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Optical waveguide sensor based on silica nanotube arrays for label-free biosensing

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ABSTRACT

Label-free biosensing based on optical waveguide spectroscopy of silica nanotube (SNT) arrays is realized with high sensitivity. The SNT arrays fabricated using a porous anodic alumina (PAA) template assisted by surface sol–gel (SSG) method showed a high value of 552 reciprocal refractive index unit as the sensing figure of merit by exchanging the sensing environment with water and ethanol. A standard biotin–streptavidin affinity model was tested using the SNT arrays which support a TM_1 mode and the fundamental response of the system was investigated. Results show that the response of the SNT arrays for adsorption of streptavidin is higher than the one using substrate without removing the PAA template due to the larger surface area and the stronger electromagnetic field. The limit of detection (LOD) of the SNT arrays for detection of streptavidin was estimated as 93 pM, with the detection time of 40 min. Additionally, the Fresnel calculations suggested higher potential sensitivity of the current system compared to that of the conventional SPR sensors. Thus, the SNT arrays may be used as a versatile platform for high-sensitive label-free optical biosensing due to the high performance and the large potential of the surface functionality.

1. Introduction

Highly sensitive and specific label-free biosensors are essential in many areas since target molecules are not labeled and are detected in their natural forms (Fan et al., 2008; Goda and Miyahara, 2013; Hunt and Armani, 2010; Li and Sailor, 2014; Narayanaswamy and Wolfbeis, 2004). Fluorescence-based methods, in the contrast, may significantly interfere with the original function of biomolecules by labeling analytes with fluorescent molecules, and can be expensive and time consuming, although they are extremely sensitive (Fan et al., 2008; Schultz et al., 2008). In the past 10 years, label-free biosensors based on different types of nanostructures have been intensively studied to improve the sensor performance (Awazu et al., 2007; Cui et al., 2001; Gitsas et al., 2010; Hotta et al., 2010, 2012; Kabashin et al., 2009; Lau et al., 2004; Maehashi et al., 2007; Yi et al., 2005; Zhang et al., 2008). For example, biosensing using plasmonic gold nanorod arrays as optical waveguide at infrared region has been demonstrated (Kabashin et al., 2009). Gitsas et al. (2010) have accomplished the first example of the integration of optically transparent nanorod arrays into device architectures enabling optical waveguide spectroscopy. Both of the two examples have shown enhanced sensitivity over the conventional surface plasmon resonance (SPR) systems due to the efficient adsorption of analytes and further enhanced electric field. Optical sensing systems facilitating application in real-life conditions would ideally be operated using visible light, thus a highly stable nanoarrays composed of relatively low refractive index materials enabling efficient overlap of the waveguide mode with the sensing zone formed in the nanostructure is highly desirable (Skivesen et al., 2004). However, it has been a challenging task to fabricate such an array despite a lot of efforts have been made.

Herein, we present a simple surface sol–gel (SSG) method to fabricate the silica nanotube (SNT) arrays on Au substrates using porous anodic alumina (PAA) templates and demonstrate the use of such arrays as efficient and sensitive waveguide (WG) label-free sensors (referred as SNTWG) operated with visible light. SSG is a kind of layer-by-layer method and allows to precisely control the film thickness in nanometer scale. The preparation of uniform and smooth nanotubes/nanowires has been demonstrated (Kovtyukhova et al., 2003). This allowed one to tailor the nanostructure of the waveguide layer for better waveguide coupling and finally to achieve high

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sensitivity. The silica are popular materials that extend the range of possible chemical modifications, including silane chemistry (Allara et al., 1995; Velleman et al., 2009) and physical adsorption, and even multi-functionality in the waveguiding layer can be achieved by differing inner and outer surface chemistries of the SNT arrays (Mitchell et al., 2002). Moreover, the tube array offers a large adsorption area by exposing both of the outside surface and additional inside surface for adsorption of analytes, which can be further enlarged by increasing the porosity and the height of the PAA template (Hotta et al., 2012). Besides, the enhanced electromagnetic field of the waveguide modes confined within the waveguiding layer due to the lower refractive index (RI) of silica (Xi et al., 2004) can also improve the sensor performance. These characters of the SNTWG will amplify the changes of the RI of the surrounding medium or the effective RI of the SNT arrays due to immobilization of molecules to give higher sensitivity based on the very sharp resonance of the waveguide mode. A high value of 552 reciprocal refractive index units was achieved as the sensing figure of merit by exchanging the sensing environment with water and ethanol, which is higher than other angular based sensors (Gitsas et al., 2010; Homola et al., 1999). A streptavidin affinity model was tested using a SNTWG which supports a TM_1 mode to further demonstrate the feasibility of our approach and the Fresnel calculations suggested the higher potential sensitivity compared to that of the conventional SPR sensors. SNT arrays may be used as a versatile platform for biosensors.

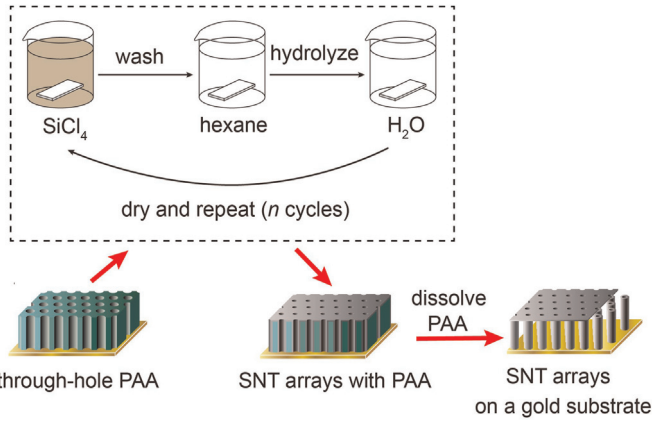
2. Materials and methods

2.1. Materials

A K9 square glass slide ($22 \times 22 \times 0.5\ mm^3$) were purchased from Dongguan City Hong Cheng Optical Co., Ltd. (China) and used as the substrate for SNT arrays. (3-Mercaptopropyl)trimethoxysilane (3-MPS) and 3-aminopropyltriethoxysilane (APTES) were purchased from Alfa Aesar China. The Sulfosuccinimidyl-6′-[biotinamido]-6-hexanamido-hexanoate (Sulfo-NHS-LC-LC-biotin) was obtained from Pierce, Inc. (Rockford, IL). Streptavidin was purchased from Beijing Biosynthesis Biotechnology Co., Ltd. (China). A buffer solution (10 mM phosphate buffer, 100 mM NaCl, a pH of 7.4) was used to prepare the following solution: 0.5 mM of Sulfo-NHS-LC-LC-biotin solution, and a series of concentrations of streptavidin. Milli-Q water (18.2 MΩ cm, Millipore Corp., Bedford, MA) was used throughout the experiments. All the other organic solvents and chemical reagents were purchased from Shenzhen Tianxiang Huabo Co., Ltd. (China) and Xiya reagents (Sichuan, China) and were used as received without further purification.

2.2. Fabrication of through-hole PAA template on Au substrates

The glass slide was sonicated in acetone substrate surface. After rinsing the glass substrate with Milli-Q water and drying it under a nitrogen gas stream, a 40 nm Au layer was magnetic sputtered on the glass slide with a 5 nm Cr as the adhesion layer between the Au layer and the glass slide. Then, the substrate with the Au layer (Au substrate) was immediately immersed in a solution of 4 mM 3-mercaptopropyltrimethoxysilane (3-MPS) for 1 h followed by hydrolysis of the 3-MPS in a 0.1 M HCl solution for 1 h to modify the Au surface with hydroxyl groups. The modified Au substrates were kept in a desiccator before use. Then, a highly pure aluminum foil (99.999%) was anodized via the classic two-step anodization (Byun et al., 2010; Masuda and Fukuda, 1995) with the second anodization at 40 V in a 0.3 M oxalic acid solution at 15 °C to fabricate the uniform PAA template. After the pore diameter was widened in a 5 wt% phosphoric acid solution at 34 °C and the remaining aluminum was detached from the PAA layer in a saturated $HgCl_2$ solution, the barrier layer of the PAA template was removed by floating on a 5 wt% phosphoric acid solution at 30 °C for 35 min. Then, the through-hole PAA template was then carefully transferred to the hydroxyl groups modified Au substrate and interacted with the hydroxyl groups on the Au layer to give excellent adhesion between the PAA template and the Au layer (left panel in Scheme 1).



Scheme 1. Illustration (not to scale) of fabrication SNT arrays on a gold substrate. First, a through-hole PAA layer was attached on a gold substrate as the template. Second, SNT arrays were synthesized using the surface sol–gel method. Third, SNT arrays were exposed by removing the PAA template in the aqueous solution of phosphoric acid.

2.3. Synthesis of SNT arrays on Au substrate

SNT arrays were synthesized using the surface sol–gel (SSG) method (He et al., 2006; Kovtyukhova et al., 2003). The PAA template with through-holes on an Au substrate was first immersed in a 99.5% $SiCl_4$ solution for 2 min. After subsequently washing with hexane to remove the unbound molecules from the pore surface, the substrate was then soaked in hexane/methanol (1:1 v/v) for 3 min and pure ethanol for 5 min followed by washing with ethanol and dried in a N_2 stream. Next, the $SiCl_4$ molecules adsorbed on the surface of PAA template was hydrolyzed for 5 min in water to form SiO_2. This is one SSG cycle and six cycles were carried out for the synthesis of the SNT arrays in the following experiment (middle panel in Scheme 1). Then, the PAA template was dissolved in a 12 wt% aqueous solution of phosphoric acid for at least 24 h at room temperature to obtain the SNT arrays on the Au substrate (Fig. 1 and right panel in Scheme 1).

2.4. Optical waveguide spectroscopy

Optical waveguide spectroscopy was carried out using the Kretschmann prism coupling technique (Fan et al., 2012; Hotta et al., 2010, 2012; Lau et al., 2004) (Fig. 2) and the reflection spectra of the SNT arrays were measured as the function of the incident angle. A He–Ne laser light ($\lambda = 632.8$ nm) was directed at the SNT/Au multilayer film attached to a K9 equilateral prism via index matching fluid ($n = 1.515$) after passing through a polarizer and modulated by a light chopper. S- (transverse electric) or p- (transverse magnetic) polarized light can be adjusted using the polarizer. The reflected light from the film was detected by a photodiode detector after its polarization was set to be the same with the incident one using an analyzer. The sample solutions were allowed to be in contact with the SNT arrays using a poly (tetrafluoroethylene) flow cell with a volume of 2 μL (16 mm × 1.3 mm × 100 μm) connected to a homemade pump system and a polyfluoroalkyl vinyl ether (PFA) tube.