Cell Patterning

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Rapid Patterning of Cells and Cell Co-Cultures on Surfaces with Spatial and Temporal Control through Centrifugation**

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The ability to spatially and temporally control the interactions between cells and materials is important for a wide variety of applications, ranging from tissue engineering[1] and cell-based microarrays[2] to fundamental studies of cell growth, adhesion, and migration.[3] Although the patterning of one cell type onto a range of materials has become routine, the ability to pattern multiple cell lines with spatial and temporal control of cell-population interactions remains technically challenging, and unavailable or impractical for access by the larger biological community.[4] A simple, fast, and inexpensive strategy to generate multiple-cell patterned arrays would greatly expand the current scope of cell biology research and generate new co-culture array screens, tissue-patterning materials, and cell-based devices.

Herein, we report a methodology that combines surface chemistry with soft lithography[5] and centrifugation for the rapid, inexpensive, and complete patterning of cells and cell co-cultures on surfaces with spatial and temporal control. By employing microcontact printing in conjunction with polydimethylsiloxane (PDMS) masks and centrifugation, we achieved the patterning of co-cultures on substrates with feature sizes as small as 30 μm. This strategy can routinely and rapidly immobilize single cells that are separated by as little as 100 μm, which provides exquisite spatial control for autocrine and paracrine signaling studies.[6] In addition, this methodology allows the preparation of geometrical patterns of co-cultures, for studies of the influence of shape and cell population on cell behavior.

First, we show that this strategy is able to pattern a single cell type rapidly and efficiently on small features to generate a cell microarray (Figure 1). As our surface, we used self-assembled monolayers (SAMs) of alkanethiolates on gold. Microcontact printing of 1-hexadecanethiol onto the gold surface was performed to generate hydrophobic patterns on the surface. These substrates were then soaked in a solution (1 mM) of tetraethylene glycol terminated alkanethiols (12 h) to complete the monolayer. The ethylene glycol group is essential to prevent nonspecific cell adhesion to the surface.[7] After completion of the SAM, an extracellular matrix protein, fibronectin (0.1 mg mL^{-1}), was added to the substrate (2 h) and was adsorbed onto the hydrophobic regions.[8] This promoted cell adhesion in the patterned regions and left the remainder of the surface inert.

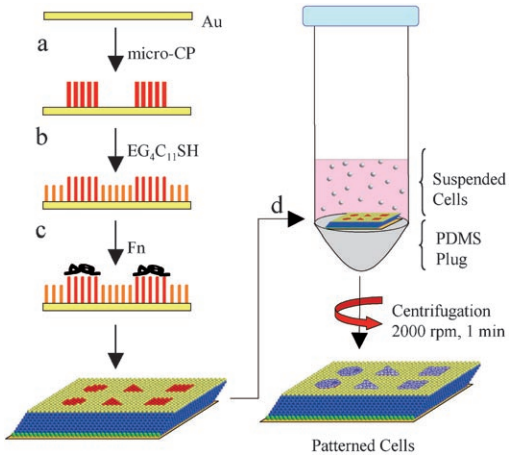

Figure 1. Methodology to rapidly pattern cells on SAMs. a) Gold substrates were patterned with hexadecanethiol by microcontact printing. b) The patterns were then backfilled with tetraethylene glycol (EG_4)-terminated alkanethiolates to create inert and hydrophobic regions on the surfaces. c) Fibronectin (Fn) was selectively adsorbed to the hydrophobic regions to present ligands for cell adhesion. d) The substrate was placed in a centrifuge tube and a minimal volume of cells in medium was added. Upon mild and rapid centrifugation, the cells adhered only to the patterned surface.

Centrifugation is a standard technique that is used in several fields, including biochemistry and cell biology, to separate components based on their size, shape, or molecular weight.[9] We employed centrifugation with SAMs to rapidly pattern cells and other materials (unpublished results) to surfaces. In particular, the centrifugation-assisted patterning strategy enables cells to adhere to substrates that present nonideal surface properties. For example, the standard procedure calls for the addition of large volumes of medium containing a high density of cells to a substrate, and then Brownian motion causes the cells to contact the surface infrequently, which eventually results in cell attachment.[10] Our novel strategy generates a layer of cells that are forced to sample the entire surface simultaneously. With this approach, cells can interact with small features and low densities of immobilized ligands on the substrate to ensure adhesion. To determine the centrifugation conditions, minimal volume of medium, and cell-seeding densities required for rapid pat-

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terning, we chose to first immobilize a single cell type on small (30-μm) pattern features.

Cells (Swiss 3T3 fibroblasts) were suspended in serum-free medium (100 μL) at a concentration of 100000 cells per mL and added to the patterned substrate in the centrifuge tube. To provide a flat surface for the substrates during centrifugation, a PDMS plug, made from an elastomer kit, was created at the base of the concave centrifuge tube. One round of centrifugation at 2000 rpm for 1 min was all that was required to generate a completely filled array of cells on the surface with no empty regions (Figure 2). The complete

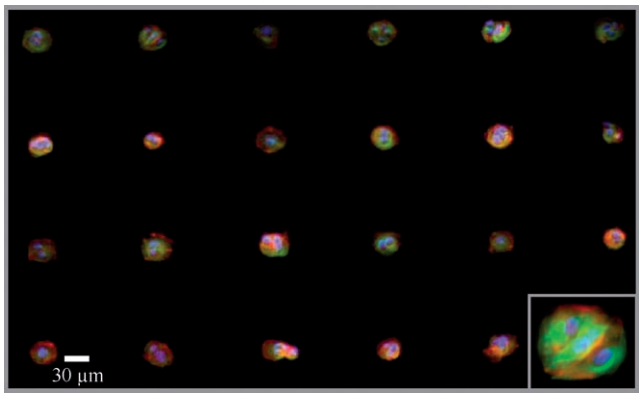

Figure 2. Fluorescence micrograph of cells adhered to a patterned array of 30-μm circles by centrifugation. Complete patterns of cells on arrays of small features are readily and rapidly generated by centrifugation. Inset: one feature that has three immobilized cells (5-fold magnification).

pattern of cells is an important result in itself, because the standard method of seeding cells onto a surface to generate small patterns requires much longer durations (hours) and greater volumes of medium with cells (5–10 mL). It is also difficult to generate a complete array of patterned cells on small features—there are usually several empty regions because of incomplete cell attachment on the surface.[11]

To extend this strategy to generate co-cultures with precise spatial control, we introduced the use of a PDMS mask to selectively hide and reveal adhesive regions on the substrate. This allows for sequential centrifugation and, therefore, the adhesion of different cells to the surface with spatial and temporal control (Figure 3). Surfaces were patterned with 200-μm fibronectin-coated circles and trapezoid features. A PDMS lift-off membrane was generated and placed onto the patterned surface, thus blocking the trapezoidal patterns and exposing only the circular regions. Medium (100 μL) containing serum and fibroblasts (50000 cells mL^{-1}) was added to the substrates, which were centrifuged at 2000 rpm for 1 min and then placed in an incubator overnight.

Cell patterns were clearly recognizable by phase-contrast imaging (Figure 4a). Before the second round of centrifugation, a fluorescent dye (CellTracker Green CMFDA) was added to the cells patterned on the gold substrates (round patterns in Figure 4). To generate co-cultures, the cell-patterning process was then repeated without the PDMS lift-off membrane. In the absence of the mask, a new adhesive

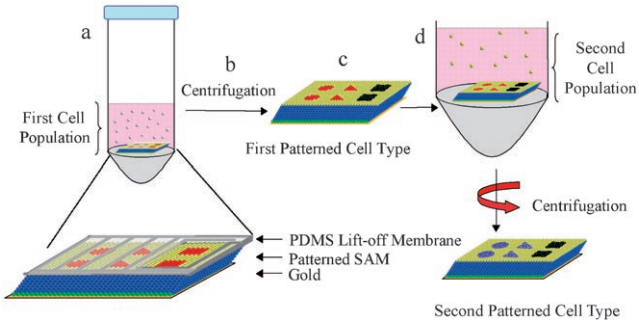

Figure 3. Schematic diagram of centrifugation of multiple cell types onto a surface. a) A masked substrate was placed in a Falcon tube that was corked with PDMS to create a flat surface. A minimal volume of cells in medium was added to the substrate. b) The sample was centrifuged for 1 min at 2000 rpm. c) The mask was removed from the substrate to reveal a new region for cell adhesion. d) Centrifugation was repeated with a second cell line, which produced a spatially controlled co-culture.

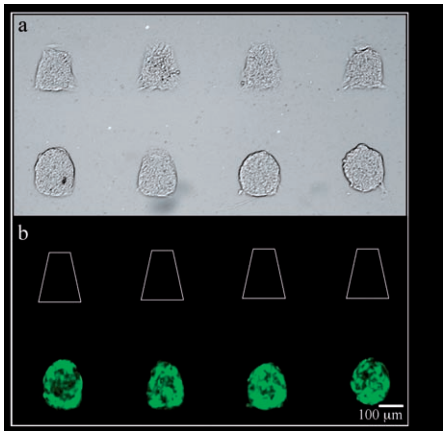

Figure 4. Sequential patterning of fibroblasts onto SAMs. a) After two rounds of centrifugation, two lines of cells are present and form a complete set of geometrical patterns. b) By fluorescence microscopy, only the first cell population is visible because these cells were exposed to a fluorescent dye.

region (trapezoidal patterns) was available for rapid cell patterning by centrifugation. After allowing the second population of cells to grow into the patterns, newly formed geometries and co-cultures were generated on the surface. Based on the ability to seed a second cell line, we also discovered that the PDMS mask, despite being in physical contact with the surface, did not damage the integrity of the substrate for subsequent cell adhesion. The SAM and the fibronectin both remained fully functional during the rapid centrifugation process. Furthermore, because the first cell pattern is complete there are no regions available for the second cell type to mix with the first patterned cell type. This may allow the ability to pattern a diverse set of cells across a wide range of surfaces.

To demonstrate that this co-culture strategy is also compatible with various other cell lines and surfaces, we repeated the spin-down patterning with a green fluorescent protein (GFP)-transfected *Drosophila* cell line. Triangular features were prepared on the surface by microcontact

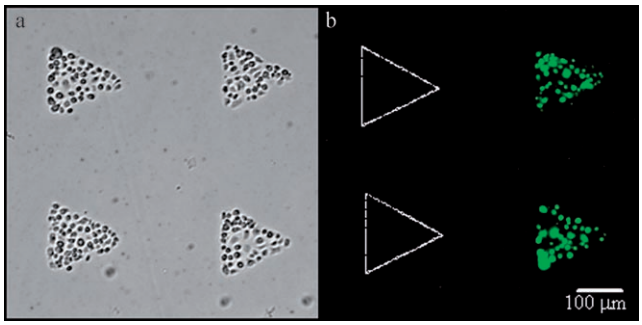

Figure 5. Sequential patterning of GFP-transfected *Drosophila* S2 cells and wild-type cells onto SAMs. a) An array of triangles demonstrates that cells were patterned. b) Only the cells that express GFP are visible by fluorescence microscopy, which confirms the presence of a co-culture.

printing, and a lectin (concanavalin A, 0.3 g mL^{-1}, 1 h) was immobilized on the patterns to induce *Drosophila* cell adhesion.[12] A PDMS membrane was placed on the surface, which blocked a portion of the triangle pattern array, and GFP-transfected *Drosophila* cells were centrifuged onto the surface (100 μL, 50000 cells mL^{-1}, 2000 rpm for 1 min), thus producing patterns immediately. Subsequent removal of the PDMS mask and centrifugation of wild-type *Drosophila* cells onto the surface showed a clear co-culture of the two cell lines (Figure 5). This result shows that the strategy is compatible with other cell lines, adsorbed proteins, and potentially high-throughput co-culture screening arrays.

The methodology described herein can be used to study interactions between different cell populations with exquisite control of pattern feature size, geometry, and proximity. We have demonstrated the ability to reproducibly immobilize as few as one or two cells in patterns and co-cultures. In addition to studying cell–cell interactions, these surfaces can be used for high-throughput co-culture screening assays. Cell-based microarrays have become powerful tools for drug discovery, gene-function assays, cell-migration and -growth studies, and tissue engineering material screens. This strategy is complementary to many cell-based assays and provides the potential for a straightforward and inexpensive approach to creating new types of microarrays for homotypic/heterotypic, autocrine, and paracrine signaling interactions.[6,13] This approach may also be used to pattern or immobilize nanomaterials, biomolecules, and cells to nonideal surfaces, because centrifugation forces the molecules to interact and sample all parts of the surface. As the substrates are composed of SAMs, the surface properties can be tailored to mimic many different types of biological systems.

In summary, we have developed a straightforward, simple, and inexpensive method for creating both co-cultures and cell-based microarrays. By sequentially masking regions of the surface with a PDMS mask, centrifugation allows the patterning of multiple cell lines while maintaining spatial control. Patterns were created with feature sizes as small as 30 μm, to immobilize one or two cells per feature. This technique also allowed different cell lines to be spaced as close as 100 μm apart. The utility of this method is general and it can be applied to many fields of study other than biology. The rapid centrifugation/masking technique can be used in material coating development, electronics, and other nanomaterial patterning or extrusion applications. The mask provides an extremely flexible and inexpensive way to selectively expose regions of a substrate, while the centrifugation ensures the complete deposition of a nanomaterial from solution.

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