Single Cell Array of Biotinylated Cells Using Surface Functionalization and Microcontact Printing

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This paper describes a versatile method for generating single cell arrays on a glass substrate, which could be applicable to any arbitrary cell types, by a combination of surface functionalization, biotinylation of cells, and microcontact printing (μCP).

There has been a great deal of interest in the controlled attachment of cells onto the defined surface areas (cell patterning), which is an important step toward the development of high-throughput cellular analysis system, bioelectronics, tissue engineering, and cell-based biosensors.[1] A variety of techniques have been developed for creating micrometer-sized cell-adhesive and cell-resistant regions on substrates in order to pattern extracellular proteins and cells. These techniques include photolithographic patterning of siloxanes with selective functional groups,[2] photochemical immobilization of biomolecules,[3] microcontact printing (μCP) of self-assembled monolayers (SAMs),[4] patterning of polyelectrolyte multilayers (PEMs),[5] patterning through microwalls with elastomeric membranes and poly(ethylene glycol) (PEG),[6] and patterning using three-dimensional microfluidic channels.[7]

The micropatterns of cells have been generated mainly by employing adhesion receptor ligands such as RGD peptides[8] and fibronectin.[9] It has been, therefore, difficult to extend this strategy to other cell types and microorganisms because suitable receptor ligands, which can be used for the pattern generation through biospecific interactions, have not been identified yet. For example, important cell types, such as certain types of stem cells, nonadherent lymphocytes and certain tumor cells, are weakly adherent or nonadherent. Researchers including us recently reported the pattern generation of nonadherent cells through biospecific interactions.[10] Hammond and collaborators generated a pattern, composed of streptavidin and anti-CD44 antibody, on surfaces via polymer-on-polymer stamping (POPS) of poly(allyamine)-*g*-poly(ethylene glycol) onto multilayers of poly(ethylenimine) and poly(acrylic acid). The patterned areas containing both streptavidin and anti-CD44 antibody were then used as a template for the attachment of biotinylated B cells through the biospecific interactions, biotin-streptavidin and CD-44–anti-CD44 antibody.[10a] In addition, we have previously reported the pattern generation of nonadherent spores by a combination of spore surface display technique and μCP.[10b] In particular, the patterned spores could germinate to vegetative cells by controllable external stimuli while providing advantages of long-term storage and outstanding stability. In this report, we demonstrate a generation of two-dimensional single cell arrays based on the biospecific biotin–streptavidin interaction between biotinylated cells and the glass surface which was functionalized to present the pattern of streptavidin. The procedure used in this paper involves: (1) biotinylation of cells using sulfosuccinimidyl-6-(biotin-amido)hexanoate (sulfo-NHS-LC-biotin), which provides simple biotin labeling to cells (e.g., adherent and nonadherent cells); (2) generation of the streptavidin pattern on glass substrates which can be used as a template for single cell arrays.

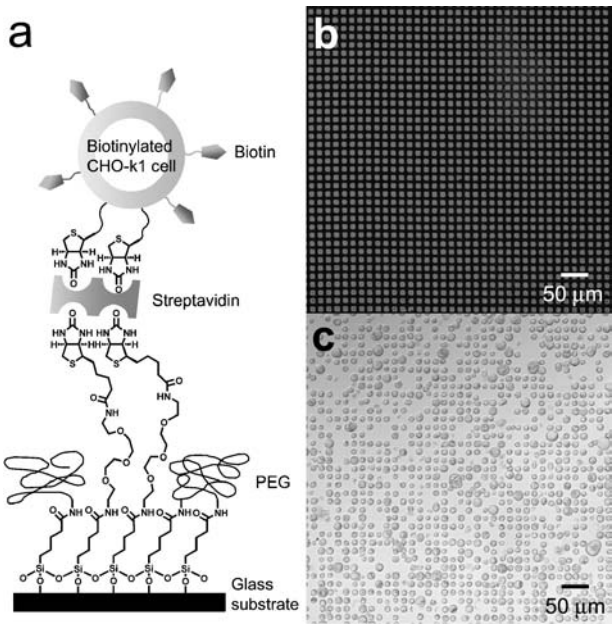

Figure 1. (a) Schematic representation of the procedure for generating a single cell array of biotinylated CHO-k1 cells. (b) Fluorescence micrograph of biotin patterns on a glass substrate. (c) Single cell array of biotinylated CHO-k1 cells.

Figure 1a shows a schematic representation of the procedure for the generation of single cell arrays of biotinylated cells.[11] To achieve the covalent attachment of amine-terminated biotin onto glass substrates, we used a method of common reactive intermediates, pentafluorophenyl (PFP) esters. We introduced the PFP ester groups onto the surface and reacted the activated surface with the amine group of the biotin ligand ((+)-biotinyl-3,6,9-trioxaundecanediamine) by μCP because the PFP ester groups were easily coupled with amines, leading to the formation of amide bonds. The PFP ester-terminated SAMs were prepared from vinyl-terminated SAMs by following the two-step surface reactions reported previously.[12] After patterning the biotin ligand on the glass surface, we passivated the other area with

mPEG-amine (MW:5000).[10b] We used the ethylene glycol-terminated amine to render underivatized regions resistant to cells because ethylene glycol is known to be the most effective functional group in minimizing non-biospecific adsorption of proteins, cells, and bacteria.[8,10b] The substrate was then incubated in a PBS solution of fluorescein (TRITC)-conjugated streptavidin containing bovine serum albumin and Tween 20.[13] Successful patterning of biotin was verified by fluorescence microscopy after complexation with TRITC-conjugated streptavidin (Figure 1b). Streptavidin has two pairs of binding sites on opposite sides. One pair was used to the complexation with the pattern of biotin on the glass substrate, leaving the other pair on the opposite side, which would be a useful binding site for further complexation of desired biomolecules.[10,12] Finally, the conjugation of biotinylated cells to the streptavidin-patterned surface led to the formation of a cell array.

To provide a specific anchor for single cells on the streptavidin-patterned surface (the patterns were square-shaped with a lateral dimension of 10 μm in width separated by 5 μm), sulfo-NHS-LC-biotin was immobilized onto the surface of cells. Sulfo-NHS-LC-biotin enables simple and efficient biotin labeling of antibodies, proteins, and any other primary amine-containing molecules and cells in solution, leading to the formation of amide bonds. We labeled live CHO-k1 cells with biotin to introduce additional high affinity of the cell-surface interaction: the biotin molecules on the cell can participate in the surface binding via complexation with the unoccupied binding sites of streptavidin on the streptavidin-patterned surface. The fabrication of single cell arrays was completed with simple washing following the seeding of biotinylated CHO-k1 cells onto the streptavidin-patterned surface. The optical micrograph shows the resulting cell arrays: although CHO-k1 cells were arranged over a large area, we observed some imperfections, such as empty spots presenting no cells and the aggregation of cells in interstitial spaces (Figure 1c). The increase of the cell seeding density eliminated some empty cell sites, but the high seeding density also had some drawbacks due to the increased occurrence of cell aggregation formation. Once the aggregation occurred, even bound cells were detached from the surface with the aggregates, leaving empty spots and the clumps of cells on the cellular array. Another factor of the aggregation could be the space between the squares (5 μm): the aggregate of cells might be capable of being attached to several spots simultaneously because the space was not sufficiently separated. However, the method demonstrated herein, utilizing the biospecific streptavidin-biotin interaction to attach biotinylated cells to the substrate, can easily be generalized to a large number of cell types by the immobilization of biotin onto the cell surface, and is of great interest because of its flexibility. A control experiment was also conducted with trypsinized CHO-k1 cells (i.e., non-biotinylated cells). In this case, we did not obtain a reproducible single cell array of CHO-k1 cells, and the cells were detached from the streptavidin-presenting arrays irreproducibly, which clearly showed the importance of the biotinylation in increasing the binding affinity of the cells to the surface.

As a demonstration that the pattern of streptavidin can also be used for controlling the spatial distribution of cells, the biotinylated CHO-k1 cells were attached onto the streptavidin-patterned surface (the lateral dimension of 50 μm separated by 50 μm) and they were cultured for 24 h. Figure 2 shows phase contrast images of the 24-h cultured cells: the cells are confined only in the areas presenting streptavidin.

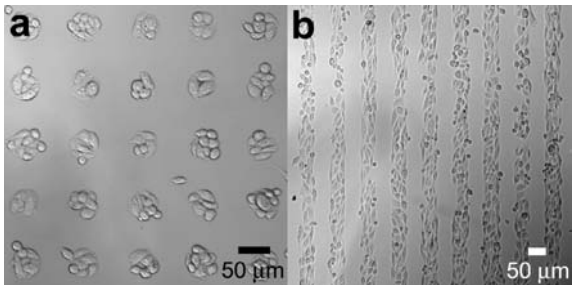

Figure 2. Optical micrographs of cell patterns.

In summary, we demonstrated a simple method for generating a single cell array based on the biotinylation of cells and surface functionalization. The method could be applicable to a wide variety of cells that are amenable to the biotinylation. It will be the next step to investigate the effect of the space between streptavidin-presenting areas on the generation of uniform single cell arrays.

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