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Surface plasmon resonance biosensor modified with multilayer silver nanoparticles films

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ABSTRACT

We alternately deposited negatively charged Ag-(3-mercaptopropionic acid) (Ag-MPA) sol and positively charged poly-(diallyldimethylammonium) (PDDA) on gold substrate modified with 4-aminothiophenol (4-ATP), through electrostatic layer-by-layer (LBL) self-assembly. We characterized the prepared three-dimensional Ag/PDDA multilayer films by surface plasmon resonance (SPR) and atomic force microscope (AFM). The thickness of each film in the multilayer films, the deposition effect of Ag nanoparticles, and the processing of DNA adsorption are characterized by SPR. AFM characterization shows that DNA/3(PDDA/Ag)/4-ATP composite is uniformly and firmly distributed on the surface of gold films. Compared with other sensors, gentamicin could be highly sensitively measured by DNA/3(PDDA/Ag)/4-ATP/Au sensor. There is a good linear relationship in the concentration range of 5×10^{-8} to 1×10^{-4} mol/L. The linear equation is found to be $\Delta\theta_{SPR} = 1.3521 \times 10^{-5}c + 0.08641$ (the correlation coefficient is 0.9983) with detection limit of 1×10^{-9} mol/L. Since such LBL assembly film is simple to prepare, the work described here provides an effective method for studying small molecule drugs on SPR.

1. Introduction

The construction of functional and organized multilayer biomolecule-containing films is one of the focal subjects in biomaterials and biotechnology [1]. This kind of films could be potential biomaterials for fabrication of biomolecule devices. Their applications are envisaged in areas such as bioelectronics, biosensors, biooptics [2,3], artificial biomembranes and biocomputing [4]. Furthermore, integration of functional inorganic nanoparticles into functional and organized ultrathin multilayer films has been found potential applications. For example, Au nanoparticle-based ultrathin films can be used in advanced microelectronic devices, nonlinear optics, electrochemical sensor and bioanalysis [5].

Extensive studies have been carried out to assemble ultrathin multilayer films mainly by Langmuir–Blodgett (LB) techniques and self-assembly methods [6]. Particularly, introduction of inorganic nanoparticles to form ultrathin multilayer films can be realized by the well-established layer-by-layer (LBL) self-assembly of inorganic nanoparticles, which is based on alternating adsorption of oppositely charged polyions on charged substrates [5]. This method was proposed by Decher and co-workers [7]. It is a simple, yet powerful approach for constructing different compositions into ultrathin multilayer films with controlled thicknesses on essentially arbitrary solid substrates. In addition, the manipulation is very easy and one does not need to label biomolecules during the measurement and thus avoids the deactivation of the biomolecules. In the past decades, many composites, including polymer [8], inorganic nanoparticles (such as Au, Ag, and other metal or metal oxide) [9], and biomolecules, including enzymes [10], DNA [11] have been used to prepare multilayer composite materials with this method.

SPR is an automated, rapid, sensitive and label-free surface-sensitive spectroscopic technique, which has been utilized in the rapid measurement of molecules and in the study of real-time monitoring interaction processing between molecules. Unfortunately, its application has so far been limited mainly to large molecules. This is because low molecular weight compounds do not have sufficient mass to cause a measurable change in refractive index, which hampers its applications to small molecules.

Several methods have been developed to enhance the sensitivity of measuring small molecules. Polymers can be used as conductance to increase the refractive index of SPR caused by small molecules [12]. Alternatively, original high polymers or proteins can be replaced with small molecules to enhance the response of SPR [13]. In addition, competitive immunoassay can also be used to study small molecules [14]. In the recent decades, some researches have been done on the sensitive SPR detection of small

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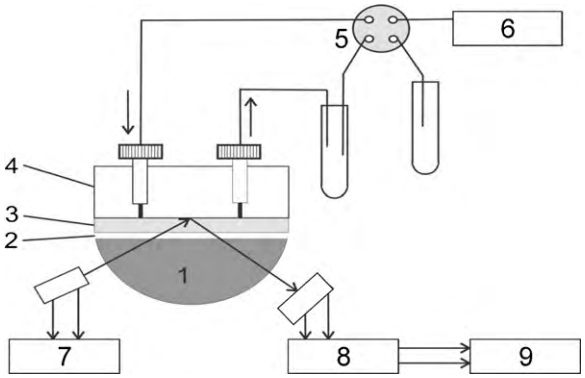

Fig. 1. The configuration of an SPR detector combined with flow injection. 1: a half-cylindrical lens; 2: gold film; 3: Teflon gasket; 4: flow channel; 5: six-way valve; 6: injector; 7: laser driver; 8: digital instrument; 9: digital processor.

molecules [15–17]. Furthermore, metal nanoparticles have been used to enhance the sensitivity of small molecule measurement by SPR, due to the inductance coupling interaction between metal nanoparticles deposited on sensors and Au films, which increases the refractive index of SPR [18]. The strength is closely related to the size of nanoparticles and the distance between nanoparticles and Au films [19].

Ag arrayed nanostructure has been extensively studied and become one of the best established systems, particularly for investigation of size and shape effects on SPR phenomenon on biosensing, e.g. surface enhanced Raman scattering (SERS). Incorporation of Ag nanoparticles into a film is also an efficient way to construct antibacterial biomaterial [19,20].

In this paper, we report a new SPR biosensor to sensitively measure the change of small molecules. We have prepared Ag/PDDA multilayer films using the LBL self-assembly method and observed the assembled processing in real-time. This technique is capable of increasing the response of DNA adsorbed on the multilayer surface of the SPR sensor, facilitating real-time monitoring of the interaction processing between DNA and small molecule drugs. It can be potentially applied to the analysis of real samples by taking advantage of catalytic amplification of Ag nanoparticles.

2. Materials and methods

2.1. Material

All solutions were made with double glass-distilled water. The OD_{260}/OD_{280} of Calf thymus DNA is more than 1.8 measured by UV spectra, which shows the interferent of protein does not exist in it. 4-Aminothiophenol (4-ATP, >97%), poly-(diallyldimethylammonium) (PDDA, >97%) and gentamicin were obtained from Sigma, USA; 3-mercaptopropionic acid (3-MPA, 99%) from Alfa Ltd.; sodium borohydride ($NaBH_4$, 96%) from Shanghai Shanpu Chemical Ltd.; silver nitrate ($AgNO_3$, 99.8%) from Tianjin Yuanda Photographic Ltd.; potassium ferricyanide ($Fe(CN)_6$) from Beijing Chemical Reagent Factory. Tris–HCl buffer (pH 7.0) was used as electrolyte for electrochemical experiment. All chemicals were of analytical grade unless otherwise specified.

The SPR instrument was assembled by Prof. Zhou Fei-meng, who is an expert in this area. The structure is shown in Fig. 1.

The gold films with the thickness of ~45 nm were sputtered on one side of glass slide for the excitation of surface plasmons. An underlayer of chromium (5 nm) was used to ensure mechanical stability of the gold film. Gold films were annealed by hydrogen flame for about 5 min to remove the filth on the surface and make the structure be more compact. Au films were pressed on the base of a half-cylindrical lens (BK_7, $n = 1.51$) via an index matching oil (wako, Japan, $n = 1.51$). Linearly *p*-polarized light having a wavelength of 670 nm from a diode laser was directed through the prism onto the gold film. The position of the prism was adjusted to make the refracted light produce plasmon resonance on gold surface. Charge-coupled device was used to receive the signal.

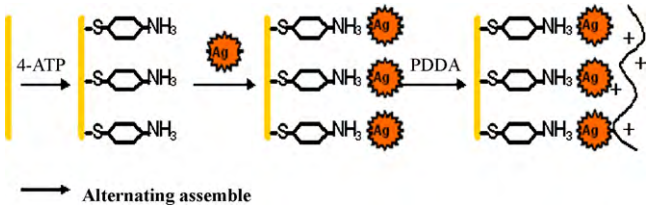

Fig. 2. Schematic diagram of the formation of the multilayer structure of 3(PDDA/Ag)/4-ATP/Au multilayer.

2.2. Methods

2.2.1. The preparation of Ag nanoparticles

We prepared Ag nanoparticles according to Lee's [21] experiments. 10 mL of 2×10^{-2} mol/L $NaBH_4$ was used as reducer, and 3-MPA was used as dispersant. The solution contained 50% (c/c) of Ag ion. 10 mL of 1×10^{-3} mol/L $AgNO_3$ solution was added immediately when they were ultra-surged at freezing point, and then keep it surging for 10 min. Finally, hyaloplasmic burgundy sol was obtained, which could be conserved for a month at 4 °C. The diameter of Ag nanoparticles was 40 ± 5 nm measured by the laser particle sizer (Malvern Zetasize nano ZS90, England).

2.2.2. The detection method of SPR

The detector features a polarized wedge-shaped light beam from a light-emitting diode which is reflected in the gold film on the sensor chip and detected on a diode array. SPR occurs in the gold film and can be detected as extinction of light in the reflected beam for a special angle of incidence. This SPR angle varies with refractive index changes on and in the vicinity of the surface as a result of adsorption of molecules on the multilayer surface of the SPR sensor.

3. Results and discussion

3.1. Assembly of Ag/PDDA multilayer

The electrostatic attraction between negatively charged Ag-MPA sol and positively charged PDDA is the driving force for the buildup of the three-dimensional Ag nanoparticles multilayer films. The *in situ R–t* mode of SPR measurements was used for the observation of adsorption kinetics of Ag and PDDA to determine the time of adsorption saturation. Fig. 2 illustrates the assembly process. Unless otherwise specified, the flow rate of the electrolyte (4-ATP solution, Ag-MPA solution and PDDA solution) and the double glass-distilled water was set to be 0.06 and 2 mL/h, respectively. The experimental procedures were as follows. The sensor was first immersed in 1 mL of 1×10^{-5} mol/L 4-ATP solution until the adsorption curve became stable. Subsequently, 1 mL of 5×10^{-5} mol/L Ag-MPA solution was injected till the baseline was stable. Finally, 1 mL of 5×10^{-5} mol/L PDDA solution was injected into the sensor. After each injection, we used the distilled water to remove the physical adsorption and the excess of assembling materials, until the SPR signal become stabilized, then begin the next assembly to make sure the assembling base are the same in each experiment. Besides, we chose the layer of PDDA/Ag film by cyclic