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Rapid Prototyping of Patterned Poly-L-Lysine Microstructures

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Abstract— **For applications in cell biology, the ability to produce patterns of adhesion proteins for directing cell patterning is of particular interest. Often though, these patterns require extensive clean room facilities and intricate chrome masks to achieve very small feature sizes. We have developed a modified lift-off method for rapid prototyping of simple PLL structures that have features on a micron scale. The lift-off method is simple and easily adaptable to a variety of biological applications.**

I. Introduction

Microtechnology, although rooted in semiconductor fabrication, has had an increased influence on biological science over the last decade. The ability to pattern features on the micron scale make it possible to influence biology on the cellular scale. Many chemical and physical approaches have been utilized to pattern biological molecules. The patterning of a self-assembled monolayer (SAM) is a chemical method to allow the positions and dimensions of attached cells to be controlled. Microcontact printing (μCP) using a poly dimethylsiloxane (PDMS) stamp is one of the most popular method to repeat patterns of SAMs [1, 2], but it needs many process steps and is hard to align multiple patterns. Microstructures having a vertical dimension can physically confine the placement or alignment of cells. Anisotropic topographic features have been shown to induce many cell types to migrate along the direction of the anisotropy [3]. Physical cages, such as a "Neuron well", have also been used to constrain the movement of cells [4]. All these physical approaches yield successful results they usually require various cleanroom facilities.

The photolithographic lift-off technique is commonly used in microfabrication for patterning metals. Only limited biomolecule patterning research for biological applications have been reported using this method [5, 6], because organic solvents are used for removing the photoresists during the lift-off process, which prevent proteins from stabilizing on the surface.

The culturing of neural cells on surfaces requires chemical modifications and deposition of cellular adhesion proteins, such as extracellular matrix proteins. Poly-L-Lysine (PLL), a highly positively charged amino acid chain, is commonly used as a coating agent to promote cell adhesion. In this work, we demonstrate various microstructure patterning of PLL by using a rapid-prototyping lift-off technique. This process uses conventional lithographic techniques, combined with rapid mask generation techniques to make structures with dimensions down to 10 microns. Unique structures with sub-micron sidewalls were then built by applying different drying conditions. In this way, small feature sizes with high aspect ratios were fabricated solely from PLL, without other physical support structures. These features have the unique capability of providing both chemical and physical features, with small feature sizes produced with rapid prototyping processes.

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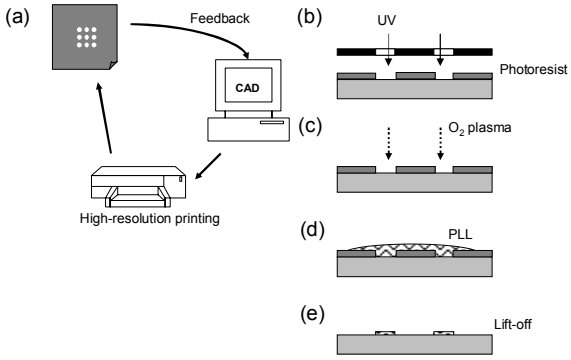

Fig. 1. A schematic description for the protein patterning by the lift-off technique. (a) Rapid prototyping of photomasks. (b) Photolithography. (c) Oxygen plasma treatment. (d) PLL soaking until drying out. (e) Lift-off.

II. Materials and Methods

A. Photolithography

The details of protein patterning process are illustrated in Fig. 1. The photomask for photolithography was designed and printed on a transparency film from a high-resolution laser printer (Silverline Studio, Madison, WI). The substrates were cleaned with Acetone, isopropyl alcohol (IPA) and rinsed by deionized water. The cleaned substrates were spin-coated with photoresist (Shipley 1813) at 4000 rpm for 30 seconds and prebaked at 115°C for 1 min. The photoresist-coated substrates were exposed with a Karl Suss MJB3 contact aligner to make patterns for 20 seconds. The photoresist was developed for 40 seconds in developing solvent (Microposit MF 321) and rinsed in deionized water.

B. Protein patterning by lift-off technique

The patterns were treated in oxygen plasma for 1 min in order to remove unnecessary photoresist residues and

facilitate better adhesion with proteins. Poly-L-Lysine hydrobromide (Sigma Aldrich), and FITC labeled Poly-L-Lysine (Sigma Aldrich) for fluorescent images, was diluted in deionized water to a final concentration of 0.5 and 2 mg/mL each. Approximately 100 µl of the solution was placed on the pattern (Fig. 1(d)) and allowed to evaporate (either at room temperature or at 50°C) until dry.

The lift-off process was performed by placing the pattern first in a spin-coater, and then transferred to an ultrasonic bath. Acetone was sprayed on the substrate during spinning for 30 sec, and then remaining photoresists were removed carefully in acetone in the ultrasonic bath.

III. Results and Discussion

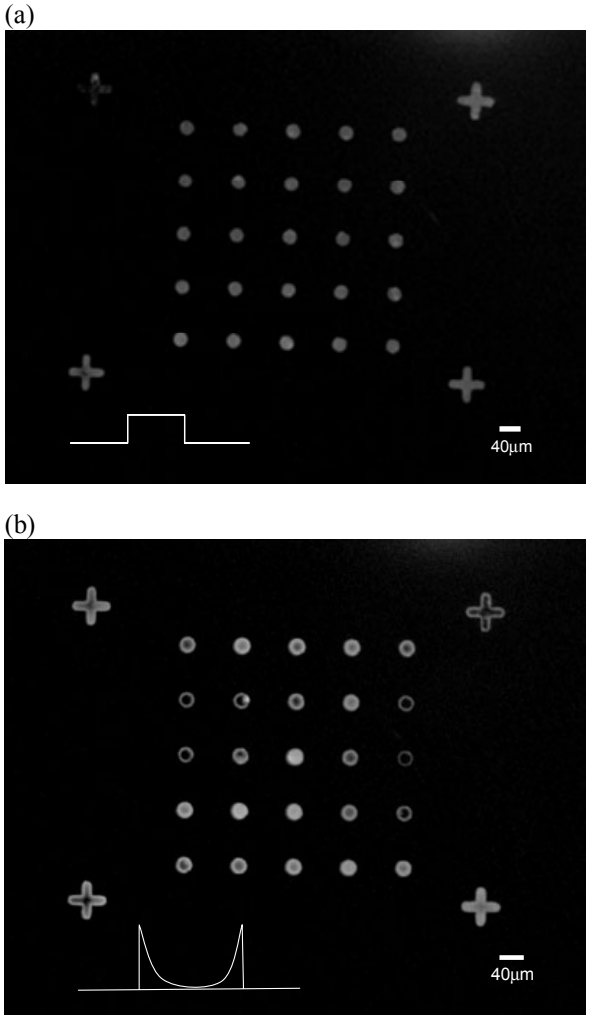

Fig. 2. Fluorescent images of PLL microstructures (a) heating up to 50°C and (b) no heating during drying.

Figure 1(a) shows the general concept of rapid prototyping using high resolution photomasks. Prototyping of masks using a high-resolution printing is very useful in biological laboratories as they do not need to make high-resolution chrome photomasks [7]. It is also a very flexible technique that can rapidly transfer new designs of a pattern on to a substrate and get relatively fast feedback and design cycles.

Fluorescent images of PLL microstructures with 25 µm diameter are shown in fig. 2. A 2 mg/ml FITC labeled PLL was used to get brighter fluorescent images. The feature size in photolithography mostly depends on the resolution of the photomask. Using high resolution printing, the transparency photomask can make patterns under 10 µm [7]. The height is affected by the drying conditions and the amount of PLL solution placed on the photoresist patterns. The PLL is practically limited by one third of the photoresist thickness after spin-coating [8].

Natural evaporation of PLL solution on the photoresist patterns was compared with heated drying at 50°C. Figure 2(a) shows cylinder-type microstructures with a flat top. Proper heating increases the rate of the water evaporation, and it accelerates the precipitation of PLL on the bottom surface. In natural evaporation, without heating, the agglomeration of PLL occurs not only on the bottom, but also on the sidewall in fig. 2(b). This demonstrates a convenient way to build submicron scale structures with a lift-off technique. The thin side wall structures with a high aspect ratio are extremely hard to make using conventional etching processes. In fig. 2(b), thin side walls with over 10:1 aspect ratio were successfully formed even in the sharp corner of the alignment marks. These parabolic microstructures have potential for various biological applications. For example, a number of cupped features, which could be used to isolate only one or a few neurons inside, can be fabricated at once easily, composed entirely of bio-compatible materials. Previous studies have shown that bio-compatible patterns on surfaces can be formed by microstamping, laser ablation, or casting on silicon molding [9-11], and these were made with the photolithography and lift-off method which are fast, well-established process. Combined with rapid prototyping of photomasks, various design ideas can be materialized and

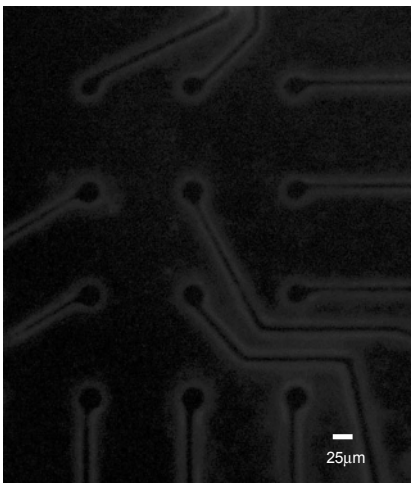

Fig. 3. Fluorescent image of PLL microstructures along the metal lines of MEAs.

tested on demand within a few days.

Figure 3 demonstrates an application of the PLL lift-off technique. Microelectrode Arrays (MEAs) have been used to record extracellular electrical activity from electrogenic cells, and recently various chemical patterning methods have been combined to improve recording efficiency [12, 13]. The PLL microstructures were constructed along the metal lines using the same, negative photomask, which is very convenient to align. Using negative photoresist, an additional photomask is not necessary to make this additional pattern. Moreover, these negative patterns using the negative photoresist (or negative photomask) can expand further the application of the lift-off technique. In contrast with positive PLL microstructures, the inside wall of the negative photoresist pattern becomes very steep after removing the photoresist. This may make the technique useful to pattern additional chemicals or proteins due to the very sharp borders that can be obtained.

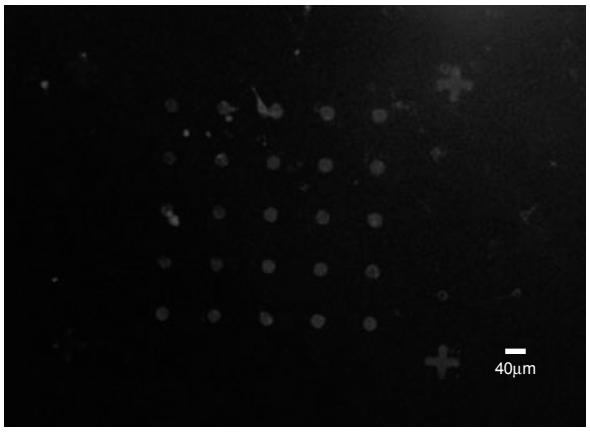

Fig. 4. Fluorescent image of PLL microstructures after 5 days in cell culture medium.

PLL dissolves well in polar solvents, like water, due to its own positive charge. Fluorescent images were retaken to test the durability of PLL microstructures in cell culture medium after 5 days (Figure 4). The fluorescent intensities were decreased, but their shapes were still consistent with the original structures. The fact they endured even the lift-off process in acetone ultrasonic bath means it is not just simple powder settlement during the evaporation of PLL solution. A brief treatment of the exposed surfaces with oxygen plasma generates a more hydrophilic surface and removes photoresist residues [14]. The microstructures may result from the strong bond between a positively charged PLL and a negatively charged hydrophilic surface. This stability in solution makes it possible to perform experiments in microfluidics systems, such as those for controlling the microenvironment of cells or delivering chemicals selectively to portions of a cell culture. In our previous, the possibility of time-varying dynamic fluid and local temperature control for *in vitro* neural recording has been demonstrated [15, 16]. The processing methods in the current study are directly applicable to those types of studies, as the micro-cylinder structures have similar features sizes as the microelectrode sensors used for neural recording.

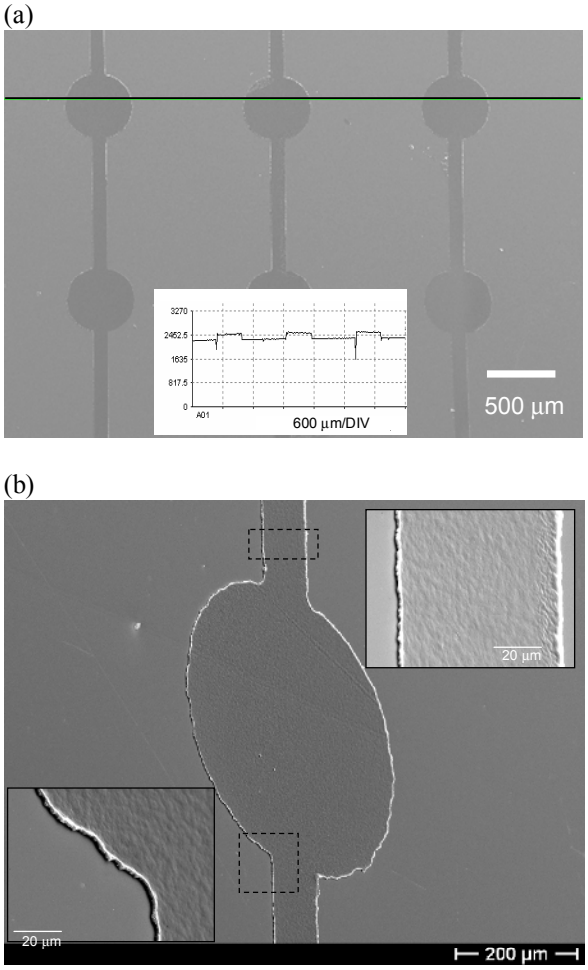

Fig. 5. SEM images of PLL microstructures (a) with the profile graph by the line scan. (b) with 45° tilt.

Scanning Electron Microscopy (SEM) images of patterns of circles connected with narrow linear tracks are shown in fig. 5(a). A cell culture grade (0.5 mg/ml) PLL hydrobromide was used to fabricate these patterns. The microstructure features matched the photomask patterns with sub-micron tolerances. The inset profile graph, which was obtained by a line scan of the SEM, shows uniform heights of 200 nm for the PLL pattern. To investigate three dimensional structures in detail, 45° tilted images were obtain in fig. 5(b). The lateral dimension is clearly confirmed from the shadow on the left side of the structures. Particularly, the jagged outline along all boundaries is distinctive in magnified images. In SEM images, a brighter area in the same material usually means higher height.

Using the advantage of rapid prototyping using high resolution printed photomasks, thinner lines with <15 µm width and more complex patterns were also tested. These were generated clearly, but the time and intensity in the acetone ultrasonic bath becomes critical on these smaller scales. In general, smaller feature sizes can drastically affect the interaction between heating, evaporation and surface tension. It may be possible that optimization of these factors will allow for precise control of the small features produced along the sidewalls of the liftoff pattern. In this way, we hope

to establish a technique to reproducibly create sub-micron patterns without the need for cleanroom facilities, while still taking advantage rapid prototyping capabilities.

In addition, it is possible to make multilayer structures easily in the lift-off process. After the completion of drying the first material solution, the second material can be applied on the established microstructures before the lift-off step. In that case, a laminated structure can be built by repeating soaking and drying steps (Fig. 1(d)).

IV. Conclusion

We have demonstrated a lift-off method for rapid prototyping of simple PLL structures with features on a micron scale. The lift-off method is simple and easily adaptable to a variety of biological applications. Many cell biology applications are likely to utilize both the larger (100's of microns) patterns as well as the much smaller patterns (10's of microns or sub-micron) that are obtainable within this process. The feature size in photolithography mostly depends on the resolution of the photomask. This technique utilizes the interactions between surface forces and evaporation processes to produce high aspect ratio features with sub-micron features, made exclusively out of biological proteins, produced using rapid prototyping methods.

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