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Direct growth of coupled gold nanoparticles on indium tin oxide substrate and construction of biosensor based on localized surface plasmon resonance

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ABSTRACT

An effective strategy for the immobilization of coupled gold nanoparticles (CGNPs) on an indium tin oxide (ITO) glass substrate without using any peculiar binding molecules was investigated by applying a seed-mediated growth method. The effects of preparation conditions, such as immersion time, growth time, and temperature, were examined. The connectivity of gold nanoparticles was analyzed by extinction spectroscopy and scanning electron microscopy. Under optimal experimental conditions, the CGNPs occupy a dominant position on the ITO substrate surface with an aspect ratio of 2.5 ± 0.4. The CGNPs display a transverse and a longitudinal localized surface plasmon resonance (LSPR) band in the extinction spectrum. Taking the advantages of this assembling manner, the resulting CGNP-based LSPR sensor demonstrated rather high stability and refractive index sensitivity. The sensing performance of the sensor was studied using biotin–streptavidin as a model. The wavelength shift in the longitudinal band establishes a good relationship with the streptavidin concentrations. All the results demonstrate the reliability of the CGNP-based LSPR sensor and the feasibility of this assembling strategy.

1. Introduction

It is well known that localized surface plasmon resonance (LSPR) is the collective oscillation of the free electrons in metal nanoparticles [1,2]. The LSPR of noble metal nanoparticles can exhibit peaks at visible-NIR wavelengths, and their LSPR peaks generally red-shift as the refractive index of the surrounding environment increases. The LSPR wavelengths of noble metals are highly sensitive to the refractive index of surrounding media, which forms the basis of LSPR sensing [3–7]. Gold nanostructures are excellent candidates for the fabrication of LSPR-based biosensors due to their biocompatibility and higher chemical stability.

According to the Mie theory [3], the shape and size of the particles play important roles in the LSPR extinction spectrum. Chen et al. [8] demonstrated a systematic study on gold nanoparticles with different shapes and sizes, including nanospheres, nanocubes, nanobranches, nanorods, nanostars and nanobipyramids, to investigate the response of their surface plasmon peaks to the refractive index of the surrounding medium. The refractive index sensitivities were found to be dependent on both the shape and the size of the gold nanoparticles. Because of this, many researchers have dedicated their attention to fabricating highly sensitive nanoparticle-based LSPR biosensors by using various nanoparticle morphologies, such as nanospheres [9–14], nanorods [15–19], nanobipyramid [20], and nanoislands [21,22]. There are some nice reviews in the literature related to LSPR-based biosensors. For example, Szunerits and Boukherroub [23] discussed the different methods used to fabricate plasmonic nanosensors. Polavarapua and Liz-Marzan [24] summarized the development in the fabrication of flexible nanoplasmonic devices for sensing applications. However, most of the assembling methods are based on attaching nanoparticles to the substrate using binding agents, such as thiols and polymers. Disregarding the tediousness of the immobilization procedures and the effects due to the nature of the attachment, the long-term stability of the assembled nanostructure films will not always be satisfactory, for high stability is crucial to the application of a biosensor. Therefore, the challenge exists in constructing an assembly method that will overcome the technological barrier and meet all the criteria. Our team previously proposed a simple and low-cost strategy for the fabrication of a label-free LSPR biosensor based on electrodeposition of twin-linked or connected gold nanoparticles onto indium tin oxide (ITO) glass surface without any template or surfactant [25,26]. The twin-linked gold nanoparticles display a transverse and a longitudinal LSPR band in the

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extinction spectrum, which is similar to that of gold nanorods. With the connecting of gold nanoparticles more than two particles linked together, the longitudinal band red-shifts, and the refractive index sensitivity is increased. The resulting "clean" surface of the twin-linked gold nanoparticles easily allows for further modification and applications.

In this work, we proposed an effective strategy for assembling coupled gold nanoparticles (CGNPs) on the ITO substrate surface by a seed-mediated growth method, without using any peculiar binding molecules. The aggregation of gold nanostructures was examined by extinction spectroscopy and scanning electron microscopy. Furthermore, we demonstrated that CGNP-based LSPR sensors assembled in this assembling manner yielded advantages that include increased stability and higher sensitivity.

2. Experiment

2.1. Materials and apparatus

Tetrachloroauric acid trihydrate ($HAuCl_4{\cdot}3H_2O$), hexadecyltrimethylammonium bromide (CTAB), trisodium citrate, sodium borohydride ($NaBH_4$), and L-ascorbic acid (AA), dimethyl sulfoxide (DMSO) were all purchased from Sinopharm Chemical Reagent Co., Ltd. Biotin ((+)-biotin N-hydroxysuccinimide ester) and (3-aminopropyl)-trimethoxysilane (APTMS) were purchased from J&K Chemical Ltd. (Beijing, China). Streptavidin was purchase from Beijing Biosynthesis Biotechnology Co., Ltd. All other chemicals were analytical grade. Double-distilled water was used throughout the experiment. ITO glass (1.1 mm thickness, 100 Ω resistance) was purchased from Suzhou NSG Electronics Co., Ltd. (Suzhou, China).

The extinction spectra of the nanoparticles were recorded using a TU-1810 spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.) at room temperature. The morphology of the assembled CGNPs was characterized with an S-4700 scanning electron microscope (Hitachi, Japan) at an accelerating voltage of 15 kV.

2.2. Assembly of CGNPs on ITO substrate surface by seed-mediated synthesis

The seed solution, containing approximately 55 nm gold nanosphere particles, was prepared by mixing 45 mL of water with 1.25 mL 0.01 M trisodium citrate and 1.6 mL 0.01 M $HAuCl_4$, and then 1.25 mL fresh ice-cold 0.1 M $NaBH_4$ aqueous solution was added. The colloidal solution was stirred for an additional 5 min and left undisturbed for 2 h at 25 °C, and then the seed solution was stored in a dark bottle for over 24 h before use. Under these conditions, the nanoparticles are stable for several months.

The growth solution was prepared by mixing 5 mL of 0.1 M CTAB, 0.14 mL of 0.01 M $HAuCl_4$, and 0.285 mL of 0.1 M AA.

Prior to construction, the ITO glass substrate (5.0 cm × 0.5 cm) was ultrasonically cleaned in acetone, ethanol, and distilled water for 15 min, followed by drying with a stream of nitrogen. The modification of the ITO surface was done in two steps using a seed-mediated growth synthesis. In the first step, the clean ITO substrate was immersed in a gold seed solution for a certain amount of time at 25 °C. Following the seeding procedure, the substrate was immersed in the growth solution at a controlled temperature for a certain amount of time, and then the substrate was taken out of the solution and thoroughly rinsed with distilled water, and dried with nitrogen.

2.3. Measurements of extinction spectra

The extinction spectra of various gold nanostructures were recorded against bare ITO glass as a reference. To measure the effect of solvent medium, about 3 mL of individual solvent were placed in a quartz cuvette, in which the CGNPs/ITO substrate was inserted along the inner wall. The extinction spectra were recorded against a bare ITO substrate immersed in the same solvent, as a reference. After each measurement in the presence of a solvent, the cuvette and CGNPs/ITO substrate were washed and dried.

2.4. Preparation of biotin-functionalized CGNPs/ITO and streptavidin binding studies

The APTMS sol–gel preparation was following a sol–gel preparation method [27]. Briefly, 400 μL of APTMS and 332 μL of 0.1 M HCl were added into 33 mL of water. The solution was vigorously stirred for at least 1 h. The sol–gel solution shall be used the same day.

A piece of CGNPs/ITO glass was first left in APTMS sol–gel solution for 20 min, and then it was immersed in 40 μg/mL biotin solution for 1 h (biotin was dissolved in DMSO to a concentration of 1 mg/mL and this was diluted with PBS to 40 μg/mL). Those biotins that were only loosely retained were removed by rinsing with water. The surface modification process of the CGNPs/ITO was shown in Scheme 1. The biotin-CGNPs/ITO substrate was treated with different concentrations of streptavidin for 0.5 h at room temperature. After the incubation, the glass substrate was washed with water several times to remove unbound streptavidin. It was then used to detect the red shifts of the longitudinal LSPR band.

3. Results and discussion

3.1. Morphology of CGNPs on ITO substrate and the LSPR extinction spectrum

The quality and density of gold nanostructure film on ITO substrate surface were confirmed by scanning electron microscopy (SEM) (Fig. 1(A)). As can be seen, the CGNPs predominantly occupy the surface, the average particle size is 120 ± 5 nm, and the aspect ratio of the CGNPs is 2.5 ± 0.4. Fig. 1(B) is the corresponding extinction spectrum of CGNPs on an ITO substrate. Because the chain structure of CGNPs is similar to that of gold nanorods, similar LSPR bands with two distinct extinction peaks are typically observed: a peak near 535 nm and another at 745 nm. Gold nanospheres, which inevitably grow on the ITO substrate, also have a peak at around 535 nm. Hence, the differences in relative intensity exhibited between the two peaks are due to the proportion of isolated spheres and coupled nanoparticles. It should be noted that the longitudinal band in the LSPR extinction spectrum was sharper and more intense, which indicates that the resulting LSPR sensor could achieve good analytical performance.

3.2. Effects of seed-mediated method on fabrication of CGNP film

The direct growth of CGNPs on the ITO substrate surface uses the seed particles that are absorbed on the surface of the ITO substrate, and then reducing agents promote anisotropic growth [28,29]. The size, density, and distribution of the particles mainly depend on the competition between seed particles and anisotropic growth. By carefully controlling the growth conditions, the aggregation of CGNPs on ITO substrate surface was controllable.

3.2.1. Influence of immersion time in seed solution

Fig. 2 shows the effect of varying the immersion time of the ITO substrate in the seed solution (all at the same growth time of 20 h). It can be see that when immersing for 0.5 h, the longitudinal peak occurs near 745 nm (curve a). When the immersion time is increased, the longitudinal peak becomes enhanced. When the immersion time reached 1 h, the intensities of the transverse