PATTERNING ADHERENT CELLS WITHIN MICROCHANNELS BY COMBINATION OF ELECTROCHEMICAL BIOLITHOGRAPHY AND DIELECTROPHORESIS
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ABSTRACT
We report a method to create cell-adhesive regions and to position adherent cells on the newly created regions in sequence within a microfluidic channel. One of the microelectrodes fabricated at the channel wall was used for locally electrogenerating hypobromous acid that renders the opposite face of the channel protein-adsorptive. After the fibronectin was immobilized on the treated region, an ac voltage was applied to the microelectrodes array in the presence of suspended HeLa cells. Since a repulsive force of negative dielectrophoresis directs the cells toward the weakest region of the nonuniform electric field, the cells were positioned on the fibronectin-patterned region to allow the cell adhesion, even in the presence of fluid flow.

KEYWORDS: Biolithography, Cell adhesion, Dielectrophoresis, Microfluidic device

INTRODUCTION
Recently, we developed a method for locally creating cell-adhesive regions in preassembled microfluidic devices by taking advantage of the electrochemical biolithography [1,2]. This approach is much suitable to pattern cell-adhesive proteins (subsequently cells) on the channel surface because such unstable materials are not subjected to denaturing conditions such as desiccation and heat usually accompanied with the device assembly. However, since cells introduced into the microchannel are randomly distributed, only cells that happen to settle on the adhesive regions can attach to the surface. To efficiently induce patterned cell adhesion on the channel surface, it will be required for cells to be placed only on the adhesive regions. Cell manipulation by dielectrophoresis (DEP), wherein ac electric fields from shaped electrodes produce forces by coupling to the induced electric dipole moments of the cells, is readily applicable to microfluidic system by assembly of

Figure 1. Schematic representation of cell patterning within a microchannel by sequentially applying electrochemical biolithography and negative DEP.
the device with microelectrode arrays [3].

In this paper, we describe a method to create cell-adhesive regions and to position adherent cells on the newly created regions in sequence within a microfluidic channel by combination of electrochemical biolithography technique with negative DEP (Fig. 1). Since the electrodes fabricated on the channel surface can be used both for electrogenerating the oxidant and for producing DEP forces, the electrodes configuration is very simple enough not to sacrifice miniaturization of microfluidic systems.

EXPERIMENTAL

We prepared a microfluidic device with a parallel array of three Pt microband electrodes (20 µm band width, each separated by 50 µm) at the upper wall of the channel (Fig. 1a). The channel was 400 µm in width and 100 µm in depth. After an antibiofouling layer of polyethyleneimine and heparin (PEI/hep) was formed on the channel surface, phosphate buffered saline (PBS) containing 25 mM KBr was introduced into the channel and a potential pulse of 1.7 V vs. Ag/AgCl with a period of 0.5 sec was applied to the central electrode of the parallel array (Fig. 1b). This procedure resulted in electrochemical oxidation of Br⁻ to Br₂ (subsequently HBrO) at the electrode to give a protein-adsorptive region on the opposite face of the channel [1]. Then, a solution of fibronectin was added, followed by a wash with PBS (Fig. 1c). Subsequently, GIT medium (13 mS cm⁻¹) containing HeLa cells (2 x 10⁶ cells mL⁻¹) was introduced into the channel, and an ac voltage (20 Vpp, 1 MHz) was applied to the electrodes array with a function generator to concentrate cells on the fibronectin-patterned region by a repulsive force of negative DEP (Fig. 1d). After the DEP operation for 5 min, cell-free medium was flowed through the channel to remove nonadherent cells (Fig. 1e).

RESULTS AND DISCUSSION

Figure 2 shows HeLa cells patterned within a sealed microchannel according to the procedure shown in Fig. 1. The electrochemical lithographic treatment was first con-

Figure 2. (a) Micrograph of a parallel array of three microband electrodes fabricated at the upper wall of the microchannel. (b, c) Fluorescence images of (b) fibronectin (labeled with Cy3) locally immobilized on the channel bottom wall and (c) HeLa cells (stained with CellTracker Green) adhered on the fibronectin region after applying the DEP force. (d) Plot of the numerically calculated electric field in the horizontal plane at the channel bottom wall when the potential difference between the central electrode and the other two electrodes at the upper wall was set at 20 V. The electric field distribution was simulated using the finite element method.
ducted to create cell-adhesive region on the channel surface by using the central electrode of the parallel array at the upper wall (Fig. 2a). As seen in Fig. 2b, line pattern of Cy3-labeled fibronectin (line width, 100 µm) was formed on the bottom wall, opposite face of the electrode. Subsequently, a suspension of HeLa cells was introduced into the channel, followed by the application of ac voltage between the central electrode and the other two electrodes. Since HeLa cells experience a repulsive force in GIT medium under the 1 MHz ac electric field, cells rapidly moved to regions of weaker electric fields within 15 sec, especially to the fibronectin-immobilized region just below the central electrode. It was observed that the cells adhere on fibronectin after incubation for 5 min under the ac voltage application (Fig. 2c). Meanwhile, cells concentrated on other regions of weaker electric fields (the foot of each peak in Fig. 2d) were removed by flowing the medium since they could not adhere to the antibiofouling layer of PEI/hep.

Figure 3 shows the plots of the capture efficiency of HeLa cells in the fibronectin-immobilized region with (●) or without (○) applying the DEP force versus the flow rate. The capture efficiency was evaluated as a rate of the number of cells attached to the number of whole cells flowing for 5 min. With applying the DEP force, the cell capture efficiency was almost 100% at flow rates lower than 0.1 µL min⁻¹. Therefore, after the first cell type was patterned on the channel surface, the second cell type could be captured in the fibronectin-immobilized region under the fluid flow by using another set of the microelectrodes array without cross-contamination of these cell types (Fig. 4).

REFERENCES