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Enhancing Cell Adhesion and Confinement by Gradient Nanotopography

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The vitality and proliferation of mammalian cells critically depend on their adhesion on a surface. This adhesion and the subsequent cell activities (spreading, focal adhesion, migration, and proliferation) are highly sensitive to the surface chemistry[1] and its physical environment, including the stiffness of the materials[2] and topography of the surfaces on which cells adhere,[3] as well as the geometry of chemical patterns on surfaces.[4] While surfaces with structural features at nanometer-scale are common in living systems,[3] the study of cell activities on nanostructured surfaces has been challenging because the ubiquitous adsorption of protein often sabotages the nanofeatures on man-made surfaces.[5] In this work, we demonstrate the use of a nanostructured gold film possessing a gradient in surface topography to enhance the surface chemistries that support and resist the adhesion of mammalian cells.

Self-assembled monolayers (SAMs) of alkanethiols on gold films have been demonstrated to be a powerful system for supporting biospecific interactions by resisting protein adsorption and cell adhesion.[1a,5] On nanostructured gold films,[6] SAMs exhibit a wide range of novel interfacial phenomena such as uniform alignment and controlled switching of liquid crystal orientation.[7] However, the effect of the nanostructures of the gold films on cell adhesion has not been studied. A gradient of continuously changing nanometer-scale topography can be introduced in the gold films by creating a continuous increase in the angle of incidence of gold atoms during vapor deposition (Figure 1A).[7c,8] Due to self-shadowing, gold films deposited on a flat surface from an oblique angle afford an anisotropic polycrystalline surface structure where the roughness at nanometer-scale is higher measured in the direction parallel to gold deposition than perpendicular to gold deposition.[7c] Detailed studies by atomic force microscopy reveal a nanometer-scale corrugation of the gold film that is of characteristic wavelengths of 10−30 nm and amplitudes of 1−2 nm.[7c] Past studies using optical second harmonic generation and infrared−visible sum frequency spectroscopy also revealed gold films with anisotropic polycrystalline structure with respect to the different angles of incidence of the gold atoms.[7a,b]

To study the adhesion of mammalian cells on this gradient of nanotopography, we patterned SAMs of different shapes of HS-$(CH_2)_{14}CH_3$ surrounded by $HS(CH_2)_{12}(OCH_2CH_2)_3OH$ to create confinements of localized cell adhesion (on methyl-terminated SAMs; Figure 1B). As the tri(ethylene glycol)-terminated SAM provides a bioinert chemistry that resists protein adsorption and cell adhesion,[1a,5] we examined the confinement of albino 3T3 fibroblasts on the entire gradient gold film (~7.5 cm) over 25 days. The cell adhesion was recorded on the same positions on the gradient gold film over time. Figure 2 shows the cell adhesion on the 18th day of cell culture on 15 out of 32 microcontact printed patterns equally spaced on the gold film deposited at a changing angle of incidence from 38° to 56°. Each position represents an increment of 0.5° in the angle of gold incidence. While cells remained confined in the printed patterns on the high topography

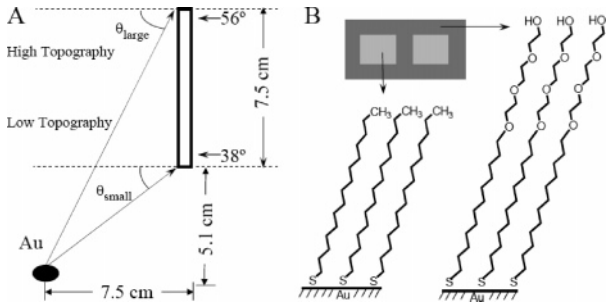

Figure 1. (A) Experimental setup for fabricating gold films with gradient nanotopography. (B) Schematic representation of patterned SAMs of HS-$(CH_2)_{14}CH_3$ surrounded by $HS(CH_2)_{12}(OCH_2CH_2)_3OH$ on gradient gold films.

region (gold films deposited from 51° to 56°), cells started to grow out of the patterns in the region of gold films deposited from 43° to 50°. At low topography (gold films deposited from 38° to 41°), the cells are widely spread out of the confined patterns. The extent of cell growth outside the confinements decreases gradually and continuously from low to high topography.

Next, we examined the cell confinement over time on four selected positions on the gradient gold film (Figure 3). We made three observations at different stages of cell culture. First, at the onset of cell adhesion (Day 1), there was more cell attachment on the high topography (region IV) than on the low topography (region I). Second, cell adhesion reached confluency in the cell adherent regions earlier on the high topographic regions (around Day 2) than on the low topographic regions (around Day 3). Third, after reaching confluency, cells spread out of the confinement on the *low* topographic region much earlier than on the high topographic region. This result is interesting as cell adhesion reached confluency earlier in the patterns on high topography than on low topography, and thus should have had a stronger overpopulation pressure to proliferate into the surrounding bioinert areas.

Figure 4 shows the percentage of patterns having peripheral cell outgrowths in a 1 mm^2 area over time at the four positions. The result shows that the higher the angle of gold deposition, the longer the confinement of cell adhesion. On gold films deposited at high oblique angle (56°), cells are confined up to 25 days by tri(ethylene glycol)-terminated SAMs, which, in contrast, confine cell adhesion for only 6−8 days on gold films deposited on glass slides being rotated on a planetary.[1a]

The mechanism for this enhanced resistance to cell adhesion, like other novel phenomena on nanostructured gold films, likely involves multiple factors, including roughness, defect structures, polycrystallinity, and surface anisotropy at nanometer-scale. For the increased cell attachment and early confluency of cell adhesion on the high topographic region, we compared the true microscopic surface area in the high and low topography region by measuring capacitance current over a fixed projection area.[10,11] Over a constant projection area of 0.178 cm^2, the high topography (gold deposited at 56°) affords a capacitance current of 3.84 ± 1.16 μA and the

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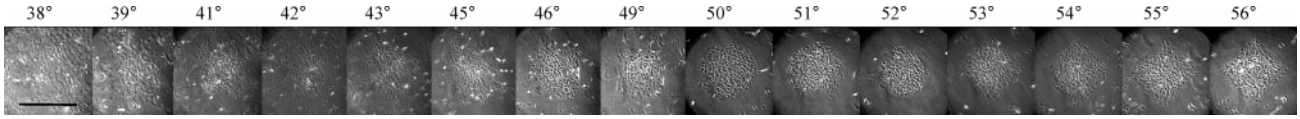

Figure 2. Micrograph of albino 3T3 fibroblasts on the gradient nanotopography of a gold film deposited on a 7.5 cm glass slide. Images were taken on the 18th day of cell culture. The angle of gold deposition is listed above the micrograph. Scale bar = 600 μm.

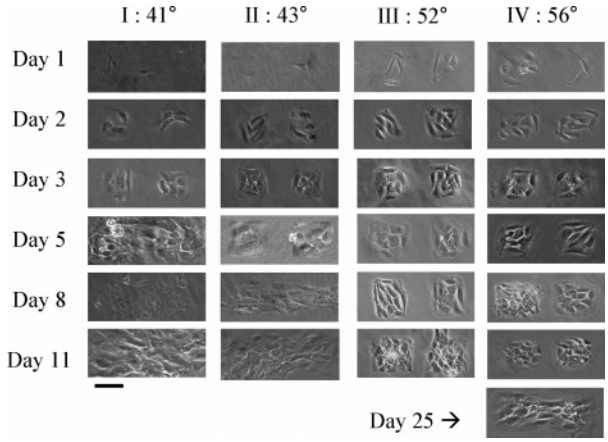

Figure 3. Micrograph of albino 3T3 fibroblasts on patterned SAMs on gradient gold film. The angle of gold deposition is shown above the micrograph; the number of days of cell culture is indicated on the left. Scale bar = 125 μm.

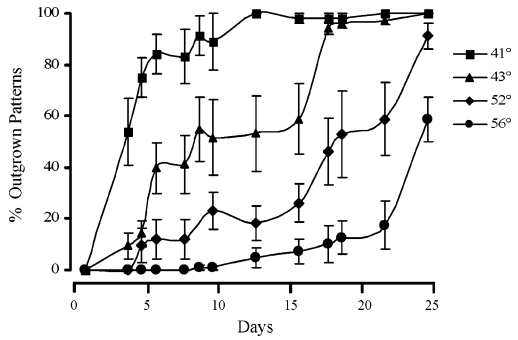

Figure 4. Percentage of out-grown patterns of cell adhesion on four positions of the gradient nanotopography.[9]

low topography (gold deposited at 38°) affords 2.29 ± 0.42 μA (see Supporting Information). This larger surface area on the high topography region is consistent with the observation of early confluency of cell adhesion on high topography.[9]

We also note that the thickness of gold film decreases as the angle of deposition increases on a single slide (Figure 1). By examining the patterned cell adhesion on gold films with different fixed angles of deposition (45° and 60°) but the same thickness, and gold films with fixed angle of deposition but varied thicknesses, we observed that the high angle of deposition plays a predominant role in enhancing the resistance to cell adhesion (see Supporting Information).

To conclude, the most significant result of this work is the discovery that gold films deposited at a high oblique angle significantly increase the resistance to cell adhesion of tri(ethylene glycol)-terminated SAMs, about 4 times longer than previously reported on isotropic gold films.[1] Thus, introducing nanostructures into gold films provides a means to enhance bioinert surface chemistry of SAMs on gold. This continuous variation in influencing cell adhesion will also be useful for studying cell activities that depend on a surface-associated gradient such as axon guidance and cell migration.

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Supporting Information Available: Effect of gold thickness, estimation of the true surface areas, details of the long−term cell confinement, and synthesis of alkanethiols. This material is available free of charge via the Internet at http://pubs.acs.org.

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