Novel Micropaterned Surface Fabricated from Heterobifunctional Poly(ethylene glycol)/polylactide Block Copolymers for Patterned Cell Culture

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Keywords: heterobifunctional poly(ethylene glycol), micropatterned surface, biodegradable polymer, rat primary hepatocyte, bovine aortic endothelial cells

1. Introduction

The spatial control of cell adhesion and growth is a critical issue in many areas of biotechnology and especially biosensing using whole cells. The goal of cell biosensing is to use molecular recognition and biochemical pathways inherent in cell function to sense complex analytes, for example, chemical/biological warfare agents and pathogens in food. Thus, micropatterned surfaces described here could potentially be used as a template to direct the function of multiple cell types into addressable arrays.

Because cellular adhesion and spreading is regulated by protein adsorption, patterning of proteins responsible for cellular adhesion leads to spatially directed cellular adhesion. Surface adsorption of patterned adhesion proteins can be accomplished by exposure of substrates with high protein affinity to protein-containing media. Several studies have demonstrated cellular adhesion by this procedure, and it has been found that surface properties such as hydrophilicity and hydrophobicity, surface charge, and surface roughness affect protein adsorption. Poly(ethylene glycol)(PEG) is one promising material that has been shown to be quite efficient to inhibit adsorption of proteins responsible for cellular adhesion. Biodegradable polymer substrates with specific chemical micropatterns were fabricated from polylactide(PLA) and diblock copolymer of lactose-ended poly(ethylene glycol) and polylactide (lactose-PEG/PLA). Particularly, the substrate was composed of the glass surface and thin film of lactose-PEG/PLA in which they supported and inhibited, respectively, adhesion of cells. A plasma-etching through micro-patterned material made by photolithographic treatment was used to define arrays of circular (diameter of 100 µm) glass domains surrounded and separated by regions (width of 100 µm) of lactose-PEG/PLA (Figure 1).

![Figure 1](image_url)
are potentially useful to make three-dimensional cell systems for application in a bioartificial liver device and for studying xenobiotic drug metabolism in biosensing.

2. Experimental Section

2.1 Materials Commercial tetrahydrofuran (THF), 3,3-diethoxy-1-propanol (Aldrich), and L-lactide (LA) (Aldrich) were purified by conventional method [4]. Ethylene oxide (EO) (Saisan) was dried over calcium hydride and distilled under an argon atmosphere. Potassium naphthalene was used as a THF solution, whose concentration was determined by titration. Other reagents were used as received. Water used in this study was purified by a Milli-Q System (Nihon Millipore Co., Tokyo, Japan) to have a specific conductivity of less than 0.1 \( \mu \)S cm\(^{-1} \).

2.2 Synthesis of Acetal-PEG/PLA Block Copolymers. \( \alpha \)-Acetal-PEG/PLA was synthesized by a one-pot anionic ring-opening polymerization of EO followed by LA initiated with potassium 3,3-diethoxypropanolate (PDP) as an initiator at room temperature under argon (Scheme 1). Because the detailed procedure was described elsewhere [5], only a brief description is presented here; One mmol of 3,3-diethoxypropanol and 1 mmol of potassium naphthalene were added to dry THF to form PDP. After stirring, an appropriate amount of EO was added to the PDP solution. The polymerization of the EO proceeded for two days at room temperature. Potassium naphthalene was added to stabilize the living chain end. LA solution in THF (c=1.10 mol/l) was then introduced and the mixture stirred for 120 min. The polymer was recovered by precipitation in cold isopropyl alcohol and centrifuged, followed by freeze-drying from benzene. The obtained PEG was treated with aqueous media adjusted to pH 2 to transform an acetal group at the PEG-chain-end into an aldehyde end group. Further, lactose (Lac) groups were successfully reacted with the distal PEG chain through reductive amination reaction of PEG terminal aldehyde (transformed from acetal group [6,7]) and corresponding sugar derivatives having p-aminophenyl moieties at the C-1 position (p-aminophenyl-\( \mu \)-D-lactopyranoside).

2.3 Polymer Characterization. The molecular weight of PEG segment was determined by both gel permeation chromatography (GPC) and MALDI-TOF-MS measurements at the end of EO polymerization. The molecular weight of the PLA segment was determined using \(^1\)H NMR spectrum by estimating the ratio of methine protons in the PLA segment and methylene protons in PEG segment based on the number-averaged molecular weight (Mn) of PEG determined from the MALDI-TOF-MS results.

2.4 Spin Coating of Lactose PEG/PLA The glass substrates, which were cleaned by a Piranha etch, were placed in 2 % (v/v) solution of 3-(Trimethoxysilyl)propyl methacrylate/ethanol. The glass substrates were dried at 160 °C for 24 h under vacuum. The PEG-brushed layer was constructed on this silanized glass surface by the spin coating of toluene solution of PLA(4 % (w/v)), followed by the acetal-PEG/PLA(2 % (w/v)).

2.5 Microfabrication Photoresist patterns were defined by photolithography technique. Round, 100 \( \mu \)m diameter holes separated by 100 \( \mu \)m distance each other were used to mask a \( \text{N}_2+\text{H}_2 \) plasma etch, forming the patterned lactose-PEG/PLA surface.

2.6 Characterization of the PEGylated Surfaces. Scanning electron microscopy with energy-dispersed analysis of X-rays (SEM/EDX). The surface of patterned substrate was subjected to SEM (Hitachi S4200) with an EDX (KEVEX Super Dry) attachment. The working distance was set for 15 mm and the EDX detector was located at the takeoff angle of 45°. X-ray Photoelectron Spectroscopy (XPS) analysis. The elemental compositions of the patterned lactose-PEG/PLA surfaces were determined using XPS. Spectra were acquired using a VG Escalab MK II electron spectrometer (VG Scientific, East Grinstead, Sussex, U.K.) with a Mg-K \( \alpha \) (1253.6eV) ionizing radiation used for a photoexcitation source. The operating conditions of the Mg-K \( \alpha \) source were fixed at 15 kV and 20mA, and the sample surface was oriented 90° relative to the direction through the entrance of the analyzer.

3. Results and Discussion

3.1 Synthesis of \( \alpha \)-acetal-PEG/PLA and Lactose-PEG/PLA Block Copolymers. After the EO polymerization, a PEG/PLA block copolymer with an acetal group at the PEG chain end can be prepared, because potassium alkoxide has the ability to initiate LA polymerization. A PEG/PLA with an methoxy moiety at the \( \alpha \)-terminus is also synthesized using 2-methoxyethanol as an initiator. The molecular weight of PEG and PLA segment was determined to be 5.0K and 8.0K, respectively. The acetal group was confirmed to be able to be
deliverized to an aldehyde group, by the moderate acid treatment, which provides the site for chemical immobilization of functional molecules such as proteins and peptides.[6-8]

3.2 SEM/EDX analysis. SEM/EDX is one of the electron probe microanalysis methods that have been used extensively to characterize the size, morphology, and elemental composition.

![SEM image](image)

**Figure 2.** (a) SEM photogragh and (b) image showing Si distribution by EDX on the surface of lactose-PEG/PLA after plasma-etching.

Figure 2 shows (a) SEM image of the patterned lactose-PEG/PLA spin-coated on silanized glass substrate and (b) image demonstrating the Si distribution on the same region as (a). The image contrast in Figure 2(b) is directly proportional to the density of Si atom. The Si distribution in EDX image nicely corresponds to high contrast SEM image of patterned substrate, indicating that the glass surface was certainly appeared by the plasma etching. This observation is also confirmed by the elemental analysis using EDX (Table. 1).

### Table 1 Elemental composition corresponding to regions A and B in Fig. 2.

<table>
<thead>
<tr>
<th>Element</th>
<th>Region A(%)</th>
<th>Region B(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>45.82</td>
<td>31.85</td>
</tr>
<tr>
<td>O</td>
<td>27.15</td>
<td>28.44</td>
</tr>
<tr>
<td>Na</td>
<td>4.07</td>
<td>5.37</td>
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<tr>
<td>Si</td>
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</tr>
<tr>
<td>K</td>
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<td>2.47</td>
</tr>
<tr>
<td>Ca</td>
<td>0.00</td>
<td>6.12</td>
</tr>
</tbody>
</table>

3.3 XPS analysis. The binding states and elemental compositions of patterned surfaces were examined by survey-scans of XPS.

![XPS spectra](image)

**Figure 3.** XPS survey-scan spectra on both region A and B in Figure 2.

By comparing Fig. 3(a) with Fig. 3(b), the appreciably increasing Si2s/Si2p/O1s signal are
assignable to glass substrate and responsible for the plasma etching.

3.4 Microfabricated Co-cultures. Cellular adhesion onto a surface is normally proceeded by adsorption of proteins. Accordingly, it is reasonable to assume that if protein adsorption can be controlled, then cell adhesion and spreading will also be controlled. As reported earlier, PEG has been shown to resist adsorption by both cells and proteins.[6-8] The fraction of lactose-PEG/PLA spin-coated onto the silanized glass surface of the patterned substrate should inhibit protein adsorption, resulting in the lessen cell adhesion. On the other hand, proteins should adsorb from the serum-containing solution on the glass surface appeared by plasma etching.

Patterned substrate surfaces were visualized using bovine plasma fibronectin (FN) adsorbed from an aqueous solution and immunostained in situ, followed by the staining with isothiocyanato-FITC reacted with secondary amine of p-aminophenyl-β-D lactopyranoside at the PEG-chain end. Briefly, FN in Dulbecco’s phosphate-buffered saline (PBS) solution was plated on the substrate at a desired concentration and incubated overnight. After washing with PBS the adsorbed FN was detected with anti-FN antibody and rhodamine-conjugated anti-rabbit IgG antibody. The surface was then stained with isothiocyanato-FITC and observed under a fluorescence microscope. Pattern formation was confirmed by several experiments including SEM/EDX, XPS, and adsorption of the extracellular matrix protein (FN).

The detailed study of cell culture, distributed as micropattern, will be the subject of a separate paper.

4. Conclusion

Biodegradable polymer substrates with specific chemical micropatterns were fabricated from polylactide (PLA) and diblock copolymer of lactose-ended poly(ethylene glycol) and polylactide (lactose-PEG/PLA). A plasma-etching through micro-patterned material made by photolithographic treatment was used to define arrays of circular (diameter of 100 μm) glass domains surrounded and separated by regions (width of 100 μm) of lactose-PEG/PLA. Pattern formation was confirmed by several experiments including SEM/EDX, XPS, and adsorption of the extracellular matrix protein (FN).

Acknowledgements

The authors gratefully thank Mr. S. Adachi, The University of Tokyo, for plasma etching the substrate. We also acknowledge Mr. T. Nakamura and M. Nakamura, The University of Tokyo, for taking TEM and SEM/EDX micrographs. This study was supported by a JSPS, The Japan Society for the Promotion of Science, "Research for the Future Program" (JSPS-RFTF96100201).

References