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Synthesis of Novel Copolymer for Selective Biomolecule Immobilization on Gold Surface

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Various types of nanoparticles such as quantum dots, magnetic nanoparticles, and liposomes have been studied for numerous biomedical applications. Among these nanoparticles, gold nanoparticles have been extensively exploited for various applications including drug delivery,[1-4] imaging agents,[5-8] biosensors,[9-12] and computed tomography (CT) contrast agents[13-17] because they have many advantages such as low cytotoxicity, easy size control, high x-ray absorption, unique optical and electrical properties. Stability of gold nanoparticles under physiological conditions is key step for biomedical application. Another key is the immobilization or conjugation of bioactive molecules on gold nanoparticles for targeted drug delivery, molecular imaging, and *in vitro* diagnosis. To satisfy these key requirements, we developed a novel random copolymer composed of a "surface anchor part" (thiol group),[13,14,18-20] "antibiofouling part" (PEG),[13,15,21-25] and "bioreactivity part" (carboxyl group) for gold surface modification in particular.

A random polymer to modify the gold surface for targeting ligand conjugation or SPR (surface plasmon resonance) chip was synthesized using radical polymerization. The chemical structure of poly(SVE-*r*-mPEGMA-*r*-MA) is shown in Figure 1(a). This polymer consists of three parts: a S-4-vinylbenzyl ethanethionate (SVE) monomer as the surface-anchor part, poly(ethylene glycol) methyl ether methacrylate (mPEGMA) as the protein-resistant part, and methacrylic acid (MA) monomer as a functional group to conjugate biomolecules. The initial feed ratio (SVE: mPEGMA:MA, 2:2:1) was chosen for synthesizing polymer that have high antibiofouling effect and strong surface binding capacity. After radical polymerization, unreacted monomers were removed by dialysis. The structure of the polymer was confirmed using NMR. Peaks for the methoxy of the mPEGMA and the methyl of the SVE monomer were observed at 3.4 ppm and 2.3 ppm, respectively (Figure S1). The molecular weight of the polymer was Mn = 16 000, as measured by gel permeation chromatography (GPC). Next, we examined the ability of the polymer to coat the gold surface. First, the acetylated thiol of the polymer was deprotected using hydroxylamine to yield a free thiol group. Because several thiol groups in the polymer provide multi-point attachments to the gold surface, the polymer spontaneously forms monolayers on a gold surface. Because of the multiple attachments, the stability of the polymeric monolayers can be significantly improved when exposed to a buffer with a non-physiological pH, or oxidative chemical and electrochemical environments. A polymeric self-assembled monolayer (SAM) of the polymer on the gold surface was prepared by immersing the substrates in an ethanol solution of 0.2 wt % polymer. The polymer monolayer thickness was measured using ellipsometry. The ellipsometric thickness increased after immersion times of up to 4 h. After 4 h, the thickness of the films remained unchanged, and the thickness of the monolayer was approximately

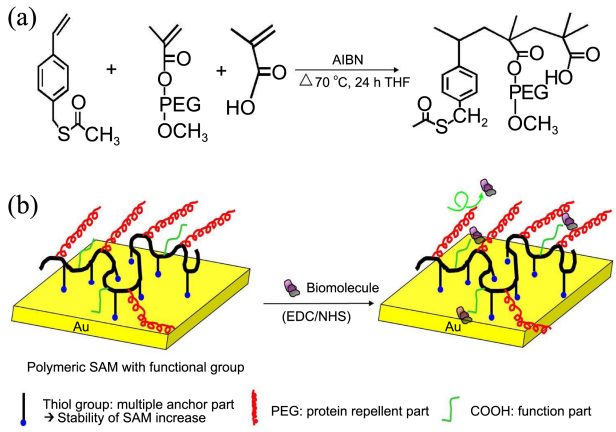

Figure 1. (a) Chemical structure of the polymer (b) schematic representation of the procedure for immobilizing biomolecules on a gold surface.

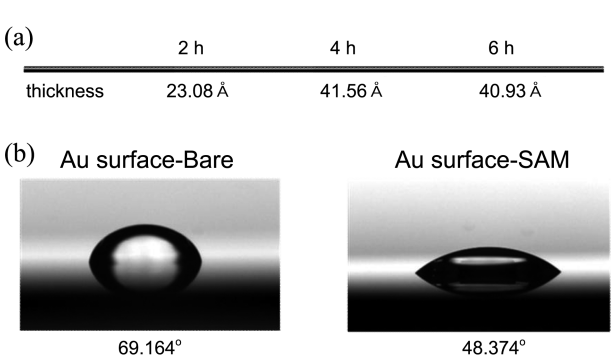

Figure 2. (a) Thickness (b) Static water contact angle of self assembled monolayers (SAM) constructed on gold surface.

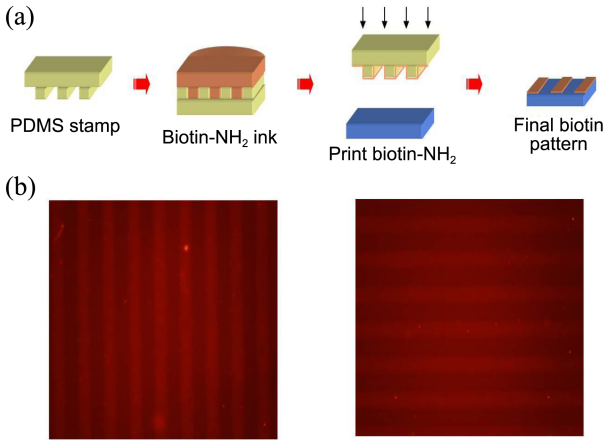

Figure 3. (a) Schematic diagram of μCP. (b) Fluorescence microscope image of TRITC-labeled streptavidin patterns prepared following μCP.

41 Å (Figure 2(a)).

The wettability of the SAM was attained using static water contact angle measurement at ambient temperature. The water contact angles decreased drastically, from 69° for the bare gold substrate to 48° for the polymer-coated gold surface (Figure 2(b)). These results indicated that polymer layers had formed and suggested that the hydrophilic PEG was exposed. To examine the feasibility of using the polymer-coated gold surface for the immobilization of biomolecules, we prepared a micropattern of biomolecules using μCP. In the first step, the carboxylic group on the polymer-coated gold surface was converted into the NHS ester by treatment with EDC/NHS reagents. Then, amine-terminated biotin ink was contact-printed onto the reactive polymer layers using a positive PDMS stamp. Because the amine group of the biomolecules could also react with the activated NHS ester group of the polymeric surface, in the subsequent step, the resulting biotin-patterned gold surface was immersed in borate buffer to hydrolyze any unreacted NHS ester on the surface and then incubated with a solution of TRITC-labeled streptavidin. Figure 3(b) shows a fluorescence microscopic image of the pattern of the TRITC-labeled streptavidin, in which streptavidin was selectively immobilized on the biotin-functionalized areas exclusively, indicative of specific interactions between them.

In conclusion, we developed a novel polymer for the selective immobilization of biomolecules on a gold surface. This polymer could easily be coated on a gold surface by forming polymeric SAM. Biomolecules could be efficiently immobilized onto the polymer-coated gold surface with high specificity, as well as little non-specific adsorption. Taken together, these results indicate that the polymer described here may be suitable for a variety of gold nanoparticle applications in the fields of biosensing, targeted drug delivery, and molecular imaging.

Experimental Section

Materials. 4-Vinylbenzylchloride, thioacetic acid, poly(ethylene glycol) methyl ether methacrylate (mPEGMA, Mn = *ca.* 475), methacrylic acid (MA), *N*-(3-dimethylaminopropyl)-*N'*-ehtylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), and 2,2'-azobisisobutyronitrile (AIBN) were purchased from Aldrich Chemical Co. (Milwaukee, WI). All of the organic solvents were used as received without further purification.

Measurement. ^{1}H NMR (300 MHz) spectra were recorded on a JEOL JNM-LA 300WB FT-NMR (Tokyo, Japan). Organic phase gel permeation chromatography (GPC) was performed using a Waters 1515 series isocratic pump and a Rheodyne model 7725 injector with a 100 μL injection loop at a flow rate of 0.4 mL/min. The thicknesses of the monolayer films were measured using a Gaertner L116A ellipsometer (Gaertner Scientific Corporation, IL) at a 70° angle of incidence. A film with a refractive index of 1.46 and a three-phase model were used to calculate the thickness. A Phoenix 300 contact angle & surface tension analyzer (Surface Electro Optics, Kyonggi, Korea) equipped with a video camera and monitor was used to measure the contact angle.

S-4-Vinylbenzyl Ethanethionate (SVE) Synthesis. 4-Vinylbenzylchloride (1 mmol) and thioacetic acid (1.5 mmol) were added to a vial; and to this, dichloromethane (3 mL) followed by triethylamine (1.5 mmol) were added and stirred for 1 h. The resulting solid in the solvent was vacuum filtered, and the solvent was evaporated. The crude mixture was purified using silica column chromatography, and the product was eluted with hexane/ethyl acetate (30:1 v/v).

Synthesis of Poly(SVE-*r*-mPEGMA-*r*-MA). Prior to polymerization, neat mPEGMA was flowed through the inhibitor removal column (Sigma-Aldrich, Milwaukee, WI). SVE (2 mmol, 2 equiv), PEGMA (2 mmol, 2 equiv), MA (1 mmol, 1 eq), and AIBN (0.02 mmol, 0.02 equiv) were placed in a vial and dissolved in 10 mL of THF (anhydrous, 99.9%, inhibitor-free). The resulting mixture was degassed for 20 min using a N_2 gas stream, after which the vial was sealed with a Teflon-lined screw cap. The polymerization reaction was carried at 70 °C. After 24 h reaction, unreated monomers were removed by dialysis (cut off: 10 K). The polymer was obtained as a viscous liquid after freeze dry.

Microcontact Printing (μCP) of Biological Ligands. First, the polymer-coated gold surface was treated with an EDC (400 mM)/NHS (100 mM) mixture for 20 min to convert the carboxylic acid to a reactive NHS ester. After inking, the biotin-NH_2 (10 mM in ethanol) was printed by pressing the PDMS stamp onto the polymer-coated gold surface. The sample was then immersed immediately in a borate buffer (pH 8.5) for 1 h to hydrolyze the unreacted NHS esters. After the pattern generation of the biotin, the substrate was subsequently transferred to a solution of TRITC-labeled streptavidin (0.1 mg/mL) in PBS (pH 7.4) at room temperature. After 1 h, the substrate was removed and washed several times with PBS and distilled water. The micropatterns of the streptavidin were characterized using fluorescence microscopy.

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