Facile Cell Patterning on an Albumin-Coated Surface

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Fabrication of micropatterned surfaces to organize and control cell adhesion and proliferation is an indispensable technique for cell-based technologies. Although several successful strategies for creating cellular micropatterns on substrates have been demonstrated, a complex multistep process and requirements for special and expensive equipment or materials limit their prevalence as a general experimental tool. To circumvent these problems, we describe here a novel facile fabrication method for a micropatterned surface for cell patterning by utilizing the UV-induced conversion of the cell adhesive property of albumin, which is the most abundant protein in blood plasma. An albumin-coated surface was prepared by cross-linking albumin with ethylene glycol diglycidyl ether and subsequent casting of the cross-linked albumin solution on the cell culture dish. While cells did not attach to the albumin surface prepared in this way, UV exposure renders the surface cell-adhesive. Thus, surface micropatterning was achieved simply by exposing the albumin-coated surface to UV light through a mask with the desired pattern. Mouse fibroblast L929 cells were inoculated on the patterned albumin substrates, and cells attached and spread in a highly selective manner according to the UV-irradiated pattern. Although detailed investigation of the molecular-level mechanism concerning the change in cell adhesiveness of the albumin-coated surface is required, the present results would give a novel facile method for the fabrication of cell micropatterned surfaces.

Introduction

Fabrication of micropatterned substrates to control cell adhesion and proliferation is a promising technique for cell-based technologies, including the screening of drug candidate libraries and fundamental investigations of cell−cell communication.[1] Several investigators have presented successful strategies for creating cell micropatterns on substrates.[2−8] A self-assembled monolayer (SAM) of alkanethiols is easily formed on a gold surface and shows a chemically defined surface.[9,10] Patterned substrates that allow cells to attach onto the delimited regions were prepared by using a combination of SAM and photolithography techniques or the microcontactprinting method,[11−13] and cell patterns could be formed through preferential attachment on the cell-adhesive region of the substrate. A cell micropattern was also prepared by directly delivering viable cells to the substrate using an inkjet printing technology.[14,15] Because these methods need a complex multistep process or special and expensive equipment or materials, their prevalence as a general experimental tool has been limited.

Serum albumin is the most abundant protein in blood plasma[16,17] and has characteristics to avoid other protein adsorption and cell adhesion on its coated surface. Recently, we found a method to obtain water-insoluble cross-linked albumin (cr-Alb) film without losing the native albumin properties, such as the resistance to cell adhesion and its drug binding ability.[18] Furthermore, cells were found to become attached on the surface by irradiating the cr-Alb-coated surface with UV light. Taking advantage of this property of the cr-Alb film, we will describe here a novel and facile cell micropatterning method that does not require any special and expensive equipment or materials.

Materials and Methods

Preparation of Patterned Albumin Substrate. A cr-Alb-coated surface was prepared following the method reported previously.[18] Briefly, bovine serum albumin (Sigma, St. Louis, MO) was dissolved in phosphate-buffered saline (PBS, pH = 7.4, 1.1 mM KH_2PO_4, 154 mM NaCl, 3 mM Na_2HPO_4) to give a 3% solution, and was reacted with 215 mM ethylene glycol diglycidyl ether (EGDE, Wako, Osaka, Japan) with vigorous stirring for 24 h at room temperature. The reaction mixture was dialyzed against distilled water using Spectra/Pro biotech membranes (molecular weight cut-off of 3500; Spectrum Laboratories, Inc., Rancho Dominguez, CA) for four days at room temperature to remove unreacted EGDE. The mixture of EGDE−cr-Alb and 0.5% glycerol was filtered through a 0.22 μm filter for

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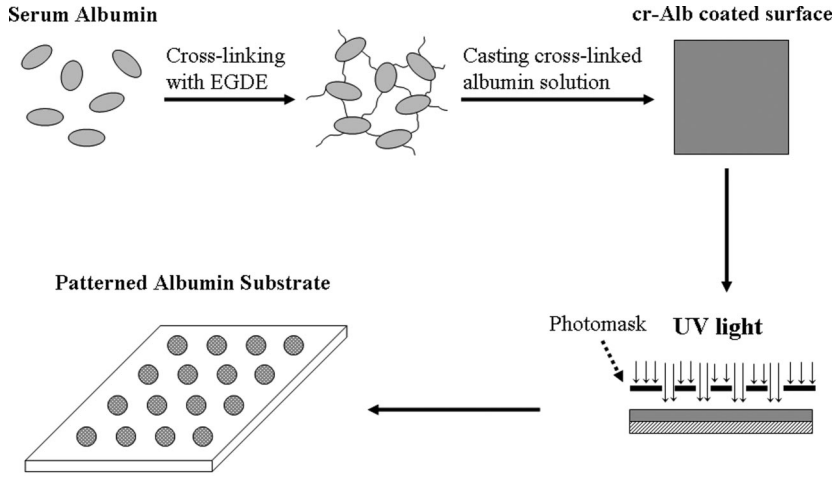

Figure 1. Schematic representation of the preparation method of the micropatterned albumin substrate.

stabilization. One milliliter of solution was poured into a 3.5 cm cell culture dish (Iwaki, Tokyo, Japan) and dried at room temperature overnight for the preparation of the cr-Alb-coated surface. To fabricate the pattern on this cr-Alb-coated surface, the cr-Alb-coated surface was exposed to a UV sterilization lamp (sterilization lamp GL15, 15 W, 254nm, Toshiba, Tokyo, Japan) equipped in a clean bench through a stainless steel mask with the desired pattern at room temperature for 5 h.

Contact Angle Measurement. The water contact angle was measured using a contact angle meter (Model CA-V, Kyowa Interface Science Co., Ltd., Saitama, Japan). A droplet of distilled water (about 1 μL) was deposited on the cr-Alb-coated surface with or without UV irradiation, and the drop shape was captured by a CCD camera. The measurement was conducted at 1 s, and then every 5 s until 176 s after the water drop was placed on the sample's surface. Measurements on five different regions of each sample were averaged.

Protein Adsorption. Bovine plasma fibronectin (Invitrogen, Carlsbad, CA) was labeled using a peroxidase labeling kit (Dojindo Laboratories, Kumamoto, Japan). The patterned albumin substrates were immersed in labeled fibronectin solution for 30 min, and subsequently washed three times with PBS. Then, the substrates were treated with 0.1% 3, 3′-diaminobenzidine in imidazole−HCl buffer containing hydrogen peroxide (pH = 7.5, Dako Cytomation, Inc., Carpinteria, CA) for 10 min. The samples were examined using a microscope (IX71; Olympus, Tokyo, Japan). All procedures were performed at room temperature.

Preparation of the Cellular Micropattern. L929 mouse fibroblast cells were grown on culture dishes in minimum essential medium (MEM, Invitrogen) supplemented with 5% fetal bovine serum (FBS, Invitrogen), 100 U/mL penicillin, and 100 μg/mL streptomycin (Nacalai, Kyoto, Japan). The cells were collected by the addition of a 0.25% trypsin/EDTA solution and resuspended in MEM supplemented with 5% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. A single cell suspension was plated over the micropatterned albumin substrates at a density of 3×10^5 cells/cm^2. After 5 h of incubation, the cells were washed five times with PBS to remove unattached cells, and then the cells were cultured for two days in a humidified 5% carbon dioxide incubator at 37 °C.

Results and Discussion

A method for the preparation of patterned albumin substrates is schematically shown in Figure 1. Albumin was cross-linked with EGDE, and was then cast on a cell culture dish. Cells did not attach to the obtained cr-Alb-coated surface, similarly to the case on the native albumin-coated surface. The cr-Alb-coated surface was subsequently exposed to UV light through a stainless steel photomask with the desired pattern to give the cell-adhesive surface. The intensity of UV light equipped in a safety cabinet for sterilization is sufficient to render the cr-Alb surface cell adhesive.

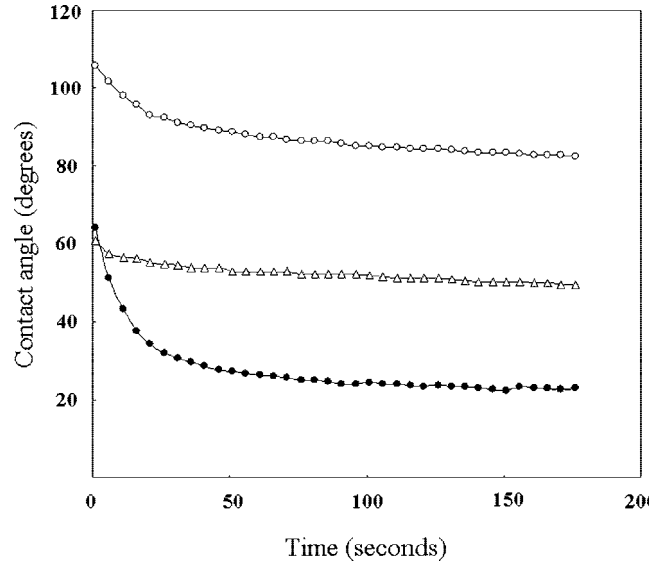

Figure 2. Water contact angle showing the wettability change of the cr-Alb-coated surface upon UV irradiation. The water droplet was placed on the cr-Alb-coated surface with (closed circle) or without (open circle) UV irradiation and on the culture dish (open triangle).

The effect of UV irradiation was analyzed by contact angle measurement, as shown in Figure 2. A water drop spread to a stationary angle within 25 s on the cr-Alb-coated and intact cell culture dishes. The water contact angle of the intact culture dish was about 53°, while that of the cr-Alb-coated dish was 85°, indicating that the cr-Alb surface is more hydrophobic than the culture dish. The wettability of the cr-Alb surface was dramatically changed from hydrophobic to hydrophilic upon UV irradiation. In addition, spontaneous spreading of the water drop took a longer time on the UV-irradiated surface as shown in Figure 2. The contact angle of the UV irradiated cr-Alb surface sharply decreased in 30 s after contact with the water drop, and gradually reached an equilibrium value (about 23°) after 70 s, suggesting that a significant structural change occurred in the albumin molecules. Since the presence of an albumin molecule on the surface after UV irradiation was confirmed by protein staining with Coomassie brilliant blue (see Figure S1, Supporting Information), the change of the surface property upon UV-irradiation is not due to removal of the cr-Alb from the surface.

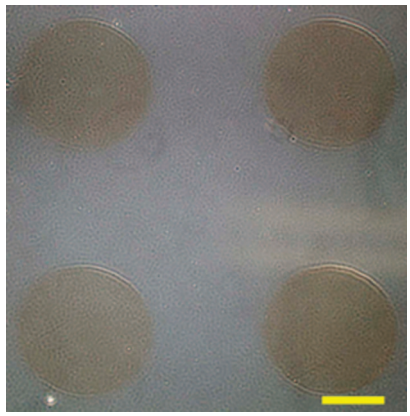

Figure 3. Selective absorption of fibronectin on the patterned albumin surface. Labeled fibronectin solution was added to the patterned albumin surface, which was prepared using a stainless steel mask with four circular windows of 1 mm diameter, and then the absorbed fibronectin was stained. Scale bar: 500 μm.

Kubasek et al. reported that photodegradation of proteins took place upon UV irradiation.[19] It is, therefore, possible that UV irradiation on cr-Alb surfaces caused partial cleavage of the polypeptide chains resulting in an increase of their mobility, which enables hydrophilic groups in albumin to migrate more easily to the surface from the interior to minimize the surface free energy when the surface contacted water. Such a surface structural rearrangement was reported to occur in cross-linked poly(2-hydroxyethyl)methacrylate hydrogel. When the surface of the hydrogel had contact with water, hydrophilic hydroxylethyl moiety migrated to the surface. Meanwhile, hydrophobic methyl groups oriented to the surface in air.[20]

Cell adhesion molecules such as fibronectin, vitronectin, and laminin play important roles in cellular function.[21–23] Hence, the cellular response, including adhesion and proliferation on the surface, is significantly affected by these proteins adsorbed on the surface. The fibronectin adsorption was compared between UV-irradiated and nonirradiated regions of the cr-Alb surface using peroxidase-labeled fibronectin. As shown in Figure 3, fibronectin adsorption took place on the UV-irradiated region, but not on the nonirradiated surrounding area. Because the native albumin-coated surface is well-known to be resistant to protein adsorption, the cr-Alb surface was thought to considerably retain the native albumin structure. The structural change induced by UV irradiation might enable fibronectin adsorption, although detailed investigation of the relationship between albumin structure and protein adsorption on the albumin is required.

When mouse fibroblast L929 cells were seeded on the patterned albumin substrate, cells attached to the UV-irradiated regions in a highly selective manner, forming circular arrays corresponding to the pattern (Figure 4a). Thus, cell adhesion on the UV-irradiated region is thought to occur through selective attachment of cell adhesion molecules such as fibrinogen. As seen in Figure 4b, cells grew well to become almost confluent on the UV-irradiated

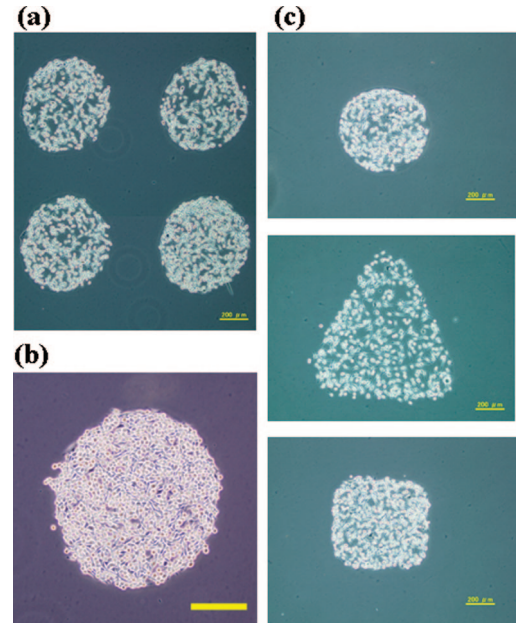

Figure 4. Patterned attachment of the mouse fibroblast cell on the albumin substrate. L929 cells were seeded on the micropatterned albumin surface, which was prepared by using a stainless steel mask with the desired pattern. The culture time and design of the mask were as follows: (a) 5-h culture; a mask with four circular windows of 600 μm diameter, (b) 2-day culture; a mask with a circular window of 600 μm diameter, (c) 5-h culture; a mask with a circular window of 600 μm diameter, with a triangular window (one side of the triangle is 900 μm) or with a square window (one side of the square is 600 μm). Scale bars: 200 μm.

regions after cultivation for two days. In addition, micropatterns formed by cells could be freely changed using a mask with the desired pattern. Figure 4c shows circular, triangular, and square micropatterns of cells. Taken together, although a detailed mechanism has not been elucidated clearly, we hypothesize that the cellular patterning on a cr-Alb-coated substrate is achieved as follows: (1) UV irradiation induces a significant structural change of albumin in the coated cr-Alb. (2) Cell adhesion molecules contained in FBS adsorb specifically on the albumin, whose native structure was disrupted. (3) Cell attachment occurred on the adsorbed cell adhesion molecules.

Conclusions

Here, we have shown a novel facile fabrication method for the cellular micropatterned substrate that does not require any special equipment and expensive materials by utilizing the UV-convertible cell adhesive property of cr-Alb. We could obtain the cell-micropatterned surface simply by coating the culture dish with water-insoluble cr-Alb and subsequent UV irradiation through a photomask. This facile and economical method is applicable to various materials' surfaces, and would contribute to the development of cell-based technologies including biosensors for the screening of drug libraries as well as the fundamental investigation of cell−cell communication.

Supporting Information Available: Methods and results of the Coomassie brilliant blue staining of a cr-Alb-coated surface before and after UV irradiation. This material is available free of charge via the Internet at http://pubs.acs.org.

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