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High-Contrast Imaging of Biomolecular Interactions Using Liquid Crystals Supported on Roller Printed Protein Surfaces

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In this study, we report a new method for the high contrast imaging of biomolecular interactions at roller printed protein surfaces using thermotropic liquid crystals (LCs). Avidin was roller printed and covalently immobilized onto the obliquely deposited gold surface that was decorated with carboxylic acid-terminated self-assembled monolayers (SAMs). The optical response of LCs on the roller printed film of avidin contrasted sharply with that on the obliquely deposited gold surface. The binding of biotin-peroxidase to the roller printed avidin was then investigated on the obliquely deposited gold substrate. LCs exhibited a non-uniform and random orientation on the roller printed area decorated with the complex of avidin and biotin-peroxidase, while LCs displayed a uniform and planar orientation on the area without roller printed proteins. The orientational transition of LCs from uniform to non-uniform state was triggered by the erasion of nanometer-scale topographies on the roller printed surface after the binding of biotin-peroxidase to the surface-immobilized avidin. The specific binding events of protein-receptor interactions were also confirmed by atomic force microscopy and ellipsometry. These results demonstrate that the roller printing of proteins on obliquely deposited gold substrates could provide a high contrast signal for imaging biomolecular interactions using LC-based sensors.

Key Words : Liquid crystals, Orientational transition, Roller printing of proteins, Protein-receptor interactions, LC-based sensors

Introduction

In the past decade, liquid crystal (LC)-based sensing techniques have drawn widespread interest for the sensitive amplification and transduction of chemical and biological interactions into optical outputs visible by the naked eye.[1-10] The orientational property of LCs enables the LC-based sensor to be a simple, effective and promising tool for real-time and label-free detection with high spatial resolution. Compared with conventional analytical methods, the LC-based sensing technique does not require the use of labeled molecules, complex instrumentations or highly-qualified technical staff.[11-13] A broad range of biomolecular interactions have been screened through ordering transitions in LCs. Examples include protein-receptor interactions, enzymatic reactions, DNA hybridization and cellular signal events.[14]

Several different types of strategies have been published to monitor protein-receptor interactions at surfaces with nanostructured topographies. For example, Kim *et al.* demonstrated that the specific binding of anti-BSA IgG to the film of mechanically rubbed BSA erased the anisotropic topography induced within the film of BSA by rubbing, and caused an orientational transition of LCs from uniform to nonuniform state.[15] Luk *et al*. investigated protein binding events on the obliquely deposited gold surface with nanometer-scale topographies.[16] The orientation of LCs changed from uniform to nonuniform state after the binding of proteins to the surface-immobilized receptor. Tingey *et al.* reported orientational transitions of LCs after transferring proteins captured by affinity microcontact printing onto amine-terminated surfaces, which might be employed to detect proteins without requiring the use of matched pairs of antibodies or other labels on surfaces.[17] Han *et al.* designed a polymer substrate containing nanometer-scale topographies with periodic sinusoidal wave patterns and observed a uniform-to-nonuniform transition in the orientation of LCs after binding proteins to the surface that was covalently immobilized with receptors.[18]

Recently, we have investigated the orientational behavior of LCs supported on the uniformly deposited gold surface decorated with a film of roller printed proteins.[19] We observed that the surface decorated with roller printed avidin caused uniform orientation of LCs; while specific interactions between biotin-peroxidase and roller printed avidin disturbed the nanometer-scale topography of the surface-immobilized avidin and induced an orientational transition of LCs from uniform to nonuniform state. These results suggested that roller printed films of proteins could be used as substrates on which specific biomolecular interactions can be imaged by LCs.

Our previous approach holds promise for building a new type of LC-based sensors, but the spatial resolution of optical response might not be sufficiently high when designing roller printed protein chips with microarrays since LCs supported on the protein-free area of the uniformly deposited gold surface would adopt a nonuniform orientation. In this study, we report a new method for the high contrast imaging

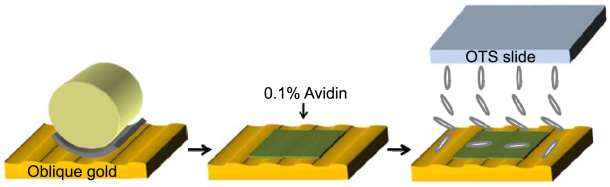

Figure 1. Schematic illustration of the procedure used for roller printing and the orientation of LCs on the obliquely deposited gold surface decorated with avidin.

of biomolecular interactions at the obliquely deposited gold surface decorated with a film of roller printed proteins using LCs. Figure 1 describes a schematic illustration of the procedure for roller printing and fabrication of optical cells using LCs. Avidin absorbed on a PDMS stamp is roller printed at an angle of 45° to the direction of the incidence of gold deposition. The binding of biotin-peroxidase to the surface-decorated avidin erases the nanometer-scale surface topography and induces orientational transition of LCs from uniform to nonuniform state. Since LCs supported on the protein-free area of the obliquely deposited gold surface adopt a uniform orientation, there is a high contrast in the optical response on the protein chip after the specific interactions between avidin and biotin-peroxidase. This approach suggested that the roller printing of proteins on obliquely deposited gold substrates could provide a high contrast signal for imaging biomolecular interactions using LC-based sensors.

Experimental Details

Materials. Titanium (99.999%) and gold (99.999%) were obtained from International Advanced Materials (NewYork, NY). The glass microscope slides were Fisher's Finest, premium grade slides obtained from Fisher Scientific (Pittsburgh, PA). The nematic liquid crystal 4-cyano-4'-pentylbiphenyl (5CB), manufactured by BDH, was purchased from EM Industries (Hawthorne, NY). The polished silicon (100) wafers were from Silicon Sense (Nashua, NH). The PDMS stamps were prepared from Sylgard 184 (Dow Corning, Midland, MI). Octyltrichlorosilane (OTS), 3-aminopropyltriethoxysilane (APES), succinic anhydride (SA), 11-mercaptoundecanoic acid, *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), avidin, biotin, and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich. All protein solutions were made from PBS (pH 7.4). All aqueous solutions were prepared with high purity deionized water (18 MΩ cm) using a Milli-Q water purification system (Millipore, Bedford, MA).

Preparation of Octyltrichlorosilane (OTS)-Treated Glass. The glass microscope slides were cleaned using a "piranha solution" (70% H_2SO_4/30% H_2O_2, Caution: "piranha solution" reacts violently with organic materials and should be handled with extreme caution; do not store the solution in closed containers) for 30 min at 80 °C. The slides were then rinsed with water, ethanol, and methanol, and dried under a stream of gaseous N_2, followed by heating to 120 °C overnight, prior to the OTS deposition. The "piranha-cleaned" glass slides were immersed into an OTS/*n*-heptane solution for 30 min. The slides were then rinsed with methylene chloride and dried under a stream of N_2. Any slide not displaying homeotropic alignment was discarded.

Oblique Deposition of Gold. For use in combination with LCs, semitransparent films of gold with thicknesses of 200 Å were deposited onto clean glass microscope slides mounted on stationary holders by using an electron beam evaporator. Obliquely deposited gold films were prepared at a fixed angle of incidence of 30°. A layer of titanium (thickness of 80 Å) was used to promote adhesion between the glass microscope slide and the film of gold. The rates of deposition of gold and titanium were 0.2 Å/s. The pressure in the evaporator was $< 5 \times 10^{-7}$ Torr during deposition.

Preparation of SAMs. Self-assembled monolayers (SAMs) were formed on the surfaces of obliquely deposited gold films by immersing the films in ethanolic solutions containing 2 mM 11-mercaptoundecanoic acid ($HOOC(CH_2)_{10}SH$). After 2 h of immersion, the slides were rinsed with ethanol and dried under a stream of gaseous N_2. Following this, an aqueous solution of NHS/EDC (50 mM/200 mM) was used to activate the carboxylic acid-terminated SAMs formed on the gold surface before proteins were roller printed.

Preparation of Stamps. PDMS stamps were prepared by casting Sylgard 184 on a silicon master placed in a Petri dish and cured overnight at 80 °C. The master was silanized with (tridecafluoro-1,1,2,2,-tetrahydrooctyl)-1-trichlorosilane vapor (overnight under vacuum) to aid in the release of the PDMS. The PDMS was cut into 15 mm × 3 mm stamps and then exposed to oxygen plasma (Covance, Femto Science) to form a thin silicon oxide layer on the surface of the stamp.

Microcontact Printing of Proteins. The PDMS stamp was inked with antibodies by immersing the entire stamp with 1mg/mL avidin in phosphate buffer saline (PBS) for 30 min. Next, the inked stamp was briefly rinsed with water, dried under a stream of gaseous N_2, and then wrapped around a glass cylinder using double-sided tape. The avidin layer was printed by rolling the stamp onto the obliquely deposited gold surface with a carboxylic acid-terminated monolayer that was pre-activated with NHS ester. During the printing process, pressure was gently applied to ensure the contact between the PDMS stamp and the SAMs on the gold surface. Subsequently, the NHS-activated carboxylic acid groups were quenched through immersing the avidin-decorated gold surface with an aqueous solution of ethanolamine (20 mM) in PBS for 1 h. After this procedure, the surface was washed with PBS, DI water, and dried under a stream of gaseous N_2.

Binding of Proteins in Aqueous Solutions. Specific and nonspecific binding of proteins to the roller printed films of covalently immobilized avidin were performed by incubating the substrates in PBS solutions of proteins for 2 h at 38 °C. Aqueous solutions of 1 mg/mL biotin-peroxidase or bovine serum albumin (BSA) in 10 mM PBS were used. The substrates were sequentially rinsed with 0.01% Triton X-100

in PBS, PBS, and DI water. They were then dried under a stream of gaseous N_2.

Fabrication of Liquid Crystal Cells. Optical cells were prepared by pairing the avidin-decorated surface with an OTS-treated glass microscope slide. The two slides were aligned facing each other, and kept apart by inserting a film spacer (thickness of ~12 μm) at the edge of the surfaces. The cells were held together using mini binder clips. Next, they were heated to ~40 °C by placing them on a warm plate and with a heat gun. 5CB, heated up to its isotropic phase (> 35 °C) within a glass syringe, was dispensed onto the edge of the optical cells. LCs were drawn into the space between the two surfaces by the capillary force. The optical cells were then slowly cooled to room temperature over a period of 1 h. Upon cooling, 5CB transited from the isotropic to the nematic phase.

Polarized Light Microscopy. A polarized light microscope (ECLIPSE LV100POL, Nikon, Tokyo, Japan) was used to image the optical textures formed by polarized light transmitted through the optical cells filled with nematic 5CB. All images were obtained using a 4 × objective lens between crossed polarizers. Imaging the optical appearance of LCs was made with a digital camera (DS-2Mv, Nikon, Tokyo, Japan) that was attached to the polarized light microscope. The images were captured at a resolution of 1600 × 1200 pixels, a gain of 1.00 ×, and a shutter speed of 1/10 s.

Ellipsometry. Ellipsometric thicknesses were measured using an Elli-SE(UV)–FM8 (Ellipsotechnology) at a wavelength of 380-1100 nm and an angle of incidence of 70°. All measurements were performed on silicon wafers that were treated with the same procedures employed to prepare the roller printed films of avidin on the glass microscope slides. The increase in optical thickness of the organic layer after the binding event of proteins was calculated using a simple two-layer model (organic layer/effective substrate of SiO_2/Si) by assuming the refractive index of the organic layers to be 1.18.

Atomic Force Microscopy. Images of the surface topographies before and after the protein binding event on the obliquely deposited gold surface were obtained by AFM using a Veeco Nanoscope IIIa instrument (Santa Barbara, CA) operated in tapping mode. Samples were imaged under ambient conditions using silicon tips with an average radius of ~10 nm. Images were acquired at a scan rate of 1.0 Hz with 256 sample points per line.

Results and Discussion

Orientation of 5CB on the Obliquely Deposited Gold Surface Immobilized with the Roller Printed Films of Avidin. Figure 2(a) shows the optical response of a film of nematic 5CB in contact with the self-assembled carboxylic acid-terminated monolayers immobilized on films of gold which were obliquely deposited on the glass microscope slide before and after the roller printing of avidin that was absorbed on a cylindrical PDMS stamp. We observed that

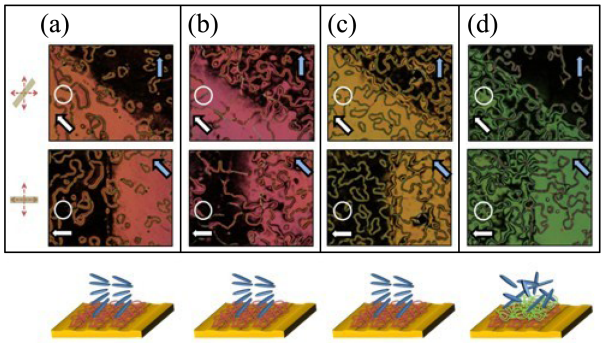

Figure 2. Polarized light microscopy images of LCs supported on the obliquely deposited gold surface decorated with roller printed avidin: (a) prior to further treatment, (b) incubated with 10 mM PBS for 2 h, (c) immersed with 1 mg/mL BSA in PBS for 2 h, (d) in contact with 1 mg/mL biotin-peroxidase in PBS for 2 h. The lower images were obtained after rotating the optical cell shown in the upper images by 45°. The white circles indicate the roller-printed side and the white arrows indicate the direction of LC alignment on each surface. The horizontal dimension of each image was 3.2 mm.

the optical appearance of LCs supported on the obliquely deposited gold surface decorated with SAMs modulated between dark and bright when rotating the optical cell between crossed polarizers. Since LCs aligned on the gold surface in a direction parallel to that of incidence of gold during deposition, the orientation of 5CB was planar and uniform. Furthermore, the optical response of LCs were observed to support on the obliquely deposited gold surface roller printed with avidin modulated between bright and dark when the optical cell was rotated between crossed polarizers. This also evidently represented the planar and uniform orientation of 5CB. However, the optical appearance of LCs on the roller printed films of avidin contrasted sharply to that on the avidin-free area of the gold surface. Since avidin was roller printed on the obliquely deposited gold surface at an angle of 45° to the direction of incidence of gold deposition, the azimuthal orientation of LCs was parallel to the direction of roller printing with an angle of 45° to that of LCs on the avidin-free area of the gold surface.

Previously, we have demonstrated that the uniformly deposited gold surface decorated with SAMs caused the random and nonuniform orientation of LCs; while the roller printed film of chemically immobilized avidin on the uniformly deposited gold surface induced the planar and uniform orientation of LCs with a direction parallel to that of the roller printing of avidin. Thus, we could observe a distinctive change in optical response of LCs before and after the roller printing of avidin on the uniformly deposited gold surface. However, the optical response of LCs exhibited a much higher contrast before and after the roller printing of avidin on the obliquely deposited gold surface. The obliquely deposited gold surface decorated with SAMs resulted in the planar and uniform orientation of LCs, while the roller printed avidin film also led to the planar and uniform orientation of LCs but with an angle of 45° to that of LCs on the gold surface without the roller printing of

avidin. These results suggest that the roller printing of proteins on obliquely deposited gold substrates provides a higher contrast signal, which might be used to develop LC-based sensors for the detection of biomolecular interactions.

The Specific Binding of Biotin to the Roller Printed Avidin. In this section, we investigated the specific binding of biotin to the roller printed film of avidin and examined the orientation of 5CB on the obliquely depositied gold surface decorated with the complex of avidin and biotin. A roller printed film of covalently immobilized avidin was incubated with an aqueous solution of 1 mg/mL biotin-peroxidase in PBS at 38 °C for 2 h. The surface was then rinsed sequentially with 0.01% Triton X-100, PBS, DI water, and dried under a stream of gaseous N_2. Figure 2(d) shows an optical response of 5CB that was in contact with the avidin-biotin complex immobilized on the obliquely deposited gold surface. The optical response of LCs revealed a bright appearance without the presence of reproducible modulation in the intensity of light transmitted through the optical cell that was rotated between crossed polarizers, representing the random and nonuniform orientation of LCs. The specific binding of biotin-peroxidase to the immobilized avidin erased the anisotropic surface topographies generated by the roller printing of avidin, thereby inducing the orientational transition of LCs from uniform to nonuniform on the obliquely deposited gold surface.

To further confirm that the oirentational transition of LCs was caused by the formation of the avidin-biotin complex that erased the anisotropic surface topographies derived from the roller printing of avidin, we conducted two control experiments, including the incubation of the roller printed avidin surface with a PBS solution and the incubation of the roller printed avidin surface with an aqueous solution of 1 mg/mL BSA in PBS at 38 °C for 2 h. Before the fabrication of optical cells, these surfaces were also washed sequentially with 0.01% Triton X-100, PBS, DI water, and dried under a stream of gaseous N_2. The optical response of LCs were observed to support on the avidin-decorated surface pre-incubated with aqueous solutions of PBS or BSA modulated between bright and dark when optical cells were rotated between crossed polarizers (Fig. 2(b) and Fig. 2(c)), indicating the planar and uniform orientation of LCs. These results suggest that the nonspecific adsorption was not sufficiently enough to induce orienational transition of LCs from uniform to nonuniform state. Thus, interactions between avidin and biotin could be specifically monitored on the obliquely deposited gold surface decorated with roller printed avidin.

With an aim to confirm the feasibility of our approach to image biomolecular interactions on a protein chip containing microarrays, we investigated the binding event of proteins on the obliquely deposited gold surface with small square patterns of roller printed avidin. Figure 3(a) shows the optical image of LCs supported on a SAM formed on a gold surface that was roller printed with small square patterns of avidin. Rotation of the optical cell between crossed polars showed an alternate modulation in the optical appearance of LCs between the avidin-printed and the avidin-free areas.

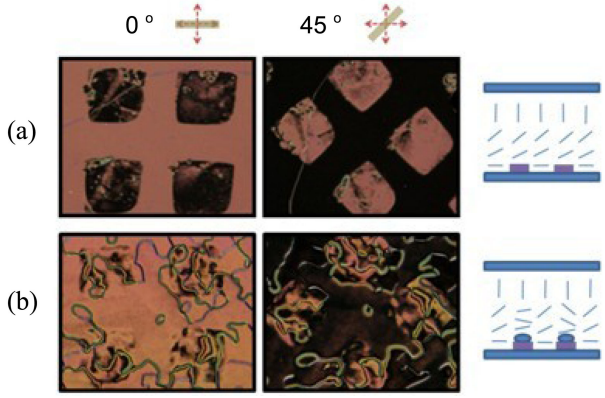

Figure 3. Optical images (crossed polarizers) of nematic 5CB: (a) in contact with the obliquely deposited gold surface which was roller printed using a pattern stamp that had been incubated with an aqueous solution of avidin, (b) in contact with the roller printed avidin surface with small square patterns in an aqueous solution of 1 mg/mL biotin-peroxidase.

Figure 3(b) displays the optical image of LCs after the incubation of the obliquely deposited gold surface containing small square patterns of avidin with an aqueous solution of 1 mg/mL biotin-peroxidase. The orientation of LCs adopted a nonuniform alignment on the area decorated with the complex of avidin and biotin; while the orientation of LCs adopted a uniform alignment on the protein-free area. These results suggest the possibility of using our strategy to design protein microarrays involving LC-based sensors with very high contrast signal.

Ellipsometric Measurement and Topographical Analysis of Protein Binding to Roller Printed Films of Avidin. In order to further confirm that the orientational transition of LCs was induced by the specific binding event of proteins on the obliquely deposited gold surface decorated with films of roller printed avidin, we examined nanometer-scale topographies of the flat gold surface using AFM before and after the incubation of proteins. First, we observed the topographical image of roller printed avidin. Figure 4(a) displays the root mean square (RMS) roughness of the surface which was 1.39 nm. Next, we incubated the roller printed avidin-decorated gold surface with an aqueous solution of 1 mg/mL biotin-peroxidase for 2 h and conducted the topographical analysis. The RMS roughness of the surface after the binding of biotin-peroxidase to the surface-immobilized avidin was measured to be 1.97 nm (Fig. 4(d)), which shows a significant increase in the RMS roughness of the surface topography. We then tested the effect of PBS on the topography of the roller printed avidin-decorated gold surface. The RMS roughness was measured to be 1.18 nm (Fig. 4(c)), which suggests that the incubation of the surface with PBS induced no significant changes of the topography. We also examined the topography of the roller printed avidin-decorated gold surface incubated with an aqueous solution of 1 mg/mL BSA for 2 h. The RMS roughness of the surface was 1.11 nm (Fig. 4(c)), representing that the non-specific interaction between BSA and the surface-immobilized avidin

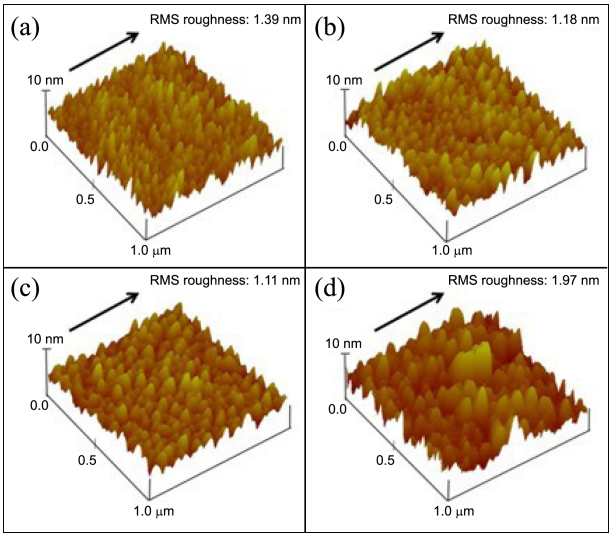

Figure 4. AFM images of roller printed films of avidin: (a) prior to incubation, (b) after incubated with PBS, (c) after immersed with an aqueous solution of 1 mg/mL BSA, and (d) after in contact with an aqueous solution of 1 mg/mL biotin. The black arrows in each image indicate shear direction.

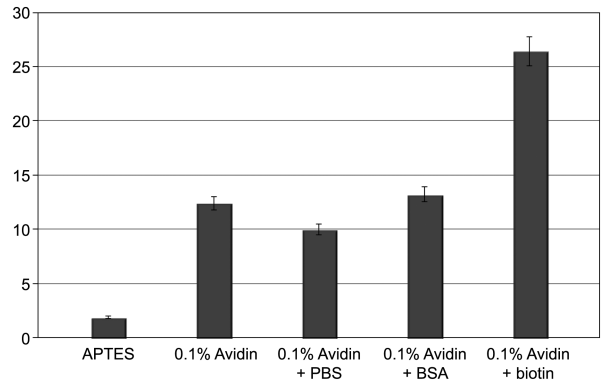

Figure 5. Ellipsometric thicknesses of proteins adsorbed onto the roller printed films of avidin.

also did not cause significant changes of the topography. These results suggest that the elimination of the anisotropic structure of the roller printed avidin surface after the specific binding event of proteins induced the orientational transition of LCs from uniform to nonuniform state.

We also conducted the ellipsometric measurement to further confirm the orientational transition was caused by the specific binding event of proteins (Fig. 5). Avidin was roller printed and covalently immobilized onto the surface of a silicon wafer functionalized with the NHS-activated carboxylic acid, which was produced using 3-aminopropyltriethoxysilane (APES) and succinic anhydride (SA). We observed that the ellipometric thickness of the surface changed significantly after the specific binding of biotin to the surface-immobilized avidin; while the optical thickness did not change greatly after the nonspecific adsorption. These results indicate that biotin was specifically bound to the roller printed avidin surface.

Conclusion

In summary, we confirm that the roller printing of proteins on the obliquely deposited gold substrate could provide a high contrast signal for imaging biomolecular interactions using LC-based sensors. The azimuthal orientation of LCs on the avidin-decorated area is parallel to the direction of roller printing with an angle of 45° to that of LCs on the avidin-free area of the gold surface. Thus, the optical response on the roller printed films of avidin contrasts sharply to that on the avidin-free area of the gold surface. The specific binding of biotin-peroxidase to the immobilized avidin erased the anisotropic surface topographies generated by the roller printing of avidin, thereby inducing the orientational transition of LCs from uniform to nonuniform state. Furthermore, we investigated the specific binding of biotin to the roller printed avidin with small square patterns on the obliquely deposited gold surface. This approach shows the feasibility of imaging biomolecular interactions on the roller printed protein chip containing microarrays with high contrast using the LC-based sensor.

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