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Model membrane-mediated cell alignment through surface hydrophobicity

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ABSTRACT

We show a realization of well-controlled alignments of human lung fibroblast cell parallel to the guided direction achieved by selectively patterned model membranes. The supported lipid bilayer membranes (SLBMs)-mediated cell alignment and selective growth in particular underlying surface properties were achieved through peeling-off of the polydimethylsiloxane (PDMS) stamp. We used the PDMS not only as a microfluidic channel to reconstruct model membrane on a hydrophilic surface, but also as a self-inking stamp for the substrate-surface modification by transfer of outmost low-molecular weight PDMS oligomers (S-PDMS). We found that surface hydrophobicity of solid supports is controlled by thermally-assisted stamping methods and this is crucial for the cell adhesion in lipid-free regions.

KEYWORDS

Surface hydrophobicity; human lung fibroblast; Supported lipid bilayer; cell alignment; Bio-array

1. Introduction

Cell migrations and their alignments contribute to life-sustaining biological activities. Cells interact with neighboring cells in the process of healing of partially wound-cells, cancer metastasis, and embryo development [1, 2]. These multicellular processes promote cellular regeneration accompanied with morphological changes in cell shape and compositional reorganization of the cell components. For example, collective migrations of the cancer cell re-fills partially wounded-site where an extracellular matrix (ECM) is lost, and in which the blood vessels build new ECMs into the void spaces [3]. To understand the multicellular activities by mimicking multicellular environments *in vitro*, reconstruction of versatile bio-array with quantitatively engineered, manageable, and high-throughput platform accompanied with incubation of diverse cell-types has been explored in numerous ways. Migrations of living cells under guided directions *in vitro* have been majorly explored by chemotaxis [4], durotaxis [5], and haptotaxis [6] in environments devoid of cellular membranes. However, practical approaches in reconstituting systematic platforms to realize multicellular activities have rarely been reported due to major technological challenges on cell adhesions and migrations on an engineered template. A lack of platforms has restricted a systematic investigation of multicellular activites, in particularly, on membrane regeneration of injured cells by neighboring cells. In regard to serving a cellular environment, a reconstruction of in- jured

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cell membrane is the challenging key requirements. Alternatively, the supported lipid bilayer membrane (SLBM) serves systematic platforms as one of promising candidates by providing a supportive environment similar to that of a living cell due to its capability to tailor the membrane compositions [7–9] and patched shape [10] in a controlled manner, enabling to exploit the physicochemical roles of membrane environments on cell migrations and alignments by the surface charge of SLBM [11] and by the compositional hydrophobicity disparity between surface properties of proteins and lipids [12].

In this work, we present a viable, surface hydrophobicity-mediated cell adhesion and lipid membrane-directed selective migration and alignment of human lung fibroblast (HLF) cell parallel to the SLBM in a microfluidic device. Selectively addressed SLBMs were attained through microfluidic channels. Here, the polydimethylsiloxane (PDMS) stamp plays a bifunctional role as a surface hydrophobicity modifier and a micro-channel forming SLBM. By stamping the PDMS mold, we found that the substrate-surface property converted from hydrophilic to hydrophobic which provides an excellent platform for selective HLF cell migration restricted to controlled alignment guided by SLBM-produced environment.

2. Materials and methods

2.1 Fabrication of PDMS stamp

To provide the surface property in which cells can survive *in vitro*, we uses PDMS material. Before coating the negative photoresist (SU-8 2050, MICROCHEM), a glass substrate was cleaned in an ultra-sonicator by acetone for 30 min followed by deionized (DI) water for 10 min. After drying the substrate, SU-8 was coated onto the substrate according to vendor's instruction. The 50-μm-thick of photoresist was chemically patterned through standard photolithography using mask aligner (MA-6, EVG). Elastomeric material, Sylgard 184 silicone elastomer (Dow Corning Corp.), mixed with a curing agent in 10:1 ratio by weight, was poured into the prepared substrate and cured for $>$ 3 h at 80 °C, and then detached. Every step was carried out at room temperature.

2.2 Modification of surface hydrophobicity

To render the substrate hydrophilic, we cleaned glass substrate with piranha cleaning treatment (3:1 (v/v) H_2SO_4:H_2O_2) at 120 °C for 10 min followed by ultra-sonication in DI water for 10 min. After drying the substrate, prepared PDMS stamp was placed on the cleaned glass and heated for 3 min at 200 °C on a hot plate.

2.3 Selective patterning and imaging the SLBM

We ruptured the vesicle mixture on the hydrophilic surface to form the SLBM. The veiscle contains 1,2-dioleoyl-sn-glycero-3-phoph-ocholine (DOPC, Avanti Polar Lipids, Birmingham, AL) doped with 1 mol% of fluorescent Texas Red 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanola-mine (Texas Red-DHPE, Life Technologies, Carlsbad, CA). The rapid solvent exchange method [13] was employed to prepare the mixture of vesicles by an evaporation of the lipid mixture-contained chloroform followed by a hydration with Tris buffer (100 mM NaCl and 10 mM Tris at pH 7.2). After that, we extrude the vesicle solution 20 times through a 50 nm filter to generate small unilamellar vesicles (SUVs) in diameter regime of 50 nm. Here, we used the periodically designed PDMS stamps not only for S-PDMS inking stamp, but also

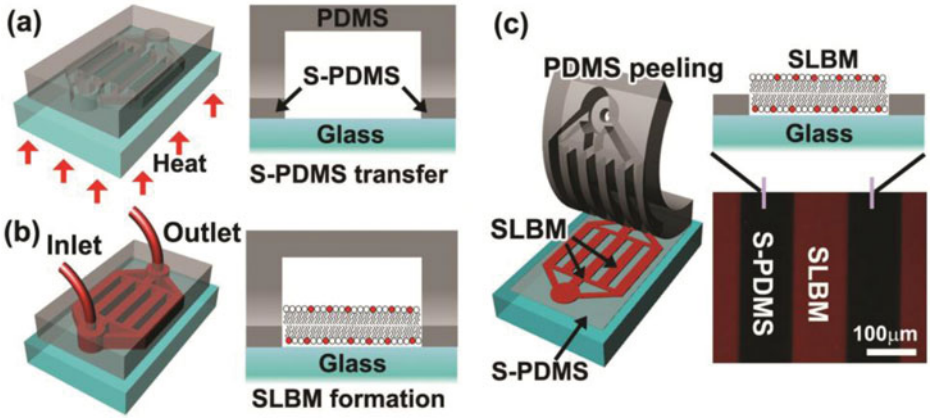

Figure 1. Schematic illustrations of selective SLBM formation by bi-funtional PDMS stamp. (a) PDMS microfluidic stamp is contacted to glass substrate and exposed to heat at 200 °C for 3 min to transfer the S-PDMS. (b) Fluorescent bilayer membrane is formed by spontaneous vesicle rupture process in the micro-channel. (c) The PDMS is peeled off leaving behind S-PDMS region. All processes were performed under aqueous conditions at room temperature.

for micro-fluidic channels where SLBM will be formed. To this end, the piranha cleaned glass with PDMS stamp was heated for 3 min on a hot plate at 200 ° (Fig. 1a) followed by injection of 0.2 mg/ml of SUV solution (DOPC/Texas Red-DHPE = 99/1) to form SLBM along the micro-fluidic channel (Fig. 1b) through via vesicle adsorption, rupture, and fusion process [14]. By replacing SUV solution with the DI water for removal of unruptured residual SUVs followed by peeling off the PDMS channel, we observed the selective patterns of SLBM were left (Fig. 1c). An epifluorescence microscopy (Eclipse E600-POL, Nikon) were used to monitor the Texas red lipids and the fluorescent molecules in SLBM were analyzed by public program (Image J, NIH, USA).

2.4 Cell culture and incubation

We used primary human lung fibroblasts (HLF, Lonza, Swiss). We cultured them in fibroblast growth medium (FGM-2; Lonza, Swiss), and incubated in at 37 °C with 5% CO_2 gas during culture. To harvest the cells, they were rinsed with phosphate buffered saline (PBS) and treated with 0.25% trypsin–EDTA (Gibco, Carlsbad, CA). After 2 min, M199 (Lonza) containing 10% fetal bovine serum was added to neutralize the effect of the enzyme. Detached cells were collected and centrifuged for 2 min in 1,100 rpm, and diluted in FGM-2 to reach a certain cell number in the suspension. All the procedures were done on the periodically patterned SLBM using PDMS self-inking methods.

3. Results and discussion

Low molecular-weight oligomericresidues atop the surface of PDMS stamp act as ink [15, 16]. We found that well-defined hydrophobic patches of the S-PDMS, corresponding to the stamp patterns, were achived over a large area in a simple manner. A couple of studies demonstrated that hydrophobic surfaces enhanced the differentiation of particular cell with low rate of cell death [17], and improved protein adhesions [15]. This obviously implies

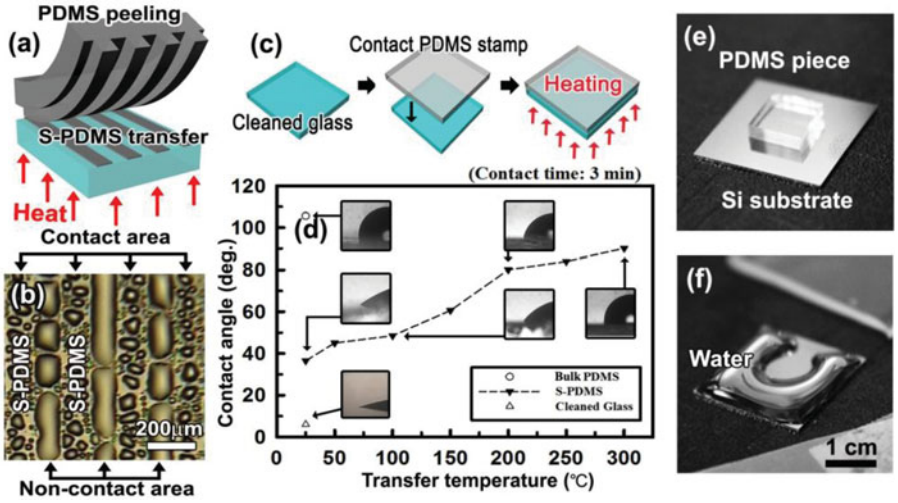

Figure 2. Surface hydrophobicity generated by transferred S-PDMS through thermally-assisted self-inking method. (a) Schematic of S-PDMS transferred from the PDMS stamp. (b) A microphotograph obtained during 'water vapor test' of alternating S-PDMS of each 100 μm-width. This shows that water condenses more on hydrophilic glass (= non-contact area) parted by alternating hydrophobic S-PDMS area. (c, d) Schematic illustrations of S-PDMS transfer methods (c) and water contact angles stamped on glass in various temperatures (d). (e, f) We also tested piranha cleaned silicon substrate (2.5 cm $\times$ 2.5 cm) using thermally-assisted stamping method at 200 °C. A water droplet was expelled from the square (1.5 cm $\times$ 1.5 cm) PDMS stamp contacted region (e) showing the distinct wettability boundary along the S-PDMS pattern (f).

potent implications of hydrophobic surface in diverse cell-based researches. Thus, reconstruction of hydrophobic surface prior to the SLBM deposition will be the first step. Contact angle (θ_c) of water on the hydrophobicity-modified surface increases monotonically with the temperature (Fig. 2c). For example, the piranha-cleaned glass surface without PDMS stamping shows water contact angle of $\theta_{RT} = 6$ °, while S-PDMS transferred at 200 °C for 3 min shows $\theta_{200\,°C} = 80$ ° (Fig. 2d). To test the high-contrast of a surface hydrophobicity across stamped/non-stamped regions, we executed the series of wetting test on the S-PDMS patterned glass surface; the condensation of water vapor test in micron-scale (Fig. 2b) and wetting test in millimeter-scale (Figs. 2e and 2f) demonstrated that high contrast of hydrophilic and hydrophobic patterns is derivable. A piece of Silicone (Si) wafer in which the outmost surface functionalized with silanol (-OH) group was also to examine substrate compatibility. Consequently, higly reactive silanol group atop of piranha cleaned Si wafer shows high contrast of surface hydrophobicity between the PDMS stamped and non-stamped regions.

To determine whether our patterned hydrophobic surface meets the primary requirements for mimicry of wounded cellular environment where healing process will occurs, we reconstructed alternating patched SLBMs under the assumption that lipid-free regions replicates wounded-site within cellular membrane. The controlled channel width (w) of the SLBMs between gaps (g) with various values of w-g was proved (Fig. 3). This demonstrates the possibility of realizing a manageable and high-throughput bio-assay in a relatively simple process. Since 1 mol% of Texas Red fluor molecules were included in the SLBMs, lipid-free (SLBM-free) areas were indicated by the black regions where the hydrophobic S-PDMS were transferred. Experiment-wise, we found that the washing-out process was crucial in achieving fine edges of the SLBM patterns, particularly in narrower gaps (lipid-free regions) between SLBMs.

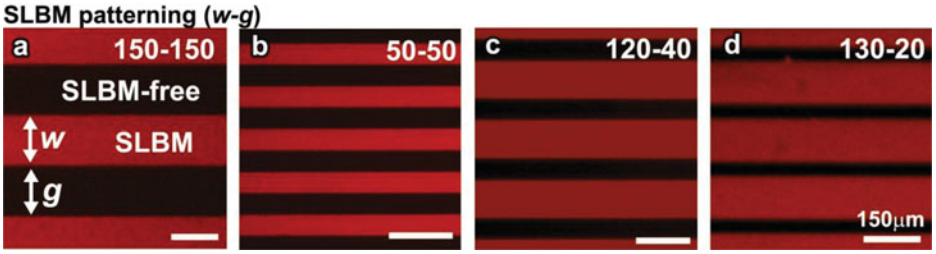

Figure 3. The patterned SLBM showing different width of channel (*w*) and gap between SLBMs (*g*). Well-defined edge was executed in various *w-g* values. (a) *w-g*; 150 μm – 150 μm (b) *w-g*; 50 μm – 50 μm (c) *w-g*; 120 μm – 40 μm, and (d) *w-g*; 130 μm – 20 μm. All scale bars: 150 μm.

Note that the PDMS stamp peel-off procedure without removal of residual SUVs obstructs formation of the SLBM patches with fine edges.

We have now prepared the engineered bio-array to explore the influence of neighboring cells for cell migration and alignment. Among various type of cells, we used primary HLF cell due to their ability to repair lung tissues, remodel tissues after injury, and respond to pulmonary inflammation [18]. Our pattenred SLBMs separated by the lipid-free regions mimic the morphochemical representation of spatially wound cellular membrane. Figure 4 shows different adhesive behavior of HLF with respect to the underlying substrate conditions. Interestingly, It is clear that existence of SLBMs prohibit cell adhesion and a growth, while lipid-free area allow cells to settle. We tested both negative (= SLBM-free) and positive (= fully SLBM covered) control experiments shown in Figs. 4a and 4b, respectively. The SLBM-free substrate were prepared by stamping flat PDMS on hydrophilic surface, while fully SLBM-covered surface were also prepared by SUV (DOPC/Texas Red-DHPE = 99/1) rupture process. From these results, It is obvious that hydrophobic surface promotes cell adhesion, albeit in random directions (Fig. 4a). By contrast, the fully SLBM-covered hydrophilic suface resists cell adhesion (Fig. 4b). Figures 4c and 4d show that HLF cells settle along the lipid-free area parallel to the SLBM on alternatively patterned SLBMs with gaps. As well known, the most of cellular membranes are negatively charged. Thus, a selective growth of the HLF cell toward SLBM-free regions may be attributed to repulsive forces between cellular membrane and compositionally 1 mol% of negatively charged pre-patterned SLBMs due to Texas-Red DHPEs. By contrast, the PDMS-inked regions promote the cell adhesion by removal of repulsion between the cell and underlying surface by covering the substrate hydroxyl group (-OH) moiety originated from the piranha cleaning process. Thus the results confirm the role of SLBMs in

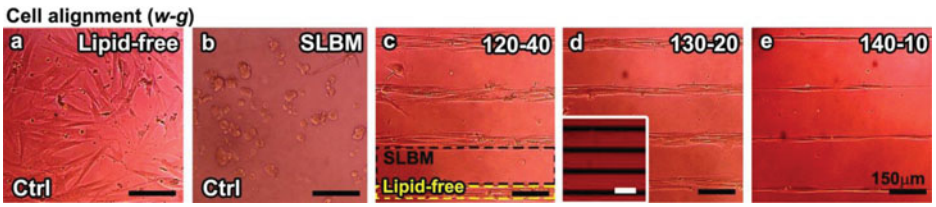

Figure 4. Incubation of HLF cells on SLBM patterned template. (a) HLF cell adhesion in random directions on S-PDMS transferred hydrophobic surface. No SLBM (lipid-free) is prepared prior to the cell incubation. (b) No adhesion of HLF cell was monitored on SLBM fully covered substrate. (c–e) Cell adhesions occur solely on hydrophobic (= S-PDMS) regions with controlled HLF cell alignment in *w-g*; 120 μm – 40 μm (c), *w-g*; 130 μm – 20 μm (d; inset for SLBM pattern), and *w-g*; 140 μm – 10 μm (e). All scale bars: 150 μm.

guiding selective adhesion of the HLF cells in scheduled direction. We also achieved different array of cell lines either in bundles (Figs. 4c and 4d) or in single assembly (Fig. 4e).

4. Concluding remarks

We developed a viable, cost-effective, one-step, site-specific bio-assays through the SLBM-directed selective incubation of HLF cells and their alignment in aqueous environment. The PDMS mold plays bi-funtional roles not only as a microfluidic channel to reconstruct the SLBM, but also as a self-inking stamp which is crucial for cell migration. Our discovery through the SLBM-based bio-array opens a frame work for understanding cell migration and cell healing prcoess by providing systematic platform that has not been realized *in vitro* system. From the viewpoint of systematic bio-assay, the reconstituted systems developed here will allow the creation of large-scale platforms to investigate cell-substrate adhesion, cell-cell interactions, as well as functional biomimetic devices for a high-throughput discovery of multicellular processes.

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