COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Patterning of DNA nanostructures on silicon surface by electron beam lithography of self-assembled monolayer

Guo-Jun Zhang,*[a] Takashi Tanii,[b] Takashi Funatsu[ac] and Iwao Ohdomari[abd]

[a] *Nanotechnology Research Center, Waseda University, Waseda Tsurumaki-cho 513, Shinjuku-ku, Tokyo 162-0041, Japan. E-mail: zhang@kaw.comm.waseda.ac.jp; Fax: +81-3-5286-9076; Tel: +81-3-5286-9067*

[b] *Department of Electronical Engineering and Bioscience, School of Science and Engineering, Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan*

[c] *Department of Physics, School of Science and Engineering, Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan*

[d] *Kagami Memorial Laboratory for Materials Science and Technology, Waseda University, 2-8-26 Nishi-waseda, Shinjuku-ku, Tokyo 169-0051, Japan*

Received (in Cambridge, UK) 25th November 2003, Accepted 16th February 2004
First published as an Advance Article on the web 5th March 2004

Nanoscale patterns of modified oligonucleotides are produced on octadecyltrimethoxysilane self-assembled monolayers at a silicon surface by electron beam lithography. DNA structures with feature sizes of the order of 250 nm were detected by epi-fluorescence microscopy.

DNA chips have been widely used as high-throughput screening assays to identify genetic variations associated with disease for discovery of new drugs.[1,2] Conventional patterning techniques, such as spot arraying[3] and photolithography,[4] have been employed to fabricate DNA arrays at sizes ranging from tens to hundreds of microns. The miniaturization of feature sizes and the spatially controlled immobilization of biomolecules on these miniaturized features on the nanometre scale become more important, mainly due to further requirements including increased density of biochip components, reduced amount and volume of a sample, and higher detection sensitivity. Therefore, arrays with smaller features lead to the development of DNA nanochips with faster analysis speed and massive parallelization.

Dip-pen nanolithography is well known for its ability to directly pattern DNA nanostructures on the metal and insulator surfaces.[5] However, it is difficult to achieve a high spatial precision since the size of the DNA pattern primarily relies on the fabrication speed and a humid environment. An atomic force microscopy (AFM) based lithography technique known as nanografting was also used to produce nanostructure of DNA on a gold surface.[6] This method offers a high spatial precision but has the limitation of low throughput. Self-assembled monolayers (SAMs) have been developed for high resolution nanofabrication techniques because of their potential use as an ultra-thin resist layer and capability of resisting the non-specific binding of biomolecules.[7] Electron beam (EB) lithography is a commonly used nanofabrication means and does not need direct substrate contact and has advantages over scanned probe lithography technique when nanostructures are patterned on SAM.

Recently, we demonstrated the immobilization of DNA on microstructured patterns fabricated by EB lithography using a conventional resist.[8] However, there is a demand for a thin electron-beam resist in the future of nanometre scale lithography because this resist deposited on SiO_2 is too thick to achieve high resolution nanofabrication. In addition, since 3-aminopropyltriethoxysilane (APTES) is a small molecule and does not form oriented self-assembled monolayers, it actually forms multilayers packed inside the amino-modified micropatterns. As soon as the resist was removed, some of the APTES molecules on the top of the multilayer were laid down onto the SiO_2 surface around the patterns, thereby leading to irregular fluorescence pattern expansion. Therefore, it is impossible to use this method to further fabricate DNA nanopatterns. In this work, we introduce a new method to produce DNA nanostructures on octadecyltrimethoxysilane (ODS) SAM at a silicon surface using EB lithography. The use of ODS SAM enables us to miniaturize the feature sizes and obtain a high signal-to-noise ratio. We also demonstrate that these DNA nanostructures can be produced with almost no detectable non-specific binding of DNA in the regions outside nanopatterns.

As illustrated in Fig. 1, the approach described here includes several steps: fabrication of nanometre-sized patterns by EB lithography of ODS SAM at a silicon surface followed by site-directed immobilization of oligonucleotides onto these nanopatterns and subsequent hybridization of fluorescently labeled target to immobilized DNA within those nanopatterns.

ODS SAM, formed through chemical bonding of molecules to the oxidized silicon substrates, can be used as an ultrathin resist for EB lithography, and a passivation layer for preventing non-specific adsorption of biomolecules as well. Typically, an ODS monolayer is deposited on SiO_2 surfaces by immersing the substrates into a solution containing ODS.[7] However, this method frequently leads to microstructural defects such as aggregates or holes at the surfaces.[9] Clearly, such inhomogeneous surfaces cannot serve as ideal templates for biomolecule modification and may cause difficulties in the characterization of subsequently assembled layers. Although the formation of ODS SAM onto cleaned SiO_2 substrates by chemical vapor deposition (CVD) has been described elsewhere,[10] nothing is known about the conditions of ODS SAM by CVD for nanofabrication using EB lithography. In this experiment, the parameters of CVD for the formation of ODS SAM of high quality were optimized. Briefly, an ODS monolayer was deposited on a SiO_2 layer at 110 °C for 3 h under N_2 by CVD and the surface was washed with chloroform for 10 min by ultrasonication. The thickness of the ODS monolayer estimated from AFM imaging is ~2.0 nm. The resulting ODS monolayer is homogeneous with low roughness and terminated with methyl groups, leading to the minimization of biomolecular adhesion.

After fabrication of the nanopatterns, the sample is immersed in a solution of 3-aminopropyltriethoxysilane (APTES) for modifying the nanodot areas with an amine group terminated APTES layer. Clearly, APTES is a smaller molecule than ODS. However, the ODS monolayer contains many pinholes, indicating that APTES can easily be deposited in the pinhole areas between two ODS molecules, resulting in a strong background outside the patterned regions. In this work, after the silicon substrate was modified with an ODS monolayer, another small molecule, ethyltriethoxysilane (ETS), was introduced to prevent APTES assembly in the pinhole

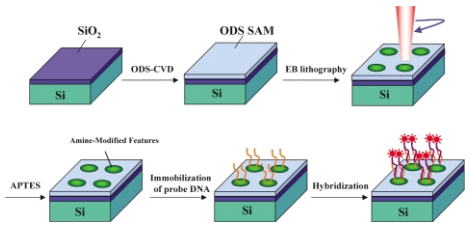

Fig. 1

DOI: 10.1039/b315278b

areas by filling up the pinholes prior to fabrication of nanopatterns by EB lithography. The surface covered with an ODS monolayer was immersed in a solution of 1 mM ETS in ethanol for 1 h. After washing with ethanol by ultrasonication, the monolayer was exposed to a dose of 960 μC cm^{-2} at 20 keV using an electron beam. All patterning was done with an electron beam lithography system (Hitachi S-4200, Japan). The patterned surface was developed using buffered hydrofluoric acid (BHF) for 60 s; subsequently, the nanopatterns on the ODS monolayer developed.

Patterns after nanofabrication by EB lithography on the ODS monolayer were imaged by tapping-mode AFM (Fig. 2). The dark dots correspond to the patterned regions. Fig. 2a shows regular submicron-sized patterns, in which the distance between the two dots is 5 μm and the diameter of the dots is 500 nm. Smaller nanopatterns are shown in Fig. 2b, in which the distance and the diameter are 2.5 μm and 250 nm, respectively. The measured depth of each dot is ~3 nm, indicating that the electron beam has penetrated through the ODS monolayer and the patterned regions were exposed with the SiO_2 surface.

To immobilize amine-modified oligonucleotides onto the nanopatterns generated by EB lithography of ODS monolayer, a typical approach using APTES and glutaraldehyde as bifunctional cross-linkers was employed according to our previous protocol.[8] Two types of sequences both complementary and non-complementary were applied to this experiment: H_2N-5′-$(T)_{15}$-CCACGGACTACTTCAAAACTA-3′ (complementary probe), H_2N-5′-ATCGATCGATCGATCGATCGA-3′ (non-complementary probe). Noticeably, a fragment of extra sequence (T_{15}) in the complementary probe was designated to introduce a long spacer (the predicted length of the fully stretched fragment is ~6 nm) between the complementary sequence and the surface because it will be able to minimize steric hindrance, increase the corresponding hybridization efficiency and thereby improve the detection sensitivity. To demonstrate the sequence specificity, patterned oligonucleotides, both complementary and non-complementary, were exposed to a solution containing a fluorescently labeled target whose sequence is: Cy 5–5′- TAGTTTTGAAGTAGTCCGTGG-3′. The patterned regions were incubated with a 1 μM Cy 5 labeled target in 2 × SSC, 0.2% Tween-20 (v/v) at 59 °C for 2 h. The patterns were then characterized by epi-fluorescence microscopy (Fig. 3). Fig. 3a–c exhibited low background, demonstrating that very little non-specific binding of biomolecules on the ODS SAM is detected. On the contrary, DNA nanostructures with dot arrays (500 nm dots, 5 μm pitch and 250 nm dots, 2.5 μm pitch) were strongly visualized in Fig. 3a and 3b, respectively. Owing to DNA–DNA interaction, homogeneous patterns of the hybridization of a target labeled with Cy 5 to the complementary probe were observed with strong fluorescence signals within the nanoscale patterns (Fig. 3a and 3b). However, as shown in Fig. 3a and 3b, some of the spots appeared brighter than the rest mainly due to unequal amounts of DNA attachment. On the other hand, in the case of non-complementary probe DNA, no fluorescence patterns could be seen because of

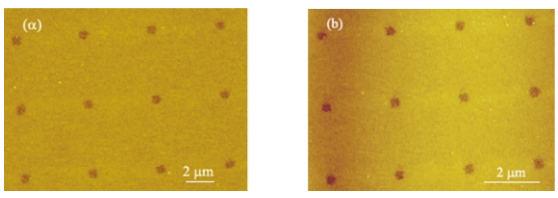

Fig. 2 Tapping mode AFM images showing nanodots after nanofabrication on ODS SAM by EB lithography: (a) dot size: 500 nm, pitch: 5 μm; (b) dot size: 250 nm, pitch: 2.5 μm.

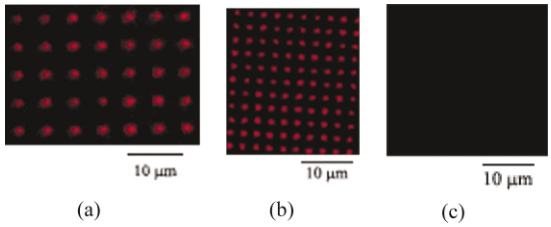

Fig. 3 Fluorescence micrographs of Cy 5 labeled target hybridized to probe DNA immobilized within nanodot areas fabricated by EB lithography on ODS SAM at a silicon surface: (a) complementary probe DNA (spot size: 500 nm, spacing: 5 μm); (b) complementary probe DNA (spot size: 250 nm, spacing: 2.5 μm); (c) non-complementary probe DNA.

unspecific binding between non-complementary sequence and target (Fig. 3c). These data indicate that probe DNA was preferentially immobilized on patterned regions of the surface and confirm the specificity of binding between the target and immobilized probe DNA.

In conclusion, this work demonstrates a novel approach for patterning DNA nanostructures on a silicon substrate based on EB lithography of ODS SAM. Through nanofabrication using EB lithography on ODS monolayers deposited on a silicon dioxide surface by CVD, nanostructures capable of being chemically modified can be generated. As a result, these nanostructures can be functionalized with amine-modified oligonucleotides, which maintain their hybridization specificity toward the complement. The procedures described here provide a protocol for creating well-defined DNA nanostructures on substrates, which will find increased applications in the field of DNA biosensors and biochips. However, sub-100 nm nanopatterns are difficult to detect by fluorescence microscopy due to the resolution limitations of the optical technique. Further work is under way to visualize several or tens of nano-scale DNA patterns by other techniques.

We thank Ms Mikiko Saito of the Nanotechnology Foundry at Waseda University for assistance with the AFM experiments. This work is supported in part by the Japan Society for the Promotion of Science (JSPS), and a Grant-in-Aid for Center of Excellence (COE) Research from the Ministry of Education, Culture, Sports, Science and Technology.

Notes and references

1 E. S. Lander, *Nat. Genet.*, 1999, **21**, 3.
2 T. R. Golub, D. K. Slonim, P. Tamayo, C. Huard, M. Gaasenbeek, J. P. Mesirov, H. Coller, M. L. Loh, J. R. Downing, M. A. Caligiuri, C. D. Bloomfield and E. S. Lander, *Science*, 1999, **286**, 531.
3 M. Schena, D. Shalon, R. W. Davis and P. O. Brown, *Science*, 1995, **270**, 467.
4 S. P. A. Fodor, J. L. Read, M. C. Pirrung, L. Stryer, L. A. Tsai and D. Solas, *Science*, 1991, **251**, 767.
5 (*a*) L. M. Demers, D. S. Ginger, S.-J. Park, Z. Li, S.-W. Chung and C. A. Mirkin, *Science*, 2002, **296**, 1836; (*b*) H. Zhang, Z. Li and C. A. Mirkin, *Adv. Mater.*, 2002, **14**, 1472.
6 M. Liu, N. A. Amro, C. S. Chow and G.-Y. Liu, *Nano Lett.*, 2002, **2**(8), 863.
7 (*a*) C. S. Whelan, M. J. Lercel, H. G. Craighead, K. Seshadri and D. L. Allara, *Appl. Phys. Lett.*, 1996, **69**, 4245; (*b*) M. J. Lercel, H. G. Craighead, A. N. Parikh, K. Seshadri and D. L. Allara, *Appl. Phys. Lett.*, 1996, **68**, 1504.
8 G.-J. Zhang, T. Tanii, T. Zako, T. Funatsu and I. Ohdomari, *Sens. Actuators B*, 2004, **97**(2–3), 243.
9 D. F. S. Petri, G. Wenz, P. Schunk and T. Schimmel, *Langmuir*, 1999, **15**, 4520.
10 K. Hayashi, N. Saito, H. Sugimura, O. Takai and N. Nakagiri, *Langmuir*, 2002, **18**, 7469.