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Surface layer reflective index changes of Au nanoparticle functionalized porous silicon microcavity for DNA detection

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ABSTRACT

A technique is demonstrated to detect DNA hybridization based on surface layer of Au/porous silicon microcavity (Au/PSM) substrate for very small amount of biomolecules. Simulations show that the increase of effective refractive index for the first layer of PSM will cause a blue shift for its reflectance spectrum, and the blue shift becomes less with the increase of refractive index for one more layers. In experiments, such a blue shift of reflectance spectrum of PSM comes from the increase of refractive index by DNA hybridization on the surface. The detection limit of Au/PSM biosensor is 15.15 nM for 19-base pair DNA, which is comparable to that of reported biosensors based on porous silicon (PS). Therefore such an Au/PSM could be very useful to develop simple, rapid and sensitive optical biosensors when the amount of target is very small.

1. Introduction

Porous silicon (PS) is a simple and efficient promising substrate for applications in drug delivery, early diagnostics and to be properly developed as a new effective miniaturized biosensor [1–5], owing to its large available surface area for molecular binding and its readily tunable pore structure formed into complex photonic crystal (PC) nanostructures, such as Bragg stacks, filters and microcavities [6–8]. In general, PCs have periodically modulated dielectric functions, resulting in light propagation dramatically different from the bulk material [9]. So photonic devices are more compact and more sensitive than other optical devices and very suitable candidates for the realization of future passive and active optical devices [10–12]. Since the first demonstration of biosensors on the PS photonic crystal platform, many groups are working with different PC sensor to demonstrate higher sensitivity [13–16]. In fabrication and detection process of biosensor based on PS materials, many chemical modifications and functionalizations of PS surface for biosensors have been reported to improve sensitivity and stability, such as hydrosilylation, electrochemical alkylation and thermal hydrocarbonization et al. [17,18]. However those methods show low flexibility in either the design of optical nanostructures or the control of pore size, and require the presence of reducing agents and stabilizers in contrast to common chemical methods. Especially in the fabrication and detection process of biosensor based on multilayer PS [14–16], when a small amount of chemical agents or biomolecule solution is dropped onto the prepared multilayer PS, such a solution probably can not penetrate uniformly into all layers and hence does not guarantee that the effective refractive index of all layers changes the same, which will affect the spectral properties and the detection accuracy of biosensor based on multilayer PS.

Gold nanoparticles (AuNPs) seem to be the most versatile and extensively studied material among the various nanoparticles resulting from large surface area, good biocompatibility and excellent electron transfer. Since 1996, DNA-directed assembly has become one of the corner stones in bionanotechnology [19]. Among bio-applications based on AuNPs, detection of double stranded genomic DNA target is of remarkable significance. This is attributable to the discovery that thiols and other functional groups have the capacity to strongly interact with AuNPs, which can be used to immobilize DNA strands on AuNPs [20–22]. The interplay effect in combining the AuNPs and PS is used in optical biosensor which is fully compatible for integrated circuit technology [23,24]. To our knowledge, there is rarely research on the biosensor based on single layer PS film with AuNPs [25]. Studies show that the change

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of the refractive index of PS will cause a reflectance spectra shift for Au/PS, but such a single PS film interference fringe is not easy to be distinguished and thus influences the sensitivity of this kind of biosensors, resulting from the absent of sharp resonance peaks.

To detect a small amount of target and improve the performance of biosensor based on Au/PS, we used one-dimension photonic structure to replace the single layer PS structure. Porous silicon microcavity (PSM) is of one-dimension photonic structure and can be obtained by inserting a Fabry-Perot cavity layer in between two symmetric Bragg reflectors. The interference of reflection of such two Braggs causes a high reflectivity stop band with one narrow resonance peak approximately in its center, which means PSM has a high value of $Q(\lambda/\Delta\lambda)$ factor. Compared with single layer cases, the narrow spectral features of the resonant modes improve the resolution of spectral, which is a possible way for a low detection limit. Au/PSM biosensor was fabricated and used for DNA detection by increasing refractive index only for the surface layer of PSM. Such a method needs neither chemical agent nor biomolecule to penetrate into more PS layers. The present research may provide an interesting way to develop a new optical label-free biosensor especially for a very small amount of biomolecule detection.

2. Experiments

2.1. Materials and characterizations

3′-thiol modifier DNA oligonucleotide, complementary DNA oligonucleotide and non-complementary DNA oligonucleotide were purchased from Invitrogen (china, www.invitrogen.com). And the following DNA strands were synthesized for assay: probe DNA, 5′-TTGTACAGCAGCGTGCACC–$(CH_2)_6$–SH-3′ (19-base), functionalized with alkane thiols at their 3′ termini; Complementary DNA, GGTGCACGCTGCTGTACAA (19-base); and non-complementary DNA, ACACGTCATCGCTCTATTG (19-base).

Chloroauric acid ($HAuCl_4 \cdot 4H_2O_2$, 48–50 % Au basis) was purchased from Aladdin Reagent Co. (China, www.aladdin-reagent.com). Phosphate buffer saline PH 7.4 (0.01 M PBS buffer solution) was obtained from Key Laboratory of Xinjiang Biological Resources and Gene Engineering.

Reflectance spectra were measured by UV–vis scanning spectrophotometer (Hitachi, U-4100, Japan) with wavelengths ranging from 400.00 to 2000.00 nm and a resolution 0.1 nm. Cross-sectional and surface images of Au/PSM substrate were obtained by a field-emission scanning electron microscope (Hitachi, S4800, Japan).

2.2. Preparation of Au/PSM substrates

PSM is a one-dimensional photonic crystal constituted of a Fabry-Perot cavity in between two Bragg reflectors (DBRs). The reflectance maximum appears at Bragg wavelength (λ_0) with a relation: $m\lambda_0/2 = n_L d_L + n_H d_H$. The multilayer PS structure was constructed with low refractive index layers (n_L) and high refractive index layers (n_H) with effective optical thickness of $\lambda_0/4$ for each DBR layer and λ_0 for the central layer. Fig. 1a shows a schematic cross-section of the PSM following $(n_L n_H)^6 n_{Lc} (n_H n_L)^6$ sequence, where n_L represents high porosity or low refractive index and n_H represents low porosity or high refractive index. PS layers were fabricated by electrochemical etching of a B-doped (100) Si substrate (resistivity 0.01–0.02 Ω cm, thickness 400 ± 10 μm) in a mixed solution composed of 49% aqueous hydrofluoric acid and ethanol (volume ratio 1:1). The effective exposed area of the Si substrate was 0.785 cm^2 in teflon electrochemical etch cell and a copper was immersed into the electrolyte as a counter electrode. The periodic multilayer structure was fabricated by using a computer program (Labview) to alternately change current density for different etching times with a 5 s pause after each layer formation. The fabrication process begun from left to right and from top down into Si substrate following a sequence $(n_L n_H)^6 n_{Lc} (n_H n_L)^6$, where for n_L the current density was 100 mA/cm^2 for 2.3 s, for n_H the current density was 40 mA/cm^2 for 3.6 s, and n_{Lc} corresponds to microcavity layer with a current density of 100 mA/cm^2 for 9.2 s.

To form mental AuNPs, the fresh PSM was immersed into alcoholic metal salt solution which can uniformly wet the H-terminated hydrophobic PS surface. The metal salt was reduced by hydrogen, which results in metal nanoparticle formation and HCl synthesis during the reaction [26,27]. The process of AuNP deposition on PS layer can be categorized within redox potential of PS according to the following chemical equation:

$$SiH_x + 2H_2O \rightarrow SiO_2 + (4+x)H^+ + (4+x)e^-.$$

The reduction reaction is

$$AuCl_4^- + 3e^- \rightarrow Au + 4Cl^-.$$

Dissolved Au from $AuCl^-_4$ behaves as an oxidizing agent, and the PS takes the role of reducing agent. These reactions favor the formation of the AuNPs. Furthermore, AuNPs should deposit on SiO_2, and the structure of AuNPs-based fresh PSM substrate is shown in Fig. 1b.

To optimize Au deposition time, AuNPs were formed on the surface of the PSM by exposing fresh PSM to a 0.5 mM $HAuCl_4$ solution in ethanol for 5 min, 10 min, 15 min, and 20 min, separately. The freshly fabricated Au/PSM is unstable in the air due to the Si–H bond of fresh PSM easy to be oxidized. So passivation of the PS layer is necessary to achieve stable PS based devices. In order to ensure the stability of optical properties of Au/PSM substrate, all freshly fabricated Au/PSM substrates were soaked in H_2O_2 (30%) for 24 h at room temperature. Then all the substrates were rinsed with deionized water and dried in the air. After one month storage in room temperature, the Au/PSM substrates had not been aggregated and their spectroscopic reflectance properties were still stable, due to the existence of Si–O–Si, O–Si–H and O_3–Si–H groups [1,2,14–16]. The preparation process of such a stable Au/PSM substrate is shown in step 2.

2.3. Functionalization of AuNPs

Probe DNA oligonucleotides were capped by thiol groups binding to AuNPs. When 30 μl 50 μM probe DNA were dropped onto the Au/PSM substrate at 37 °C for 2 h, AuNPs were functionalized with thiol-modified probe DNA, and then rinsed with PBS buffer solution three times in order to remove unbounded probe DNA and dried in the air. The structure of DNA-functional AuNP/PSM self-assemble is shown in Fig. 1d.

2.4. DNA detections

In experiments, 15 μl complementary DNA with different concentrations were added onto probe DNA modified Au/PSM substrates for 2 h at 37 °C to ensure all target segments were recognized, then they were washed with PBS buffer solution three times to remove unhybridized complementary DNA and dried in the air. When the immobilized probe DNA in the pores of PS are exposed to complementary DNA, the two DNAs hybridization causes an increase of refractive index and thus causes a shift of the reflectance resonance peak.