively by photolirradiation. Since early days, a photosensitive surface has been developed for cell surface technology [12, 13]. For this purpose, we prepared a cytocompatible polymer surface with photocleavable groups to control the cell attachment and detachment using a photochemical reaction (Fig. 1). As a polymer, 2-methacryloyloxyethylphosphorylcholine (MPC) polymer bearing photocleavable (PL) monomer unit (PMB-PL) was synthesized. The MPC units are well known as a cytocompatible and nonbiofouling property [14-16]. The irradiation with UV light removes the cell adhesive molecules on the surface. Thus, cell detachment with UV irradiation may be achieved. We would like to confirm that the photochemical reactions on the polymer surface are powerful for developing the single cell analytical devices.
2. MATERIAL AND METHODS

2.1 Reagents and materials

Most of reagents and solvents were purchased either from Wako (Osaka, Japan), Tokyo Chemical Industry (Tokyo, Japan), Sigma-Aldrich (St. Louis, MO), or Invitrogen Corporation (New York, USA).

2.2 Synthesis of photocleavable polymer (PMB-PL)

PMB-PL with an MPC, a BMA, and a PL monomer unit was prepared by conventional radical polymerization of the corresponding monomers using α, α’-azobisisobutyronitrile (AIBN) as an initiator. Mixture of monomers was dissolved in dioxane/ethanol (1/1, v/v) solution and polymerized at 65°C. The chemical structure of PMB-PL polymer was confirmed by 1H NMR (300 MHz, JEOL, Japan) and FT-IR (FT-IR 615, JASCO, Japan) measurements. The average molecular weight of PMB-PL was evaluated by gel-permeation chromatography (GPC) (column: OHpak SB-804HQ, Showa Denko K.K., Japan) with a poly(ethylene oxide) (PEO) standards.

2.3 UV-VIS spectral change of PMB-PL

An ethanol solution of PMB-PL was irradiated in a quartz cell with a 250W Deep UV lamp (UXM-Q256BY, USHIO Inc., Japan). The light power density of the cell was 80mW/cm², as determined with a UVR-T1 light meter equipped with a UD-T36 sensor (TOPCON, Japan). UV/VIS spectra were obtained with a UV-560 UV/VIS/NIR spectrometer (JASCO, Japan).

2.4 Surface modification of PMB-PL to glass slide

The glass slides (18 mm×18 mm, thickness 0.12-0.17 mm, Matsunami, Japan) were precleaned by ultrasonication in hexane, ethanol, chloroform solution at RT for 20 min and were treated with oxygen plasma. For modification of the precleaned cover glass surface 0.5wt% PMB-PL/ethanol solution was adopted.

2.5 Characterization of PMB-PL modified surface

Characterization of PMB-PL modified surface analyzed by Fourier transformed infrared reflection adsorption spectroscopy (FT-IRRAS), X-ray photoelectron spectroscopy (XPS), and static contact angle measurement. In FT-IRRAS spectrum measurement, spectra were obtained at a resolution of 4 cm⁻¹ and a scan number of 128. In XPS spectrum measurement, the surface-modified glass samples were inserted in the holder of an XPS instrument (AXIS-HSI, Shimadzu/Kratos, Japan) and were equipped with a monochromatized Mg focused X-ray source. High resolution scans for C 1s, N 1s, O 1s, P 2p, and Si 2p were acquired at a takeoff angle for 90° for the photoelectrons. The energies of all spectra were shifted by correcting with the C 1s peak at 285 eV for energy calibration. Water contact angle measurements were conducted at room temperature using a CA-W automatic contact angle meter (Kyowa Interface Science, Japan). The typical protocol involved using a constant drop volume (200 µL) of ultrapure water, which was dropped onto the surface and monitored with a charge-coupled device (CCD) camera. The captured images were analyzed using FAMAS software (Kyowa Interface Science) to determine the contact angle. The contact angle reported in this study is the average of more than three values taken at different points on the surface and it was calculated from the following (1) equation:

\[ \theta = 2 \cdot \tan^{-1} \left( \frac{h}{r} \right) \]

where \( \theta \), \( h \), and \( r \) are the contact angle, height, and radius of the water drop, respectively. At least five contact angles from different areas were measured and averaged.

2.6 Preparation of HeLa cells

HeLa cells were cultured in 100 mm cell-culture dishes at 37°C under 5% CO₂ using Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS). After HeLa cells became confluent, they were washed with 10 mL of phosphate-buffered saline (PBS), pH 7.4, were immersed for 2 min in 1 mL of trypsin to detach them. The detached cells were added to fresh DMEM and the cell suspension was centrifuged at 1000 rpm for 3 min. Finally, supernatant was aspirated and HeLa cells were resuspended in DMEM for following experiment.

2.7 Cell attachment/detachment on the PMB-PL surface

The PMB-PL modified cover glass surface was placed into a 24 well plate cell-culture dish, sterilized with ethanol, and then washed with PBS. A cell suspension (2×10⁴ cells/mL; 2 mL) was seeded on the surface-modified glass surface and incubated under 5% CO₂ at 37°C. After 4 hr of incubation, unattached cells were washed off with medium and the cells observed under a microscope. Then UV light (360 nm, 80 mW/cm²) was irradiated to the surface for 60 sec. Detached cells by photoirradiation was recovered and calculated for cell density. After irradiation culture dish plate was washed and remained cells were detached by trypsin. Detached cells were counted and cell counts were converted to cell density per area (cells/cm²).

3. RESULTS AND DISCUSSION

3.1 Characterization of the PMB-PL

PMB-PL was synthesized using MPC, BMA, and PL monomer by a conventional radical polymerization. The chemical structure of PMB-PL is shown in Fig. 2. The MPC unit was selected as a hydrophilic and biocompatible moiety, the BMA unit was selected as a hydrophobic moiety for spacer, and the PL unit has a photocleavable active ester unit to conjugate with the cell. The monomer unit composition of PMB-PL in mol% was quantitatively related to the feeding ratio by 1H NMR as MPC/BMA/PL=24/50/26. The PMB-PL could be dissolve in organic solvents as alcohol, dimethylsulfoxide, and dioxin. The weight average molecular weight was \( M_w = 1.43 \times 10^4 \) and the polydispersity index (PDI=\( M_w/M_n \), \( M_n \) is the number average molecular weight) was 1.31 by GPC based on PEO standards.
3.2 Photochemical activity of PMB-PL surface

To analyze the photochemical activity, the PMB-PL was dissolved in ethanol and its spectral change in response to UV light irradiation (λ > 200 nm) was examined. Before irradiation, the solution had absorption peaks at 300 nm and 348 nm typical for a 3,4-dimethoxy-6-nitrophenyl group (Fig. 3a, bold line) [17, 18]. After UV light irradiation, the spectrum showed a dose dependent decrease at 348 nm band while two new adsorption peaks were increased at 265 nm and 375 nm (Fig. 3). These new adsorption bands probably belong to the 4-(4-acetyl-2-methoxy-5-nitro-phenoxy) butanoic acid which is photoproduct obtained by photocleavage of ester group. This result indicates PMB-PL in bulk solution undergoes a photochemical reaction characteristic of the 2-nitrobenzyl ester.

It was also analyzed whether the PMB-PL retained its photochemical activity even after being immobilized to the substrate surface. Before photoirradiation the PMB-PL modified substrate showed similar absorption spectrum at 250-400 nm as that of PMB-PL in the bulk solution (data not shown). After photoirradiation the peak intensity at 348 nm band decreased, which was the same as the spectral change of PMB-PL in the bulk solution. Thus it can be concluded that the polymer kept its photochemical activity on the surface-immobilized state.

3.3 Characterization analysis of PMB-PL surface

Active esters are usually susceptible to hydrolysis, so IR spectroscopy was conducted to demonstrate that the methacryloyl carbonate moiety of PMB-PL survived after the surface modification. In order to obtain a clear spectrum, we used Au board of 200 nm as a substrate for the coating in this experiment. The coated Au board gel showed absorption peaks characteristic of the methacryloyl carbonate at 1740, 1780, and 1820 cm⁻¹, the nitro group at 1660 and 1290 cm⁻¹, the phosphoryl group at 1190 cm⁻¹ (data not shown). The peak positions were almost the same as those in the original PMB-PL. Although the population of the methacryloyl carbonate could not be estimated, these results demonstrate the existence of the methacryloyl carbonate on the substrate surface.

To analyze more information on the chemical state of atom XPS measurement was performed. PMB-PL modified surface had a phosphorus peak, a nitrogen peak, an oxygen peak, a silicon peak, and strong carbon peak by XPS. Before irradiation, the atomic percentages of C1s and P2p on PMB-PL modified surface were 63.9% and 5.6%, respectively. After 10 min irradiation of PMB-PL modified surface, these compositions were changed as 64.2% and 9.3%, respectively. More specifically, the ratio of P/C increased from 0.088 to 0.145 during photoirradiation process. The change of atomic composition on the PMB-PL modified surface supports the notion of photocleavage of active ester groups on the surface.

During modification of PMB-PL to quartz cell glass the surface contact angle changed from 50° to 88° which means PMB-PL coated surface is more hydrophobic than glass board. After 10 min irradiation to PMB-PL surface the contact angle changed from 88° to 101°. From this result, it is concluded that the surface chemical composition which is consequences of surface wettability was able to control under photoirradiation.

3.4 Photo-induced detachment of cells on the PMB-PL modified surface

To confirm the photo-induced detachment of cells on the surface and to evaluate the photocleavage efficiency more quantitatively, detachment of cells on the different surfaces were examined. In this experiment we used a photocleavable PMB-PL modified surface, a pre-photoirradiated PMB-PL modified surface, and a tissue culture treated polystyrene (TCPS) surface. Cells were attached from these surfaces and photoirradiated for 60 sec with 80 mW/cm². As a result, more than 90% in 4.0×10⁴ seeded cells were attached to the PMB-PL modified surface and approximately 67% of attached cells were detached by photoirradiation (Fig. 4). UV light removes the cell adhesive molecules in the PL units by photochemical reaction and nonbiofouling surface was remained. For this reason, cells bounded on the surfaces might be detached. These results demonstrate the detachment of the cells on the PMB-PL modified surface was selectively related to photochemical cleavage of PL unit by photoirradiation.

To obtain more evidence for photorelease of attached cells the difference between cell detachment on the pre-photoirradiated and non-prephotoirradiated PMB-PL modified surface was examined. Before cell attachment, one of the PMB-PL modified surfaces was
photoirradiated for 60 sec with 80 mW/cm² (pre-photoirradiated surface). After washing, the cells were seeded to both surfaces. Then, attached cells onto both surfaces were irradiated with UV light and detached cells were counted. As a result, in pre-photoirradiated surface, approximately 50% of seeded cells were attached and around 11% of seeded cells were detached by photoirradiation even when more than 67% of attached cells were detached on the non pre-photoirradiated PMB-PL modified surface. Additionally, in case of TCPS surfaces, around 5% of attached cells were detached by photoirradiation which means much closer with that of pre-photoirradiated PMB-PL surface. These results strongly support the cell detachment on the PMB-PL modified surface is related to the photochemical reaction on the PMB-PL surface.

We also examined cell biological and physical phenomena after photo-induced detachment. In this experiment, the growth rates of cells recovered from different surfaces were examined. As a result, detached cells after photoirradiation were kept their physiological phenomena as before cell attachment (Fig. 5).

4 CONCLUSION

In this study, a novel photocleavable and cytocompatible PMB-PL surface was prepared and characterized. Also, photoinduced cell detachment on the PMB-PL modified surface was discussed. As a result, more than 60% of attached cells onto PMB-PL surface were detached using photochemical reaction by single cell level. It is expected this technology can be a powerful tool for a single cell analysis system, and the expansion of this tool can be applied for various cell-based fields such as medical, pharmaceutics, and bioengineering.

REFERENCES


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