Biopatterning using PEG microstructures and polymeric thin films

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An improved technique for selecting and immobilizing biomolecules in microstructures promises more-effective patterning of proteins and cells for microtechnology applications.

Patterning biomolecules in well-defined microstructures is critical for developing biosensors, high-throughput screening apparatuses, biochips, labs on a chip, and tissue engineering. But creating microstructures capable of immobilizing biomolecules is difficult because the physical and chemical barriers separating the patterns are indistinct. One of the most frequently used methods of patterning is microcontact printing. This technique employs a microstamp to transfer molecular ‘ink’—in this case, organic self-assembled monolayers (SAMs)—to a surface. Physical barriers are created using a second technique, called capillary force lithography. These approaches have been successfully demonstrated. But they require delicate chemical steps, such as gold sputtering, microcontact printing of polyethylene glycol (PEG), and tedious surface modification of the patterned region.

This study presents a method that is similar to rapid biomolecule patterning using micromolding in capillaries (MIMIC), based on soft-lithographic fabrication of PEG microstructures and simple surface modification using polyelectrolyte multilayers (PELs). The technique creates spatial barriers that prevent nonspecific biomolecule binding. The biomolecules are selectively bound to the patterned region primarily through electrostatic interactions.

Figure 1 illustrates our approach, which consists of three main steps: preparing the polyelectrolyte-coated surface; MIMICing the PEG; and fabricating PEG microstructures via UV polymerization. MIMIC produced two distinctive regions. One contained PEG microstructures fabricated using photopolymerization, which provided physical, chemical, and biological barriers to the nonspecific binding of proteins, bacteria, and fibroblast cells. The second region was the PEL, whose positively charged surface promoted immobilization of negatively charged proteins and cells.

To prove our concept, we first tested the PEG microstructures and PEL coating using a pattern (a 50 µm-diameter circle) and a protein—FITC-BSA (1 µg/ml)—as the model biomolecule. Figure 2 clearly shows that using the PEG microstructure for the pattern background prevents nonspecific binding of proteins. FITC-BSA is patterned only on the PEL-exposed regions.

In addition, being able to selectively pattern cells is important for developing biosensors, bioelectronic devices, and cell-based microsystems. We thus focused on optimizing the effectiveness

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Figure 2. Fluorescence images of a protein (FITC-BSA) patterned on a 50µm circle.

Figure 3. Fluorescence images of cell patterns of Escherichia coli expressing green fluorescent protein (BL21-pET23b-GFP) on (a) a 100µm circle and (b) a 100µm line. (c) An optical image of a patterned mammalian cell (NIH 3T3).

of the PEG microstructures as a biological barrier to reduce non-specific binding of cells and on the PEL-coated surface to improve cell immobilization. Bacterial patterning was carried out using *Escherichia coli* that express green fluorescent protein to enable visualization of the patterns. As expected, the fluorescence signals showed that the bacteria attached correctly only onto the PEL-coated surface (see Figure 3). Bacterial patterning can easily be used to make a variety of shapes, for example, circles, squares, stars, and lines. Our approach is a simple, reproducible, and stable method for cell patterning that eliminates background noise at the micron level.

Preparation of well-ordered 2D microstructures with exposed PEL binding regions may find use in a variety of applications that require immobilization of biomolecules such as DNA, RNA, carbohydrates, proteins, cells, and tissues while preserving their 3D structures. The methods we describe in this study provide a means to implement the strict control required for such applications.

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References