A Novel Method for Accurate Patterning and Positioning of Biological Cells

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Abstract

The ability to anchor cells in predefined patterns on a surface has become very important for the development of cell-based sensors, tissue-engineering applications, and the understanding of basic cell functions. Currently, the most widely used technique to generate micrometer or sub-micrometer-sized patterns for various biological applications is microcontact printing (µCP). However, the fidelity of the final pattern may be compromised by deformation of the poly (dimethylsiloxane) (PDMS) stamps used during printing. A novel technique for accurately patterning and positioning biological cells is presented, which can overcome this obstacle.

Introduction to Research

We have developed a new technique to control the placement and growth of cultured cells on patterned silicon oxide [1]. First, we coated a hydrophobic self-assembled monolayer (SAM) derived from 1-hexadecanethiol on a patterned gold surface to prevent cell growth, and then we deposited a hydrophilic SAM derived from 3-trimethoxysilyl propyldiethylenetriamine (DETA) on the exposed silicon oxide surface to promote cell growth. The fabrication process to make the substrate for subsequent cell patterning is illustrated in Figure 1.

First, 500 Å of plasma enhanced chemical vapor deposition (PECVD) silicon oxide film was deposited on a three inch silicon wafer at 250°C. Then, a thin Cr/Au film was patterned on the surface. Next, Shipley 1818 photoresist was spin-coated at 3000 rpm to get a 2.2 µm thick film. After soft baking at 90°C for 60 s, a contact aligner was used to expose the photoresist with a dose of 76.8 mJ/cm². This step was followed by baking in ammonia for 90 min in an image reversal oven, where the whole substrate was flood-exposed for 60 s. After soft baking at 90°C for 60 s, a contact aligner was used to expose the photoresist with a dose of 76.8 mJ/cm². This step was followed by baking in ammonia for 90 min in an image reversal oven, where the whole substrate was flood-exposed for 60 s. After developing in MF321 developer for 90 s, oxygen plasma was used to remove the photoresist scum residue. Then, 50 Å of chromium was deposited on the surface followed by 500 Å of gold by an e-beam evaporator. Finally, the lift off process was performed using a Microposit Remover 1165. The Cr/Au patterns on top of the silicon oxide surface are shown in Figures 2 and 3.
We have developed a convenient and robust method to position and pattern cultured cells with single cell resolution, which is easy to align with pre-existing structures on the substrate. An additional advantage of this method is that cytophobic/cytophilic patterns can be easily visualized prior to cell culturing. The best resolution we have obtained to date is two microns. It has been established that the single cells we have utilized here – immortalized mouse hypothalamic cells – can be successfully anchored on 20 µm wide square cytophilic islands, and 2 µm wide pathways are suitable for the neurite growth. With this technique, design and fabrication of complex biosensor systems will become possible.

**References**