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Abstract

Here we describe an approach to fabricate addressable cell microarrays, which are based on the patterned switchable superhydrophobic surfaces. The switchable superhydrophobic surfaces were prepared by roughening the surfaces of fluoropolymers on the electrodes. Upon the application of 150 V to the underneath electrodes, the water contact angle on the roughened fluoropolymer surfaces could be decreased from 163° to less than 10° allowing the deposition of fibronectin, which could guide the growth of the cells. Our result indicated that it was possible to control the spatial distribution of two different cells on the cell microarrays.

Keywords

Superhydrophobic surface, protein array, cell array, nanostructure, cell adhesion

1. Introduction

In the areas of genomics and proteomics, there are increasing demands for the development of novel patterning techniques to create arrays of functional biomolecules or cells on the miniaturized devices, which could be used in various large-scale biomedical applications such as biosensing, proteomics, immunoassays or drug screening [1, 2]. Several processes have been demonstrated which are capable of patterning biomolecules with very high degree of spatial control including dip-pen lithography, inkjet printing, photolithography, nanoimprinting, etc. [3–10]. While the serial writing techniques provide individual addressability, the parallel printing processes offer an easy and fast protein patterning. However, very few of the above-mentioned techniques are capable of patterning cells. The cell microarrays, which provide the native environments for various biochemical reactions, are often used to investigate the expression of genes and the function of proteins [11]. In the past few years, many schemes have been proposed to fabricate cells microarrays

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[12]. One of the most popular approaches is to print biomolecules on a chip where the desired types of cells are cultured. However, in such type of cell microarray, the cells are not confined. The separation of different colonies sometime becomes problematic. Another approach is to employ micro-contact printing where the extracellular matrix (ECM) molecules such as fibronectin, vitronecin and collagens are first patterned on the surfaces [13]. Then the growth of cells on the surfaces is guided through binding to these ECM molecules. However, in these two cases, only one type of cells can be used on a chip. Here we report the use of switchable superhydrophobic surfaces to create cell microarrays where two or more types of cells can simultaneously be cultured on different areas of the same chip.

Superhydrophobic surfaces, whose water contact angles are larger than 150°, have been one of the most popular research topics for material scientists recently [14]. The studies on superhydrophobic surfaces allow investigation of the influence of surface nanostructures on the water-repellent behavior, similar to that observed in many living organs [15, 16]. The understanding of the origin of the water-repellent behavior may help in developing new industrial applications such as self-cleaning, anti-adhesion and oxidation resistant coatings [17, 18]. To prepare superhydrophobic surfaces, there are two general approaches: roughening the surfaces of hydrophobic materials, or coating the surface with layers of hydrophobic nanostructured materials [19–25]. In these processes, the surface hydrophobicity can be controlled *via* proper surface engineering. However, the surface wettability cannot be varied using these approaches once the materials are fabricated. A switchable surface is always desirable because of its great potential in many applications including fluidic manipulation, actuation and the study of cell adhesion [26–31]. In a previous publication [32], we have demonstrated a novel class of nanostructured materials, switchable superhydrophobic surfaces, for the fabrication of functional multi-component protein arrays where the electrowetting effect was employed to convert a superhydrophobic state into a complete wetted state, allowing fast but addressable protein deposition on the otherwise protein-resistant superhydrophobic surfaces. In such switchable superhydrophobic surfaces, the contact between protein solution and surface is minimized. Therefore, the protein deposition takes place only on the arrays, which are activated by applying voltage. As the protein solution stays on the top of device only for a few seconds, it is very unlikely that proteins would accidentally deposit on an area already patterned with other proteins. To pattern different types of cells on such device, we propose to prepare addressable cell microarrays by patterning the extracellular matrix (ECM) molecules, such as fibronectin, sequentially on the pre-determined areas, and then the microarray is cultured with the desired cell type. By repeating this process, two different types of cells can be cultured on to the same chip with spatial control.

2. Experimental Section

The detailed fabrication process for addressable superhydrophobic microarrays can be found in a previous publication [32]. In short, to fabricate addressable cell mi-

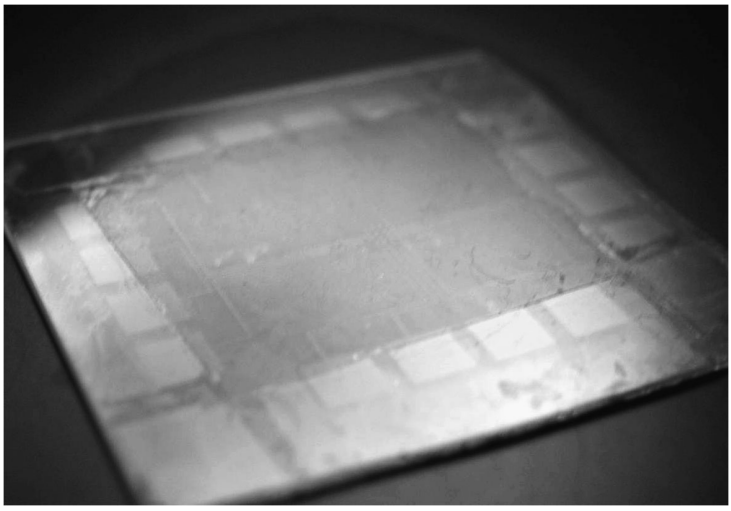
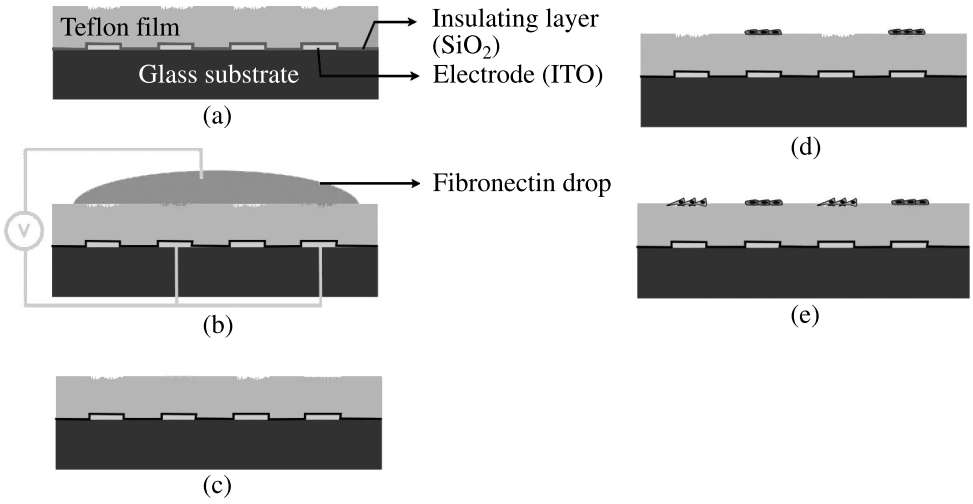


Figure 1. Optical image of an addressable chip containing 4 × 4 switchable superhydrophobic microarrays.

croarrays, the patterned switchable superhydrophobic surfaces were prepared on the ITO glass. A layer of 5 μm thick fluoropolymer poly [tetrafluoroethylene-co-2,2-bis(trifluoromethyl)-4,5-difluoro-1,3-dioxole] (Teflon AF, DuPont) was first coated on the ITO glass with pre-patterned electrodes, which were covered with a layer of silicon oxide (∼300 nm thick) for insulation purpose. Then a layer of photoresist (S1813, Shipley) was spun on top of the fluoropolymer and a photolithographic process was used to define the superhydrophobic area on the photoresist. The superhydrophobic microarray was fabricated using an oxygen plasma treatment (Oxford Plasmalab 80 Plus, 80 W) with O_2 as the gas (2 sccm) at a total pressure of 25 mTorr. After plasma treatment, the photoresist was removed by washing the surface with acetone. Only the areas exposed to the oxygen plasma exhibited superhydrophobic behavior, whose surface water contact angle was measured to be 163° and the surface roughness was 65 nm. The switchable superhydrophobic chip is shown in Fig. 1.

Shown in Scheme 1 is the patterning process for ECM molecules and cells. To guide the growth of the cells, ECM molecules such as fibronectins were patterned on the superhydrophobic microarray (Scheme 1(a)). A drop (∼15 μl) of fibronectin solution was pipetted onto the top of the microarray, which covered the whole superhydrophobic microarray. A platinum wire (0.1 mm in diameter) was inserted into the droplet, which served as the counter electrode. A 150 V voltage was applied to the selected ITO electrode for a few seconds to switch the surface wettability of individual superhydrophobic microarrays (Scheme 1(b)). After washing the chip with phosphate buffered saline (PBS) solution, the fibronectin patterned microarrays on the desired area could be obtained (Scheme 1(c)). The chip was then used to culture the first type of cells for a short time. Cells would attach to the area patterned with



Scheme 1. (a) The switchable superhydrophobic surface is fabricated by roughening a layer of fluoropolymer on the pre-patterned ITO electrodes. (b) A drop of fibronectin solution is added to the surface and a 150 V is applied to the desired electrodes. (c) Fibronectin molecules are deposited on the array with underneath electrode activated. (d) The microarray is then used for cell culture. The cells will only attach to the area coated with fibronectin. (e) The procedure is repeated to culture the second type of cells.

fibronectin (Scheme 1(d)). The process was repeated once to culture the second type of cells on other patterned areas (Scheme 1(e)).

To create cell microarrays, a 4 × 4 switchable superhydrophobic microarray was used. The dimensions for each array were 200 μm × 200 μm. Two cell lines, NIH 3T3 and HeLa, were seeded on the patterned superhydrophobic surfaces and placed in a confocal microscope (Fluoview 1000, Olympus) equipped with an incubator (MIU-IBC-IF, Olympus) at 37°C and 5% CO_2 for 6 h. The density of the cells was about 10^5 cell/ml. Before measurement, the suspension cells were removed and the Differential Interference Contrast (DIC) or fluorescence image was taken.

3. Results and Discussion

Shown in Fig. 1 is an addressable chip containing 4 × 4 switchable superhydrophobic microarrays. In a previous experiment [32], we had demonstrated that the water contact angle on the switchable superhydrophobic surface could be decreased from 163° to less than 10° by applying 150 V to the underneath electrodes and five different proteins could be selectively deposited into individual microarrays. To produce cell microarrays with different types of cells, ECM molecules were deposited into the desired area and followed by culturing the first type of cells. After the cells were attached to the desired area, the ECM molecules could be deposited into another area and followed by culturing the second type of cells.

Before using the switchable superhydrophobic microarray for cell patterning, the chip was tested by depositing two different protein solutions. To deposit proteins on

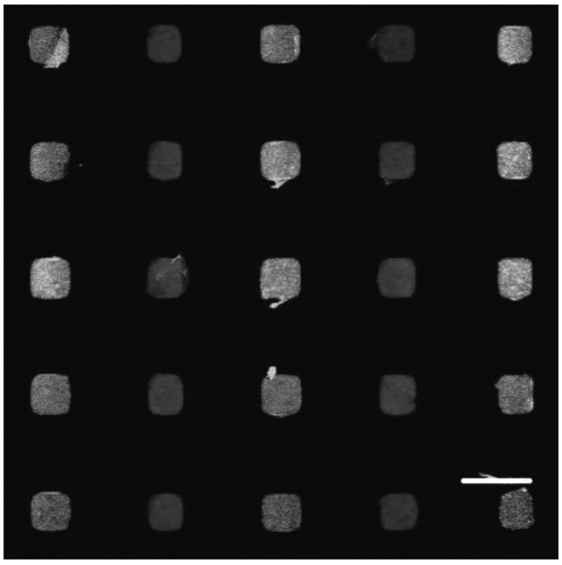


Figure 2. Fluorescence image of the patterned FITC conjugated anti-chicken IgG (green) and cy3 conjugated anti-rabbit IgG (red). Bar: 400 μm.

the switchable superhydrophobic microarray, a drop (15 μl) of phosphate buffered saline (PBS) solution containing green-fluorescent FITC conjugated anti-chicken IgG (5 μg/ml, Sigma-Aldrich) was first placed on the superhydrophobic microarray for 1 s with 150 V applied voltage, and then washed with PBS solution. A second drop of protein solution containing cy3 conjugated anti-rabbit IgG (10 μg/ml, red, Sigma-Aldrich), was then added on the chip and the procedure was repeated. The result is depicted in Fig. 2. It can be clearly seen that the areas with deposited anti-chicken IgG (green) and anti-rabbit IgG (red) were well separated and there was very little cross contamination (<2%).

Knowing that the protein could be selectively deposited on the switchable superhydrophobic microarray, the protein solution containing fibronectins (50 μg/ml) was then deposited into a 4 × 4 microarray. The chip was then placed in the cell culture dish and seeded with HeLa cells at a concentration of 10^5 cell/ml. After 6 h of incubation at 5% of CO_2 and 37°C, HeLa cells were found to attach to all the arrays patterned with fibronectins as shown in Fig. 3. Since the HeLa cells can grow even in the suspension, some HeLa cells were found to grow on the flat area (no fibronectin deposition). During the cell culture, the HeLa cells were found to migrate from the flat area to the patterned area. The HeLa cells attached to the flat area tended to aggregate. The situation was slightly different for the adherent cell line. When the fibroblast cells were seeded on the alternatively patterned microarrays, it was found that the fibroblast cells were attached exclusively on the arrays patterned with fibronectins as shown in Fig. 4. No fibroblast cell was found in the roughened region without the fibronectin deposition.

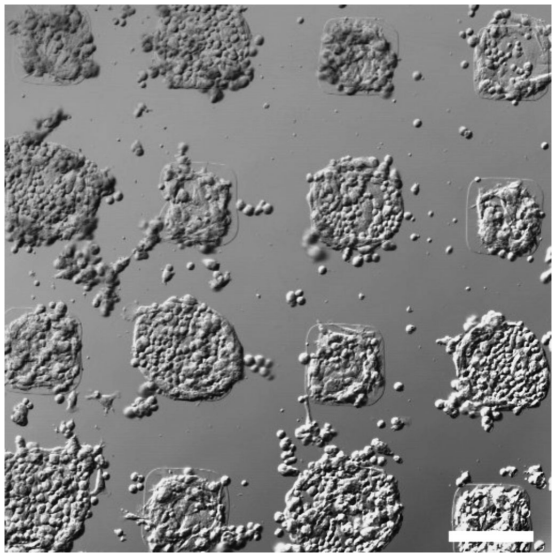


Figure 3. HeLa cells patterned on the switchable superhydrophobic microarrays. Bar: 200 μm.

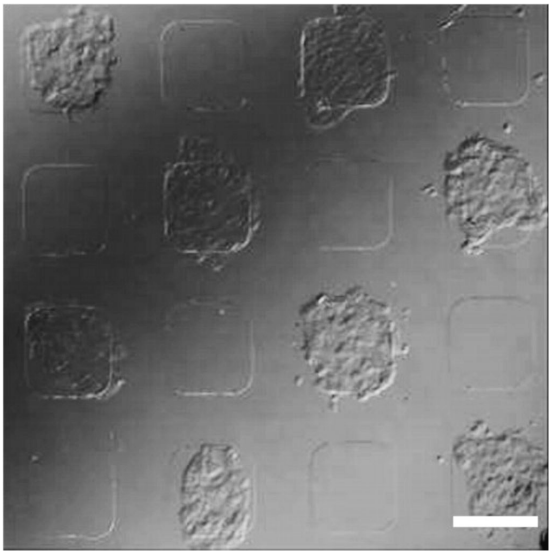


Figure 4. Fibroblast cells patterned on the switchable superhydrophobic microarrays. Bar: 200 μm.

To culture different cells on the same chip, the fibronectin solution was deposited on alternative arrays similar to those shown in Fig. 4 and then seeded with fibroblast cells. After 30 min of incubation, the fibronectin was deposited on the rest of the microarrays and the HeLa cells were added to the culture dish. To distinguish two different types of cells, the fibroblast cells were stained with a red cell tracker dye and the HeLa cells were stained with a green cell tracker dye. Shown in Fig. 5

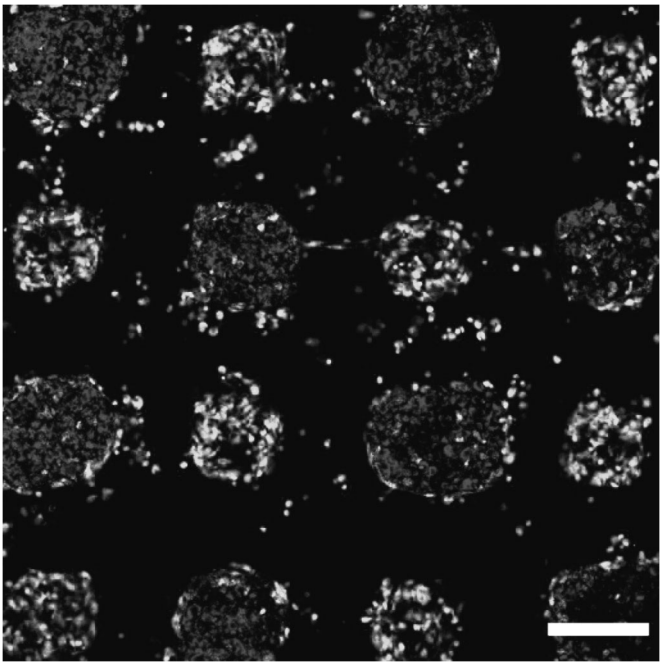


Figure 5. Fibroblast cells (red) were first patterned on switchable superhydrophobic microarray then followed by the HeLa cells (green). Bar: 200 μm.

is the fluorescence image of the cells on the switchable superhydrophobic array. It can be clearly seen that two different cells can be grown in the desired region in an addressable fashion. Therefore, we conclude that our approach can be used to co-culture two different types of cells on the same chip with spatial control. In principle, this approach can be extended to pattern more than two types of cells.

4. Conclusion

In conclusion, we have demonstrated a novel cell patterning technique using switchable superhydrophobic surfaces. It has been shown that each element on a switchable superhydrophobic microarray can be addressed individually and different types of functional biomolecules can be selectively deposited on the microarray. It has also been demonstrated that two different types of cells can be cultured on the same chip in any desired area.

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