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Macroporous ordered titanium dioxide (TiO_2) inverse opal as a new label-free immunosensor

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ABSTRACT

Photonic crystal sensing materials have been validated that they are very sensitive to refractive index changes. Herein, three-dimensionally ordered macroporous (3DOM) (>50 nm) TiO_2 inverse opal film has been fabricated by the self-assembly technique. Based on the TiO_2 inverse opal film, the optical spectrometer was established for label-free immunosensor. The sensing performance of the 3DOM TiO_2 was investigated using human IgG/goat anti-human IgG couple, which showed that the sensitivity of 3DOM TiO_2 inverse opal film could reach to 1 $\mu g\,mL^{-1}$ (equivalent to 1.5 $pg\,mm^{-2}$) of protein concentration detection limit. The 3DOM TiO_2 inverse opal has a large internal surface area, low fluorescence background and unique optical properties. These characteristics indicated the feasibility of 3DOM TiO_2 inverse opal in label-free immunoassay.

1. Introduction

Label-free immunosensors are observably desired for applications in the fields of high-throughput drug discovery, disease diagnostics, environmental monitoring, and food industry. The label-free immunosensors combine the advantages of the immunological specificity, convenience of spectral or electrochemical techniques and can operate effectively in untreated samples without separation steps. Generally, the label-free optical transduction methods can be divided mainly into two categories: optical interferometric and surface plasmon resonance (SPR) methods [1]. In these devices, biomolecules such as antibody or ligand are immobilized on a solid substrate for detecting the presence of a target antigen or receptor. Effective immobilization of the biomolecules is one of the key features for successful application. Biocompatible microcavities materials, such as titanium dioxide [2–8], have been recently demonstrated to be a sensitive sensing template for the support substrate because of its large internal surface area, good biocompatibility and wide application. The optical and electrical properties of the microcavities materials, such as the refractive index, photoluminescence, and impedance, are very sensitive to the presence of biological or chemical species inside the porous structure [9].

To fabricate titanium dioxide microcavities materials, several conventional fabrication methods, including etching techniques [10–12] and sol–gel technique [3–5,8,13,14] have been employed to generate the microcavities structures. Generally, the etching techniques require specific equipment and a controllable process in strong acid or alkali solution. Some of the sol–gel methods will result in a loss of enzyme activity due to the harsh processing conditions [15]. In addition, these techniques are limited to the minimum features size that they can achieve. Another important method of titanium dioxide microcavities structure called TiO_2 inverse opal is the template-directed method using monodispersed

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latex spheres [16–20]. In this method, the monodispersed polystyrene or polymethyl methacrylate was self-assembled as template. After the titania precursor was infiltrated into the interspaces of the spheres, the organic template was removed by the calcination or toluene-etching. Because of the controllable size of the colloidal microsphere and thickness of film, this approach is feasible in the common laboratory and received a significant interest. These titanium dioxide microcavities have been widely studied as sensor for various gases [21–24], biomolecules [4,5,8,11,25]. However, the 3DOM TiO_2 inverse opal structure used as biosensor has not been reported. The sensing properties of the 3DOM TiO_2 structure, such as protein adsorption, sensitivity, specificity and regeneration, are still not clear.

In the paper, we fabricated the 3DOM TiO_2 inverse opal films by the inversed-crystal technique and set up a biosensor using this film as a biochip. Proteins were directly immobilized on the pore surfaces of 3DOM TiO_2 substrates by physical adsorption. The reflected signal was obtained by monitoring the change of the diffraction peak shifts. The results would display the unique optical properties of 3DOM TiO_2 inverse opal for free-label biomolecules detection.

2. Experiment

2.1. Materials and equipments

Monodispersed polymethyl methacrylate (PMMA) spheres were synthesized in our laboratory. 10% 15-nm-titania and 6-nm-silicon dioxide nanoparticles were bought from Catalysts & Chemicals Ltd. Co. (Tokyo, Japan). Reagent quality sulfuric acid (95%), hydrogen peroxide (30%), and sodium dodecyl sulfate (SDS) were bought from Nanjing Chemical Company (Nanjing, China). All the chemicals used in this work were of analytical grade. Slide glasses were the products from Shanghai Glass, Inc. (Shanghai, China). Deionized water (>18 MΩ) was used for the experiments. Human immunoglobulin G (hIgG), goat polyclonal anti-human IgG (goat anti-hIgG), FITC-labeled goat anti-hIgG and FITC-labeled goat polyclonal anti-rabbit IgG (goat anti-rIgG) were purchased from KPL Company (Guildford, UK).

The lifting machine is an automation stage controlled by a computer [26]. The lifting speed can be varied over a range of 0.1–70 $\mu m\,s^{-1}$. Reflected spectrum were recorded by an Ocean Optics HR2000 spectrometer with a reflection probe R200-7 (six illumination fibers around one read fiber) (Ocean Optics, FL, USA).

Scanning electron microscope (SEM) observations were performed using a model S-3000N (Hitachi, Tokyo, Japan).

Both the fluorescence images and spectra were collected with a fluorescence inverted microscope (Olympus, Tokyo, Japan) equipped with Nikon D1X digital CCD camera and an Ocean Optics spectrofluorometer (Ocean Optics, FL, USA).

2.2. Slide glass treatment

The slide glasses used for the experiment were first immersed in a solution containing 30% (v/v_0) hydrogen peroxide and 70% sulfuric acid overnight. Then, the glass substrates were rinsed with deionized water and dried with nitrogen gas.

2.3. Fabrication of films by the vertically lifting template method

Fabrication of the three-dimensionally ordered nanoporous TiO_2, SiO_2 and polystyrene (PS) inverse opal films were similar to the method previously reported [7]. Briefly, PMMA spheres were coated on a glass substrate by the lifting method. The interstitial space between the PMMA spheres was filled by the 10% 15-nm-titania- or 6-nm-silicon dioxide-nanoparticles water suspension containing 5 mM SDS. Finally, the films were calcined at 500 °C to remove the polymer spheres and solidify the network. The temperature was raised from 25 to 500 °C at speed of 2 °C min^{-1} and then descended to room temperature at speed of 2 °C min^{-1}. For the PS inverse opal film, monodispersed silica spheres of 310 nm diameters (supplied by Catalysts & Chemicals Ltd. Co., Tokyo, Japan) were coated on glass substrate by the lifting method. The polystyrene–toluene solutions was filled with the voids of colloids and the toluene solutions was evaporated. Then, the silica spheres were etched out by the 4% hydrofluoric acid.

2.4. Label-free detection

The fabricated TiO_2 inverse opal film was used as sensing chip for reflectometry interference spectroscopy. The probe of the optical fiber spectrometer was perpendicular to the surface of the inversed TiO_2 opal film during the detection.

To immobilize proteins on the pore surfaces of 3DOM TiO_2 inverse opal films, the sensors chips were respectively exposed to various concentrations hIgG solution in 10 mmol L^{-1} phosphate buffer saline (PBS) at pH 7.4. After overnight incubation at 4 °C, the sensors chips were rinsed for three times with 10 mmol L^{-1} PBS buffer at pH 7.4. Then the sensors chip were blocked with bovine serum albumin (BSA, 5 mg mL^{-1}) in 10 mmol L^{-1} PBS at pH 7.4 for 1 h at room temperature to prevent nonspecific adsorption of proteins during subsequent steps. The sensors chips were subsequently rinsed with pH 7.4 PBS buffer and filled with the sample containing the target analyte for 1 h at room temperature. Then the sensors chips were rinsed with pH 7.4 PBS buffer and deionized water and dried with N_2 flow before the optical measurement. In situ analyses of protein immobilization, BSA blocking and analyte binding on the pore surfaces were performed using an Ocean Optics HR2000 spectrometer. All measurements were performed at normal incidence and constant regions.

2.5. Assay of protein-binding specificity

According to above method, 0.5 mg mL^{-1} hIgG was adsorbed on the pore surfaces. After the chips were blocked with 5 mg mL^{-1} BSA, the chips were incubated in solutions containing 10 μL of 0.05 mg mL^{-1} goat anti-rIgG, FITC-labeled target antibody and FITC-labeled goat anti-rIgG for 1 h in the room temperature, respectively. Then the chips were washed three