Development of Nano-sized Protein Arrays using Block Copolymer Self-Assemblies

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ABSTRACTS

The single molecule approach is potentially of great technological interest as a complementary means to obtain information about protein conformation or molecular interaction. The measurement for molecular interaction in single molecule level requires that the molecule in the question is immobilized by binding to an appropriate surface. W have developed a novel fabrication method of nano-patterned array of bio-molecules with ultra-high density, using self-assembly of block-copolymers. Compared to previous lithographic methods, self-assembly of block-copolymers makes it easier to fabrication of patterns with ~ 10 nanometer feature size and to reduce the number of processing steps.

In this work, we have detected a single molecule array on the basis of immunoreaction using protein conjugated gold nanoparticle. In this manner, BCP structure played an important role as confining molecules in single molecule level to a hole avoiding intermolecular interaction or nonspecific binding.

KEYWORDS

Block-copolymer, Bio-array, Self-assembled monolayer

INTRODUCTION

The reduction in size and integration of bio-arrays has the potential to produce structures with capabilities that exceed those of the conventional macroscopic systems and to add new functionality. In particular, patterning biomolecules at defined positions on surfaces with special control and resolution down to single protein molecules is one of the key issues in nanobiotechnology.[1-7] For this purpose, it has to be possible to fabricate template with features as small as ~ 20nm on substrates. Among various lithographic technologies, e-beam lithography,[8] scanning probe lithography[1-3] and block copolymer lithography[9-11] may have a high-resolution limit regime for making such feature size. In reality, however, pattern with feature sizes below 30nm is difficult to achieve with commercial e-beam lithography. The smallest bio-arrays feature using scanning probe techniques is reported to be ~50nm[1-3], although the method has been proved to be a powerful tool for bio-arrays with multi-component systems. Also, it takes long time to fabricate over large-area, because of serial processing. Block copolymer templates can be used as nanolithography templates to achieve single molecule resolution, since the absolute size of the domain, 10-100nm, is defined by the molecular weight of the copolymer and the strength of segmental interaction between the blocks. Moreover, this method provides low-cost and mass production possible. Nevertheless, none of approaches and developments for protein arrays via block copolymer lithography have been carried out, because of difficulty in a high degree of control and selectivity of

biomolecules at predefined positions on dense periodic arrays of BCP holes.

Here we systematically show, for the first time, how to positioning the level of individual protein molecules at defined position to produce ultra-high density bio-array using BCP nanostructures To accomplish this goal, it requires that highly ordered surface pattern, surface modification and the complementary chemistry be developed for placing protein structures on a surface of interest with nanoscale resolution, and control over the biological activity of the resulting structures.

EXPERIMENTAL

Thin film of PS-b-PMMA was prepared by spin casting from 1wt% toluene solution at 3000~4000rpm and 30~60s onto piranha-treated silicon wafer and gold chip (K-MAC Co., Korea). Film thickness was measured using SEM and X-ray reflection. The specimen was annealed then PMMA segments were selectively removed by UV (254nm) irradiation for 30min as PMMA polymer chains were degraded through radical generation. The pattern was transferred onto the substrate using reactive ion etching (RIE). The etching process was performed under a CF_4 flow. SEM work was performed with incident electron beam energy between 1 and 10kV and by collecting secondary electrons. Cyclic voltammetric measurements were carried out in a three-electrode cell using an Autolab potentiostat (Eco Chemie, Netherlands) with the running software provided by the manufacture. A gold-coated silicon substrate onto which block copolymer film was spin-coated sand subject to etching condition, served as a working electrode. The reference and counter electrodes were $Hg/HgSO_4$ and a platinum spiral wire, respectively. The reaction electrolyte (i.e., 0.5M H_2SO_4) was purged in advance with N_2 gas for 30min at least before data acquisition and kept in room temperature. The surface topological measurements were performed under ambient condition with a commercial atomic force microscope(SPA400;Seico, Japan), equipped with a 10 ㎛ scanner. All substrates were imaged in contact mode in air, using standard Si_3N_4 cantilevers which nominal spring constant is 0.08N/m.

RESULT AND DISCUSSION

The 2D nanoscopic pattern on flat solid substrate (such as silicon wafer and sputtered gold film) is fabricated by etching the thin film of PS-b-PMMA unproportionally. FE-SEM images of the etched film on silicon and cross-section are shown in Figure I-a.

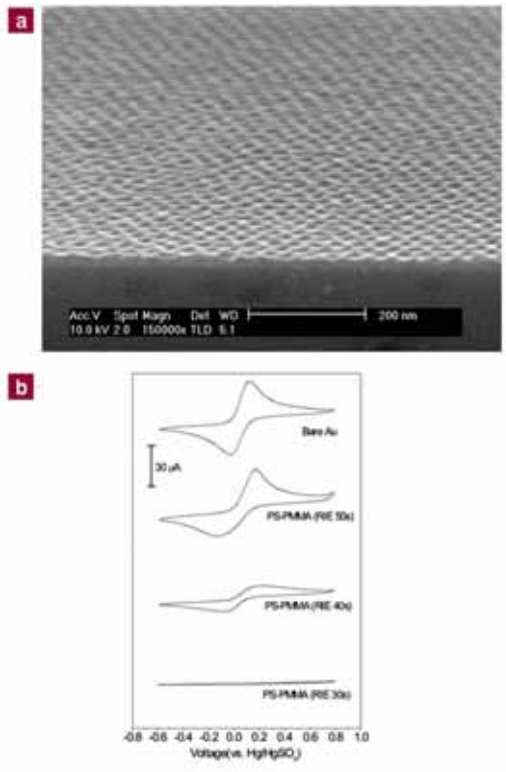

Fig. I. (a)SEM image of patterned after RIE process, (b) Cyclic voltammograms at various RIE conditions.

It is necessary to verify whether the bare gold surface is exposed before immobilizing host molecules (i.e., protein, biotin, antibody, or oligonucleotide), which is allowed to bind target molecules. Therefore, we make

an electrochemical measurement to obtain the optimum exposure time in RIE condition by observing the dynamics of electroactive chemicals at the electrode surface. Shown in Figure I-b are cyclic voltammograms as the incubation time in RIE condition varies from 30s to 50s. After 30s etching time, although most of void region is observed to be open in the AFM and SEM images, the electron transfer by $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ pair is hardly detectable. This implicates that most of the PS layer were not punctured yet in 30s incubation. As further etching is carried out (for 40s), the current substantially increases suggesting that much more gold surface becomes adjacent with electrolyte.

In order to prepare a biocompatible template, the patterned gold electrode is modified with biotin-terminated SAM. After washing substrate was then soaked with solution of streptavidin-conjugated gold colloid for 6 hrs. Judging from the AFM image in Figure II, gold nanoparticles modified with streptavidin (SA-AuNP) are successfully incorporated into patterned surface. We also tried to build another biocompatible template using another functionalized monolayer, i.e., 11-mercaptoundecanic acid (11-MUA), which is subsequently modified with protein G conjugated gold colloid (diameter ~ 6nm) via EDC/NHS modification protocol(data not shown). Protein G is covalently combined with carboxyl group through amine coupling reaction and successfully immobilized on MUA exposed on gold.

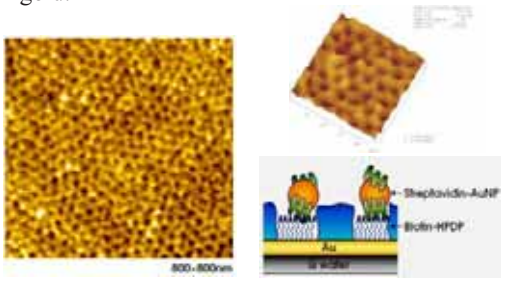

Fig. II. AFM image of the substrate was treated with streptavidin-conjugated gold colloid

CONCLUSIONS

In summary, a novel approach for positioning functional biomolecules at defined position to produce ultra-high density biomolecule array below 20 nm resolution was achieved in BCP nanostructure which has hexagonally ordered holes on substrates. Exquisite control was achieved in placing protein-conjugated gold nanparticles of a specific size within the template holes. Compared to previous fabrication methods of protein arrays, this method created not only protein array density an order as high as the recently reported procedure but also immobilization of the level of single protein molecules on defined position, leading to fundamental understanding of interactions between biomolecular structures and surfaces patterned with proteins.

REFERENCES

1. Piner, R. D., Zhu, J., Xu, F., Hong, S. & Mirkin, C. A., *Science* 283, 661-663(1999)
2. Lee, K. B., Lim, J. H. & Mirkin, C. A., *J. Am. Chem. Soc.*125, 5588-5589(2003)
3. Agarwal, G., Naik, R. R. & Stone, M. O., *J. Am. Chem. Soc.*125, 7408-7412(2003)
4. Gu, J., Yam, C. M., Li, S. & Cai, C., *J. Am. Chem. Soc.*126, 8098-8099(2004)
5. Li, H. W., Muir, B. V. O., Fichet, G. & Huck, W. T. S., *Langmuir* 19, 1963-1965(2003)
6. Bruckbauer, A., Zhou, D., Kang, D, -J., Korchev, Y. E., Abell, C. & Klenerman, D., *J. Am.Chem. Soc*.126, 8098-8099(2004)
7. Li, L., Chen, S., Oh, S. & Jiang S., *Anal. Chem*. 74, 6017-6022(2002)
8. Wallraff, G M. & Hinsberg, W. D. *Chem Rev*. 99, 1801-1822 (1999)
9. Fasolka, M. J. & Mayes, A. M., *Annu. Rev. Mater. Res*. 31, 323-325(2001)

10. Russell, T. P., Thurn-Albrecht, T., Touminen, M., Huang, E. & Hawker, C. J, *Macromol. Symp*. 159, 77-88 (2000)
11. Lazzari, M. & López-Quintela, M. A., fabricaltion, *Adv. Mater*. 15, 1583-1593 (2003)