Observation of enhanced cell adhesion and transfection efficiency on superhydrophobic surfaces†

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It was found that cells attached preferentially on the roughened area of patterned superhydrophobic surfaces allowing the formation of cell microarrays with the advantages of improved cell adhesion, natural separation of colonies and enhanced transfection efficiency.

Ever since the discovery of the importance of surface roughness to the water repellent behavior of plant leaves,[1] scientists have developed various strategies[2] to produce so-called "superhydrophobic surfaces", whose water contact angles are larger than 150°. It is generally believed that the water repellent properties of the superhydrophobic materials could reduce the water contact area on the surfaces, therefore, minimizing the adsorption of particles or molecules. In the past few years, several potential applications of the superhydrophobic surfaces have been identified including coatings for self-cleaning, fog condensation, contamination reduction, oxidation reduction, oil–water separation, and rapid water spreading.[3] However, there are very limited research activities in exploring the possibility of using the superhydrophobic materials for biological applications. The reduced contact area between the solution and surface may minimize the adsorption of biomolecules, therefore, improving the protein resistance on the superhydrophobic surface. It has been shown that the short term protein resistance on the superhydrophobic surfaces was very similar to the poly(ethylene glycol) (PEG) surfaces, a well known protein resistant coating, allowing the selective deposition of proteins on the patterned superhydrophobic surfaces.[4] The bioanalytical readout in the protein microarrays fabricated on the superhydrophobic surfaces have been greatly improved owing to the reduced protein adsorption on the superhydrophobic surfaces.[5] It was also shown that the superhydrophobic surfaces could suppress the protein adsorption and promote the flow-induced protein detachment in the microfluidic system.[6] The adhesion of blood cells was found to be minimized on the superhydrophobic surfaces.[7] Here we report a surprising observation of enhanced cell adhesion and transfection efficiency on patterned superhydrophobic surfaces, which could be used as cell microarrays.

Cell microarrays have been used to investigate the expression of genes and the function of proteins in living cells where the native environments could facilitate correct biomolecular reactions.[8] However, cell microarray technology is still far from mature. There are several challenges that need to be overcome including the improvement of cell adhesion, transfection efficiency, and colony separation.[9] In the past two decades, a lot of surface modification schemes have been proposed to promote the adhesion of cells on the surfaces. Most of them rely on self-assembly monolayers or polymers that provide ligands for cell adhesion.[10] One of the most commonly used approaches is to employ the micro-contact printing technique[11] where ECM molecules such as fibronectins, vitronecins and collagens are first patterned on the surfaces. Then the adhesion of cells could be guided through the binding to these ECM molecules. In a previous experiment,[4] we have demonstrated that different kinds of proteins can be deposited on the desired areas on the switchable superhydrophobic arrays *via* electrowetting effect. Therefore, it should be possible to create a cell array by patterning ECM molecules on the switchable superhydrophobic array. To test how cells behave on the superhydrophobic surfaces, a thin layer of fluoropolymer poly-[tetrafluoroethylene-*co*-2,2-bis(trifluoromethyl)-4,5-difluoro-1,3-dioxole] (Teflon, AF, DuPont) was spin-coated on a cover slip at 3000 rpm for 1 min. The fluoropolymer coated cover slip was then baked on a hot plate at 110° for 30 min. After these processes, the advancing water contact angle measured on the fluoropolymer was about 128°. The fluoropolymer surface were then roughened by oxygen plasma treatment (Oxford Plasmalab 80 Plus, 80 W) with a gas O_2 (2 sccm) at a total pressure of 25 mTorr for 2 to 12 min. The water contact angles on the roughened fluoropolymer surfaces were plotted in Fig. 1(a). The superhydrophobic surfaces could be obtained by 12 min of plasma treatment where the advancing and receding contact angles were measured to be 164° and 154°, respectively. Based on the XPS spectra, it was concluded that the oxygen plasma treatment did not alter the surface chemistry of fluoropolymer.[4] To create patterned superhydrophobic surfaces, a similar procedure was used where a layer of photoresist (S1813, Shipley) was spun on top of the fluoropolymers and a photolithographic process was used to define the superhydrophobic area on the photoresist. After oxygen plasma treatment and a lift-off process, patterned

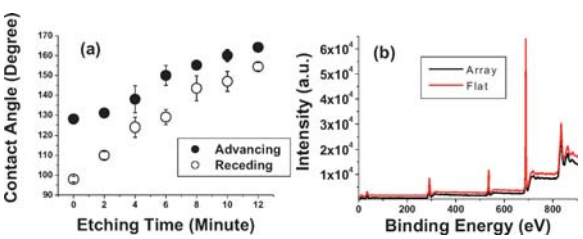

Fig. 1 (a) Advancing and receding water contact angles of roughened fluoropolymers as a function of etching time. (b) XPS spectra of patterned (black) and flat (red) fluoropolymers.

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superhydrophobic surfaces could be obtained. The XPS spectra of flat and patterned fluoropolymers are shown in Fig. 1(b) (high resolution XPS spectra can be found in the ESI†). Angle resolved XPS, XPS imaging and FTIR were also used to characterize the plasma treated patterned fluoropolymers. No significant change in surface chemical composition was observed (details of measurements can be found in the ESI†)

When the patterned superhydrophobic surfaces were used as the substrates for cell culture, a surprising behavior of cells was observed where cells adhered selectively on the patterned superhydrophobic surfaces, which exhibited short-term protein resistance.[4] Shown in Fig. 2 are the time-lapse DIC images of the NIH 3T3 cells on the patterned superhydrophobic surfaces (movie in the ESI†). The cells were randomly distributed on the surfaces when the cells were first seeded. However, more and more cells were found to migrate toward the roughened area. After 3 h of incubation, most cells were found to locate inside the roughened area. When the patterned surfaces were washed with PBS solution, the weakly adhered cells were removed as shown in Fig. 2(d). It is known that the adhesion of cells on materials depends on the surface characteristics such as wettability, surface charge, surface chemistry, chirality and roughness.[12] Since cells normally attached to the surfaces through ECM molecules, we examined the time dependent fibronectin, an ECM molecule, and the adsorption on the superhydrophobic surfaces was similar to that of those reported previously.[4] The superhydrophobic surfaces were found to resist to the adsorption of fibronectins in the first thirty minutes. However, when the fibronectin solution stayed on the superhydrophobic surfaces for more than one hour, the accumulation of fibronectin molecules on the superhydrophobic surfaces became evident and eventually surpassed those adsorbed on the flat fluoropolymers (for details see ESI†). Since the cells prefer to stay on the more adhesive surfaces, the preferential growth of the cells on the patterned area may be attributed to accumulation of ECM molecules on the superhydrophobic surfaces during the incubation period.

To further explore the relationship between the number of the adhered cells and the surface roughness, we have investigated the cell adhesion of three cell lines, CHO, HeLa and 3T3, on various roughened fluoropolymers. Shown in Fig. 3 are the DIC images of 3T3 cells cultured on the patterned fluoropolymer surfaces with surface roughness ranging from 10 to 65 nm. It can be clearly seen that the number of cells adhered on the patterned area increased with the surface roughness. The averaged numbers of cells adhered on various roughened fluoropolymers for all three cell lines are summarized in Fig. 4. It can be seen that a very small amount of cells could adhere on the flat fluoropolymer. However, the number of cells attached to the roughened fluoropolymer increased as the surface roughness increased. When the surface was in the superhydrophobic state (advancing and receding contact angles both larger than 150°), the number of cells attached to the roughened surfaces surpassed the surface coated with collagens for all three types of cells. No measurable cytotoxicity was observed for the cells grown on the superhydrophobic surfaces over seven days.

Since the spatial distribution of cells on the patterned superhydrophobic surfaces can be regulated, the roughened fluoropolymers may be used as a cell microarray. On the patterned superhydrophobic surfaces, we have shown that the adhesion has been improved and the cell colonies were already separated by their spatial patterns. The only question that remains for using the patterned superhydrophobic surfaces as the substrates of cell array is:

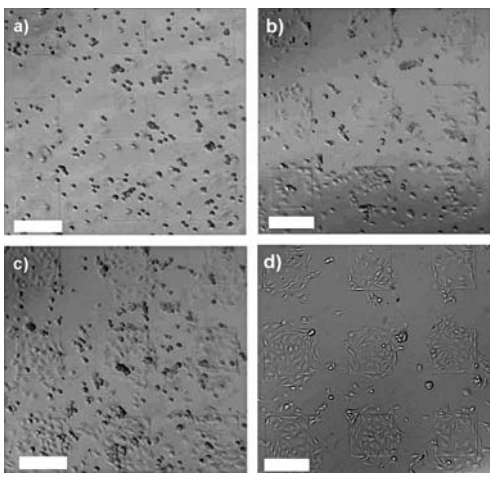

Fig. 2 The time-lapse DIC images of NIH 3T3 cells on the patterned superhydrophobic surfaces after (a) 0 min, (b) 50 min (c) 3 h of incubation. (d) After washing the sample with PBS solution. Scale bars represent 200 μm.

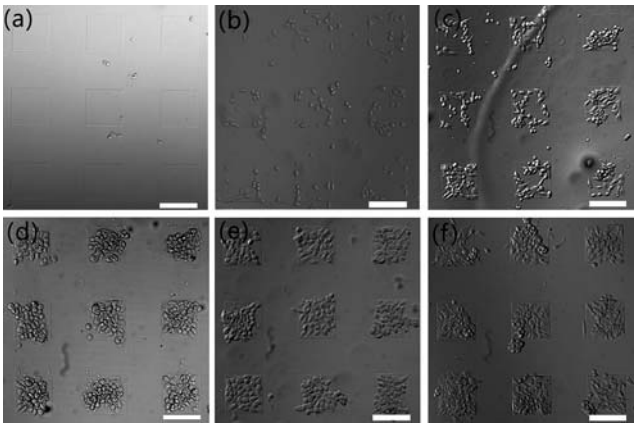

Fig. 3 3T3 cells cultured on various roughened fluoropolymers. The surface roughness on the patterned area was (a) 10 nm (b) 25 nm (c) 35 nm (d) 42 nm (e) 52 nm (f) 65 nm. Scale bars represent 200 μm.

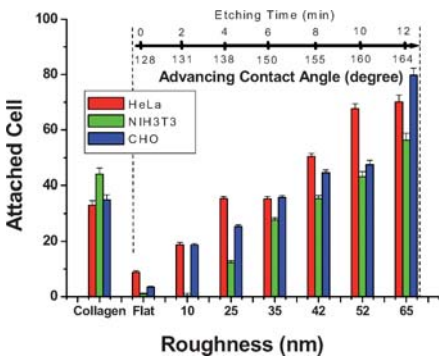

Fig. 4 The averaged cell numbers attached on the roughened fluoropolymer surfaces as a function of surface roughness. The patterned area was 200 μm × 200 μm. The collagen coated glasses and flat fluoropolymers were used as controls.

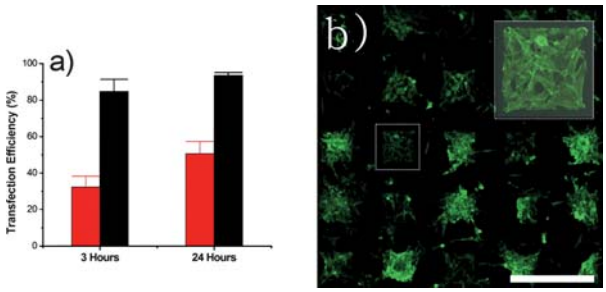

Fig. 5 (a) Transfection efficiency measured for the CHO cells on the poly D-lysine coated glasses (red) and superhydrophobic surfaces (black). (b) The NIH 3T3 cells on the patterned superhydrophobic surfaces transfected with GFP-actin plasmids. Scale bar represents 500 μm. Inset: enlarged view of the marked array.

could the cells on the superhydrophobic surfaces be transfected efficiently? To test the transfection efficiency of the cells on the patterned superhydrophobic surfaces, CHO cells were transfected by transfection reagents, PolyFect (Qiagen), using a commercial fluorescence protein construct, pKaede-MC1, which can be used to express a fluorescence protein, Kaede, in the cells. The CHO cells on the poly D-lysine coated culture dishes were used as the control. The concentration of plasmid used in this experiment was 50 μmg ml^{-1}, and 10 μl of Polyfect solution was added to 1 ml of medium. Fig. 5(a) summarizes the transfection efficiency measured on both surfaces after 3 h and 24 h of transfection. It can be clearly seen that the transfection efficiencies on the patterned superhydrophobic surfaces were much higher than those measured on the normal culture dishes over short times. A similar result was observed for 3T3 cells transfected with GFP-actin plasmid as shown in Fig. 5(b). The enhanced observed transfection efficiency may be attributed to the surface nanostructures. It has been suggested that the surface nanostructures may help to retain the cells therefore, the gene delivery.[13]

In conclusion, it was found that the cells attached preferentially on the roughened area allowing the formation of cell microarrays when the patterned superhydrophobic surfaces were used in the cell culture. It was also observed that the transfection efficiencies of the CHO cells and NIH 3T3 cells were greatly improved on the superhydrophobic surfaces. Therefore, we conclude that the patterned superhydrophobic surfaces could be used as cell microarrays with the advantages of improved cell adhesion, natural separation of colonies and enhanced transfection efficiency.

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