

LIFT-OFF CELL LITHOGRAPHY FOR HIGH EFFICIENCY AND CLEAN BACKGROUND CELL PATTERNING

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ABSTRACT

We develop a highly efficient method for patterning cells by introducing a novel and simple technique called lift-off cell lithography (LCL). This works borrows the key concept of lift-off lithography in microfabrication but utilizes a fully biocompatible process to achieve high patterning efficiency with nearly zero background defect. Using LCL, we accomplish over 60% patterning efficiency for both adherent and non-adherent cells and less than 0.15% cell defects in undesired areas.

KEYWORDS: lift-off lithography, cell patterning, poly-L-lysine

INTRODUCTION

Cell patterning is a useful tool in single cell analysis and tissue engineering [1, 2]. Many efforts have been devoted to developing various patterning techniques [3, 4]. These prior works include physical methods based on DEP, acoustic, fluid, or mechanical mechanisms, and chemical methods such as microcontact printing. Trade-off between patterning efficiency (cells in desired locations) and background defect density (cells in undesired locations) often needs to be made [5]. As a result, an efficient cell patterning approach able to realize high efficiency, low background defect density, and high resolution patterning is highly sought of, but still difficult to achieve with current methods.

Here, we demonstrate a novel and simple cell patterning technique called lift-off cell lithography (LCL) that can achieve high efficiency patterning with nearly zero background defect. Lift-off lithography is a widely applied metal patterning approach in microfabrication. This work borrows the key concept of lift-off lithography but utilizes a fully biocompatible process for cell patterning. Using LCL, we can accomplish 62% cell patterning efficiency for both adherent and non-adherent cells and only 0.15% background defect density. And compare to widely used surface chemical treatment approach, the background defect density is an order of magnitude lower.

EXPERIMENT

The fabrication process and experimental protocol for patterning cells using LCL are shown in Figure 1. First, a glass coverslip is sequentially coated with polymer poly-L-lysine (PLL) to promote cell-attachment and polyvinyl alcohol (PVA) as a sacrificial layer. Then a 5 μm SU-8 photoresist is spun-on and micropatterned via photolithography. Next, oxygen plasma etching is

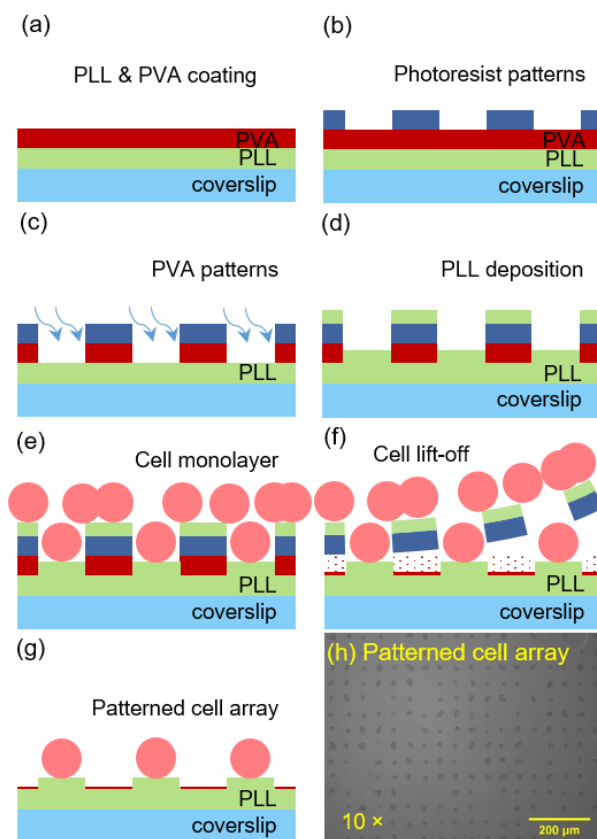


Figure 1: Illustration of the process of lift-off cell lithography (LCL). (a) PLL and PVA are sequentially coated on a glass coverslip. (b) SU-8 photoresist is spun-on and patterned via photolithography. (c) PVA is patterned through O_2 plasma etching. (d) PLL coating for the second time. (e) Cell loading for 10 min at room temperature. (f) Cell culturing for another 30 min in an incubator while PVA dissolves and enables cell lift-off on the photoresist layer. (g) & (h) The patterned array of cells.

performed to etch the PVA layer. Finally the fabrication concludes with coating the chip with PLL for the second time for another 10 minutes at room temperature. Ramos or HeLa cells suspended in media at a density $\sim 2 \times 10^6/\text{ml}$ are dispensed on the chip for 10 minute to allow cells to settle. PVA is a water soluble material. Culturing cells in an incubator (37°C) for 30 minute allows the removal of the PVA layer under SU-8. The continuous SU-8 film is thin but rigid enough to be peeled off together with all the cells on it. This leaves only cells at desired pattern locations. Since high concentration of cells can be used in the patterning process without any concern of background defects, high fill-up efficiency and clean background patterning can be achieved at the same time without trade-off.

RESULTS AND DISCUSSION

Figure 2 demonstrates the results of patterning Ramos cells using circular holes, each with a diameter of $20\ \mu\text{m}$, on a large area of $1.2 \times 1.2\ \text{cm}^2$. Calcein AM/propidium iodide (PI) is used for live/dead cell staining. The experiment is repeated three times; the

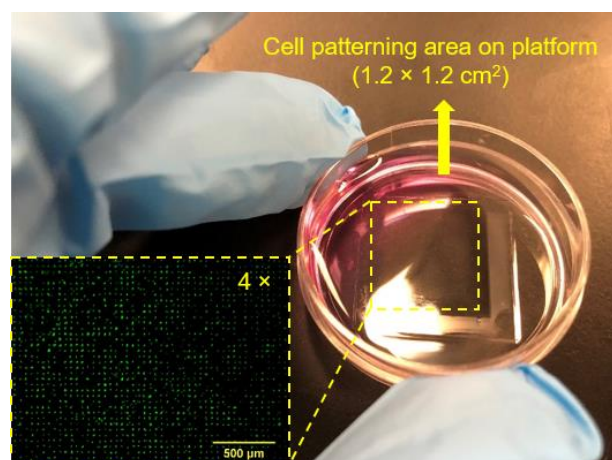


Figure 2: Image of an array of individual Ramos cells on a coverslip. The yellow dashed box marks the patterning region; the magnified insert shows large-scale cell patterning. Calcein AM/PI is used for live/dead cell staining (green/red).

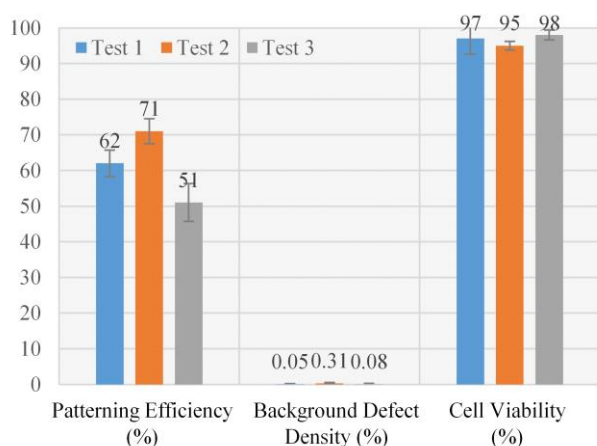


Figure 3: Patterning efficiencies, background defect densities and cell viabilities of the cell lift-off experiment using circular micropatterns.

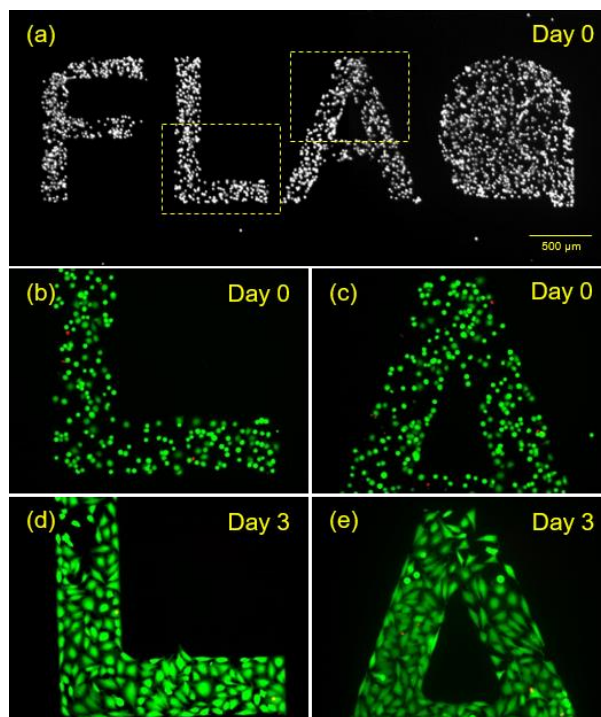


Figure 4: Fluorescence images of HeLa patterns arranged to form letters of "FLAG" immediately after cell lift-off (a, b, c) and after culturing for three days (d, e). Calcein AM/PI is used for live/dead cell recognition (green/red).

numbers of spots occupied by cells, total spots, cells in undesired areas, live cells and total cells are recorded from 10 randomly selected locations per test for estimation of the average values (Figure 3). In addition, Figure 4 shows a pattern of HeLa cells forming the letters of "FLAG" immediately after cell lift-off (4a, b, and c) and after culturing for three days (4d and e). Adherent cell attachment, division and proliferation within the confined regions have also been observed over multiple days.

CONCLUSION

We have developed a novel and simple method for cell patterning called lift-off cell lithography (LCL) that can achieve an average of 62% patterning efficiency for both adherent and non-adherent cells with only 0.15% background defect density. Also, compared with other chemical treatment approaches, LCL for arbitrary

patterning can reach a high efficiency with clean surroundings, and has potential for broad applications in cell biology and related fields.

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