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Controlled Cell Patterning on Bioactive Surfaces with Special Wettability

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Abstract

The ability to control cell patterning on artificial substrates with various physicochemical properties is of essence for important implications in cytology and biomedical fields. Despite extensive progress, the ability to control the cell-surface interaction is complicated by the complexity in the physiochemical features of bioactive surfaces. In particular, the manifestation of special wettability rendered by the combination of surface roughness and surface chemistry further enriches the cell-surface interaction. Herein we investigated the cell adhesion behaviors of Circulating Tumor Cells (CTCs) on topographically patterned but chemically homogeneous surfaces. Harnessing the distinctive cell adhesion on surfaces with different topography, we further explored the feasibility of controlled cell patterning using periodic lattices of alternative topographies. We envision that our method provides a designer's toolbox to manage the extracellular environment.

Keywords: cell patterning, wettability, superhydrophilic, surface chemistry, circulating tumor cells

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1 Introduction

The study of the interaction between cells and their microenvironment is of essence in a wide spectrum of applications ranging from cell-cell communication, co-culture systems, cellular force measurements[1–5], and drug discovery system, cell penetration-related drug delivery[6–9], cell capture, separation, and guidance[10–13]. Over the past decades, a number of techniques have been developed by Wang and many other researchers to functionally interrogate living cells with bioactive surfaces[14–23]. For many applications, it is desirable that cells can be immobilized at the predetermined locations using the physical confinement, biochemical patterning, external forces or the combination of them. For example, cells can be isolated simply by controlling the geometrical feature of surfaces. However, the immobilized cells are susceptible to undesired detachment owing to their relatively small adhesion with the underlying surfaces during washing and fluidic exchange process[24]. The manipulations of cells using external forces such as optical[25,26], magnetic[27], mechanical[28], dielectrophoretic[29], and acoustic[30], usually require complicated facilities, which are difficult to be miniaturized. Decorating biochemical molecules including PEGylation or specific biomolecules (antibody, protein, or DNA) on specified positions is an efficient way to spatially control the cell behaviors[31–38]. However, such kind of processes normally involves multi-step operations.

Arising from the advances in microfabrication, bioactive surfaces with refined texture and unprecedented precision have been reported for efficient cell capture, diagnosis, and monitoring[39–47]. The philosophy behind this is that the interactions between cells and roughened surfaces are significantly enhanced compared to those on the flat surface since cell membrane struc-

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tures are associated with nanoscale roughness. Despite extensive progress, our understanding and the ability to control the cell-surface interaction are complicated by the complexity in the physiochemical features of bioactive surfaces. The manifestation of special wettability rendered by the combination of surface roughness and surface chemistry further enriches the cell-surface interaction[48,49]. Most of bioactive surfaces with special wettability are functionalized with additional chemical composition and their chemical property might degrade during the long-term operation. Consequently, it remains unclear how cell behaviors related to adhesion, mobility, viability, and proliferation, are influenced by the topographical and chemical features[50–52]. To decouple different effects, we studied in this work cell adhesion behaviors on topographically patterned but chemically homogeneous surfaces. Harnessing the distinctive cell adhesion on surfaces with different topography, we further explored the feasibility of controlled cell patterning using periodic lattices of alternative topographies. We envision that our method provides a designer's tool box to manage the extracellular environments.

2 Methods

2.1 The flat SiO_2 and superhydrophilic nano SiO_2 structure fabrication

The SiO_2 nano structure with superhydrophilic properties was fabricated by Deep Reactive Ion Etching (DRIE) technology and thermal oxidation. Silicon nano structure arrays were formed on single crystal *n*-type silicon (100) wafer of 100 mm in diameter by two steps DRIE etching using photoresist as the mask. The etcher was operated at a power of 90 W. The flow rates for SF_6, O_2, and C_4F_8 were 150 sccm, 50 sccm, and 260 sccm, respectively. Then the silicon wafer with nano silicon pillars is thermally oxidized at 1100 ˚C for 2 h in O_2 atmosphere. After oxidation, superhydrophilic SiO_2 nano structure was obtained. Silicon wafers were also oxidized at the same conditions to obtain the flat SiO_2 for comparison. The wafers were diced into pieces of 10 mm × 10 mm in size. To characterize the wettability of the flat and nanorough surfaces, a goniometer was used to measure the water contact angle of the samples. Approximately 2 μL water droplets were gently deposited on the surfaces utilizing a microsyringe and then the profile of the water on the substrate was captured by the optical system.

2.2 Cell culture

Cell lines A549 and H1975 were cultured in growth media (RPMI-1640) supplemented with 10% newborn calf serum and 1% penicillin-streptomycin. These cells were cultured at 37 ˚C in an atmosphere of 5% CO_2. 0.25% trypsin-EDTA in Phosphate Buffered Saline (PBS) was used to resuspend cells.

2.3 Cell-culture reservoir fabrication

Cell-culture reservoir was made of PDMS and square substrate. A mixture of SYLGARD 184 PDMS prepolymer parts A and B (10:1) was homogenized and degased for 2 h. Then the mixture was poured into a blank petri dish with a thickness of 5 mm and cured at about 80 ˚C for 2 h. The solidified PDMS mold was peeled off the petri dish and diced at size of 10 mm × 10 mm. A stainless–steel punch was used to poke a 6 mm × 6 mm hole in the square PDMS mold. The reservoir was made by bonding thermally the punched PDMS mold to the 10 mm × 10 mm wafer substrate. All the reservoirs were treated with air plasma etching for 30 s to remove impurities from the substrates.

2.4 Quantification of adhesion efficiency of cells

Prior to cell assays, the cells were labelled with DiO cell-labeling reagent (for emission of 501 nm, Beyotime Institute of Biotechnology, China). The cell concentration was determined using a haemocytometer and diluted to about 12 cells per microliter with culture medium. Reservoirs were sterilized under UV light for 30 min. A volume of 120 μL cell solution was pipetted into each cell-culture reservoir. Three reservoirs were directly dried in an oven with a constant temperature of 37 ˚C, the average cell number in which was used as the initial cell number of each reservoir. After incubation at 5% CO_2 and 37 ˚C for different duration time (0.5 h – 10 h, three samples for each time), the culture media were removed from the samples, then the reservoirs were gently rinsed twice with PBS to remove floating cells. The cells that were adhered to the substrates were then fixed with 4% paraformaldehyde for 15 min, followed by rinsing with PBS. The adherent substrate-immobilized cells were then imaged and photographed using fluorescence microscopy. The pictures of the same substrate were mosaicked into a complete fluorescent cell image of the substrate. The cells were