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Nano-porous Light-emitting Silicon Chip as a Potential Biosensor Platform

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Abstract

Nano-porous silicon (PS) offers a potential platform for biosensors with benefits both in terms of light emission and the large functional surface area. A light emitting PS chip with a stable and functional surface was fabricated in our laboratory. When protein was deposited on it, the light emission was reduced in proportion to the protein concentration. Based on this property, we developed a rudimentary demonstration of a label-free sensor to detect bovine serum albumin (BSA). A serial concentration of BSA was applied to the light chip and the reduction in light emission was measured. The reduction of the light intensity was linearly related to the concentration of the BSA at concentrations below 10^{-5}M. The detection limit was $8x10^{-9}$ M.

Keywords: Nano-porous silicon; label-free biosensor; Optical sensor

1. Introduction

Light emission from porous silicon (PS) was first described by Canham in 1990. During the early 90's, exploitation of this phenomenon mostly focused on microelectronic and optoelectronic areas (Smith et al. 1992; Hamilton 1995; Canham 1997) with research being carried out by physical and material scientists. Following this initial work, the effect of nano-structures, the light emission principle and further surface characterization studies were also undertaken (Linford et al. 1995; Song et al. 1998; Parisinia et al. 2000). It was shown that the PS light-emission intensity is largely reliant on surface termination (Allen et al. 1999). Conjugating groups with phenylacetylenyl (Song et al. 1998) completely quenched the light emission, whereas isolated double bonds only quenched 40±80% of the light intensity (Buriak et al. 1998). Simple phenyl termination had little effect, as compared to the Si±Hx terminated PS (Song et al. 1998).

Since the electrical and optical properties of PS are largely determined by its nano-structure and are particularly sensitive to adsorbates on the internal surfaces, the changes in these properties following chemical interactions enables PS to operate as a basic chemical sensor (Mulloni et al. 2000; De Stefano et al. 2003a; De Stefano et al. 2003b; De Stefano et al. 2004)

Chan and co-workers (Chan et al. 2001) demonstrated a DNA sensor with Bragg reflector. Porous silicon can luminesce efficiently in the visible. When a luminescent porous

silicon layer is inserted between two Bragg reflectors, the broad luminescence band is altered and multiple, further the very narrow peaks are detected.

An interferometric biosensor using a double-layer of PS has been investigated by Pacholski et al. in 2005. It comprised of a top layer with large pores and a bottom layer with smaller pores. The reflectivity spectrum of this structure displayed a complicated interference pattern. Shifts of the Fast Fourier Transform (FFT) peaks indicate penetration of biomolecules into the different layers. A small molecule, sucrose, entered into both porous Si layers, whereas a large protein only entered the large pores. Detection was accomplished either by computing the weighted difference in the frequencies of two peaks or by computing the ratio of the intensities of two peaks in the FFT spectrum. More recently, De Stefano and co-workers (De Stefano et al. 2006) published their work on PS based optical microsensor for the detection of L-glutamine. The molecular binding between the glutamine-binding protein (GlnBP) from *Escherichia coli* and L-glutamine (Gln) was revealed as a shift in wavelength of the fringes. Due to the hydrophobic interaction with the Si-H terminated surface of the porous silicon, the GlnBP protein, which acts as a molecular probe for Gln, penetrates and links into the pores of the porous silicon matrix.

In the current work, we employed the principle of PS light intensity changes following molecule adsorption to develop a simple and low cost label-free biosensor (see the conclusion section).

2. Materials and methods

The PS was fabricated from p-type silicon with a specific resistance ρ=1-2 Ω.cm using an electrochemical etching process at 300 mA/cm^2 in hydrofluoric acid (HF) solution followed by treating with H_2O_2 to oxidize the PS surface (Linford et al. 1995).

The pore size of PS was detected by Jem-100Cex-II transmission electron microscope (TEM) from Shimadzu.

The surface chemistry was analyzed by VECTOR22 fourier transform infrared (FT-IR) from Brurker Optics.

The protein binding capacity of PS chip was achieved by immersing the PS chips into the 5mg/ml BSA solution for 2 hours at room temperature. After rinsed thoroughly with dH_2O, the protein-PS surface was etched off with sonication in 20% HF and then the protein concentration was measured using Bio-Rad protein assay. This assay is based on the observation that the absorbance maximum for Coomassie Brilliant Blue G-250 shift from 465 nm to 595 nm when binding to protein and the differential colour change of the dye in response to various concentrations of protein. The standard curve was made from a concentration series of bovine serum albumin (BSA). Samples and standards were reacted with dye for 5 minutes and then read at OD_{595}.

The relationship between the PS surface fluorescence reduction and the immobilized BSA concentration was analyzed. The adsorption was carried out by immersing the PS chips of 0.5mm^2 into BSA solutions with concentration of 0.05mg/ml，0.1mg/ml，0.5mg/ml, 1mg/ml, 2mg/ml and dH_2O for 2 hours at room temperature. The chips

were then washed with dH_2O thoroughly. After drying in air, the fluorescence intensity was measured using a RF-5301 fluorometer from Shimadzu. The chip stability was also studied by measuring the light emission from the same chips every hour, every day and every month. All experiments were undertaken in triplicates.

3. Results and discussion

The factors affecting light emission from PS are functional groups, gas, other molecules and the pore size on the PS surface. The pore radii vary from 4-12 nm for PS etched at 220 mA/cm^2 and up to 300-800 nm for PS etched at 600 mA/cm^2 (Janshoff et al. 1998). Under our working condition, the pore size of PS is about 20nm (figure 1). The PS chips are stable for more than 4 months (figure 2 and more data did not show here). When exited with UV light at about 360nm, the PS emits bright fluoresce at 569nm. The silicon dissolution involves two free carriers (electrons and holes). When PS was etched in the HF solution, the surface acquires Si-F bonds and Si-H termination and it became hydrophobic and unstable. After the surface was oxidized with H_2O_2, the PS surface was modified with Si–OH and Si–O (Figure 3). It then became hydrophilic, stable and ready for protein adsorption.

The protein binding capacity of PS chip is about 4 $\mu g/cm^2$ (see material and methods).

The results shown in Figure 4 were obtained by immersing the PS chips into different concentration of BSA solutions and then measuring the light reduction. This revealed that the intensity of fluoresce varied in inverse proportion to the

concentration of BSA. When BSA concentrations were lower than 1mg/ml, the fluorescence was reduced by about 9% for each increase in BSA concentration of 0.5mg/ml. However, when the BSA concentration was greater than 1mg/ml, the fluoresce reduction became much less, indicating that the surface of the chips were saturated with BSA. Further, the detection limit was calculation based on the data from figure 4 and it was $8x10^{-9}$ M.

The light reduction was presumably caused by the interaction between the PS surface and BSA. The ions existing on the PS surface within the nano-structure absorb energy from UV and then emit light. When BSA molecules bound to the surface, the PS surface chemistry, charges, pore size and structures are all subject to change. Consequently, the energy intake and light emission were reduced. However, we also found that some gases, biological materials, inorganic and organic molecules also change the light intensity as well (results are not shown).

4. Conclusion

We have investigated a simple and label free optical BSA sensor, based on the interaction of protein molecules with the nano-PS surface. The PS obtained under our experimental conditions has the pore size of about 20nm and area of about $500cm^2/cm^3$ and emitted fluorescence under UV light. This provided good characteristics for new biosensor development. We showed that a series of concentrations of BSA absorbed on the surface of the PS resulted in a proportional decrease in fluorescence, We also found that some gases, biological materials,

inorganic and organic molecules also change the light intensity as well. The detection limit of the sensor was about $8x10^{-9}$ M. This method for monitoring BSA is label free and uses a simple and inexpensive technique for PS preparation. As a measurement system it is simple to operate and offers high sensitivity and hence may have utility for a range of analytes provided that suitable selectivity can be introduced onto the surface.

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Legends

Figure 1. The PS pore size image of TME.

Figure 2. The PS fluorescence stability over more than two months.

Figure 3. The chemical structure (A) of PS and the FT-IF data (B).

Figure 4. The % of PS fluorescence reduction, after the adsorption with different concentration of BSA.

Figures

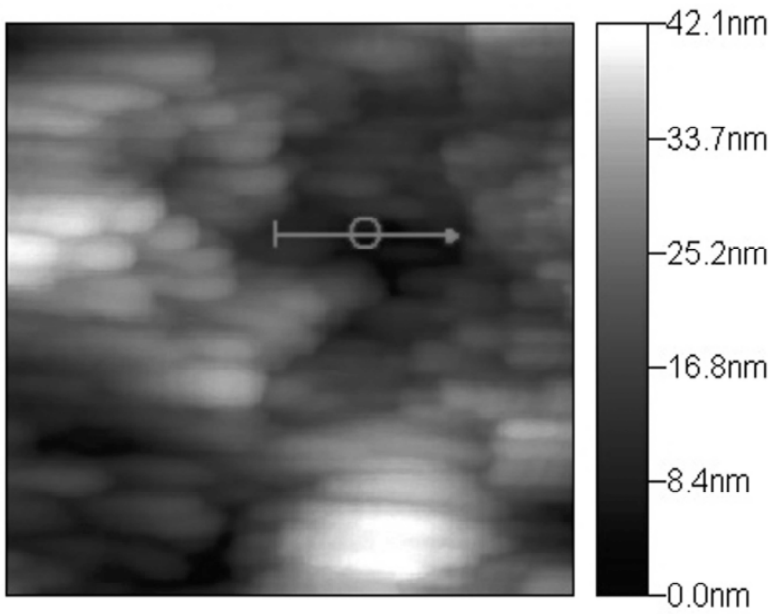


Figure 1.

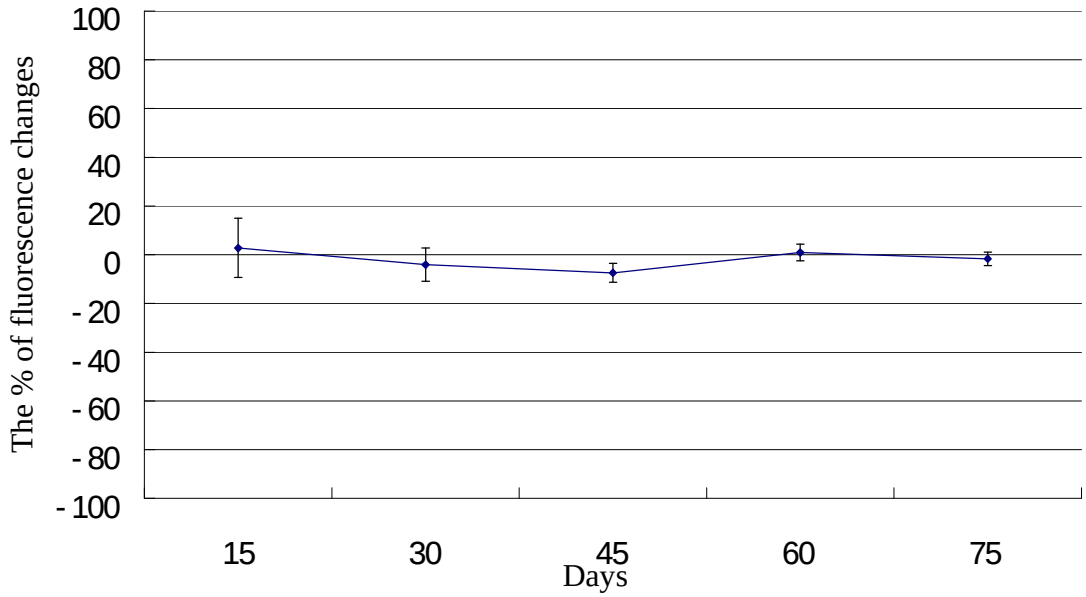
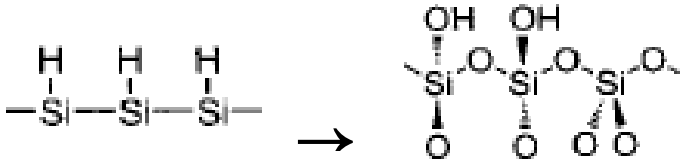

100
80
60
40
20
0
- 20
- 40
- 60
- 80
- 100
The % of fluorescence changes
15
30
45
60
75
Days

Figure 2.

(A)



In HF In H_2O_2

(B)

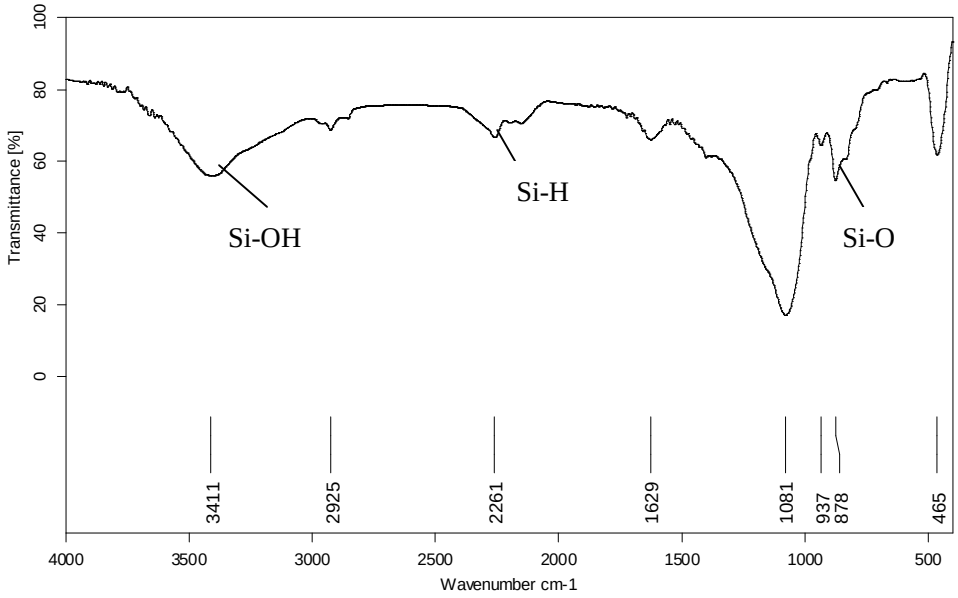


Figure 3.

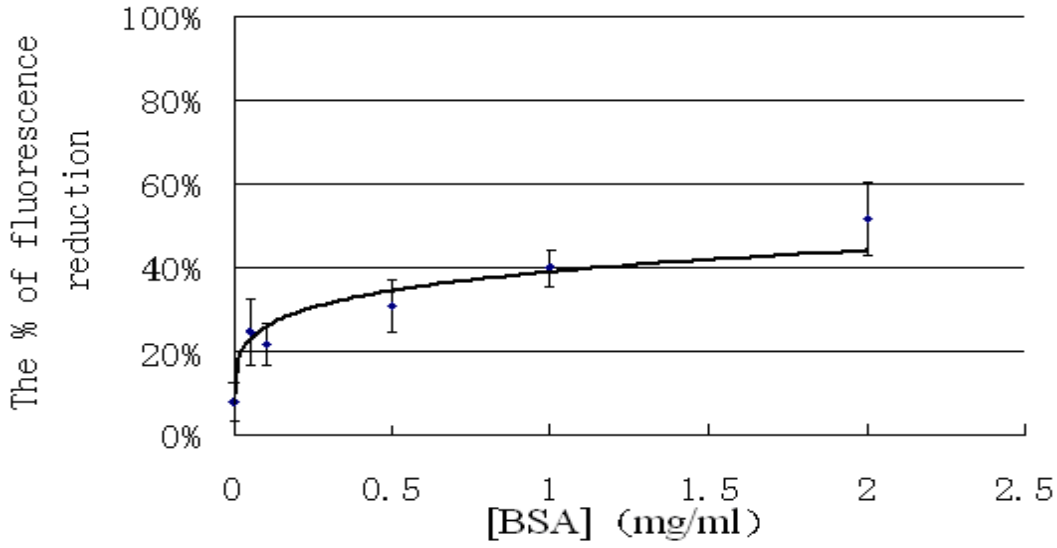

The % of fluorescence reduction
100%
80%
60%
40%
20%
0%
0
0. 5
1
1. 5
2
2. 5
[BSA] (mg/ml)

Figure 4.

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