A fluorescent porous silicon-based biosensor for small molecule detection*

LÜ Xiao-yi (吕小毅)[1], **LÜ Guo-dong** (吕国栋)[2†], **MO Jia-qing** (莫家庆)[1], **XU Liang** (许亮)[3], **and JIA Zhen-hong** (贾振红)[1]**

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

2. Xinjiang Key Laboratory of Echinococcosis, Clinical Medical Research Institute, The First Affiliated Hospital of Xinjiang Medical University, Urumqi 830000, China

3. School of Electrical Engineering, Tianjin University of Technology, Tianjin 300384, China

(Received 22 August 2016)

Fluorescent porous silicon was prepared as a stable biosensor chip substrate. The aminopropyltriethoxysilane (APTES) molecules are attached in the pores of the porous silicon with a crosslink method, and when the molecules are added into the chip, the fluorescence intensity is reduced according to the concentration of the APTES. Controlled experiments are also presented with the small molecule that cannot be covalently coupled, and the results show that this kind of sensor chip has better specificity. Compared with other conventional methods, this method is simple, quick and label-free.

Document code: A **Article ID:** 1673-1905(2016)06-0478-3

DOI 10.1007/s11801-016-6182-3

Porous silicon is one of the most promising nanostructured silicon-based chips with large surface area. Porous silicon has the unique characteristics of photoluminescence at room temperature[1], which brings a hope for the realization of silicon based optoelectronic integration. Additionally, porous silicon has been a focus of bioscience and biotechnology owing to its biocompatibility, biodegradability, and its pore size can be controlled[2]. Furthermore, porous silicon is an ideal material for various optoelectronic sensor devices, such as chemical sensors[3] and biosensors[4].

Various types of biochemical sensors based on porous silicon have been widely studied in these years. Porous silicon-based sensors offer many advantages, such as ease of preparation, label-free property and they can be made into a variety of optical structure devices[5-7]. There are many studies on the porous silicon unique fluorescence characteristics for the detection of biological and chemical molecules. However, many experiments require fluorescent labeling[8,9]. Zhang et al[10] have developed a label-free biosensor based on light emitting porous silicon chip for determining bovine serum albumin (BSA), and Sailor MJ et al[11] have also used a fluorescent porous silicon for the chemical molecules detection. The quenching of luminescence by molecular adsorbates was observed with simple detection process and higher sensitivity. However, BSA is a large molecule and it also has not shown higher specificity.

In this paper, a fluorescent porous silicon-based biosensor was fabricated, and aminopropyltriethoxysilane (APTES), which is a small molecule commonly used in biosensing experiments[12,13], has been immobilized to the porous silicon chips with a crosslink method. When the APTES is added into the biosensor, the luminescence intensity is reduced according to the concentration of the APTES. Controlled experiments were also performed and offered high specificity. This silicon-based fluorescent biosensor can also be applied in many other fields.

Fluorescent porous silicon chips were fabricated using electrochemical etching method on n-type silicon substrates (resistivity of 1—10 Ω•cm, <100>orientation). The etching solution consisted of 49% aqueous hydrofluoric acid (HF) and ethanol with the volume ratio of 1:1. The current density of 100 mA/cm^2 was applied. After etching, the chip was soaked into H_2O_2 (30%) for 2 h, and the substrates were rinsed with deionized water and dried in the air.

After being oxidized, the chips were immersed in a series of APTES solutions with different concentrations. APTES was diluted in a H_2O:methanol (1:1) mixture, and the concentration of APTES solution is between 1.25% and 20%. Then each sensor was exposed to the

* This work has been supported by the National Natural Science Foundation of China (Nos.11264038 and 62165009), the Doctor Startup Project of Xinjiang University (No.BS120122), and the Reserve Talents Project of National High-level Personnel of Special Support Program (2014).

† LÜ Guo-dong and LÜ Xiao-yi contributed equally to this work.

** E-mail: jzhh@xju.edu.cn

solutions for 20 min before being rinsed with deionized (DI) water and methanol, and then dried with nitrogen. Fluorescence spectra were measured before and after APTES adsorption by the excitation of Xe lamp (Hitachi, F-4600, and Japan) at an excitation wavelength of 370 nm.

The pore size of n-type silicon is larger, as shown in Fig.1. The average pore dimension is approximately 500 nm, making the biomolecules into pores of porous silicon easy. And according to our experimental experience, compared with different types of porous silicon, the fluorescence properties of porous silicon we prepared are stronger.

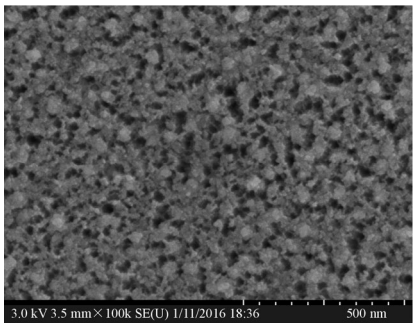

Fig.1 Top view of SEM image of the fluorescent porous silicon chip

The chemical reaction was analyzed by absorbance Fourier transform infrared (FTIR) spectroscopy. In Fig.2, the initial chip shows Si-H bonds around 2 125 cm^{-1}, 977 cm^{-1}, 611 cm^{-1} and 657 cm^{-1}[14]. After oxidation, the sample displays a band around 1 014 cm^{-1} due to Si-O bond formation[15]. After being preprocessed, the sensors were exposed to 1.25% APTES solution. As shown in Fig.3, the chip exhibits small band near 3 349 cm^{-1}, which is attributed to -NH bonds[15]. Two other bands at 2 887 cm^{-1} and 2 933 cm^{-1} can be attributed to -CH bonds, and the characteristics of APTES are easily recognized. It shows that APTES molecules were immobilized to the porous silicon chips successfully.

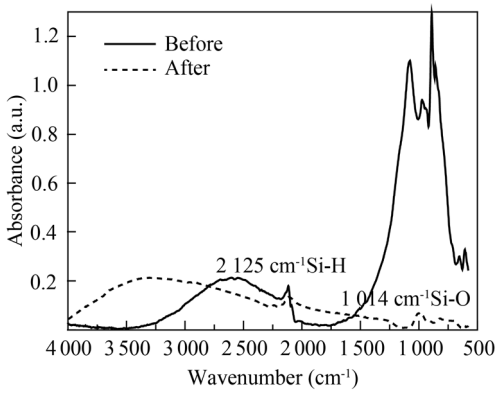

Fig.2 FTIR spectra of porous silicon before any treatment and after oxidation

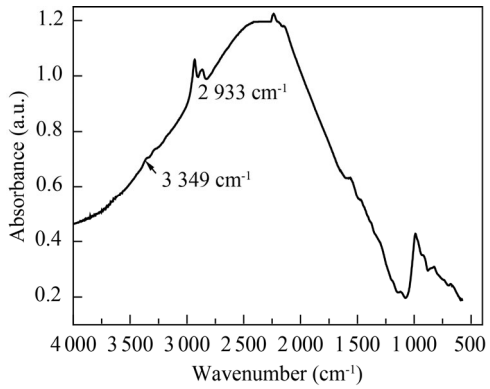

Fig.3 FTIR spectrum of porous silicon after APTES treatment

Fig.4 shows the photoluminescence (PL) intensities of porous silicon after being immersed into the 20% APTES and 10% APTES, respectively. A reduction in PL intensity was detected. The intensity reduction is 537 at APTES concentration of 20%, while 490 at APTES concentration of 10%, indicating that more molecules lead to a greater fluorescence reduction.

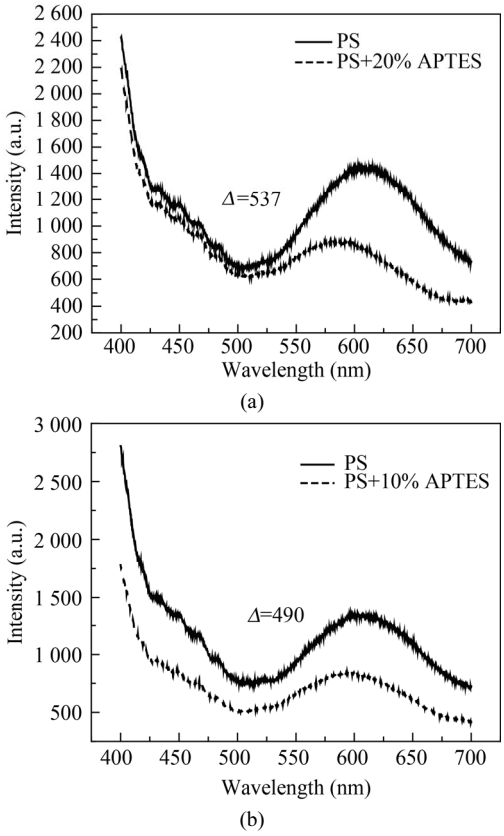

Fig.4 PL intensities of porous silicon after being immersed into the (a) 20% APTES and (b) 10% APTES, respectively

Control experiments were performed by using 10 g/L NaCl solution. Fig.5 shows that there is no detectable reduction in PL intensity after immersing porous silicon into the NaCl solution, which indicates that there is spe-

cific absorption of APTES inside the porous silicon pores. The red-shift of the PL spectrum in Fig.5 is shown, which may be related to the oxidation of the quantum dot in the center of the fluorescent porous silicon.

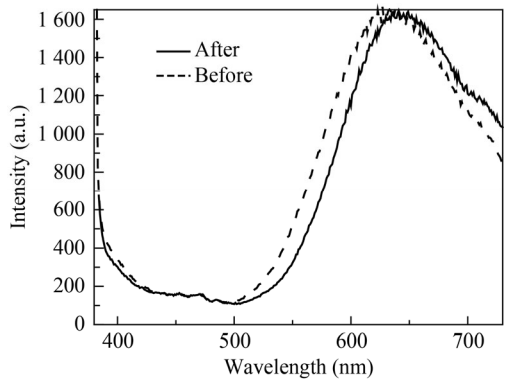

Fig.5 PL intensities of porous silicon before and after being immersed into 10 g/L NaCl solution

Fig.6 shows that the reduction of the fluorescence intensity is as a function of the APTES concentration and the fluorescence intensity decreases with increasing the concentration. All the experiments were undertaken in triplicate.

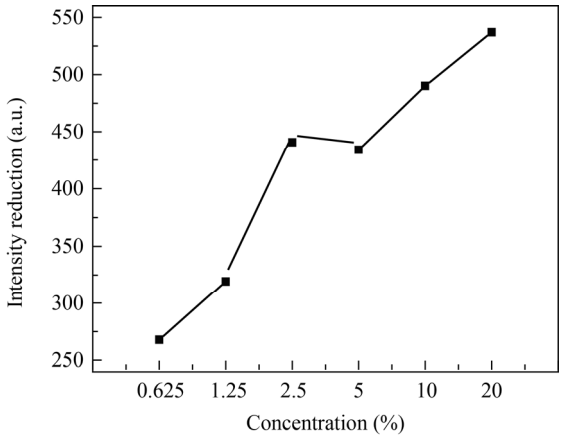

Fig.6 PL intensity reduction after immersing samples into APTES with different concentrations

The reduction in fluorescence intensity is supposedly caused by the APTES reaction on porous silicon and it is generally believed that the fluorescence quenching of porous silicon is the formation of non-radiative recombination centers of APTES on the porous silicon surface[11]. As the surface chemical property has changed, the fluorescence intensity is likely absorbed by the molecules. Optimization experiments are in progress to investigate the relationship between porous silicon fluorescence and biological molecules.

In conclusion, we have successfully fabricated a fluorescent porous silicon-based biosensor for simple and rapid small molecule detection. Compared with the conventional techniques, our sensor chip is quick, label-free and specific. The experiments can pave the way for the development of simple porous silicon fluorescence sensor that can be applied in many fields.

References

[1] Yan DT, Journal of Optical Technology **80**, 421 (2013).
[2] Park J-H, Gu L and von Maltzahn G, Nat. Mater. **8**, 331 (2009).
[3] Osorio E, Urteaga R and Juarez H, Sensors and Actuators B-Chemical **213**, 164 (2015).
[4] Lv changwu, Jia zhenhong and Liu yajun, Journal of Applied Physics **119**, 12108 (2016).
[5] Li Peng, Jia Zhenhong and Lv Xiaoyi, Optics Express **23**, 24626 (2015).
[6] Lv Xiaoyi, Chen Liangliang and Zhang Hongan, Biosensors & Bioelectronics **39**, 329 (2013).
[7] Chen WR, Jia ZH and Li P, Applied Physics B-Lasers and Optics **122**, 1 (2016).
[8] Zhang HY, Jia ZH and Lv XY, Current Applied Physics **13**, 736 (2013).
[9] Levitsky IA, Euler WB and Tokranova N, Applied Physics Letters **90**, 041904 (2007).
[10] Zhang JK, Zhang WL and Dong SM, Analytical Letters **40**, 1549 (2007).
[11] Sailor MJ and Wu EC, Advanced Functional Materials **19**, 3195 (2009).
[12] Mo JQ, Liu YJ and Liu CL, Physica Status Solidi a-Applications and Materials Science **211**, 1651 (2014).
[13] Ryckman JD, Liscidini M and Sipe JE, Applied Physics Letters **96**, 171103 (2010).
[14] Canaria C, Lees I and Wun A, Inorganic Chemistry Communications **5**, 560 (2002).
[15] Fernandez R, Bhattacharya E and Chadha A, Applied Surface Science **254**, 4512 (2008).