Label-free and Real-Time Photonic Band-Gap Silica Nanosensor

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ABSTRACT

Optical techniques have received significant attention for label-free biosensing. Technologies including surface plasmon resonance (SPR) and interference spectroscopy have been employed in label-free biosensors. SPR have advanced label-free biosensing significantly; however, its instrumental setup is expensive and complicated. In order to overcome the problems associated with the SPR systems, interference biosensors based on optically flat porous silicon thin films were developed. The advantages of using porous silicon include its easy fabrication and compatibility with conventional silicon microfabrication. However, porous silicon absorbs visible light strongly, and the strong optical absorption limits the sensitivity of the porous silicon interference biosensors.

A photonic band-gap nanosensor based on porous silica is proposed in the study. The photonic band-gap silica nanosensor is easy to fabricate and simple to use. Its optical characteristics can be easily modulated by electrochemical etching and its large internal surface area can be easily modified by different types of biomolecules. The photonic band-gap silica nanosensor can provide inexpensive, real time, simple to use and high-throughput assays for biomolecular reactions. The advantages of low cost, high sensitivity and versatile use in the photonic band-gap silica nanosensor make it surpass SPR and porous silicon interference biosensors

Keywords: label-free, interference, photonic, silica

1 INTRODUCTION

In biosensor research, there is a continuously growing interest to find out new methods and devices that would provide easy, highly reproducible, and sensitive sensing assays for biomolecular reactions. It is driven by the requirement to classify and sense biological interactions for medical applications, environmental monitoring, and basic mechanistic studies. Fluorescence labeling technology has been applied in the study of the biological interactions [1-2]; however, the procedure of the fluorescence labeling is material and time consuming. The fluorescent materials even have issues on the interference of the biomolecular interactions. Compared to the fluorescence labeling technology, label-free sensors save the work of purifying and labeling to achieve easy, inexpensive, and real time biosensing. It also eliminates the risk that labels might interfere with the biomolecules.

Optical technologies have received significant attention for the label-free biosensing and sensitive methods employing surface plasmon resonance (SPR) [3-4] and interference spectroscopy [5-6] have been the leading methods in the optical label-free biosensing. The optical label-free biosensors monitor the interaction between two biomolecules by observing the optical signal response without processing of the molecules. Both SPR and interference biosensors measure the change of refractive index in the detection system for the characterization and the quantification of binding biomolecules. SPR spectroscopy is widely used in many research institutes and performs high sensitive biosensing. However, the instrumental setup of SPR spectroscopy is expensive and complicated. Moreover, the effective sensing region in the SPR spectroscopy is limited by surface plasmon penetration depth of nearly 100nm. Interference biosensors based on porous silicon have large internal surface, allowing for easy and large quantity biomolecules immobilization. It also offers a solution to an important drawback of SPR, the limited penetration depth. However, porous silicon absorbs visible light energy (1.2eV-1.38eV) significantly, so the strong optical absorption results in optical signal decays dramatically and limits the sensitivity. On the other hand, porous silicon interference biosensors only allow sensing in reflection mode which limits the measurement facilities and surpass the design and the integration possibility to achieve a “lab-on-a-chip” system.

For solving the problems associated with SPR and porous silicon interference biosensors, a new sensor proposed in this paper offers flexible, high sensitive and easy biosensing. The proposed sensor is a photonic band-gap nanosensor based on porous silica. The photonic band-gap silica nanosensor can be fabricated on silicon substrates, allowing the integration with other silicon-based bio-microsystems such as microfluid channels and micro mixers. The photonic band-gap silica nanosensor can also be transferred to glass-based biochips or even conventional glass slides for naked-eye measurement. The photonic band-gap silica nanosensor is easy to fabricate by electrochemical etching and thermal oxidation. It has good compatibility with conventional silicon microfabrication. Moreover, it can provide inexpensive, real time, high sensitivity, simple to use and high-throughput assays for biomolecular reactions.

2 SENSING MACHENISM

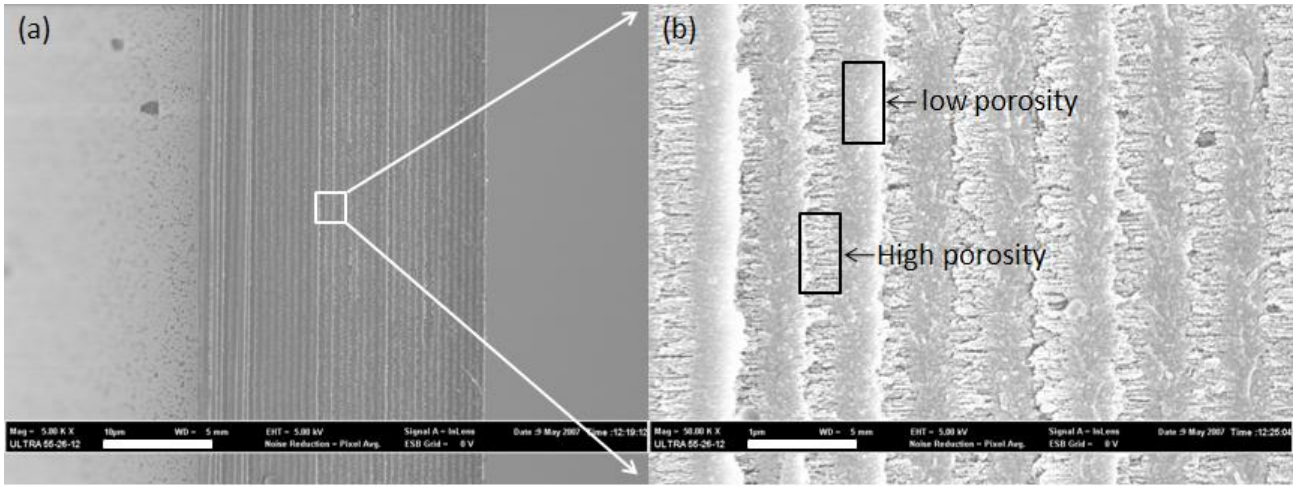

Figure 1: The FESEM photos of a free-standing photonic band-gap silica nanosensor. It consists of low porosity and high porosity layers. The physical thickness of the low porosity layers and the high porosity layers is 307nm and 448nm, respectively. The scale bar is 10μm in (a) and 1μm in (b)

The photonic band-gap silica nanosensor consists of stacks of low porosity and high porosity layers. The discrete multi-layers can be considered as layers of alternatively high and low refractive index. The photonic band-gap silica nanosensor performs a high reflectivity stop band with a peak of transmittance. The optical characteristics can be easily modulated by electrochemical etching. The center of the wavelength at which strong reflection occurs in the photonic band-gap silica film is given by the Bragg condition:

$$\lambda_B = \frac{2}{m}[d_L(n_L{}^2 - \sin^2\theta)^{\frac{1}{2}} + d_H(n_H{}^2 - \sin^2\theta)^{\frac{1}{2}}]$$

where n_L and n_H are low and high refractive indices, d_L and d_H are the correspondent layers thickness. θ is the incident angle, and m is an integer (the order of Bragg condition). When biomolecules enter into the photonic band-gap silica, both low and high refractive indices increase. The increase results in a shift toward longer wavelengths of its stop band peaks**.** The photonic band-gap silica nanosensor is designed to reflect light at visible light wavelengths and to detect biomolecules such as proteins and DNAs. Figure 1 show the FESEM photos of the photonic band-gap silica nanosensor used in this study. The photos were captured by a Carl Zeizz Ultra 55 field emission scanning electron microscope (FESEM) at a 5-kV accelerating voltage. The thickness of the low porosity layers and the high porosity layers is 307nm and 448nm, respectively. The total thickness of the photonic band-gap silica nanosensor is 28μm. Figure 2 shows the photonic band-gap silica nanosensors transferred to a glass slide and marked by 1 to 4. The different colors in the photonic band-gap silica nanosensors represent different biomolecules interactions and can be obtained by naked eyes.

3 EXPERIMENTAL

Porous silicon is first electrochemically etched into a 6" single crystalline silicon substrate (*p*-type, (100), 15mΩ). The etching solution consists of a 2:3 by volume mixture of absolute ethanol and aqueous 49% HF. Etching is carried out in a Teflon cell with a cycling pump. A current density varying between 20 and 65mA/cm^2 is applied for 40 cycles with a periodicity of 30s and 8s, respectively. The freshly etched porous silicon is then placed in an conventional furnace at 900°C for 3 hours. This step converts the porous silicon into the porous silica completely.

The obtained porous silica were immersed in a 2-propanol solution of 2% (3-aminopropyl)trimethoxysilane (APTMS) in the presence of 0.1% acetic acid for a period of 5 hours. Then, the modified porous silica were rinsed twice with 2-propanal and deionized water, respectively, and dried in following nitrogen to become the photonic band-gap silica biosensor. As-prepared photonic band-gap silica nanosensor were placed in a borate buffer solution containing 3% glutaraldehyde (GA) for 3 hours at room temperature, and were then rinsed with borate buffer solution (BBS) to make an aldehyde-terminated surface. Goat anti-h-IgG was immobilized on the sensor surface by immersing the sensor into a phosphorous buffer solution (PBS) of pH7.4 containing 100μgmL^{-1} goat anti-h-IgG and incubated for 12 hours at 4^{o}C. After incubation, the sensor was rinsed with PBS for 5 minutes. Subsequently, the sensor was placed in 0.1M L-lysine for 5 hours at 4^{o}C to block the remaining aldehyde and rinsed thoroughly with PBS of pH8.0 and dried with nitrogen gas. The sensor immobilized with anti-h-IgG were immersed in h-IgG solutions with concentration of 100ngmL^{-1} for 3 hours at 4^{o}C and rinsed twice with PBS of pH7.4.

4 RESULTS AND DISCUSSION

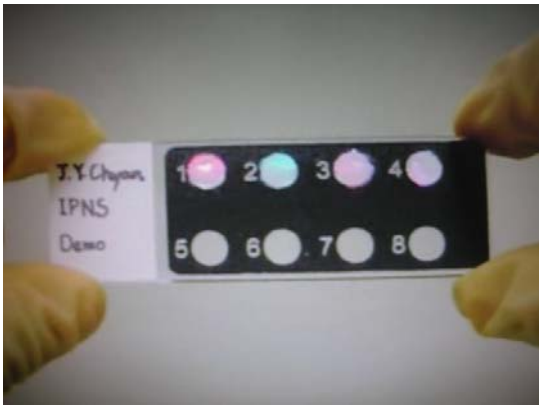

Figure 2: The photonic band-gap silica nanosensor is transferred to a conventional glass slide in the sensing area 1 to 4. It makes naked-eye detection of biomolecular interactions.

Figure 3 shows the experiment results of the photonic band-gap silica nanosensor modified by organic monolayers and biomolecules. The results meet expectation from the design theory according to Bragg condition. The transmission peak moves to longer wavelength with the increasing in binding layers as well as the increasing in effective refractive index. The wavelength shift caused by the 100μgmL^{-1} goat anti-h-IgG coating is 12nm and can be further extended to be 30nm after recognition between the goat anti-h-IgG and h-IgG. It indicates that the molecular recognition of goat anti-h-IgG and h-IgG can be detected by changes in the transmission spectra and/or color changes. Upon increasing the refractive index as well as the dielectric constant of the photonic band-gap silica nanosensor, both an obviously red-shift in wavelength and a slightly increased absorption intensity have been observed. However, the low visible light absorption in silica and biomolecules do not affect the sensitivity of the photonic band-gap silica nanosensor. These experiments clearly demonstrate the potential of the proposed nanosensor for biosensing applications.

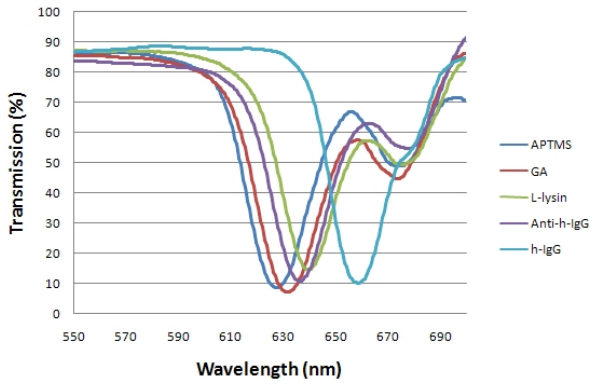

Figure 3: Transmission spectra of the photonic band-gap silica nanosensor modified with layer-by-layer organic monolayers and biomolecules.

5 CONCLUSION

We have developed an optical label-free biosensors which can provide easy, highly reproducible, and sensitive sensing assays for biomolecular reactions. The fabrication of the biosensor based on photonic band-gap silica is simple and compatible with conventional silicon microfabrication. The sensing signal of the photonic band-gap silica biosensor can be detected by both reflection and transmission mode. This flexibility gives the photonic band-gap silica biosensor more easily to integrate into silicon or glass based bio-microsystems. A wavelength shift of 30nm has been demonstrated by goat anti-h-IgG and 100ngmL^{-1} h-IgG recognition. The photonic band-gap silica nanosensor is promising as an easy and cost-effective alternative to conventional biosensing. Furthermore, it can be generally applied for the creation of colormetric sensors for many different analytes of choice.

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