Micropatterning Proteins and Cells on Substrates Using Electrochemical Lithography

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The micropatterning of proteins and living cells is important theme in biosensors, tissue engineering, and fundamental cell biology. Microfabrication techniques are used to generate patterns of biomolecules on surfaces. Especially, soft lithography such as microcontact printing has been recognized as a useful method because it has the advantages; biocompatibility, reducing cost and simplifying experiments [1]. We have achieved formation of the single-cell network of cardiac myocytes and neuronal cells by microcontact printing with the objective to developing bioassay chip devices based on functions of cellular networks for drug screening and assessment [2-4]. We have also reported the electrochemical method to draw a cellular pattern (we call this microelectrochemical lithography (μECL)), which is based on our finding that cytophobic nature of albumin-coated substrate can be rapidly switched to cell-adhesive by exposure to an oxidizing agent such as hypobromous acid [5]. In this present work, we report the recent progress and potential applications of the μECL.

We have found that besides albumin, a blocking agent such as heparin is also applicable to the μECL. Figure 1 demonstrates the micropatterning of proteins and cells on the heparin-coated substrate. Fluorescence micrograph shows that fibronectin-Cy3 adsorbed only onto the electrochemically treated area, forming a clear, circular pattern. The pattern size corresponds to the diffusion layer of the electrogenerated bromine species, which would quickly denature the heparin layer to allow protein adsorption. It was confirmed that various kinds of protein (antibody, enzyme, and protein A, and ECM protein such as fibronectin and laminin) can be patterned by using this procedure. Phase micrograph shows the HeLa cell populations cultured on the substrate where the pattern of fibronectin was prepared in advance. The cells adhered and spread within the area on which fibronectin adsorbed.

By taking advantage of the μECL, we attempt the following subjects:

Micropatterning on uneven substrates

The dependency of the pattern size on the distance of the electrode tip – substrate surface was investigated and cellular patterning on the deep-grooved substrate was demonstrated.

Multistep lithography

Muiti-cells patterning was attempted using μECL stepwisely, since this technique can be conducted under the condition as mild as cell culture. Also, cellular growth and migration was navigated into the electrochemically treated area which was nearby the as-prepared cellular pattern.

Integration into microchannel

The μECL-based cellular patterning technique was applied to the microchannel system. The formation of cellular pattern on the bottom surface of the channel was achieved by using the microelectrode array fabricated at the channel ceiling as the source of the oxidizing agent.

We have developed the simple but widely applicable technique to generate micropattern of proteins and cells. We believe it would be one of the key techniques to control the interface between biomolecules and materials.

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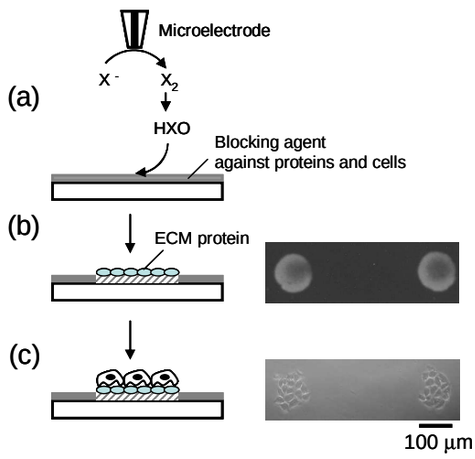

Figure 1. Example of μECL to pattern proteins and cells on a substrate. (a) A substrate is coated with a blocking agent inert to the attachment of proteins and cells. Localized treatment of the substrate by the oxidizing agent (HXO; HBrO, HClO) electrogenerated at the microelectrode, resulting in an area-selective protein adsorption. (b) The Pt disk-type microelectrode (tip diameter; 15 μm) was placed 10 μm above the heparin-coated substrate, and a potential pulse of 1.7 V vs. Ag/AgCl with 10-s period was applied to generate Br_2 (subsequently HBrO) in a 0.1 M PBS containing 25 mM KBr (pH 7.4). The patterned substrate was soaked in a solution of fibronectin-Cy3 (0.025 mg mL^{-1} in PBS) for 10 min, followed by extensive rinsing with PBS. Fibronectin-Cy3 adsorbed onto the electrochemically treated area. Subsequent cell seeding shows the selective cell adhesion onto the ECM proteins. (c) HeLa cells were patterned by culturing on the electrochemically treated substrate that was coated with fibronectin, prior to incubation with cells using the procedure described in (b).