Porous silicon biosensor for detection of variable domain of heavy-chain of HCAb antibody*

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In this paper, we produce porous silicon (PSi) by electrochemical etching, and it is the first time to evaluate the performance of label-free porous silicon biosensor for detection of variable domain of heavy chain of heavy-chain antibody (VHH). The binding of hen egg white lysozyme (HEWL) and VHH causes a red shift in the reflection spectrum of the biosensor. The red shift is proportional to the VHH concentration in the range from 14 μg·ml^{-1} to 30 μg·ml^{-1} with a detection limit of 0.648 ng·ml^{-1}. The research is useful for the development of label-free biosensor applied in the rapid and sensitive determination of small molecules.

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Variable domain of heavy-chain antibody (HCAb) or nanobody (Nb) with the prolate shape (i.e., shaped like a rugby ball) in the nanometer range (with diameter of 2.5 nm and height of 4 nm)[1] has been used to diagnose infections and treat diseases like cancer or trypanosomosis[2]. For the easy cloning of variable domain of heavy chain of HCAb (VHH) and its other properties such as small size, good solubility and high stability, VHH holds great promise in immunological modulation and therapy application[3, 4]. VHH is smaller, so there are considerable challenges for detection. Therefore, it is important to rapidly detect it in antigen-antibody reaction.

Label-free biosensors can directly measure unmodified samples, because they do not require the reporter molecules to generate a signal. Optical label-free biosensors operate based on the change of refractive index due to affinity binding events of biomolecules. Recently, porous silicon(PSi) has been widely investigated for biosensing and optical detecting of biomolecules[5-8]. PSi exhibits many interesting optical properties, and its optical behavior is extremely sensitive due to the large specific surface area. Moreover, PSi can be easily obtained by electrochemical etching in a solution of hydrofluoric acid (HF)[9,10]. After electrochemical etching, the large quantities of biomolecules can be immobilized on the internal pore surface of PSi through bioconjugation[11]. Consequently, PSi has many advantages as the biosensor platform for chemical and biological applications such as the ease of preparation[12], high sensitivity, label-free procedures and compatibility with standard microelectronics processing. In our lab, VHH is directly determined by PSi-based optical biosensor for the first time. Here we present the new progress in developing sensitive label-free optical biosensors for detection of VHH using porous silicon.

The commercial n-type (100) Si wafers with the resistivity of 0.02–0.03 Ω·cm and the thickness of 380 μm were cut to 2 cm × 2 cm squares. Before electrochemical anodization of silicon, all pieces were firstly cleaned with the solution of carbinol, alcohol and deionized water successively, and then were rinsed by deionized water. PSi layers were made by means of the electrochemical etching method in a 1:3 mixture of HF solution and ethanol. The contact area of the HF-ethanol solution with the silicon substrate is 1.0 cm^2. Electrochemical etching was carried out in an electrolytic cell made of teflon, and controlled by personal computer at a

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constant current density of 60 mA/cm^2 for 150 s. After electrochemical etching, the substrates were thoroughly rinsed in ethanol and deionized water for about 5 min, and dried with nitrogen at room temperature.

After electrochemical etching and drying, the substrates were immersed into a solution of H_2O_2 (30%) and heated for 3 h at 55 °C to oxidize the PSi surface, and subsequently dried with nitrogen. The purpose of oxidization is to increase the characteristic stability of PSi and provide hydroxyl bond (−OH) required in the silanization. Next, the substrates were transferred to a solution of the 3-aminopropyltriethoxysilane (APTES) in toluene and immersed for 50 min, and thoroughly rinsed by toluene and deionized water. Finally, the substrates were dried with nitrogen and heated for 10 min at 100 °C. Following silanization, the substrates were immersed into a solution of glutaraldehyde (3%) for 30 min at room temperature. It is important to rinse them successively with 0.05% Tween-20 in phosphate buffer silane (PBS, PH=7.4) in order to remove all excess of glutaraldehyde.

20 ul hen egg white lysozymel (HEWL) antigen was dropped onto the treated substrates at 37 °C for 2 h. Under these conditions, the glutaraldehyde groups reacted with the amino groups of the HEWL. After the immobilization, the substrates were cleaned with PBS. In order to prevent non-specific adsorption, the substrates were dipped into a 3% bovine serum albumin (BSA) solution for 2 h at 37 °C. After that, the substrates were in contact with VHH antibody in different concentrations. All antibodies and antigens were provided by Key Laboratory of Xingjiang Biological Resources and Gene Engineering, College of Life Sciences and Technology, Xinjiang University.

In order to ensure the reliability of experimental results, a piece of PSi was cut into two pieces with different VHH concentrations, and their corresponding surface morphologies were studied by field emission scanning electron microscopy (FESEM) as shown in Fig.1.

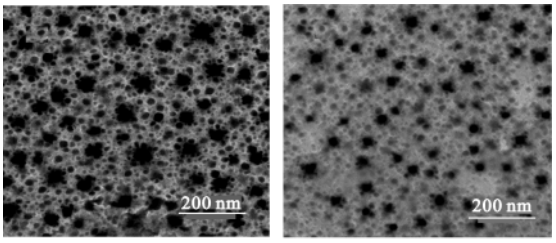


Fig.1 Surface morphologies of PSi with different VHH concentrations

From Fig.1, it is illustrated that the micropores of PSi are almost covered, and the diameter of macropores decreases significantly. By comparing the two figures, it is well recognized that there are more VHH combined with HEWL in PSi with high VHH concentration at the same time.

Fig.2(a) shows a surface image of the PSi layer sample. The pore size of PSi layer is usually about 50−300 nm in diameter. The pore distribution is random, and the porosity layers facilitate sufficient mass transport characteristics for antibody-antigen competitive binding interactions. The thickness of PSi pore wall is a function of the VHH concentration, owing to it is increased by the coupling of biological molecules in Fig.2(b).

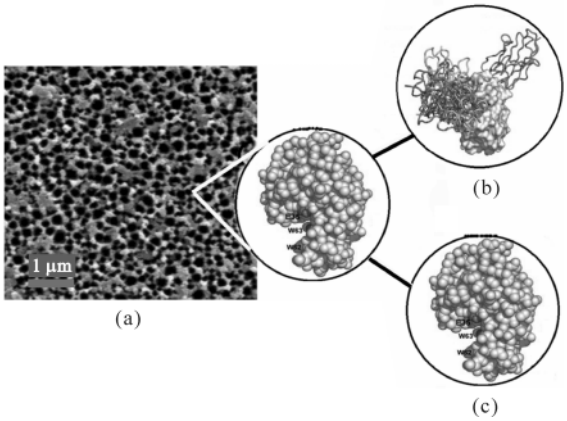
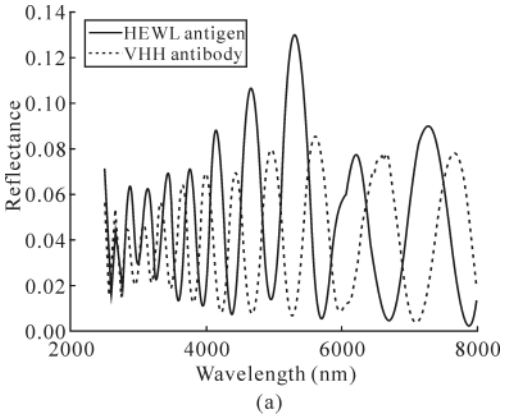


Fig.2 Principle of the PSi biosensor: (a) Top-down scanning electron micrograph of the PSi films produced by electrochemical etching; (b) VHH-HEWL complex structures; (c) HEWL antigen

As shown in Fig.3(a), the representative reflection spectra are experimentally attained, which illustrate a red shift of wavelength. Therefore, the effective index increases, and the reflection spectra of samples shift to longer wavelengths. It can be observed in Fig.3(b) that negligible wavelength shift in the reflection spectrum of PSi is observed with the addition of non-immune antibody and subsequent washing with PBS (0.05 wt%, Tween-20). Initial experiments demonstrate that there is no antibody-antigen competitive binding interaction inside the biosensor.



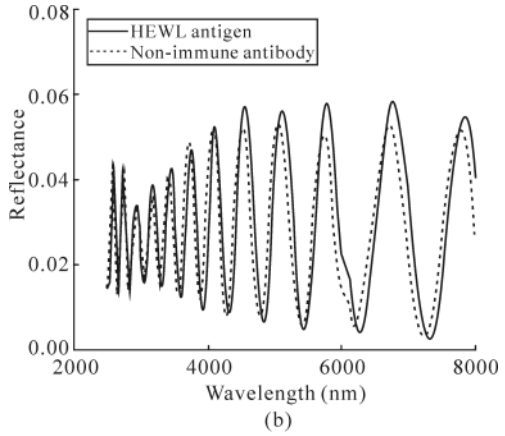


Fig.3(a) Reflection spectrum of PSi with HEWL after VHH (25 μg/ml) infiltrated into it; (b) Reflection spectrum of PSi with HEWL antigen after non-immune antibody

Fig.4 shows the relationship between the red shift of reflection spectrum and the VHH concentration. The thickness of PSi pore wall is increased by antitibody-antigen competitive binding interactions, which is followed by the increase of the refractive index of the PSi layer. The data of wavelength shift in PSi biosensor for all reactive VHH are imported into data analysis and graphing software OriginLab7.5. All data present Boltzmann distribution. There is a good linear relationship in the range from 14 μg•ml^{-1} to 30 μg•ml^{-1}, and the coefficient of correlation is 0.998. In addition, the sensitivity corresponding to the slope of the line can be determined to be 15.43 nm• μg•ml^{-1} by the linear fitting approach. Considering the resolution of device is 0.01 nm, the lowest detection limit of the biosensor can be given as 0.01 nm/15.43 nm• μg•ml^{-1}, which is equal to 0.648 ng•ml^{-1}[13].

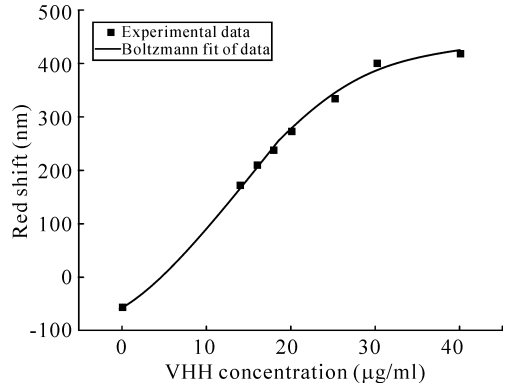


Fig.4 Relationship between the red shift of the reflection spectrum and the VHH concentration (0-40 μg/ml)

In this paper, the performance of a label-free PSi biosensor for detection of VHH is evaluated for the first time. The results show that the VHH can be well detected by measuring the red shift of reflection spectrum. The ultimate detection limit of the biosensor is 0.648 ng•ml^{-1} and the sensitivity is 15.43 nm•μg•ml^{-1}. The small molecules and the low antibody concentration can be detected by the PSi biosensor. As a biosensor platform, PSi can be used for the development of label-free biosensor and rapid and sensitive determination of small molecules as well.

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