Research on immunosensor based on porous silicon*

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Porous silicon is functionalized using thermal oxidation and silanization. The HYSA antibodies are immobilized to the porous surface in this paper. The changes in the reflection spectrum and photoluminescence spectrum are investigated when BSA are attached to the PS surface. The result shows that the luminescence peak of PS disappears. After the antigen-antibody reaction, the higher light emission is observed and the reflection spectrum also red shifts. Therefore, this research lays a foundation for the development of sensitive label-free optical immunosensor.

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Optical biosensors utilize optical techniques to detect and identify chemical or biological species. They offer a number of advantages such as the ability for principal remote sensing with high selectivity, sensitivity and immunity to isolation from electromagnetic interference. Various optical biosensors use the traditional radioactivity labeling methods for biomolecular detection by converting the molecular-recognition events into easily detectable optical signals. Unique porous structure of porous silicon and its high surface area make it advantageous over planar platforms in biosensor development[1]. Moreover, porous silicon has low cost, is easy to be obtained, and it is fully compatible with the standard IC processes. These characteristics make it an ideal optical biosensor material[2-9].

The flower of the safflower plant has been used extensively in traditional Chinese medicine for treatment of cerebrovascular and cardiovascular diseases[10-12]. Hydroxysafflor yellow A (HYSA) is the main extraction from the flower of the safflower. The traditional method for determination of HYSA is HPLC[13-15]. but it has poor reproducibility and is also a cumbersome technique. Therefore, exploration of a quick and accurate determination of such small molecules has important significance.

In this paper, we have successfully used a crosslink method linker HYSA antibody to porous silicon surface and measured the PL spectrum and reflectivity before and after biological reaction. This research lays the foundation of exploiting passive and label-free immunosensor to determine HYSA.

N-type <100>single crystal silicon (6.2-7 Ω • cm) was first cut into 2.0 cm × 2.0 cm square chip using a diamond-tripped pen. The sample were first cleaned in 3:1(v/v) concentrated H_2SO_4(30%)/H_2O_2 for 15 minutes at 80 ℃ and then immersed in 5% aqueous HF solution for 1 minute at room temperature to remove the native oxide. Then the sample was cleaned in ultrasonic bath with acetone and ethanol and deionized water, respectively. Porous silicon samples were processed by anodizing in a 50% (1:2 in volume) solution of HF (48 wt%) and ethanol (98%). The anodization was carried out with a current density of 30 mA/cm^2 for a fixed time of 15 minutes. The sample of n-PS was obtained.

Freshly anodized porous silicon has unstable silicon-hydrogen bonds that gradually oxidize in air and cause a change in the porous silicon properties over time. In order to stabilize the porous silicon and also as a prerequisite step for the biosensing functionalization, porous silicon must be oxidized [16-19]. We oxidized the samples at 900℃ for 15 minutes.

After thermal oxidation, porous silicon must undergo silanization[20-22]. First, the chips were dropped in a 5% solution of APTES and a hydroalcoholic mixture of water and methanol (1:1) for 20 minutes at room temperature. Then, we washed the sample with DI water and dried it in nitrogen steam. The silanized chips were baked at 100℃ for 10 minutes. In the second step of the chemical functionalization,

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the chip was covered with a 2.5% Glutaraldehyde solution in PBST buffer (pH 7.4) for 20 minutes and then rinsed in PBST solution(Fig.1).

The covalent bind of the HYSA antibodies on the porous silicon surface is based on a two-step process(Fig.1).

Step 1: Drop 40 μl HYSA antibodies solution (the titer of determined antiserum was 1:3200) to the PS and incubate the system at 37℃ for 2 hours.

Step 2: 40 μl of target BSA are exposed to the PS for 2 hour at 37℃, followed by rinsing in PBST buffer.

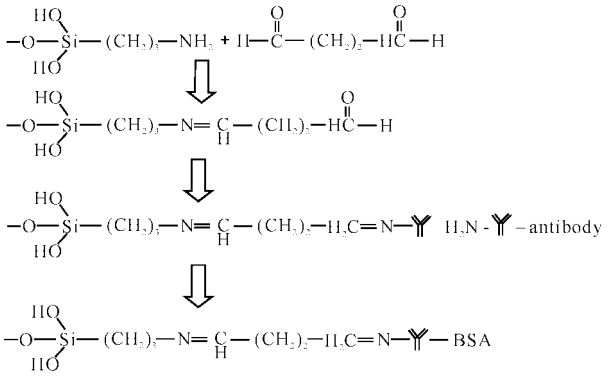

Fig.1 Schematic of silanization and cross linking of biomolecule into PS

We have monitored the chemical reactions by Fourier transform infrared spectroscopic microscopy (FTIR). Fourier-transform infrared (FTIR) spectra were obtained in absorbance mode with a Brucker EQUINOX55 spectrometer equipped with a middle infrared (MIR) source. All spectra were collected with 240 scans for the absorbance of the sample, with 4 cm^{-1} resolution. Fig.2 and Fig.3 show the FTIR spectra of the porous silicon and the silane monolayer between 500 and 3500 cm^{-1}, respectively. In Fig.3, the FT IR spectra of the porous silicon after the silanization process are shown: APTES characteristic peaks of the ethylic (at 1626, 1529 and 1397 cm^{-1}), amino (at 1060 cm^{-1}) are evident. In Fig.4 the porous silicon FT IR spectra after the GA treatment are reported, the characteristic C=N bonds (at 1627 cm^{-1}) due to the reaction with APTES are easily recognized.

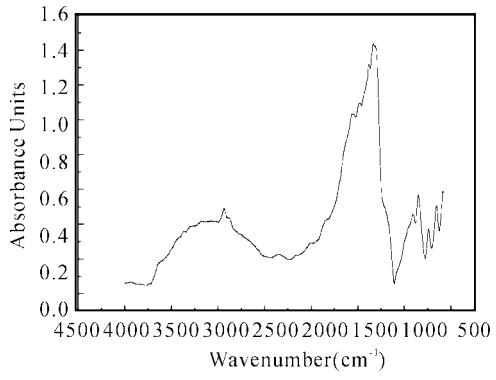

Fig.2 FT-IR spectra of PS after the APTES functionalization process

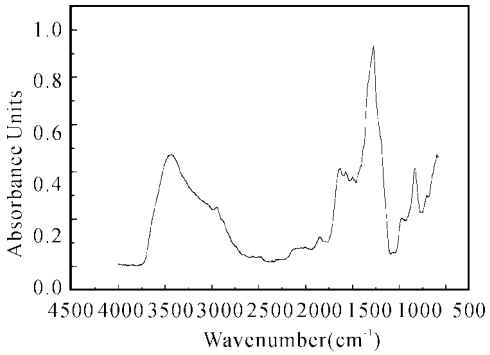

Fig.3 FT IR spectra of PS after the APTES functionalization process and after Binding GA

In order to test the biomolecule immobilization properties of the PS, we measured SEM of porous silicon before and after antigen-antibody reaction (Fig.4 and Fig.5).

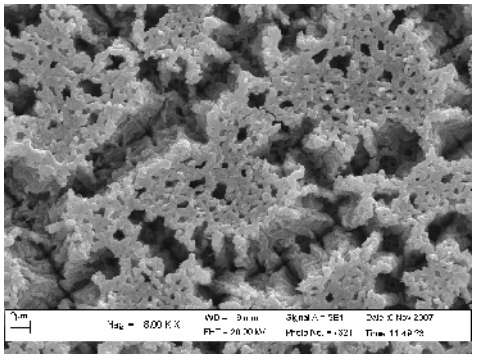

Fig.4 SEM image of porous silicon

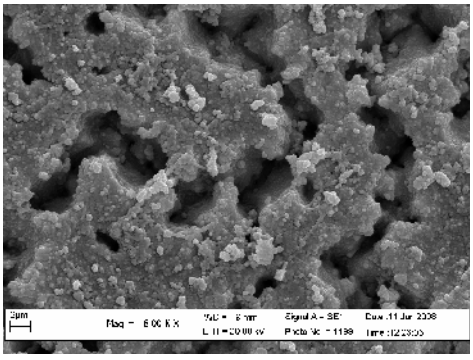

Fig.5 SEM image of porous silicon after antigen-antibody reaction

PL spectrum was tested by Hitachi F-4500 Fluorospectrophotometer. Light emission disappears when HYSA antibody immoblizes to porous silicon surface(Fig.6). Light emission increases after antigen-antibody reaction, which can be applied in biosensing. The optical reflectance spectrum was measured using Hitachi ultraviolet spectrometers U3010. Reflectivity spectra of the same sample, registered at any step of the sensing process, are shown in Fig.7. The red shift of the spectrum is due to the binding of biological material on the internal surface. This result suggests that after the interaction among biological molecules, the effective optical thickness changes, which results in the increase of the effective index of the porous silicon.

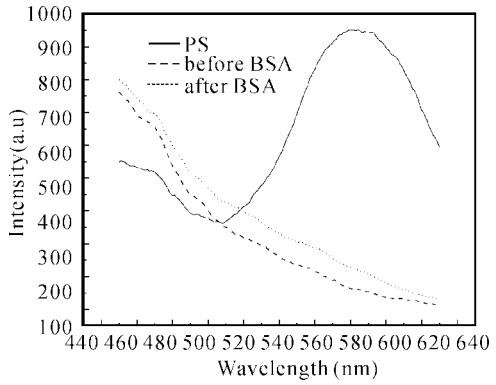

Fig.6 Photoluminescence spectrum of PS

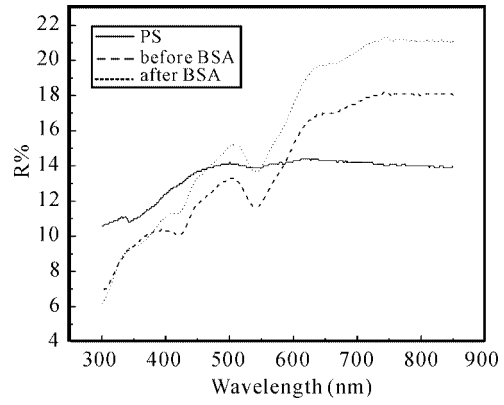

Fig.7 Reflection optical spectra of the PS

In summary, we have fabricated porous silicon-based optical immunosensor and investigated the use of PS as an immobilization and transfer matrix for monitoring antigen-antibody reaction. The result shows that after the interaction among biological molecules, the reflectance spectrum red shifts and the light emission increases. This study is expected to have extensive applications in Chinese herbal medicine quality identification.

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