Surface-Displayed Antibodies as a Tool for Simultaneously Controlling the Arrangement and Morphology of Multiple Cell Types with Microscale Precision

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ABSTRACT Cell−cell interactions are considered to play critical roles in the development and physiology of most tissues. However, it is not straightforward to analyze cell−cell interactions with conventional cell culture in which cells are randomly distributed. To overcome this limitation, we employed here an antibody display to sort different cell types onto separate regions on a single substrate with microscale precision, taking advantage of the specific recognition of cell surface markers by surface-displayed antibodies. The results obtained with two sets of cell combinations, T cell/myelomonocytoid cell and neuron/astrocyte, demonstrate that antibody displays are feasible to establish a site-addressable coculture.

KEYWORDS: multicellular • coculture • cell−cell interaction • surface marker • neuron • astrocyte

INTRODUCTION

Most biological tissues are constructed from diverse cell types that undergo heterophilic interactions with each other. Such cell−cell interactions are mediated by membrane-bound molecules, junctional complexes, and diffusible factors. Thorough investigations of cell communication are needed to gain deeper insights into the developmental processes and pathological conditions in various tissues. However, current in vitro cell culture techniques do not always permit the detailed analysis of cell−cell interactions because cells are randomly distributed in a culture.

Here we report on the novel method that allows one to establish the coculture of multiple cell types in a way that the arrangement of each cell type is controlled with microscale precision. The method is therefore expected to provide an in vitro analytical platform suitable for the investigation of heterophilic cell−cell interactions at a single-cell level. Our approach to establishing a coculture system is to utilize the spatially controlled display of different antibodies that recognize specific surface markers expressed on individual cells of interest.

The spatially controlled display of biomolecules can be readily created with the microcontact printing (μCP) method using a microfabricated elastomeric stamp (1). In particular, many studies have been made to create the micropatterns of biomolecules by the μCP method, including cell adhesive substances such as extracellular matrix components and functional polymers for establishing the micropatterned culture of mammalian cells (2−5) and an antibody for capturing *Escherichia coli* on a substrate for cell-based detection (6). In contrast to these previous studies, we utilize here the μCP technique to display multiple antibodies against cell surface markers in a spatially controlled manner on a single substrate.

In this study, we examined the feasibility of antibody displays for controlling the arrangement of multiple cell types with microscale precision. Two different combinations of cells were employed to provide a proof of principle for our strategy: One is the combination of nonadherent cell lines including T cells and myelomonocytoid cells, and the other is the combination of primary cultured hippocampus neurons and cortical astrocytes. Antibodies against the surface marker of these cells were patterned as microlines on glass plates. We show that different types of cells can be separately sorted to microscale regions on account of specific recognition of cell surface markers by printed antibodies.

EXPERIMENTAL PROCEDURES

Antibody Display. Poly(dimethylsiloxane) stamps purchased from Hitachi High-Technologies Corp., Tokyo, Japan, had stripes consisting of ledges (width and height 10 μm and length 10 mm) running unidirectionally with 100 μm intervals between ledges. One of the stamps was inked with a solution of the first antibody and contacted to the (3-aminopropyl)triethoxysilane (APTES)-treated glass slide to transfer the antibody to the glass surface. Then, another stamp inked with the second antibody solution was contacted to the glass surface in a crossing direction to the previously transferred antibody lines. We used antibodies against CD5 (CD5-Ab), CD33 (CD33-Ab), the neural cell adhesion molecule (NCAM-Ab), and CD44 (CD44-Ab). For visualization of micropatterns, antiimmunoglobulin G antibodies conjugated with a green or red fluorescent dye (IgG-Ab-G and IgG-Ab-R) were used. Glass slides with printed antibodies were blocked with 2% bovine serum albumin.

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T Cells and Myelomonocytoid Cells. T cells (CCRF-CEM) were fluorescently stained with PKH26 (Sigma), whereas myelomonocytoid cells (HL-60) were stained with PKH67 (Sigma). The fluorescently labeled CCRF-CEM and HL-60 cells were suspended in 1 mL of phosphate-buffered saline (PBS) containing 1 mg/mL human γ-globulin and 0.53 mM ethylenediaminetetraacetic acid. Cells were plated to the glass plate with an antibody display at a density of 3.0×10^5 cells/cm^2 and incubated for 30 min to effect cell binding. Then the plate was gently washed with PBS to remove unbound cells. Cells were fixed with a 4% glutaraldehyde solution and observed with an epifluorescent microscope.

Neurons and Astrocytes. The hippocampus and the cerebral cortex were isolated from the fetus of Wister rats (E18) and dissociated into single cells by trypsin digestion (7). All animal experiments were conducted according to the guidelines of the Animal Welfare Committee of the Institute. Dissociated cells from the cerebral cortex were cultured in a type I collagen-coated polystyrene dish (8) and passaged 2−3 times to enrich astrocytes. Astrocytes harvested from the dish were stained with PKH67 and plated to the substrate displaying crossing microlines with NCAM-Ab and CD44-Ab. After a 1-day culture, the astrocytes were treated with the conjugate of poly(ethylene glycol) (M_w = 5000) with 1,2-dipalmitoyl-*sn*-glycerol−3-phosphatidylethanolamine [poly(ethylene glycol) (PEG)−lipid] (9) to tether PEG chains to the cell membrane. Then the medium was replaced with a suspension of PKH26-stained hippocampus neurons. The cells were further incubated for 2 days and observed with an epifluorescent microscope.

RESULTS AND DISCUSSION

The simultaneous positioning of multiple cell types is not straightforward by the individual and serial cell seeding onto micropatterns of cell adhesive extracellular matrix components or functional polymers, such as laminin and poly-L-lysine, because these proteins and polymers nonspecifically interact with various types of cells. We implement here the display of different antibodies specific for cell surface markers to simultaneously control the arrangements of multiple cell types on a single substrate.

As a basement substrate, a glass plate modified with APTES was used because antibody microlines were most uniformly printed on this amine-functionalized surface compared with surfaces with other chemistries (Figure S1 in the Supporting Information). As shown in Figure 1a, the uniform distribution of antibodies is seen for both stripes with a clear contrast at the edge of the printed regions and with no sign of cross contamination between antibodies. The exception was the crossing areas where two antibodies were superimposed. Each antibody type may have different abilities to survive the microcontact printing step, which has an influence on the density of captured cells.

Figure 1b shows the micrograph of fluorescently stained CCRF-CEM and HL-60 seeded to the substrate on which CD5-Ab and CD33-Ab were displayed as a crossing stripe pattern. Flow cytometric analyses showed that CCRF-CEM expresses CD5 but not CD33, whereas HL-60 exhibits an opposite expression profile (data not shown). As is obvious from Figure 1b, CCRF-CEM cells were trapped on CD5-Ab microlines, while HL-60 cells were on CD33-Ab microlines, with a negligible number of cells on the regions between microlines. The observed cell arrangements imply that one can individually locate two types of nonadherent blood cells with microscale precision on a single substrate taking advantage of the interactions between surface antigens and printed specific antibodies.

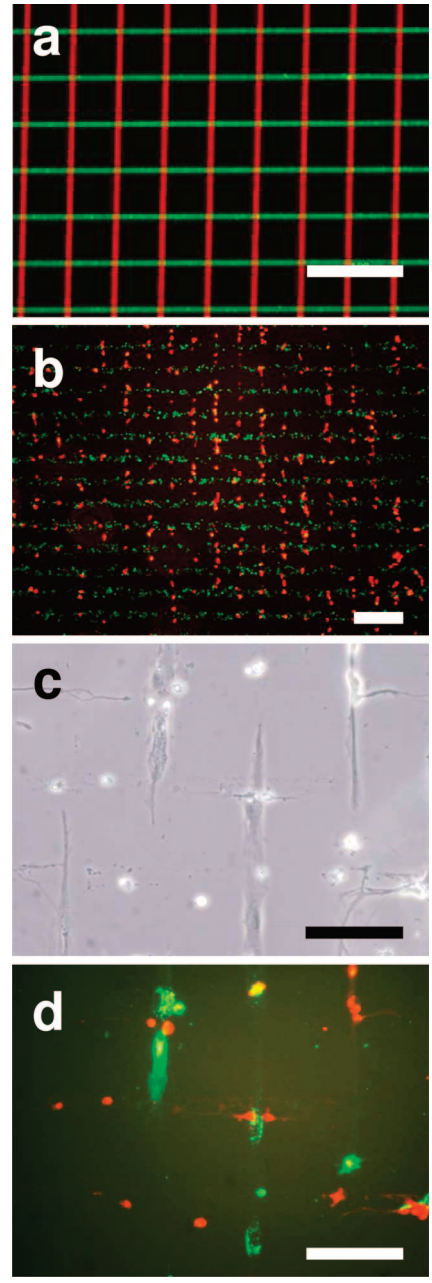

FIGURE 1. Micrographs of (a) an antibody display and (b−d) cellular arrangements. (a) Merged fluorescent micrograph of crossing micropatterns prepared by stamping IgG-Ab-G (horizontal microlimes) and IgG-Ab-R (vertical microlines) to the APTES-treated surface. (b) Merged fluorescent micrograph of fluorescently stained CCRF-CEM (red) and HL-60 (green) seeded to the substrate displaying CD5-Ab (vertical) and CD33-Ab (horizontal) microlines. (c) Phase-contrast and (d) fluorescent images of neurons (red) and astrocytes (green) cocultured on the substrate displaying NCAM-Ab (horizontal) and CD44-Ab (vertical) microlines. Previously patterned astrocytes were treated in situ with a PEG−lipid conjugate, and then neurons were seeded. Micrographs c and d were acquired for the same sight. Bars: (a and b) 200 μm; (c and d) 100 μm.

The feasibility of our technique was further examined with primary-cultured hippocampus neurons and cortical astrocytes. When the mixture of neurons and astrocytes was plated to the micropattern that consisted of crossing micro-

lines of NCAM-Ab and CD44-Ab, astrocytes were sorted selectively on the CD44-Ab lines, while neurons attached frequently to the astrocytes instead of NCAM-Ab lines (Figure S2 in the Supporting Information), plausibly because of neuron−astrocyte association mediated by cell adhesion molecules (10). To weaken such cell−cell interactions, astrocytes adhering to the micropattern were treated with PEG−lipid to tether PEG chains to the cell membrane followed by seeding of neurons. As shown in parts c and d of Figure 1, neurons and astrocytes were sorted to respectively NCAM-Ab and CD44-Ab microlines. Neurons extend neurites on NCAM-Ab microlines, while astrocytes are constrained on CD44-Ab microlines. Importantly, some of the neurons and astrocytes have heterologous cell−cell contacts although the density of the contacts is not very high.

NCAM, a member of the immunoglobulin superfamily cell adhesion molecules, is known to be expressed strongly on neurons (11). Although astrocytes are also known to express NCAM, the expression level is modulated during their maturation (12) and long-term culture (13). On the other hand, CD44, a hyaluronic acid receptor, is expressed specifically on astrocytes (14). Such specificities are the basis of our technique for cell arrangement. For practical applications, it is worth quantitatively studying the density of captured cells and cell−cell contacts as a function of time, as well as their fidelity to surface patterns. We harvested neurons and astrocytes by trypsin digestion, which might have affects on the reactivity of surface antigens. However, no adverse effect of the typsin treatment was observed in the present study, being similar to our previous observation (15).

In this letter, we demonstrate that the display of different antibodies specific for cell surface markers can be suitably used to control the arrangements of different cell types on a single substrate. It may be possible that our method will be extended for establishing coculture with more than three different types of cells. Though no practical metrics are shown in this letter, the method described here provides a technical basis for the study of heterologous cell−cell interactions. To allow more effective cell−cell contacts, micropattern design should be optimized in compliance with the specific purpose of an analysis.

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Supporting Information Available: Details of the experimental procedures, the images of antibody displays, and the images of simultaneously seeded neurons and astrocytes. This material is available free of charge via the Internet at http://pubs.acs.org.

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