Protein Nanopatterning on a Highly-Oriented Lamellar Surface

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Statement of Purpose: The nano- or micro-patterning of proteins is of great interest not only for fundamental biological research involving cell biology, but also for applications in high-throughput screening. In general, two types of approaches, the top-down and bottom-up methods, have been used for the fabrication of protein patterning. Top-down approaches have been historically developed using various lithography techniques, typically microcontact printing, photo lithography and nanoimprint lithography. These lithographic techniques have advantages in terms of low cost and time saving, however precise patterning at the nanometer scale is difficult to achieve with these techniques. Bottom-up approaches, for example dip-pen lithography, ink-jet printing and scanning probe patterning, have been employed to pattern proteins onto solid surfaces. Although these approaches can immobilize proteins at the desired positions with high resolution, the control of experimental parameters such as humidity, substrate roughness and ink viscosity is necessary. Recently, a self-assembled regular lamellar pattern of diblock copolymers is a valuable candidate to control of the alignment of the hydrophobic domains (protein adsorption domains) for a lamellar morphology. However, it is difficult to fabricate perfect periodic domain ordering over a large area (the micrometer-scale), and defects exist at the edges of the grain boundaries.

We have recently reported the defect-free, well-aligned nanopatterns of a block copolymer, poly (styrene-block-methyl methacrylate) (PS-b-PMMA), at the tens of micrmeter-scale by directed self-assembly and hierarchical self-assembly methods[1)]. In this study, we report that control the spatial resolution of the adsorbed serum and cell adhesive proteins on the nanometer scale by site-selective adsorption using well-aligned nanopatterns of a block copolymer, PS-b-PMMA[2)].

Method: The well-aligned lamellar film of the symmetric PS-b-PMMA (Mn: 1.04 x 10^5 g mol^{-1}, Unit numbers of PS and PMMA blocks are 500 and 520, respectively) was prepared via solution dropping method. Briefly, a droplet of block copolymer solution was dropped over a 45° tilted, neutrally silicon wafer. The well-aligned lamellar area, having a straight-line shape, was prepared after drying. The lamellar surface was deposited 0.4 μg ml^{-1} γ-globulin in phosphate buffered saline (PBS) for 10 min at 25 °C and was then rinsed with ultra pure water. The AFM images were obtained with a JSPM-5400 (JEOL, Japan) that was operated in tapping mode in air at room temperature.

Results: Fig. 1a shows AFM images of lamellar films of 5 μm x 5 μm area, and the height profile of the nanopatterned surface. The hydrophobic PS domains were lower than the hydrophilic PMMA domains by 1.5 nm. Fig. 1b shows the nanopatterning of γ-globulin, one of the major serum proteins (Mw: 60~80 kDa), on the aligned lamellar surface. The γ-globulin molecules were selectively adsorbed without any aggregation onto hydrophobic PS domains, and the unidirectional orientation of γ-globulin nanoarray agreed well with the 48 nm periodic lamellar spacing. The accumulation of γ-globulin molecules in the PS regions leads to an inversion of the AFM topographic contrast: the bright, and therefore higher, areas in Fig. 1b correspond to γ-globulin molecules assembled on PS domains, which were initially 1.5 nm lower in height than the neighboring PMMA domains. γ-globulin molecules on the PS domains exhibited an average height of 1.8 nm greater than the neighboring PMMA domain regions. Other proteins such as fibrinogen and fibronectin were site-selectively adsorbed on the well-aligned lamellar films similar to the γ-globulin. In particular, type I collagen showed a unique meshwork conformation like an iron grille on the well-aligned lamellar films.

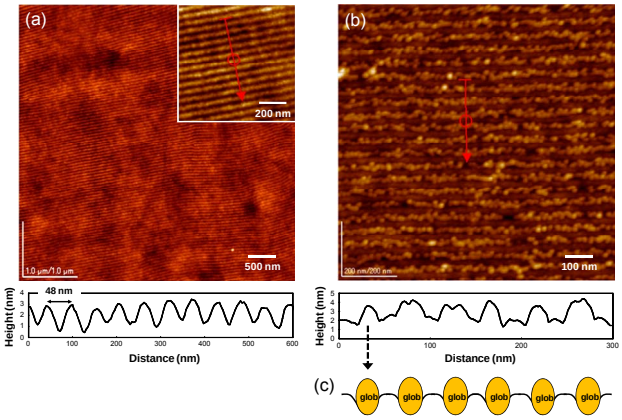

Fig. 1 (a) AFM image of well-aligned PS-*b*-PMMA copolymer film of 5 μm x 5 μm area. The inset shows a magnified image. The height profile was taken along the red line indicated in the inset image. (b) AFM image of the lamellar surface of 1 μm x 1 μm after the deposition of 0.4 μg ml^{-1} γ-globulin in phosphate buffered saline (PBS) for 10 min at 25 °C. The height profile was taken along the red line in the image. (c) Schematic cross-section al image of the adsorbed γ-globulin on the PS domains.

Conclusions: Novel protein nanoarrays were successfully fabricated at the tens of micrmeter-scale using well-aligned lamellar films. Protein nanopatternings might be valuable for biological assays.

References:

1) Kim BH. et al. Adv Funct Mater, 2009; 19: 2584-2591.
2) Matsusaki M, Omichi M, et al. submitted.