Electronic Supplementary Information

Protein Nanoarrays on a Highly-Oriented Lamellar Surface

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The electronic supplementary information contains the following sections:

1. Materials

All reagents were used without further purification. Poly(styrene-*block*-methyl methacrylate) (PS-*b*-PMMA; M_n: 1.04 x 10^5 g mol^{-1}, M_n of PS block: 5.2 x 10^4 g mol^{-1}, M_n of PMMA block: 5.2 x 10^4 g mol^{-1}) and polystyrene-*ran*-poly(methyl methacrylate) random copolymer (PS-*r*-PMMA; M_n: 9.5 x 10^4 g mol^{-1}) were purchased from Polymer Source, Inc. (Montreal, Canada). γ-globulin from bovine blood, fibrinogen fraction I type I-S, and fibronectin (FN) from bovine plasma were purchased from SIGMA-Aldrich (St. Louis, USA). Acid freeze-dried type I porcine atelocollagen (Col I) was purchased from Nippon Meat Packers (Tokyo, Japan). Recombinant human thrombomodulin (hTM; ART-123) was kindly donated from Asahi Kasei Phama Corporation (Tokyo, Japan).

2. Methods

2.1 Fabrication of the well-aligned lamellar films on silicon wafer

The surface of a silicon substrate was neutrally modified by a PS-*r*-PMMA copolymer brush so as to provide an identical interfacial tension for the PS and PMMA components.[8e] First, the silicon wafer was immersed in a piranha solution (7:3 mixture of H_2SO_4 and H_2O_2) for 1 hour at 110 ^oC. The acid-treated silicon wafer was subsequently washed with deionized water. The PS-*r*-PMMA neutral brush layer was deposited by spin-coating a thin film of the brush upon the silicon wafer, and thermal annealing at 160 ^oC for 48 hours in a vacuum, and spin-washing physisorbed random copolymers. After neutral treatment, thickness-modulated films of a PS-*b*-PMMA were prepared by a simple method of solution dropping. A droplet of block copolymer solution (2 wt%, dissolved in toluene) was dropped over a 45 ^{o} tilted, neutrally treated silicon wafer. One bump, having a straight-line shape, was prepared after drying. After film preparation, thermal annealing was conducted at 220 ^oC to produce a well-aligned lamellar morphology in the thickness-modulated region. The obtained lamellar morphology was characterized by atomic force microscope (AFM) using a scanning probe microscope JSPM-5200 (JEOL, Japan). As shown in Fig. S1, the edges of the straight-line provided the well-aligned lamellar morphology, and the outside of this area showed random lamellar morphology.

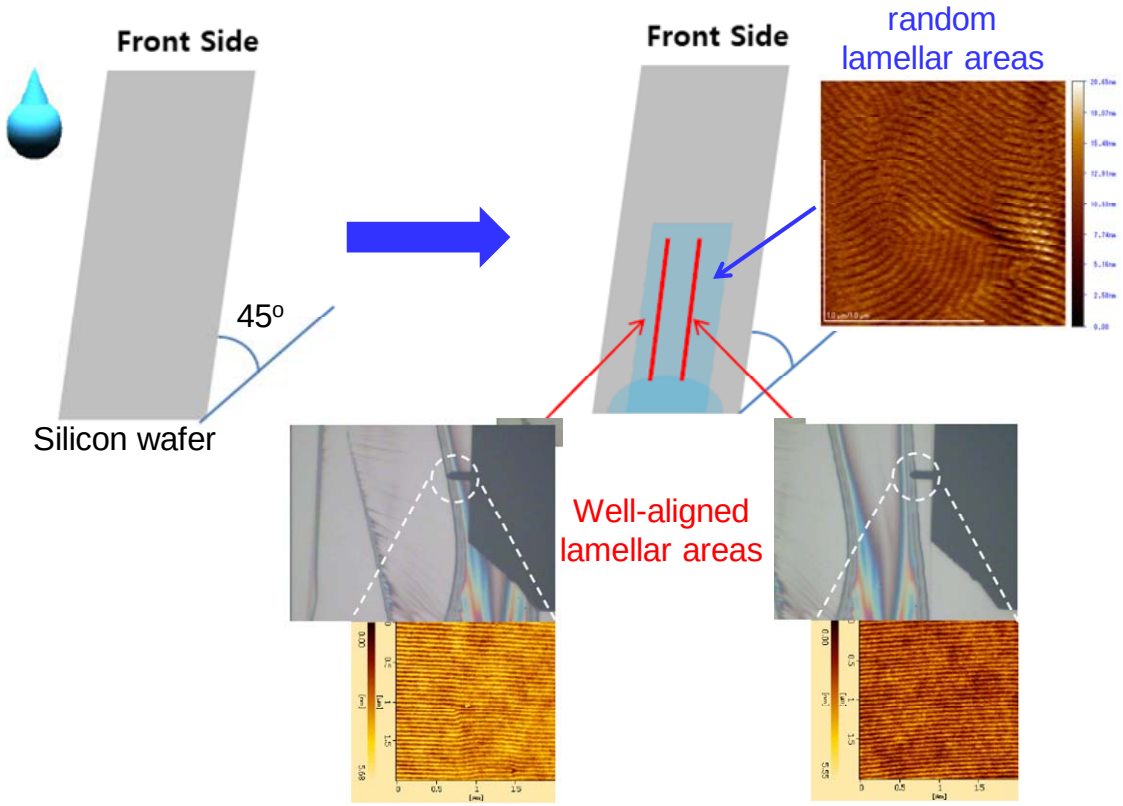

Fig. S1. Schematic illustration of fabrication process of the well-aligned lamellar surfaces. The red lines are the aligned lamellar areas and the outside of these lines reveal random lamellar areas as shown in blue arrow.

2.2 Protein adsorption on the aligned lamellar films

Protein adsorption on the aligned lamellar films was performed by following procedure. Proteins were dissolved in phosphate buffered saline (PBS) at varied concentrations such as 4.0 μg ml^{-1} for γ-globulin, 10 μg ml^{-1} for hTM, 1.0 μg ml^{-1} for fibrinogen, 0.4 μg ml^{-1} for FN and 4.0 μg ml^{-1} for Col I, respectively. The protein solutions were dropped onto the lamellar surfaces for prescribed times at 25 ^{o}C, and subsequent washing with PBS was performed for three times. Finally, the protein adsorbed films were washed with ultrapure water, and then it was dried by dry nitrogen gas. The morphology of the adsorbed proteins on the aligned and random lamellar areas was evaluated by atomic force microscope (AFM) using a scanning probe microscope JSPM-5200 (JEOL, Japan).