DETECTION OF CRP BASED ON DBR POROUS SILICON INTERFEROMETERS

Sungyong Um, Bomin Cho, Honglae Sohn*
1 Department of Chemistry, Chosun University, Gwangju, Korea,
* Corresponding author (hsohn@chosun.ac.kr)

A simply modified biosensor based on anti-C-reactive Protein (CRP)-modified distributed Bragg reflectors (DBR) porous silicon (PSi) chip for the detection of C-reactive protein has been developed. The fabrication, optical characterization, and surface derivatization of DBR PSi are investigated. The sensor system studied consist of multi-layer of porous silicon modified with anti-CRP. The sensor is operated by the measurement of the reflection peak in the white light reflection spectrum. Molecular binding is detected as a shift in wavelength of reflection peaks.

Keywords: Porous Silicon, C-Reactive Protein, Interferometer, Biosensor,

1 Introduction
Recently, the nanostructured porous silicon (PSi) has been received a great interest in optoelectronic devices. The PSi has a high surface area, which has shown to be useful for many applications, such as chemical and biological sensors,1 switching devices,2 implantable biomaterials,3 drug delivery,4 and in high-throughput screening.5 For applications in biosensors, label-free biosensors would be important due to the advantage of easy sample preparation. Biosensor based on PSi interferometer has a great advantage due to a large surface area matrix for immobilization of a variety of biomolecules such as enzymes,6 protein,7 and DNA fragments.8 Recently, the double-layers of PSi can also be used as the transducer of biomolecular interaction in biosensor application.

C-reactive protein (CRP) is an acute-phase protein found in human serum.9-11 Concentrations of CRP can be elevated up to 1000-fold (200 μg/mL) in response to inflammation, injury, or infection.11,12 Recent studies have demonstrated that CRP can be used to predict the risk of acute events in patients with atherosclerosis.11,13-19 CRP is known to predict risk of future events in patients with acute coronary syndromes, stable angina, and coronary artery stents.11-23 Therefore many investigations for high-sensitive CRP detection techniques based on fluorescent nanoparticle,24 thin-layer immunoaffinity chromatography,25 surfactant-enhanced latex particle immunoassay,26,27 and polymeric nanoparticles,28 have been reported. In the present work, a simply modified biosensor for the detection of C-Reactive Protein (CRP) based on anti-CRP-modified DBR PSi interferomerer is reported.

2 Experimental Details

2.1 General Information
THF was distilled from sodium benzophenone ketyl immediately prior to use. Triethylamine was distilled under nitrogen and stored over sodium hydroxide in a dark, cold place before use. Silicon wafer (boron doped, polished on the <100> face, resistivity of 0.8–1.2 mΩ•cm) was purchased from Siltronix Inc. Ethanol, toluene, methylene chloride, acetone, phosphate-buffered solution (PBS, pH = 7.4), 48% hydrofluoric acid (ACS reagent), (3-aminopropyl)trimethoxysilane (99%), biotinamidohexanoyl-6-amino-hexanoic acid N-hydroxy-succinimide ester (95%), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimidehydro-chloride (EDC), and streptavidin, were all purchased from Aldrich and used as received without further purification. C-reactive protein (from human plasma) and anti-human C-reactive protein (anti-CRP, developed in rabbit, ~90 mg/mL) were purchased from Sigma.

2.2 Preparation of DBR PSi

The DBR PSi was prepared by an electrochemical etching of Si wafer (boron doped,
polished on the <100>face, resistivity of 0.8~1.2 mΩ•cm, Siltronix, Inc.). The etching solution consisted of a 1:3 (v/v) mixture of absolute ethanol and aqueous 48% hydrofluoric acid. Galvanostatic etching was carried out in a Teflon cell by using a two-electrode configuration with a Pt mesh counter electrode. DBR PSI was prepared by using a periodic pseudo-square wave current between 250 mA•cm$^{-2}$ for 1 s and 100 mA•cm$^{-2}$ for 5 s with 50 repeats. The anodization current was supplied by Keithley 2420 high-precision constant current source controlled by a computer allow the formation of PSI. To prevent the photogeneration of carriers, electrochemical etching was performed the anodization in the dark. All DBR PSI samples were then rinsed several times with ethanol and dried under argon atmosphere.

2.3 Biotin functionalization of DBR PSI

Thermal oxidized DBR PSI samples were obtained by heat treatment in a furnace (Thermolyne F6270-26 furnace equipped with controller) using the following parameters: initial ramp rate, 5 ºC/min to 300 ºC, hold time, 3 h and passive cooling to ambient temperature.

For the functionalization of oxidized DBR PSI, the oxidized DBR PSI sample and 1.8 mL of (3-aminopropyl)trimethoxysilane were placed in Schlenk flask and refluxed for 20 hrs in a dry argon atmosphere. After functionalization with amine group, the DBR PSI sample was rinsed successively with methylene chloride, acetone, and ethanol and subsequently dried under a stream of nitrogen.

For surface modification of biotin-derivatized DBR PSI sample, 10 mg of biotinamidohexanoyl-6-amino-hexanoic acid N-hydroxy-succinimide ester was dissolved in 10 mL of THF. The mixture solution was stirred vigorously for 10 min. The amine-functionalized DBR PSI chip, EDC (20 mg, 0.1 mmol), and triethylamine (TEA, 0.9 mL, 6 mmol) were added to the mixture solution. The reaction mixture was allowed to stir at room temperature for overnight. After removal of the solution, samples were rinsed by ethanol, methylene chloride, and acetone, dried under a reduced pressure, and stored in nitrogen atmosphere prior to use.

2.4 Anti-CRP functionalization of DBR PSI

Biotin-terminated DBR PSI was placed in 20 μM streptavidin PBS solution (pH 7.2), and allowed to bind for 30 min at room temperature. Streptavidin-terminated DBR PSI chip was rinsed with PBS solution several times and was incubated in 20 μM anti-CRP PBS solution and biotinamidohexanoyl-6-amino-hexanoic acid N-hydroxy-succinimide ester PBS solution for 30 min. Free biotin molecules were subsequently removed by rinsing with PBS solution.

2.5 Instruments and Data Acquisitions

Interferometric reflectance spectra of DBR PSI were recorded by using an Ocean Optics S2000 spectrometer fitted with a bifurcated fiber optic probe. A tungsten light source was focused onto the center of DBR PSI surface with a spot size of approximately 1–2 mm. Spectra were recorded with a CCD detector in the wavelength rang 400–1200 nm. The illumination of the surface as well as the detection of the reflected light was performed along an axis coincident with the surface normal. FT-IR instrument in the diffuse reflectance mode (Spectra-Tech diffuse reflectance attachment), with diffuse reflectance absorption spectra are reported in absorbance units. The morphology of DBR PSI film was observed with cold field emission scanning electron microscope (FE-SEM, S-4700, Hitachi).

3 Results and Discussion

A DBR PSI exhibits a high reflectivity band with the Bragg wavelength, $\lambda_{\text{Bragg}}$, depending on the thickness of the layers ($d_1$, $d_2$) and the corresponding refractive indices ($n_1$, $n_2$). The $m^{th}$ order of the Bragg peak is given by

$$m\lambda_{\text{Bragg}} = 2(d_1n_1 + d_2n_2). \quad (1)$$

Typical etch parameters for the DBR PSI structure involve using a periodic square-wave current between low and high current densities. In this work, the applied current densities for the fabrication of DBR PSI was varied between 100 and 250 mA•cm$^{-2}$.
The surface and cross sectional morphology of DBR PSi were obtained with cold FE-SEM and shown in Fig. 1. The pore size of prepared DBR PSi was about 20–30 nm. The cross-sectional images of DBR PSi displayed that the DBR PSi had a depth of about 20 microns.

The schematic diagram for the preparation of biotin-functionalized DBR PSi was shown in Scheme 1. Freshly prepared DBR PSi was thermally oxidized at 300 °C to convert the hydrogen-terminated PSi into hydroxy-terminated PSi. Amine-terminated DBR PSi was obtained from the reaction of OH-terminated DBR PSi and (3-aminopropyl)trimethoxysilane. This amine moiety reacted with biotinamidohexanppyl-6-aminohexanoic acid N-hydroxy-succinimide ester in the presence of EDC and TEA to give the desired biotin-functionalized DBR PSi.

This step-by-step addition process was monitored by FT-IR spectroscopy and shown in Figure 2. After oxidation, Si-H stretching vibrational frequencies observed at about 2115 cm⁻¹ of as-prepared DBR PSi disappeared, however OSi-H and Si-O stretching vibrational frequencies appeared at about 2263 and 1100 cm⁻¹, respectively. For amine-terminated DBR PSi, N-H and C-H stretching vibrational frequencies were observed at about 3360 and 2975 cm⁻¹, respectively. Biotin-terminated DBR PSi displayed an amide vibrational frequency of biotin moiety at 1662 cm⁻¹.

The optical setup for the biosensing experiments was shown in Figure 3 and consisted of
a biotin-terminated DBR PSi mounted in a transparent flow cell. Reflectance spectra were collected and transmitted through fiber optic cables. Aqueous PBS buffer solution was flushed to perform an initial measurement of reflectivity. The surface was rinsed thoroughly with PBS buffer solution to ensure covalent attachment to the surface and to check stability of the reflectivity measurement. Biotin-terminated DBR PSi chip was then incubated in the cell with 20 μM streptavidin PBS solution and then followed by 20 μM biotinamidohexanoyl-6-amino-hexanoic acid N-hydroxy-succinimide ester PBS solution and 20 μM anti-CRP PBS solution for 30 min, respectively. Finally, the cell was then flushed with a constant flow of 0.8 mL/min of 20 μM CRP PBS buffer solution which coupled to the anti-CRP-modified DBR PSi surface.

The change of optical reflectivity in the reflection spectra depending on the surface modification of DBR PSi was shown in Figure 4. When the biotin-terminated DBR PSi was placed in PBS solution, the reflectivity of 596 nm shifted to longer wavelengths by 24 nm due to the increase of refractive indices upon introduction of PBS solution into the pores of DBR PSi. The subsequent introduction of streptavidin, biotin derivatives, anti-CRP to the DBR PSi resulted in the reflectivity at 620 nm shifted to longer wavelengths by 9, 27, and 7 nm, respectively. This result is due to the increase of additional refractive indices. For binding studies, the exposure of 20 μM CRP to the anti-CRP-functionalized DBR PSi resulted in an increase of the reflection wavelength in the reflectivity spectrum by 4 nm, indicative of a change in refractive indices induced by binding of CRP into the anti-CRP-derivatized DBR PSi.

The change of reflective intensity at the fixed wavelength of 663 nm observed from anti-CRP modified DBR PSi was measured to determine the detection limit for CRP and shown in Figure 5. Different decreases of reflective intensity were obtained according to the loaded quantity of CRP. A dramatic decrease of the reflectivity, resulting from the red-shift, was observed in the reflectivity spectrum, indicating that a binding of CRP to anti-CRP took place within 10 s. With this technique, the CRP detection limit is as low as 100 pM.
Fig. 5. Change of optical reflective intensity of anti-CRP-modified DBR PSi at the fixed wavelength of 663 nm according to the loaded quantity of CRP.

4 Conclusion
A simple and very sensitive immunoassay method based on anti-CRP modified DBR PSi chip has been developed. The anti-CRP functionalized DBR PSi resulted in the reflectivity shifted to longer wavelengths by the introduction of CRP, indicative of binding of anti-CRP and CRP.

References


