Biological reaction signal enhancement in porous silicon Bragg mirror based on quantum dots fluorescence*

LI Yan-yu (李彦宇)[1], **JIA Zhen-hong** (贾振红)[2]**, **WANG Jia-jia** (王佳佳)[1], **and LÜ Chang-wu** (吕长武)[1]

1. School of Physical Science and Technology, Xinjiang University, Urumqi 830046, China

2. School of Information Science and Engineering, Xinjiang University, Urumqi 830046, China

(Received 18 February 2017)

In this paper, we mainly study the preparation of an optical biosensor based on porous silicon (PSi) Bragg mirror and its feasibility for biological detection. The quantum dot (QD) labeled biotin was pipetted onto streptavidin functionalized PSi Bragg mirror samples, the affinity reaction between QD labeled biotin and streptavidin in PSi occurred, so the QDs were indirectly connected to the PSi. The fluorescence of QD enhanced the signal of biological reactions in PSi. The performance of the sensor is verified by detecting the fluorescence of the QD in PSi. Due to the fluorescence intensity of the QDs can be enhanced by PSi Bragg mirror, the sensitivity of the PSi optical biosensor will be improved.

Document code: A **Article ID:** 1673-1905(2017)03-0172-3

DOI 10.1007/s11801-017-7034-5

In 1997, J. Sailor Micheal used porous silicon (PSi) as the substrate material of the sensor, then the research on PSi biosensors grew up[1,2]. The advantages of PSi as the base material of optical biosensor include adjustable pore size and structure, ease of preparing all kinds of photonic devices, large specific surface area to enhance the capacity of the unit area of the signal, good biological compatibility and adsorption, and non-toxic property[3-5]. There are two main detection mechanisms of PSi optical biological sensor. One is based on the detection of the refractive index change caused by the biological entry into the PSi hole[6]. The second kind is to detect the fluorescence changes caused by biological reactions[7].

Quantum dot (QD) as a kind of nano-luminescent material can be used in the fluorescent labeling of biologics. QDs have strong fluorescence stability, narrow and symmetrical fluorescence spectrum, high quantum yield, adjustable size and resistance to photobleaching, and water-soluble QDs can be chemically modified for labeling or detection of specific biomolecules[8-10]. The efficiency of fluorophores can be further enhanced by using PSi photonic crystal[11,12]. Therefore, the unique optical properties of QDs can be combined with the excellent properties of PSi which can be used to fabricate an optical biosensor with high sensitivity[13]. QDs via electrostatic adsorption can be connected to the pore wall of PSi to achieve the QDs fluorescence enhancement, and to be used for fluorescence detection of biomolecules[14].

The biotin and streptavidin have strong non-covalent interactions. Once the combination is not affected by the pH environment, reagent concentration, protein denaturing agent or other organic solvents, the conjugation has strong specificity and good stability. Under the action of protein cross-linking agent, the activated biotin can be coupled with protein, lipid, nucleic acid, polysaccharide and other biological macromolecules, so they are good biological bridges[15,16].

A piece of p-type silicon wafer (boron doped amorphous silicon <100>, resistivity of 0.03—0.06 Ω·cm) was cut into 2 cm×2 cm square, followed by acetone, ethanol, deionized water (DI), ultrasonic cleaning for 10 min. The electrochemical anodic oxidation method was used to etch the silicon wafer, the cleaned silicon wafer was soaked in the etching solution of ethanolic HF (volume ratio of 1:1), and the corrosion conditions are strictly controlled by LabVIEW (shown as Fig.1). The samples were rinsed thoroughly with ethanol, DI and dried in nitrogen. The fresh PSi samples are not stable, and easy to be oxidized in the air. In order to achieve more stable optical properties of PSi, the PSi samples were oxidized with 30% H_2O_2 solution for 24 h at room temperature.

The PSi samples after oxidation were soaked in a freshly prepared 4% 3-aminopropyltriethoxysilane (APTES) solution (volume ratio of APTES: DI: methanol is 10:10:1) for 1 h, then rinsed thoroughly with ethanol and DI and dried in nitrogen, and put in a vacuum drying oven at 100 °C for 10 min. The silanized PSi samples were soaked in 2.5% cross-linker glutaraldehyde solution for 1 h, the PSi samples were repeatedly washed with the phosphate buffer solution (PBS, pH:7.4) and DI to remove the excess glutaraldehyde molecules, and then

* This work has been supported by the National Natural Science Foundation of China (Nos.61575168 and 61665012), and the Xinjiang Science and Technology Project (No.201412112).

** E-mail: 315877668@qq.com

dried in nitrogen.

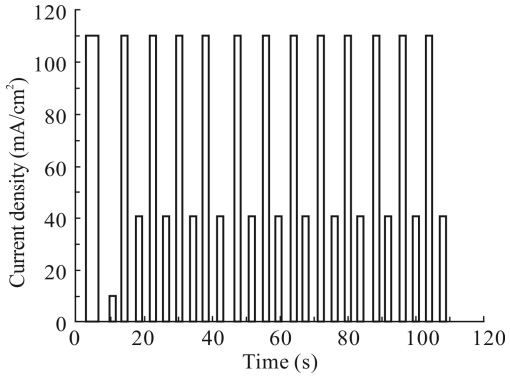

Fig.1 Current density applied in the PSi etching experiment

The target molecule streptavidin was pipetted onto the functionalized PSi samples at 37 °C incubator for 2 h, to combined streptavidin molecules and aldehyde molecules of PSi, the excess of unreacted biomolecules were thoroughly rinsed with PBS and DI and dried in nitrogen. The PSi samples were soaked in 3 mol/L ethanolamine with pH adjusted to 9.0 at 37 °C incubator for 2 h, to close any unreacted aldehyde groups for minimizing non-specific binding, the PSi samples were repeatedly washed with PBS and DI in a shaker, dried in nitrogen.

In this study, we mainly used the water-souble carboxyl CdSe/ZnS QDs labeled biotin (OD-biotin), whose size is about 7 nm, and the fluorescence peak is about 530 nm. The OD-biotin, biotin and PBS were pipetted onto the streptanidin functionalized PSi in 37 °C incubator for 2 h, to realize sufficient reaction between streptavidin and biotin, then washed repeatedly with PBS and DI in a shaker to remove the unreacted OD-bioti and biotin, and dried in nitrogen.

The scanning electron microscope (SEM) image of the multilayer PSi can be seen from Fig.2. The multilayer PSi is mainly composed of three parts, the surface layer and the intermediate layer, which are composed of a single layer PSi with different pore sizes and thicknesses, and a Bragg mirror with periodic variation of high and low refractive indices. Fig.3(a) and (b) are SEM images for the surface layer and the intermediate layer of the sample. In Fig.3(a), we can see that the pore size of the surface layer of PSi is about 30 nm, and the holes on the surface of PSi are distributed evenly without impurities clogging, which is beneficial for the penetration of the QD-biotin and biotin. The pore size of the intermediate layer of PSi is about 10 nm, and most of the QD-biotin can be distributed in the first part of the PSi instead of further penetration.

As shown in Fig.4, we find that only the reflection spectrum of oxidized PSi presents blue shift, and the remaining steps of the functionalization make the reflection spectrum of PSi red shifted, which is due to the increased optical thickness of PSi after the reagent or biological molecules were attached to the inner surface of PSi, leading to the effective refractive index of PSi increasing. The oxidized PSi shows a blue shift due to silicon particles or silicon wire was oxidized to form SiO_2 whose refractive index is much lower than that of silicon, while the geometric thickness of PSi is not changed, so that the optical thickness of PSi is reduced. Each movement of the PSi reflection spectrum indicates that the functionalization of the PSi is effective.

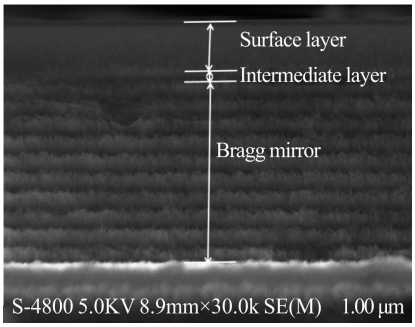

Fig.2 Cross-sectional SEM image of multilayer PSi

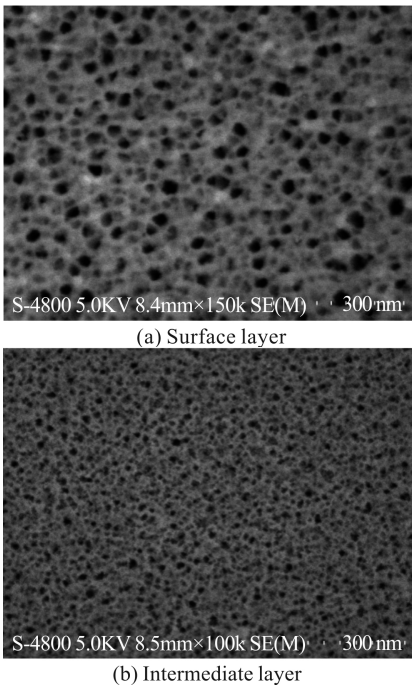

Fig.3 Top-view SEM images of PSi sample

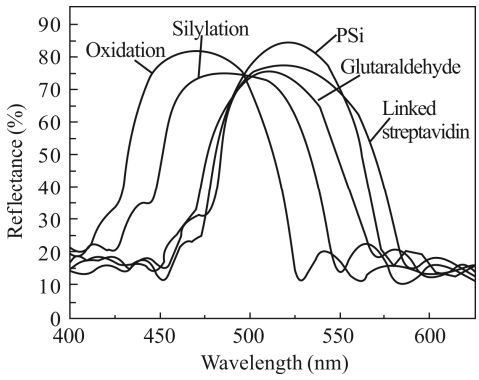

Fig.4 Reflection spectra of PSi through oxidation, silylation, glutaraldehyde and linked streptavidin treatment

The fluorescence spectrum of PSi was detected by the

fluorescence spectrophotometer F-4600, and the results are shown in Fig.5. The PBS pipetted onto the streptanidin functionalized PSi has no fluorescence signal, so it cannot interfere with the experimental phenomenon. The biotin pipetted onto the streptanidin functionalized PSi also has no fluorescence signal, but we cannot directly determine whether the affinity reaction between biotin and avidin occurred in PSi through the experimental results. The fluorescence signal of QD-conjugation (conjugation reaction of QD-biotin and streptavidin) in PSi appears. When the QD labeled biotin reacts with the streptavidin in the PSi, the QDs are indirectly connected to the PSi, so that the QD-conjugation in the PSi can produce fluorescence signal. By means of QDs fluorescence signal, the affinity reaction between QD-biotin and streptavidin in PSi can be explained. The fluorescence peak of the QD-conjugation is located in the high reflection band of PSi. The high reflection band of Bragg mirror can be utilized to reflect the fluorescence of QD-conjugation, which can lead to an enhancement of the fluorescence intensity in PSi[12]. Multilayer PSi structure makes it superior to other substrates. The large specific surface area of PSi can be connected to more biological molecules, the intermediate layer can prevent most of QD-biotin into the Bragg mirror to restrict the QD-biotin in the surface layer of PSi, so that the fluorescence intensity of QD-conjugation can be enhanced again.

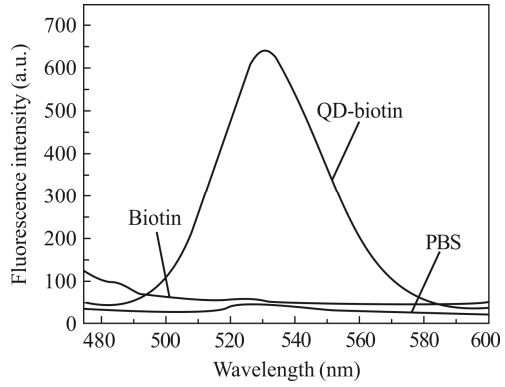

Fig.5 The fluorescence spectra of streptavidin functionalized PSi connected with different substances

In this study, a highly sensitive optical biosensor was fabricated by combining the QD with the PSi for the detection of streptavidin. The fluorescence of QD can enhance the signal of biological reactions between streptavidin and biotin in PSi. The system can also be used for other biological testing.

References

[1] Lin V. S., Motesharei K., Dancil K. P., Sailor M. J. and Ghadiri M. R., Science **278**, 840 (1997).

[2] Xiao-yi LÜ, Guo-dong LÜ, Jia-qing MO, Liang XU and Zhen-hong JIA, Optoelectronics Letters **12**, 478 (2016).

[3] Stein N. E., Hamelers H. V. M. and Buisman C. N. J., Sensors & Actuators B Chemical **s171–172**, 816 (2012).

[4] Niu S., Zhao M., Hu L. and Zhang S., Sensors & Actuators B Chemical **135**, 200 (2008).

[5] Yakovleva M., Bhand S. and Danielsson B., Analytica Chimica Acta **766**, 1 (2013).

[6] Gaur G., Koktysh D. S. and Weiss S. M., Advanced Functional Materials **23**, 3604 (2013).

[7] Jenie S. N., Prieto-Simon B. and Voelcker N. H., Biosensors & Bioelectronics **74**, 637 (2015).

[8] Ledentsov N. N., Ustinov V. M., Shchukin V. A., Kop'Ev P. S., Alferov Z. I. and Bimberg D., Semiconductors **32**, 343 (1998).

[9] Midgett A. G., Hillhouse H. W., Hughes B. K., Nozik A. J. and Beard M. C., Journal of Physical Chemistry C **114**, 17486 (2010).

[10] Lord S. J., Lee H. D. and Moerner W. E., Analytical Chemistry **82**, 2192 (2010).

[11] Dorfner D. F., Hürlimann T., Abstreiter G. and Finley J. J., Applied Physics Letters **91**, 4648 (2007).

[12] Mo J. Q., Jiang J., Zhai Z. G., Shi F. G. and Jia ssssssZ. H., Optoelectronics Letters **13**, 10 (2017).

[13] Gaur G., Koktysh D. and Weiss S. M., Mrs Online Proceeding Library **1301**, 463 (2011).

[14] Liu C., Jia Z., Lv X., Lv C. and Shi F., Physica B Condensed Matter **457**, 263 (2014).

[15] Zimbron M J., Zimbron Malcolm Jeremy, 2012.

[16] Reyes F. D., Romero J. M. F. and Castro M. D. L. D., Analytica Chimica A cta **436**, 109 (2001).