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Controlled Cell Adhesion onto Photochemically Micropatterned Perfluoroalkyl Isocyanate Monolayer on Oxidized Aluminum

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A superhydrophobic surface of a perfluoroalkyl isocyanate (R^F-NCO) layer on oxidized aluminum ($^{BW}Al^{Al_2O_3}$) was photolithographed using masked 172-nm vacuum ultraviolet light to fabricate a superhydrophobic/superhydrophilic micropattern. Cell-adhesion experiments revealed complete cell repellency on the superhydrophobic region of the R^F-NCO layer and cell adhesiveness on the superhydrophilic region of $^{BW}Al^{Al_2O_3}$ surface.

Microarray technology has become a crucial tool for large-scale and high-throughput biological science and technology, allowing fast, easy, and parallel detection of thousands of addressable elements in a single experiment under identical conditions.[1] Such microarrays of DNA, protein, and cells are important for genomics, proteomics, and cellomics. Cell microarrays (single or multiple) have recently received considerable attention because of their important roles in fundamental cell biology, cell-based biosensors, and bio-microelectromechanical systems (Bio-MEMS).[2] One effective method for creating cell microarrays is based on the micropatterning of protein-repellant chemicals such as poly(ethylene glycol) (PEG) on substrates to reduce the nonspecific adsorption of cell-adhesive proteins such as fibronectin and vitronectin from culture media. PEG is a hydrophilic and neutrally charged polymer and is known to decrease the attractive forces between surfaces and proteins because of the highly hydrated polymer chains and steric stabilization forces.[3] On the other hand, Ino et al.[4] reported the potential application of a patterned ultra-water-repellent surface for cell microarrays, which represents a different strategy for blocking the adsorption of proteins and adhesion of cells. For these cell microarrays, generally glasses are used as substrates, while only a few metals have been so far used for this purpose. Metals have different physical properties that glasses do not have, such as high light reflectivity and electric conductivity. Therefore, metals including aluminum are considered potential substrates, especially for cell microarrays capable of detecting light or electric signals from arrayed cells effectively. In the present study, in order to develop cell microarrays on metal substrates, we examined cell adhesion on a photochemically micropatterned surface composed of superhydrophobic and superhydrophilic regions on oxidized aluminum ($^{BW}Al^{Al_2O_3}$).

The superhydrophobic surface of the perfluoroalkyl isocyanate layer on oxidized aluminum was fabricated, as described in some previous studies.[4] An aluminum plate (purity: 99.999%, Sigma-Aldrich) was electrochemically polished in 20 wt % perchloric acid/ethanol solution for 30 min at 5 V. We abbreviate this polished aluminum as $Al^{Al_2O_3}$. A piece ($10 \times 10 \times 0.5\,mm^3$) of $Al^{Al_2O_3}$ was treated with boiling Milli-Q water for 5–30 min. We abbreviate this oxidized aluminum as $^{BW}Al^{Al_2O_3}$. The cleaned surface of $^{BW}Al^{Al_2O_3}$ contained pebble-like features, 10–15 nm in size as confirmed by atomic force microscopy (AFM), exhibiting superhydrophilicity with a water-contact angle value of less than 5° (data not shown). Then, a perfluoroalkyl isocyanate layer (SAM) was prepared on the surface of $^{BW}Al^{Al_2O_3}$ by chemical vapor deposition (CVD) of 1*H*,1*H*,2*H*,2*H*-perfluorodecyl isocyanate ($CF_3[CF_2]_7CH_2$-CH_2N=C=O, i.e., R^F-NCO, Aldrich) at 150 °C for 1–24 h (Figure 1). A typical AFM image of the surface morphology of the R^F-NCO layer on $^{BW}Al^{Al_2O_3}$ of which thickness was 1.2 nm estimated by XPS is shown in Figure 2A. The surface of $^{BW}Al^{Al_2O_3}$ deposited with R^F-NCO molecules exhibited an unchanged topography, with a root mean square (RMS) of the surface roughness within the 10–15 nm range. The advancing/receding water-contact angles of the R^F-NCO layer on $^{BW}Al^{Al_2O_3}$ were 167°/165° respectively, indicating that R^F-NCO deposition on $^{BW}Al^{Al_2O_3}$ with nanosize topographical features induced superhydrophobicity with low hysteresis (Figure 2B).[5]

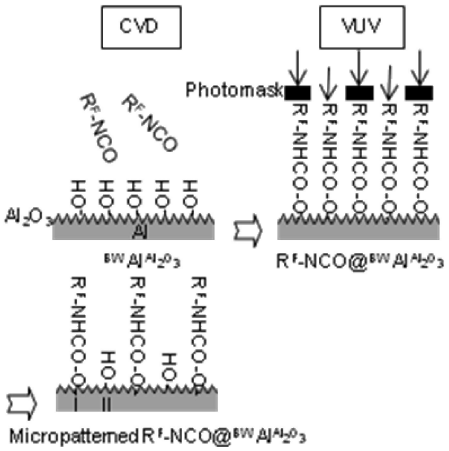

Figure 1. Fabrication of a micropatterned R^F-NCO layer on $^{BW}Al^{Al_2O_3}$ surface. $^{BW}Al^{Al_2O_3}$ was modified with R^F-NCO via CVD and microsized pattern (I and II) was fabricated using VUV photolithography.

The R^F-NCO layer was then photolithographed using 172-nm vacuum ultraviolet (VUV) light[6] through a photomask having circular windows of 500-μm diameter (Figure 1). The surface was exposed to VUV light generated from an excimer lamp (Ushio Inc., UER20-172V; $\lambda = 172$ nm, 10 mW cm^{-2}) for 30 min under a reduced pressure of 10^3 Pa. An energy-dispersive X-ray spectrometer (Horiba EMAX 6853-H EDS system)

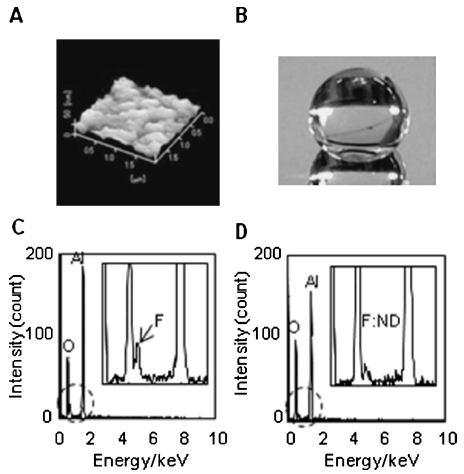

Figure 2. Typical AFM images of the R^F-NCO layer on $^{BW}Al^{Al_2O_3}$ surface (A), behavior of a water droplet on the R^F-NCO layer on $^{BW}Al^{Al_2O_3}$ surface (B), EDS analyses of a masked region (I) of the R^F-NCO layer on $^{BW}Al^{Al_2O_3}$ surface (C) (Arrow: the fluorine peak.), and region (II) exposed to VUV irradiation (D) (ND: not detected).

detected the fluorine peak at 677 eV (Figure 2C) on a masked region, while no peak on an exposed region to the VUV light (Figure 2D), indicating that R^F-NCO molecules were completely removed by the VUV light irradiation. Consequently, the contact angle of the R^F-NCO layer surface dramatically decreased from 167° to less than 5°, suggesting that the continuous superhydrophobic surface was micropatterned to two regions with the absolute opposite chemical characteristics of superhydrophobicity and superhydrophilicity.

After photochemical micropatterning, the substrate was sterilized by immersing into 70% EtOH for 30 s in preparation for cell culture. Mouse osteoblast-like cells (MC-3T3-E1) obtained from the Riken Bioresource Center (Tsukuba) were plated at densities of 5×10^4 cells/cm^2 onto the micropatterned substrate composed of a superhydrophobic region covered with the R^F-NCO layer and a superhydrophilic region of $^{BW}Al^{Al_2O_3}$. An n-type Si(100) wafer (10 × 10 mm, Shin-etsu Handoutai) cleaned and hydrophilized with 172-nm VUV light for 30 min under a reduced pressure of 10^3 Pa was used as a control surface for the cell adhesion. The cells on the substrates were cultured in α-minimum essential medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and antibiotics in a humidified 5% CO_2-balanced-air incubator at 37 °C.[7] After 2 h incubation, the surface was washed to remove the unattached cells with serum-free medium, and the culture was continued using serum-free medium to examine initial cell attachment occurring during 2 h incubation. After 1-day culture, the cells were washed with phosphate-buffered saline (PBS), fixed for 10 min with 3.7% formaldehyde in PBS, and permeabilized with 0.1% TritonX-100 in PBS. The actin filaments and nuclei of the attached cells were costained with rhodamine phalloidin (Invitrogen) and 4,6-diamidino-2-phenylindole (DAPI, Invitrogen) for 20 min at room temperature, respectively. Each fluorescence image of actin filaments and nuclei was obtained using a fluorescence microscope (Olympus BX51, Tokyo) and

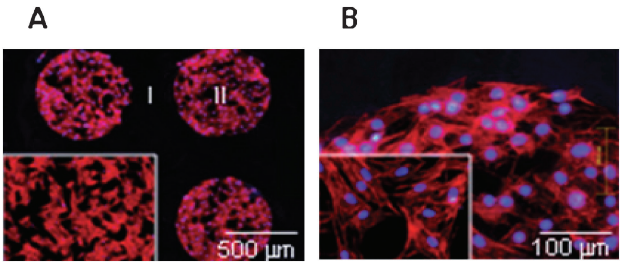

Figure 3. Cell adhesion after 1-day culture on the micropatterned R^F-NCO layer on $^{BW}Al^{Al_2O_3}$ surface composed of a superhydrophobic region (I) and a superhydrophilic region (II). Magnification × 4 (A) and × 20 (B); Insets: cells attached on hydrophilized Si surface.

electronically combined to generate a colocalized image (Figure 3). As shown in Figure 3A, during 2 h incubation, MC-3T3-E1 cells specifically attached to the superhydrophilic region (II) of $^{BW}Al^{Al_2O_3}$ but not to the superhydrophobic region (I) covered with the R^F-NCO layer. The density of the attached cell on the superhydrophilic region (II) of $^{BW}Al^{Al_2O_3}$ was almost as great as that on the hydrophilized Si surface (inset). The complete cell repellant property of the R^F-NCO layer is clearly observed in the magnified image in Figure 3B, indicating that the cells attached to the superhydrophilic region (II) formed a strict border with the superhydrophobic region (I), which became unclear after 3 days because of the decreasing of the live cells under serum-free culture.

In conclusion, a R^F-NCO layer was deposited on the surface of the metal substrate, $^{BW}Al^{Al_2O_3}$, and subsequently photolithographed using masked 172-nm VUV light to fabricate a superhydrophobic/superhydrophilic micropattern on $^{BW}Al^{Al_2O_3}$. The initial cell-adhesion experiments revealed complete cell repellency on the superhydrophobic region of the R^F-NCO layer and cell adhesiveness on the superhydrophilic region of $^{BW}Al^{Al_2O_3}$, showing the possibility of fabricating cell microarrays on metal substrates. Having higher light reflectivity and electric conductivity than any other materials, metals would be more preferable substrates for cell microarrays which need effective detection of weak light or electric signals emitted from cells, especially arrayed in a low cell population.

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