Transactions of the Materials Research Society of Japan 30[4] 1211-1214 (2005)

Micropatterned Carbohydrate and Protein Display via Self-Assembly of Glyco-polymers

Yoshiko Miura,* Hajime Sato, Takahiro Yamauchi, Osamu Takai, Kazukiyo Kobayashi
Department of Molecular Design and Engineering and Department of Materials Processing Engineering, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan
Fax: +81-52-789-2528, E-mail: miuray@mol.nagoya-u.ac.jp

We report a novel strategy for micropatterned carbohyrate and protein display on substrates. This method exploited the hydrophobic-hydrophilic microtemplate by photolithography, and the subsequent selective self-assembly of glyco-polymers onto the template. Protein micropatterned self-assembly by molecular recognition on the carbohydrate substrates was also successful.
Key words: glyco-polymer, self-assembly, lectin

INTRODUCTION

Micropatterned biomacromolecules on solid surfaces have been fabricated for a variety of applications to support the progress of biotechnology [1]. For instance, micropatterned DNA and proteins are extensively exploited for biochip and biosensors. Micropatterned carbohydrates on solid surface are expected to analyze carbohydrate-protein interactions and to fabricate the scaffolds of cell cultivation based on the important roles of carbohydrates in numerous intercellular recognition processes [2]. Since carbohydrate-protein interactions are usually weak and amplified with multivalent effects, it is important to exploit a strategy for micropatterning of carbohydrate on solid surfaces with the multivalency [3].

Both "bottom-up" (self-assembly of molecules) and "top-down" (lithography of substrates) processes have been developed to fabricate micropatterned biomolecules on solid surfaces. Self-assembled monolayers (SAMs) on surfaces are paid much attention for fabrication of nanomaterials due to their well-defined structures and applicabilities [4]. Photolithography of substrate is most practical among various patterining methods [5]. Combinations of the self-assembly of molecules and lithography of substrates are promising methods for fabricating microstructures on solid surfaces.

In this paper, micropatterned display of carbohydrate and protein on substrate have been accomplished by a combination of "bottom-up" and "top-down" approaches according to the process illustrated in Figure 1 [6]. Here, the well-ordered SAM of octadecyltrimethoxysilane (ODS) on substrate was micropatterned by photolithography using vacuum ultraviolet (VUV) light. The well-defined hydrophobic-hydrophilic micropattern was modified by self-assembly of amphiphlic glyco-polymers, and the protein was also micropatterned by following carbohydrate-lectin recognition. The micropattern was visualized by fluorescein isothiocyanate (FITC)-labeled lectins.

EXPERIMENTAL

Chemicals. Glyco-polymers were synthesized by our previous methods (Figure 2) [7]. Concanavalin A (Con A; α-Glc binding lectin), *Ricinus communis* agglutinin 120 (RCA_{120}; β-Gal binding lectin) (Honen Co. Ltd., Tokyo), and bovine serum albumin (BSA) (Amersham Bioscience, Uppsala, Sweden) were used as received.

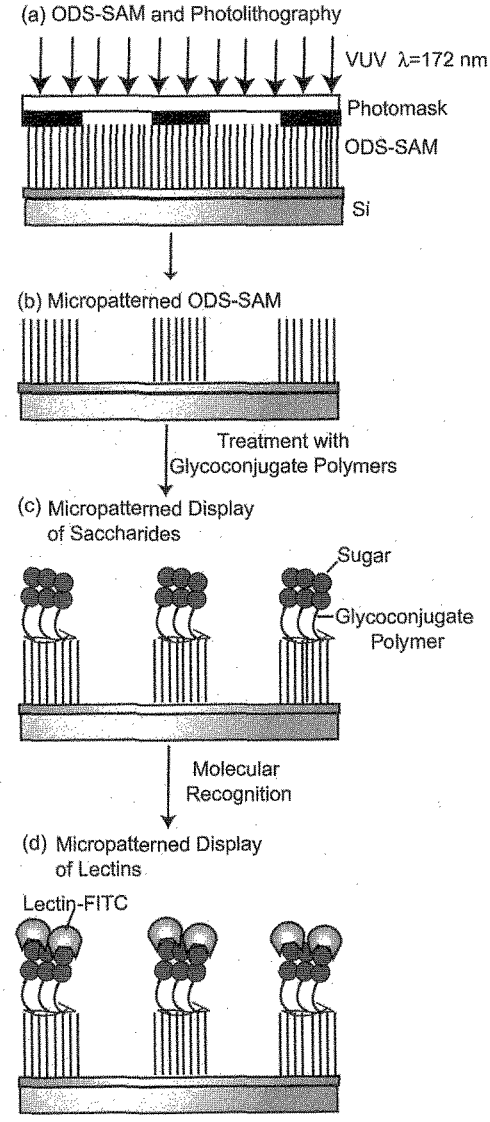

Figure 1 Schematic illustration of micropatterned display: (a) photolithography on ODS-SAM, (b) micropatterned ODS-SAM, (c) micropatterned display of carbohydrate, and (d) micropatterned display of lectin.

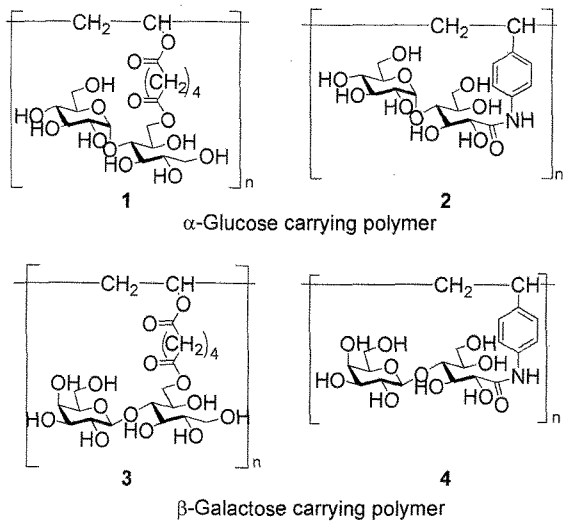

Figure 2 Molecular structure of the glyco-polymers.

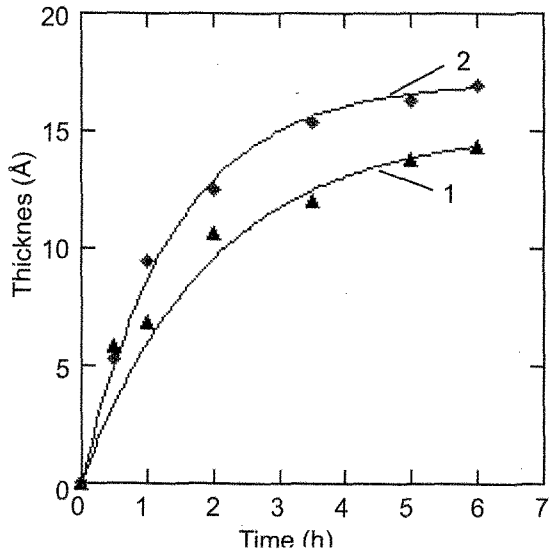

Figure 3 Thicknesses of the glyco-polymers (**1** and **2**) of ODS-SAM on Si aubstrate estimated by ellipsometery: (-) calculation according to Langmuir isotherms.

ODS-SAM was prepared by chemical vapor deposition. Micropatterned SAM samples were prepared by photolithography. A selected region of SAM was photochemically decomposed and removed by irradiation with VUV light (excimer lamp, Ushio Inc., UER20-172V, λ= 172 nm and 10 mW/cm^2) through a photomask under a reduced pressure of 10 Pa for 30 min.

Glycoengineering and Protein Binding on the Micropatterned Surface. The patterned ODS-SAM substrates were incubated in an aqueous solution of the glyco-polymers (0.5 mg/ mL) at room temperature for 5 h. The substrate was rinsed thoroughly with distilled water and dried in a steam of nitrogen gas and under vacuum. The resultant polymer-coated substrate was incubated with FITC-labeled lectin (Con A and RCA_{120}) in 1mg/mL phosphate buffered saline (PBS) solution at room

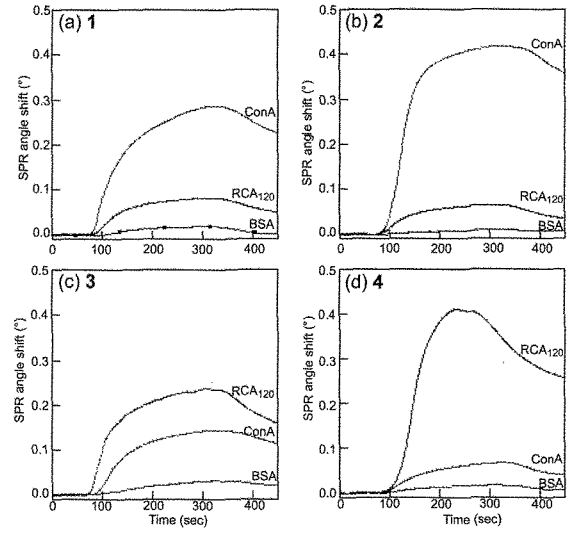

Figure 4 SPR analyses of Con A, RCA_{120} and BSA affinities to glyco-polymer-coated substrates (**1-4**) at 25 °C.

temperature for 2 h, and then washed with PBS to remove weakly bound substance. The fluorescence image was recorded with a fluorescence microscope and analyzed with Scion image software (ver. 4.02β, Scion Co., Fredeick, MD).

RESULTS AND DISCUSSION

Adsorption of the Glycoconjugate Polymers on SAMs. The adsorption behaviors of the glyco-polymers were studied with ODS-SAM without photolithography. Glyco-polymers were polymer substituted α-glucose (**1** and **2**) and β-galactose (**3** and **4**), which interacts with Con A and RCA_{120}, respectively. Polyvinyl alcohol substituted polymers (**1** and **3**) have biodegradabilties. Polystyrene substituted polymers (**2** and **4**) are used in the cell cultivation. Immersing an ODS-SAM substrate in an aqueous solution of glyco-polymers decreased the contact angles on the substrate. The thicknesses of the polymer layers estimated by ellipsometry were increased with the immersion to reach 15-20 Å after 5-6 h irrespective of the polymer backbones and sugar structures, and the time courses of the polymer adsorption were followed by the Langmuir equation (Figure 3).

Typical bands due to carbohydrates of the polymer-coated SAM on Si were observed with all glyco-polymers by FTIR and XPS [8]. On the other hand, the glyco-polymers were hardly adsorbed on bare hydrophilic Si. It is suggested that the glyco-polymers were specifically adsorbed on the hydrophobic ODS-SAM.

Lectin Recognition on Substrates. The affinities of lectins to glyco-polymer-coated substrates were estimated by SPR (Figure 4). Strong and specific response of Con A to **1** and **2**, and RCA_{120} to **3** and **4** were observed in the SPR shift angles. Interestingly, the affinities of glyco-polymers to lectins were dependent on the polymer-backbone. The sensorgrams of **2** and **4** with polystyrene backbone showed a more rapid rise in the association phase and a more rapid downturn in the dissociation phase than those of **1** and **3** with poly(vinyl alcohol) backbone. Polystyrene substituted

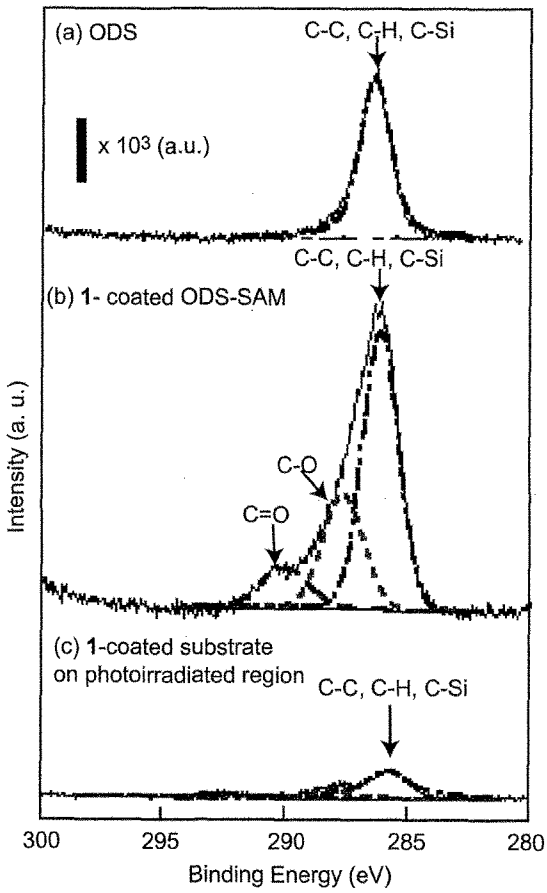

Figure 5 XPS spectra on Si substrate: (a) ODS-SAM, (b) **1**-coated ODS-SAM, and (c) **1**-coated substrate on photoirradiated region.

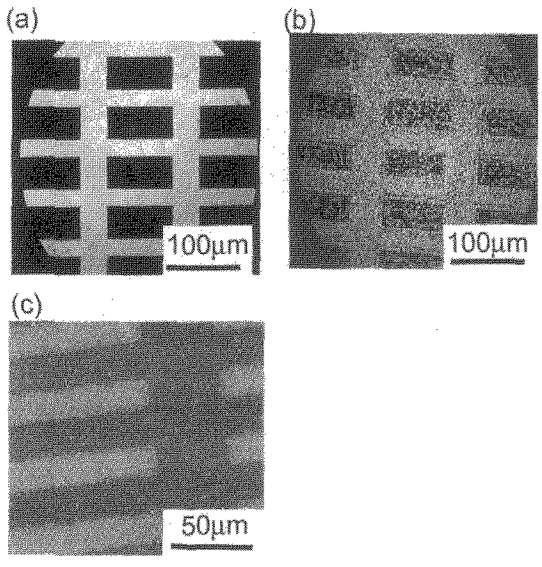

Figure 6 Optical micrographs on Si substrae: (a) photomask used , (b) the micropatterned ODS-SAM stained with water vapor, and (c) SEM of **1**-coated substrate.

glyco-polymers showed stronger and faster affinities, because of the stable and favorable conformation for lectin recognition in an aqueous solution [9]. The specificity of the polymer to proteins was observed in the

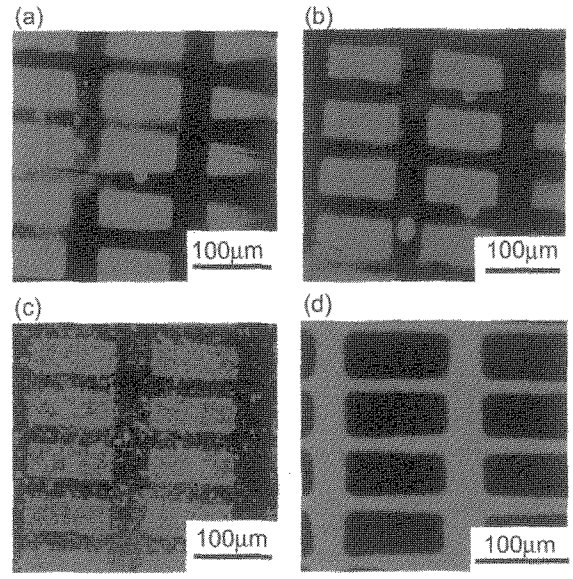

Figure 7 Fluorescence micrographs on Si substrate: (a) Con A on **1**-coated ODS-SAM, (b) Con A on **2**-coated ODS-SAM, (c) RCA_{120} on **3**-coated ODS-SAM, and (d) RCA_{120} on **4**-coated ODS-SAM (negative pattern).

SPR response. Typically, the maximum SPR shift angle of Con A to **2** was 7 times that of RCA_{120} to **2** and 50 times that of BSA to **2**.

Micro-patterned Carbohydrate Displays. Hydrophobic-hydrophilic micropatterned ODS-SAM was prepared by photolithography using VUV at 172 nm. The decomposition of ODS-SAM by photoirradiation was confirmed by XPS and visualized by optical micrographs. Then the micropatterned ODS-SAM was immersed in aqueous solutions of glyco-polymers. XPS spectra of the glyco-polymer coated substrates indicated the selective adsorption on the hydrophobic region (Figure 5). The glyco-polymer adsorbed region showed shoulders in the C1s correnponding C-O of saccharides and C=O of esters and amide. The intensities of C-O and C=O peaks on the photodegraded region were almost 10 % of those on ODS-SAM, indicating the selective adsorption of the polymers on the micropatterned substrate.

Micropatterned Protein Displays on the Glyco-polymer Substrate. The protein recognition of the micropatterned carbohydrates was visualized by fluorescecnce microscopy using FITC labeled lectins and. Fluorescence images were observed alone the micropatterns, and the patterning was specific to the combination between carbohyrate and proteins (**1** and **2** to Con A, and **3** and **4** to RCA_{120}). The protein patterning to glyco-polymer coated substrate on ODS-SAM was assessed by fluorescent intensity. The fluorescent intensity ratios of the micropatterned region were over 3.0 for the specific combinations. However, the contrast of patterning of the lectins was not complete, compared to the high contrast or selective adsorption of the glyco-polymers to ODS-SAM was suggested by XPS peak intensity ratio. The shapes of the fluoresecne images were also slightly different from each other due to the nonspecific adsorption of protein. The blocking method is required to attain the better lectin patterning.

CONCLUSION

We have proposed a new method for micropatterned carbohydrate and protein displays, which are achieved by the self-assembling of the glycopolymer on hydrophobic micropatterned prepared via photolithography of ODS-SAM, and the molecular recognition of the corresponding carbohydrates.

This patterning strategy is based on the self-assembly of biomacromolecules, and is applicable for a variety of substrates. The micropatterning via templates is useful for the fabrication of micropatterned biomacromolecules and nanomaterials. The combination of the top-down material processing (lithography) and the bottom-up processing (self-assembly of the biomacromolecules) is one of the key methodologies for the bio-nanomaterials.

ACKNOWLEDGEMENT

This work was supported by the Industrial Technology Research Grant Program in 2003 from the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

REFERENCES

[1] (a) J. Ziauddin and D. M. Sabatini, *Nature*, **411**, 107-110 (2001). (b) M. Mrksich and G. M. Whitesides, *Trends Biotechnol.*, **13**, 228-235 (1995).

[2] M. E. Tayler and D. Kurt, "Introduction to Glycobiology" (2003) Oxford University Press, New York.

[3] Y. C. Lee, *Carbohydr. Res.*, **67**, 509-514 (1978).

[4] A. Ulman, " An introduction to ultrathin organic films: from Langmuir-Blodgett to self-assembly" (1991) Academic Press, Boston.

[5] (a) H. Sugimura, T. Hanji, O. Takai, T. Masuda and H. Misawa, *Electrochim. Acta*, **47**, 103-107 (2001). (b) H. Sugimura, K. Ushiyama, A. Hozumi and O. Takai, *Langmuir*, **16**, 885-888 (2000).

[6] (a) R. Simghvi, A. Kumar, G. P. Lopez, G. N. Stephanopoulos, D. I. Wang, G. M. Whitesides and D. E. Ingber, *Science*, **264**, 696-698 (1994). (b) Y. Xia, M. Mrksich, E. Kim and G. M. Whitesides, *J. Am. Chem. Soc.*, **117**, 9576-9577 (1995). (c) J. Aizenberg, A. J. Black and G. M. Whitesides, *Nature*, **398**, 495-498 (1999).

[7] (a) A. Kobayashi, T. Akaike, K. Kobayashi and H. Sumitomo, *Macromol. Chem., Rapid Commun.*, **7**, 645-650 (1986). (b) Y. Miura, T. Ikeda and K. Kobayashi, *Biomacromolecues*, **4**, 410-415 (2003).

[8] M. Ishihara, *Trends Glocosci. Glycotech.*, **5**, 343-354 (1993).

[9] I. Wataoke, H. Urakawa, K. Kobayashi, T. Akaike, M. Schmidt and K. Kajikawa, *Macrmolecules*, **32**, 1816-1821 (1999).

(Received December 24, 2004; Accepted June 30, 2005)