Research Article

Nanostructured Surface with Tunable Contact Angle Hysteresis for Constructing *In Vitro* Tumor Model

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Contact angle hysteresis (CAH) is an important phenomenon in surface chemistry. In this paper, we fabricated nanostructured substrates and investigated the relationship between roughness and CAH. We demonstrated that by patterning well-tuned CAH in superhydrophobic background, we can pattern droplets with controlled sizes. We further showed that our system could be used in fabricating complex hydrogel architecture, allowing coculture of different types of cells in three-dimensional way. This CAH-based patterning strategy would provide *in vitro* models for tissue engineering and drug delivery.

1. Introduction

Contact angle hysteresis (CAH), defined as the difference between advancing and receding contact angles, has continuously been one of important topics in surface chemistry. Enormous theoretical and experimental efforts have been devoted to how CAH correlates to topographical or chemical surface heterogeneities [1, 2]. Topological heterogeneities, for example, nanoscale roughness, have shown to largely affect surface CAH. Many studies have been devoted to mechanisms underlying CAH [3]. However, the applications of CAH are yet to be explored. In nature, CAH is crucial during feeding of water by shore birds, as it can overcome the gravity on the water droplet in the beak of birds [4]. Thus, the exploration of CAH could be beneficial in controlling the liquid behavior on surface.

Herein, we propose a CAH-based strategy to control the size of droplets in patterns and further constructed complex hydrogel architecture for three-dimensional (3D) cell coculture. Patterning droplets have been an important issue in many fields, such as biochips, microlens, and digital microfluidics [5–7]. Among various techniques developed for droplet patterning, wettability contrast-based method is

widely employed. In this case, a hydrophilic/hydrophobic (or superhydrophilic/superhydrophobic) patterned substrate is fabricated. During dip-coating, hydrophilic area can capture liquid droplets [8, 9]. In our study, we show that CAH can be utilized for patterning droplets. By tailoring the CAH, we fabricated patterned droplets with various sizes. We further fabricated hydrogel droplets with complex architecture. We constructed an *in vitro* tumor model using cell-encapsulated prehydrogel solutions. We believe that the tumor model could find potential applications in mimicking tissue *in vivo* and could serve as an *in vitro* model for drug delivery.

2. Materials and Methods

2.1. Preparing CAH-Varied Surface. Silicon wafer was cut and cleaned in ethanol and acetone solution and boiled in $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ solution (3:1, v/v. *Caution! The harmful solution should be treated carefully!*). The substrates were dried under nitrogen gun and etched in five different etching solutions in AgNO_3 and HF solution [10]. The original etching solution was prepared by dissolving 0.15 g AgNO_3 in 15 mL HF and 55 mL deionized water mixed solution and further diluted in different ratios (i.e., 0-, 0.2-, 0.4-, 0.6-, 0.8-, and

FIGURE 1: Scanning electron microscopy images of silicon substrates with increasing nanoscale roughness by various etching solutions.

1-fold). The substrates were immersed in various etching solutions for 20 s. HNO_3 solution (30%, v/v) was employed to dissolve the silver. Finally, silicon substrates were dried in oven.

2.2. Fabrication of CAH-Varied Substrates with Superhydrophobic Background. Silicon substrates with various roughness were achieved using the methods above. The substrates were patterned using photolithography and further etched (photoresist-patterned regions were protected from etching) in the original etching solution. By modifying substrates using octadecyltrichlorosilane (OTS), the background of substrate was rendered superhydrophobic. Finally, the substrates were rinsed in acetone to remove the patterned photoresist.

2.3. Fabrication of In Vitro Tumor Model. Hela cancer cells and NIH 3T3 fibroblasts were suspended in alginate solution

(1 wt% in PBS). To visualize the cells in the model, Hela cells were prestained in green and NIH 3T3 cells in red. The substrates were immersed firstly in Hela cell solution for 30 s and pulled out. The cell-encapsulated droplets captured in substrates were gelled by calcium chloride solution (5 wt%). Subsequently, the substrates were immersed in NIH 3T3 cell solution for 30 s and pulled out. Also the solution on substrates was gelled by calcium chloride solution.

3. Results and Discussions

3.1. Characterization of Surface Morphology and Calculation of CAH. We modified the surface CAH by varying surface roughness. We observed the morphologies using scanning electron microscopy (SEM). From Figure 1, we obtained that the roughness of silicon increases with the increase of the

FIGURE 2: (a) Static contact angles, advancing contact angles, and receding contact angles measured on substrates with different roughness. (b) The calculated cosine values of contact angle hysteresis increased with the increase of surface roughness.

reaction concentration. We obtained the root-mean-square roughness 0.20, 1.30, 2.03, 3.87, 6.23, and 9.40 nm for solutions between 0-, 0.2-, 0.4-, 0.6-, 0.8-, and 1-fold of original solution. The high concentration of etching solutions provided more deposited Ag^+ ions as etching sites compared to low ones, resulting in a rougher surface. We calculated the CAH of different substrates after etching. For static contact angle and advancing contact angle (θ_a) of substrates, the values did not vary much in response to different roughness. However, receding contact angles (θ_r) decreased with the increase of roughness. As a result, the calculated CAH increased in response to surface roughness. The decreasing receding contact angle is implied from theoretical predictions. In their theory, receding contact angle is more sensitive to the proportion of surface defects [11]. We also calculated the difference between the cosine of values of advancing and receding contact angle $\cos(\theta_r) - \cos(\theta_a)$ and obtained that $\cos(\theta_r) - \cos(\theta_a)$ was positively related to surface roughness (Figure 2).

3.2. Controlling Liquid Size. The tunable CAH can be utilized to control liquid size on substrate. We fabricated substrates with CAH-controlled patterns in superhydrophobic background (Figure 3(a)) and examined the size of water droplets that were captured in patterns. We obtained patterned water droplets by dip-coating. We immersed the substrates in deionized water and pulled them out vertically at a speed of about 1 mm/s. For convenience, we measured the projected area of droplets just after pulling out substrates. For substrates with CAH ($\cos(\theta_r) - \cos(\theta_a)$) smaller than 0.4, sizes of droplets were close to zero. Some of the patterns contained no droplets after dip-coating (for droplets smaller than 0.02 mm^2 , some evaporated quickly before imaging). For CAH larger than 0.4, larger droplets were obtained in

the patterns. In previous studies, for patterning water, high wettability contrast between patterns and background was employed, for example, superhydrophilic/superhydrophobic patterns. In our experiment, we show that making patterns with CAH contrast is also effective in patterning droplets, with even more capabilities to control droplet sizes.

3.3. Patterning Hydrogel Droplets with Complex Architecture. Based on the control over liquid size using CAH, we constructed hydrogel droplets with complex architecture by dual dip-coating and gelling cell-encapsulated prehydrogel solutions. For prehydrogel solution, we employed 1% sodium alginate solution. Sodium alginate is a natural macromolecule and its hydrogel can be used for cell culturing [12]. Alginate solutions can be rapidly turned into hydrogel by adding Ca^{2+} ions as the cross-linking reagent. According to this, we fabricated complex hydrogel structure by performing primary dip-coating, gelation, secondary dip-coating, and gelation. After secondary dip-coating, the size of droplet from second pulling was almost equal to the size of patterns. We believe that after primary dip-coating, gelled droplets contributed to the adhesion force of the whole patterns. As a result, the whole pattern areas were covered by the secondary alginate solutions.

3.4. Construction of In Vitro Tumor Model. To construct an *in vitro* tumor model, we employed two types of cells, cancer cells (Hela cell) and normal cells (NIH 3T3 fibroblast). We suspended the cells in separate alginate solutions. We used Hela cells for the primary solution and NIH 3T3 fibroblasts for the secondary. By serially dip-coating two solutions and gelation, we obtained the coculture of different cells with Hela cells in the inner part and fibroblasts in the outer part of the hydrogel droplet (Figure 4). Controlling spatial distribution

FIGURE 3: (a) The design of substrates with superhydrophobic background and CAH-controlled patterns (top view). The schematic of dip-coating process of substrates (side view). (b) Sizes of water droplets captured after dip-coating the substrates with various CAH.

FIGURE 4: Confocal image of tumor models. Two types of cells were employed. Red cells were NIH 3T3 cells and green ones were Hela cells. Inset was the schematic of three-dimensional tumor model.

of heterogeneous types of cells is an important issue in tissue engineering and regenerative medicine. Our method provides a convenient method to fabricate complex structures for 3D cell coculture, with less dependence on functional materials and equipment and minimal harm to cells. We believe that our method could be applied in studying cell performance in tissue-level, mimicking microenvironments

in tumor, and constructing models for drug delivery and screening [13, 14].

In conclusion, we examined the relationship between contact angle hysteresis and surface roughness and demonstrated a CAH-based patterning strategy for patterning droplets with controlled sizes. We showed that droplets sizes were affected by the CAH of the surface. We fabricated

complex architecture and patterned different cells in spatially controlled way. We believe that our work would provide useful tools for tissue engineering and drug delivery.

Competing Interests

The authors declare that there are no competing interests related to this paper.

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