Photolithographic process for the patterning of quantum dots

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Recently, quantum dots have been used as molecular probes substituting for conventional organic fluorophores. Quantum dots are stable against photobleaching and have more controllable emission bands, broader absorption spectra, and higher quantum yields. In this study, an array of ZnS-coated CdSe quantum dots on a slide glass has been prepared by photolithographic method. The array pattern was prepared using a positive photoresist (AZ1518) and developer (AZ351). The patterned glass was silanized with 3-aminopropyltriethoxysilane (APTES), and carboxyl-coated quantum dots were selectively attached onto the array pattern. The silanization was examined by measuring contact angle and the surface of the array pattern was analyzed using AFM and fluorescent microscope.

1. Introduction

Recently, ordered nanostructures with micorscale featured size on the surface of material have generated considerable interests owing to their unique electronic, optical, and biological characteristics. The conventional lithographic techniques are commonly used for the fabrication of such devices [1]. There have been numerous applications of these techniques in electronics and bio-chemical researches such as specific detection of biomolecular interaction which is most important for future drug and diagnostic development [2]. Semiconductor nanoparticle quantum dots are luminescent inorganic fluorophores which can be excited with a single light source for multicolor light emission. They are attractive especially in the area of biosensors and biomarkers due to their long-term photo stability and efficient continuous monitoring. Colloidal semiconductor quantum dots are single crystals a few nanometers in diameter and their size and shape can be controlled by the reaction time, temperature, and ligand molecules used for their synthesis. Over the past several years, quantum dots have been tested in most biotechnological applications which use fluorescence, including DNA array, immunofluorescence assays, and cell and animal biology [3]. Several methods have been attempted to modify the surface of quantum dots for those applications by functionalization including ligand exchange [4], encapsulation [5], and bioconjugation [6]. In this study, CdSe/ZnS quantum dots [7] have been immobilized on the microarray (300µm in diameter) of glass substrate for the application of biosensor. The array pattern of quantum dots was prepared by combining photolithographic process and vapor phase silanization, followed by the activated succinimide ester process. The immobilized quantum dots on microarray pattern were visualized by using fluorescent microscopy.

2. Experiment

2.1. Materials and characterizations

N-hydroxysuccinimide (NHS), sodium tetraborate decahydrate, boric acid, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) were purchased from Sigma-Aldrich. Positive photoresist (AZ1518), MIF developer and PR remover 700 were obtained from AZ Electronic Materials. Qdots® S85 ITK carboxyl quantum dots solution were purchased from Invitrogen (Carlsbad, CA, USA). Phosphate buffered saline (pH 7.4) was obtained from Welgene.

Contact angle was measured by the sessile drop technique using tensiometer (KRÜSS G10 MK2) under ambient condition. Zeta-potential of solid surface was measured by electrophoretic light scattering system (ELS-8000, Otsuka). The excitation spectra and fluorescence image were obtained using IM-1/2005 Ratio Fluorescence Imaging System (Photon Technology International) equipped with xenon lamp which is connected with a fluorescent microscope (Olympus X71). The excitation wavelength was scanned via monochromator with a 590 nm emission filter. The surface morphology of the Qdots immobilized array pattern was studied by means of atomic force microscopes (AFM, XE-100, PSIA Inc.). The AFM image was obtained in non-contact mode with a cantilever (910M-NSC15, PSIA Inc.). The scan was performed at the surface with an average scanning speed of 0.5 Hz.
2.2. Array patterning via photolithographic process

Glass substrates were cleaned for 2 h at 70 °C in piranha solution consisting of 3:1 volume ratio of 50% (w/v) aqueous solution of sulfuric acid and 30% (w/v) hydrogen peroxide, and rinsed with deionized water and dried with nitrogen gas. The photoresist was spin coated on the substrate with a spin speed of 1800 rpm for 15 s. After soft baking at 95 °C for 30 min, UV exposure (12 mW/cm²) through emulsion mask, and developing in MIF developer for 3 min, the array pattern was prepared and hard baked in a convection oven (120 °C, 30 min).

2.3. Silanization by vapor phase deposition

The array patterned glass substrate was silanized by vapor phase deposition of APTES [8]. The substrate was placed inside of vacuum chamber with 3 ml APTES. The chamber was evacuated for 5 min using a vacuum pump and sealed for 1 h at room temperature. The pressure was reduced to approximately 70 cm Hg below atmosphere. APTES was vaporized inside chamber, and the vapor was deposited on substrate. The glass substrate was then removed and annealed at 120 °C for 2 h at ambient condition.

2.4. Immobilization of quantum dots on microarray and PR removal

CdSe/ZnS quantum dots with carboxyl group were immobilized on the array pattern by selectively reaction between

![Diagram](image)

Carboxyl coated Qdot

Fig. 3. The activated succinimide ester process with EDC and NHS.

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Fig. 1. The excitation fluorescent spectra of carboxyl quantum dot 585. The excitation spectra were measured at the emission window of 590 nm.

Fig. 2. Fluorescence images of immobilized quantum dot on glass substrate with various concentrations of EDC and NHS: (a) EDC 0 mM, NHS 0 mM; (b) EDC 50 mM, NHS 50 mM; and (c) EDC 100 mM, NHS 100 mM.
carboxyl group of Qdots and silanol on glass substrate. The coupling reaction was performed with EDC and NHS in borate buffer. Eight nanomolars of carboxyl-coated quantum dots were kept on top of the substrate for 24 h at room temperature. Borate buffer (pH 7.4) was prepared by mixing 7.07 g/L boric acid and 8.17 g/L sodium tetraborate decahydrate. After immobilization, the glass substrate was washed with borate buffer and rinsed with deionized water and dried with nitrogen gas [9]. The photoresist was removed by dipping the substrate in PR remover 700 for 5 min. The array patterned substrate with CdSe/ZnS quantum dots are rinsed with borate buffer again and dried.

3. Results and discussion

In order to functionalize the glass surface prior to reaction with CdSe/ZnS quantum dots, the silanization was initiated under vacuum atmosphere to add a free amine functional group. This amine group is to react with the activated carboxylic group on the quantum dot. The surface modification of glass substrate by silanization was confirmed by measuring surface wettability. The static contact angle, $\theta$, was measured for sessile droplets of pure water. The contact angle after piranha cleaning was $8.2^\circ$. Low values of the contact angle correspond to high surface wettability. After silanization with APTES, the contact angle increased to $55.9^\circ$ which corresponds to lower surface wettability. Zeta-potential of the glass surface was measured in 1 mM NaCl solution using Helmholtz–Smolukowsky equation, and was $-12.63$ and $93.82$ mV for glass substrate after piranha cleaning and silanization, respectively. Fig. 1 shows the fluorescence excitation spectra of the CdSe/ZnS quantum dots. The excitation wavelength was determined to 428 nm, at which glass substrate does not effect the fluorescence emission. Fig. 2 shows the effect of EDC and NHS concentration on the immobilization of quantum dots. Quantum dots are immobilized by the activated succinimide ester process with EDC and NHS which is depicted in Fig. 3. As the concentration of EDC and NHS increases, more quantum dots are immobilized on to the glass substrate. The fluorescent image of the immobilized quantum dots was obtained using fluorescence microscope with excitation wavelength of 428 nm and emission window at 590 nm. Fig. 4 shows fluorescent image of the quantum dots in array pattern. Fig. 4(a) shows the array pattern after developing, (b) shows fluorescence image from quantum dots immobilized on the pattern, and (c) and (d) show the fluorescent image from two circular patterns in (b) with higher magnification. The array pattern was prepared by photoresist process. When PR is removed after silanization, only the inner circle area of the array pattern can immobilize the quantum dots. The figure shows that not enough quantum dots are immobilized, which may be due to the loss of amine group after PR removing. The best array pattern was obtained with EDC and NHS concentration of 100 mM each and PR stripping time of 5 min. Fig. 5 shows AFM images of the immobilized quantum dots. Fig. 5(A) shows the boundary of the quantum dot immobilized area, (B) shows the quantum dot immobilized area with higher magnification, and (C) shows glass substrate without quantum dots. The figure shows that the quantum dots are disordered and agglomerated with very rough surface structure. This shows why the fluorescence images from the array pattern of quantum dots are poor.

4. Conclusion

Semiconductor quantum dots are promising in the field of bioassay owing to their unique optical and physical properties. In this report, we immobilized quantum dots in microarray pattern on
glass substrate by the reaction of carboxyl group in quantum dots and amine group in silanized glass substrate. The array pattern was prepared by using conventional photolithographic process and glass surface was functionalized with APTES. The silaization was confirmed by measuring the contact angle of water droplet on the glass substrate and zeta-potential in NaCl solution.
The number of immobilized quantum dots was highly dependent on the concentration of EDC and NHS. The fluorescent microarray images from the immobilized quantum dots were obtained using fluorescent microscopy, but the image was poor due to the loss of amine functional group after PR removing and disordered and agglomerated quantum dots in array pattern.

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References