Biosensors and Bioelectronics 50 (2013) 137–142

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Sensitivity improved plasmonic gold nanoholes array biosensor by coupling quantum-dots for the detection of specific biomolecular interactions

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ARTICLE INFO

Article history:
Received 13 March 2013
Received in revised form
28 May 2013
Accepted 10 June 2013
Available online 18 June 2013

Keywords:
Large-scale
Nanosphere lithography
Gold nanoholes array
Label-free SPR biosensors
Enhanced detection sensitivity
Quantum dots

ABSTRACT

In this paper, we focused on the large-scale fabrication of gold nanoholes array capable of supporting surface plasmonic resonance (SPR) via the developed nanosphere lithography (NSL) technique, which could be used as high performance biosensor for the detection of specific streptavidin–biotin interactions. Direct UV–vis absorption mode measurement was used to monitor the SPR peak shift. For the better immobilization of biotin, the surface of gold nanoholes array was functionalized with 3-mercaptopropyl trimethoxysilane (MPTS) and 3-aminopropyl triethoxysilane (APTES). After the streptavidin binding to the biotin, the SPR peak position showed an 11 nm wavelength shift due to the refractive index change caused by the biotin–streptavidin binding. The sealing treatment was performed by using bovine serum albumin (BSA) to eliminate the influences of nonspecific adsorption for more accurate detection. Interestingly, the detection sensitivity of the gold nanoholes array could be further enhanced by coupling the water-soluble CdSe/ZnS quantum dots (QDs), which showed four-fold improvement in detection sensitivity as compared to the gold nanoholes array biosensor without the coupling of QDs. The mechanisms for the enhancement of detection sensitivity were also discussed. This would provide new capabilities for the highly sensitive measurements of biomolecular binding.

1. Introduction

The need to probe biomolecular interactions such as biotin/streptavidin binding, antibody/antigen interactions, sugar/lectin interactions, DNA hybridization, and other analyte/receptor interactions is an important motivation for the understanding of fundamental biological processes (Baleviciuteet et al., 2013; Heo et al., 2013; Jin, 2012; Y. Kim et al., 2012; Liu et al., 2013; Tawa et al., 2011; Yao et al., 2011). The fluorescence-based methods have proven to be the most charming platforms suitable for the detection of such biomolecular interactions. However, the complexity and cost have been widely recognized as their inherent shortcomings. Moreover, the label itself may potentially alter the binding properties of biomolecules and cause other significant problems like background binding and autofluorescence (Cooper, 2002; Yang et al., 2008). In contrast, label-free detection methods provide more accurate quantitative and kinetic measurements by monitoring the binding of analytes in their natural forms, which can be realized based on the transduction of plasmonic, optical, electrochemical, or mechanical signals (Sriram et al., 2011; Yanik et al., 2010). Among these label-free biosensing platforms, the SPR is one of the most widely used methods today (Abbas et al., 2011; Law et al., 2011; Park et al., 2010; Pernites et al., 2010). SPR is the optical resonance in electromagnetic radiation due to the coupling with electrons in nanoscale metallic structures. The SPR spectral peak position (λ_{max}) is highly dependent on the local refractive index at the surface of metallic structures. Since the excited surface plasmon modes are very sensitive to the local refractive index changing caused by the binding of specific biomolecules to the metallic structures, it can be used as label-free biosensor with a high sensitivity exceeding 10^3 nm per refractive index unit (RIU) by monitoring the peak shift of λ_{max} (Guo et al., 2010).

In conventional SPR biosensor, light needs to couple to surface plasmon polaritons (SPP) using the Kretschmann configuration, which requires a prism and complicated optical alignment (Hunta and Armani, 2010). In contrast, plasmonic resonances can be excited by direct illumination without any special arrangements for the nanostructured metal films and can be monitored using conventional transmission mode spectroscopy (Nakamoto et al., 2012; Yang et al., 2008). Despite being less sensitive than Kretschmann configuration, the optical transmission configuration enables multiplexed detection and offers better opportunities to develop low-cost biosensor devices with integrated microfluidics for rapid bioanalytical measurements. This significantly simplifies the experimental setup, which is one of the reasons for the increasing popularity of nanostructured SPR

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http://dx.doi.org/10.1016/j.bios.2013.06.023

biosensors. Based on the recent advances in fabrication techniques such as electron beam lithography (EBL), focused ion beam lithography (FIBL), and phase-shifting lithography (PSL), a wide range of patterned metal nanostructures including nanotriangles, nanodisks, nanoholes, bowtie antennas, and nanorings have been fabricated as the SPR based biosensors with high sensitivity (Correia-Ledo et al., 2012; Rodríguez-Fortuño et al., 2011; Sharpe et al., 2008; Yu et al., 2012). Among them, metal nanoholes array can provide smaller foot-print, denser integration, increased potential for multiplexing and simplified collinear optical detection, in which analyte binding is determined directly due to the plasmon-mediated extraordinary optical transmission (EOT) effect (Escobedo et al., 2010). Several groups have shown the potential of such metal nanoholes array for label-free kinetic SPR biosensing. Yu and Golden (2007) adopted EBL to construct 270 nm thick gold nanoholes array and the distribution of cytochrome was characterized by surface enhanced Raman scattering (SERS). Nakamoto et al. (2012) reported the fabrication of gold nanoholes array by nanoimprinting and vacuum deposition, where they obtained the synchronized responses of current and SPR optical signals in association with the redox change of the Os complex. Sharpe et al. (2008) had characterized and demonstrated the production of a nanoholes array detection system through EBL using cortisol as the model analyte. Immunochemical binding interactions have been sensed using a new cortisol-linker-thiol derivative for surface modification.

However, the above patterned metal nanoholes have been fabricated only with a finite area (always tens to hundreds of micrometer square) by using the slow and expensive top-down fabrication method. This would result in a spectrum change due to the significant contribution from the edges. In general, the desirable nanofabrication technique for substrate-bound nanostructures would have some of the conspicuous features, low-cost fabrication, large-area patterning, reproducibility, and tunability of their optical properties (Lee et al., 2011). Therefore, the adequate, large-scale and affordable nanofabrication methods are highly desired for the real application of such nanostructured SPR biosensors. On the other hand, we should note that the SPR resonance mode is always broad for the nanostructured SPR biosensors due to the large absorption in the metal film, which restricts its detection sensitivity and precludes its use for the detection of low surface coverage of bound molecules (Guo et al., 2010). Improving such biosensor's detection sensitivity by new strategy is still the key point for the further advances of the metallic nanostructured SPR biosensors.

In this paper, we focus on the design and large-scale fabrication of gold nanoholes array using the developed NSL technique, which utilizes a hexagonally closely-packed nanosphere mask that permits direct metal deposition onto a substrate through the interstitial regions of the mask. The large-scale feature of the gold nanoholes array allows us to detect the interaction of biomolecules through the direct UV–vis absorption mode measurement. In order to detect the specific affinity reaction of the biotin–streptavidin based on our gold nanoholes array SPR biosensors, the layer-by-layer chemical modification was conducted to make the biomolecule firmly immobilized on the surface of gold nanoholes array. BSA was used to reduce the nonspecific adsorption. The detection sensitivity of the gold nanoholes array biosensor can be further enhanced by coupling the core–shell CdSe/ZnS QDs to streptavidin.

2. Materials and methods

2.1. Large-scale fabrication of gold nanoholes array on the glass substrate

Slides glass substrate was treated by piranha solution (95% H_2SO_4:30% H_2O_2=3:1 by volume) for 30 min to clean the surface. After this, the slides glass substrate was rinsed with ethanol, acetone, and water. Then, it was blow-dried with nitrogen gas. The polystyrene (PS) spheres with a diameter of about 330 nm were synthesized using the surfactant-free emulsion polymerization. The hexagonal closely-packed PS monolayer template was fabricated on the pre-cleaned slides glass substrate using the modified float-transferring technique (Cheng et al., 2012a). In brief, the water/ethanol dispersion containing monodisperse PS spheres was dropped onto the pre-cleaned slides glass. Subsequently, the slides glass was immersed into the water filled Petri dish with a tilt angle of 30–40°. The dispersion spread on the water surface, resulting in a self-assembled PS monolayer film. Then the PS monolayer film was picked up by another slides glass. The diameter of PS spheres was decreased slightly by the reactive ion etching (RIE) treatment (150 W, 15 s) to expand the voids between them. The etched PS monolayer on the slides glass was taken out from the RIE chamber and then thermo-treated at 110 °C for 2 min in order to increase the binding force between the PS spheres and slides glass. The gold deposition was performed at 200 V, 0.2 A for 30 s using a magnetron sputtering system. After the gold sputtering, the PS sphere template was thoroughly removed from the slides glass substrate by dissolution in tetrahydrofuran under a gentle ultrasonic bath for 1 min. Finally, the large-scale gold nanoholes array was obtained on the slides glass substrate. The gold nanoholes array showed a large-scale uniformity as can be seen from Fig. 1a.

2.2. Surface functionalization of gold nanoholes array

The surface of gold nanoholes array was modified for better immobilization of biotin via the layer-by-layer method. Firstly, the gold nanoholes array was immersed into 20 ml solution containing 1% MPTS, 95% methanol, and 4% 1 mM acetic acid for 30 min. Such process resulted in the formation of a silane-terminated MPTS self-assembled monolayer (SAM) on the gold surface. After thoroughly rinsed with water, the slides glass was ultrasonicated in ethanol and water solution successively and was dried by nitrogen gas flow at the room temperature. Secondly, the MPTS modified gold nanoholes array was silanized in APTES solution. In detail, the solution containing 3 ml ultrapure water and 23.5 ml ammonia was added to the other solution containing 25 μl APTES in 23.5 ml absolute ethanol and mixed homogeneously. Subsquently, the MPTS modified gold nanoholes array was immersed into the mixed solution for 15 min. After being taken out from the mixed solution, the gold nanoholes array was thoroughly rinsed with absolute ethanol and dried by nitrogen gas flow.

2.3. Biotin immobilization and bovine serum albumin sealing treatment

Biotin–N-hydroxysuccinimide (biotin–NHS) was anchored onto the surface of functionalized gold nanoholes array via the following process. Firstly, the MPTS and APTES functionalized gold nanoholes array was immersed into the mixed solution of 300 μl biotin–NHS (150 μM) and 29.7 ml phosphate buffer solution (PBS) (pH 7.2, 0.01 M) for 24 h at 4 °C. After this, a large amount of PBS buffer solution and water were used to flush away the unreacted biotin from the gold nanoholes array and dried by nitrogen gas flow. Secondly, the biotin bonded to the gold nanoholes array was sealing treated in order to eliminate the influences of nonspecific adsorption for the more accurate detection. In detail, the antibody immobilized gold nanoholes array was immersed into the solution consisting of 300 μl BSA (1 mg ml^{-1}) and 29.7 ml PBS (pH 7.2, 0.01 M) for 30 min. Finally, nitrogen gas dried sample was placed into the mixed solution containing 500 μl streptavidin and 4.5 ml