Ultra-Precise Cultured Cell Patterning Using Molecular Vapor Deposition of Self Assembled Monolayers and Lift-Off Technique

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Abstract:

Patterned growth of cultured cells is a technique gaining importance in a variety of different applications and fields, such as cell-based sensors, neurobiology and tissue engineering. In all applications, the fundamental idea is to form a pattern of alternating surfaces which are either permit (cytophilic) or inhibit (cytophobic) cell growth. The most frequently used technique to generate such patterns is microcontact printing (µCP). Microcontact printing does have some drawbacks, in spite of the fact that this technique is very simple and cost-effective. The major drawbacks that we have observed are the inability, using this technique, to create printed patterns precisely aligned to pre-existing structures on the substrate, and the inability to visualize the printed pattern prior to cell culturing. We developed a new cell patterning technique that is simple, effective and which eliminates the primary problems exhibited by its predecessors [1].

Introduction to Research:

The fabrication process for the cell-culturing substrate is illustrated in Figure 1 and is as follows. First, 100 nm of silicon oxide was deposited on a 3 inch silicon wafer at 130°C, and positive photosist Shipley 1818 was patterned by photolithography. Then, a stack of two self-assembled monolayers was created by MVD. Heptadecafluoro-1,1,2,2-tetrahydrodecyltrichlorosilane (FDTS) was used as the organo-silane functional monolayer. The resulting FDTS film was annealed after deposition at 100°C for 30 minutes. FDTS SAM can be deposited directly on a silicon wafer.

In the subsequent MVD coating, a vapor deposited oxide layer is initially deposited, followed by a SAM. The silicon oxide functions as an adhesion layer and was created by the hydrolysis of silicon-chloride and water within the MVD reactor just prior the introduction of the FDTS SAM. The thickness of the adhesion oxide can be controlled to create sufficient optical contrast so that the pattern is visible under the microscope.

Figure 1: The patterning of GT1-7 cells using molecular vapor deposition of self-assembled monolayers and lift-off technique.
We chose to deposit a 10 nm thick oxide adhesion film on the top of a silicon wafer. Following MVD, the photoresist was stripped with acetone for 5 minutes, and the wafers were cleaned in ethanol by an ultrasonic cleaner. The wafer was then immersed in a 9 mM solution of trimethoxysilyl propyldiethylenetriamine (DETA) (Gelest, Inc., PA) for 1 hour, which has been shown to be favorable for cell growth. This step created a cytophilic surface wherever the MVD-deposited silicon oxide/cytophobic SAM bilayer was not already present. Then, the substrate was rinsed with ethanol and DI water, and dried with a stream of dry nitrogen to remove any excess solvent.

The prepared substrates were put into six-well cell culture plates (BD Biosciences, CA) and immortalized mouse hypothalamic neurons (GT1-7) were cultured in DMEM medium (Gibco, NY) with 10% fetal calf serum (FBS, Gibco, NY) at 37°C in an incubator with 8% CO2. After 48 hours of incubation, the GT1-7 cells were visualized with an E800 microscope (Nikon Inc, NY). The scale bars for all relevant figures were calibrated with a stage micrometer (OB-M 1/100, Olympus Inc, NY), and scale bars were added with the SPOT imaging software (Diagnostic Instruments, MI).

Figure 2 illustrates the cell patterning results we have obtained so far, and the pre-existing SAM patterns are included for comparison. In the upper panel of Figure 2, the first number on the left depicts the size of the square island suitable for cell growth (in this case, 20 µm, which we have determined is an appropriate size for anchoring a single GT1-7 cell). The second number depicts the width, in microns, of the pathway between two islands. For example, the width “0” denotes that there is no pathway between the islands, and that the islands are isolated.

On the left-hand side on Figure 2, one can see that eight out of sixteen available sites are populated by neurons. The right hand side of the same figure shows a well-developed neuronal network, with cell bodies occupying most of the 20 µm islands, with a network of neurites growing neatly along 5 µm-wide, cytophilic pathways.

One can see that we have obtained a faithful replica of the SAM pattern in the cultured neuron pattern.

**Summary:**

We have developed a simple and effective method for cell patterning, with single-cell resolution, utilizing molecular vapor deposition of a seed oxide/SAM bilayer. The patterns can be easily aligned with previously existing patterns and structures since the patterning alignment depends on photolithographic mask exposure rather than imprecise alignment during stamping. The primary benefit of this technique is that the SAM pattern is visible prior to cell culturing, which is beneficial for targeting sources of error in individual cell patterning steps. Furthermore, the hydrophobic surface is highly uniform and stable because of the silicon oxide seed layer which is a benefit for extended cell culturing times. Finally, this technique avoids the disadvantages of more traditional cell patterning techniques, such as deformation of the PDMS stamp fabricated using conventional molding process.

**References:**