Facile Synthesis of Au Nanoparticles Supported on TiO$_2$ Inverse Opals with Biosensor

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Abstract. In this paper, a three-dimensional porous structure of TiO$_2$ inverse opals loaded the large area gold nanoparticles (AuNPs) was used as an advanced material for detection of L-cysteine and Avidin molecule concentrations. We think that functionalized gold nanoparticles combined with TiO$_2$ inverse opals of low cost can couple out a characteristic reflection peak. After biomolecules were successively linked to the gold particles supported on TiO$_2$ inverse opals, it is found that the peak position linearly red shifted with a change of avidin concentration. The lower limit of detection concentration can be up to 2x10$^{-7}$M. This maybe provide a kind of biosensor for detecting biomolecules in the future.

1. Introduction

In the past decades, gold nanoparticles have attracted much attention due to their excellent physical[1] and chemical[2] properties, including the large specific surface area, excellent biocompatibility, unique optical properties and various applications[3, 4]. The available diameter range of AuNPs is from one to more than 120 nm, and visible absorption of their plasmon bands can be observed easily[5, 6]. Based on the excellent characteristics, AuNPs has been used in the detection of variety of target analytes including metal ions[7], organic molecules[2], proteins[8], and nucleic acids[9]. But most of them used the colorimetric method to achieve the purpose of sensing[10]. Moreover, AuNPs can offer a suitable platform for versatility with a wide range of organic or biological ligands for the selective binding and detection of small molecules and biological targets[2, 11, 12].

However, there are few methods reported about biosensor detection via titanium oxide inverse opals. As far as we know, the local surface plasmon resonance (LSPR) of gold nanoparticles due to the collective oscillation of surface electrons can respond to visible light and has great potential to extend the wide-bandgap light absorption range of semiconductors. Simultaneously, incident light in the photonic band gap (PBG) wavelength range can’t propagate in the TiO$_2$ inverse opals and can modulate its PBG due to Bragg diffraction and scattering[1, 13, 14]. Based on the unique structure and material of the TiO$_2$ inverse opals, it maybe provides a broad application space for biosensor detection.

In order to increase the sensitivity of the detection and decrease the cost of sensor, we attached more gold nanoparticles to the inside and outside surface of the three-dimensional porous TiO$_2$ inverse opals. And we found that the reflection peak under the combined action of gold nanoparticles and titanium...
oxide may be used to detect biomolecules. Simultaneously, this view is confirmed by FDTD simulation and experimental results. Therefore, we proposed a novel and convenient approach to detect biomolecules amounts of L-cysteine and Avidin based on the structure of TiO₂ inverse opals loaded gold nanoparticles.

2. Experimental Section

2.1. Chemicals and Solutions
H AuCl₄·H₂O stock (Tianjin Chemical Plant, China), Polyvinyl Pyrrolidone (PVP, China), Sodium phosphate monobasic (Aladdin), Sodium phosphate dibasic dodecahydrate (Aladdin), Avidin from egg white (Aladdin), L-Cysteine (MACKLIN). All the experimental utensils were soaked with detergent for 48 hours, scrubbed with cotton balls, ultrasonicated with deionized water for 30 min twice, then ultrasonically spun with alcohol for 30 min twice, and blown dry with a hair dryer in turn.

2.2. Synthesis of TiO₂ Inverse Opals
The colloidal crystal templates were fabricated using polystyrene colloidal spheres via vertical self-assembly method (figure 1(a)). Firstly, the clean indium tin oxide (ITO) glasses were put vertically into the weighing bottles with 10mL water solution containing 0.25% colloidal spheres with diameters of 181nm, 238nm, 302nm and 372nm. Then the weighing bottles were placed in an incubator setting at 55°C for 5 days. Then, three dimensions polystyrene colloidal crystals covered on ITO glass substrate was shown in figure 1(b). Next, TiO₂ layers were deposited on the prepared colloidal crystal templates using the thermal ALD cycles system. The oxidant precursor (H₂O) and the titanium precursor (titanium tetrachloride) were pulsed for 0.03s, held in the chamber for 4s, purged with nitrogen for 60s, and then evacuated for 20s, respectively[15]. After 100 cycles of ALD, the prepared PS/TiO₂ opals (figure 1(c)) were placed in calcine setting at 400°C for 4h. Finally, polystyrene spheres were removed, and three dimensions orderly arranged TiO₂ inverse opals were shown in figure 1(d)[16].

2.3. Aunps Loaded to TiO₂ Inverse Opals
AuNPs were loaded in the TiO₂ inverse opals inside and outside surface via hydrothermal stirring redox approach. The TiO₂ inverse opals were attached to the wall of the reaction beaker with about 80ml deionized water. Then the reaction beaker was placed on the heating agitator setting at 1500 r/min and 70 °C. 2mL HAuCl₄ solution (0.02 M) was quickly put in the mixture of 2 mL NaBH₄ (0.1M,0.15M,0.2M) and 0.5g PVP[17]. After the hydrothermal reduction process about 10 minutes, the samples were washed with deionized water, cooled down to room temperature, and dried in air. Generally speaking, PVP can enhance the dispersion of gold nanoparticles to prevent their agglomeration. Therefore, a large number of gold nanoparticles were uniformly distributed on the inside and outside surface of TiO₂ inverse opals (figure 1(e)).

2.4. Biomolecules Modification to Aunps
On the other hand, L-cysteine has a thiol group, which functions as a reducing group and is not only a targeting group for this compound but also for many bio-thiol compounds[10]. L-cysteine has a thiol group, which functions as a reducing group and is not only a targeting group for this compound but also for many bio-thiol compounds. And we took the advantage of the fact that gold nanoparticles readily were bound to thiol group. The prepared samples were placed in L-cysteine solution of
different concentrations (100uM, 1Mm, 10mM), respectively. The pH of the solution was adjusted to about 6.0 by using 0.2M phosphate buffered solution (PBS). Next, reaction vessel was placed in a vacuum circulation system for 16h to prevent oxidation of biomolecules. After self-assembly of cysteine molecules on the surface of gold nanoparticles, excess L-cysteine solution from the surface of the sample was slowly removed with a phosphate buffer. Then, Avidin solution of different concentrations were put in the reaction vessel for 30min[18]. Finally, a new kind biosensor for detection was shown in figure 1(f).

2.5. Biomolecules Modification to Aunps

The morphology and elemental analysis of the samples were characterized by high-resolution scanning electron microscopy (SEM, ZEISSG300) and Energy Dispersive Spectrometer (EDS), respectively. The spectra of the samples were characterized by Fiber optic spectrometer and UV-310 absorption.

Figure 1. (a) Ps microspheres were deposited on the ITO glasses by vertical self-assembly method. (b) PS microsphere template obtained after 5days. (c)TiO$_2$ layers were deposited on the prepared colloidal crystal templates by a thermal ALD system. (d)TiO$_2$ inverse opals obtained by removing PS microspheres. (e)AuNPs were loaded on the surface of TiO$_2$ IO inside and outside in hydrothermal stirring redox approach. (f)Different kind of molecules were linked on the surface of AuNPs

3. Result and Discussion

3.1. Characterization of TiO$_2$ Inverse Opals and TiO$_2$ Modified with Au NPs

Figure 2(a) shows SEM image of titanium oxide inverse opals structure. The thickness of TiO$_2$ layer was controlled with ALD cycles and measured by the stylus profiler as 0.58 Å/cycle. It can be observed that TiO$_2$ inverse opals structure maintained well after the hydrothermal reduction process. The thickness of the TiO$_2$ inverse opals structure was about 4.5um, and the average diameter of the TiO$_2$ hollow spheres was approximately 92% of the original size of the polystyrene spheres[15]. AuNPs of the average diameter approximately 3-10 nm were uniformly distributed and anchored along the outside (figure 2(b)) and inside (figure 2(c)) surface of the TiO$_2$ hollow spheres. The important thing is that the surface density of AuNPs was estimated around $4\times10^{10}$ cm$^{-2}$. Figure 2(d) shows the SEM images of different molecules modified AuNPs. The smoother samples surface covered with a layer of molecules become rough.
3.2. The Spectra Analysis of TiO$_2$ IO and TiO$_2$ Modified with Au NPs

The prepared gold nanoparticles sol by experiment was cooled, and the absorption spectrum (figure 3(a)) of gold nanoparticles solution in cuvette was measured by UV-310IPC absorption spectrometer. It is found that the so-called plasmon resonance band (PRB) observed approximately 530 nm with changes of gold nanoparticles diameter[2]. Simultaneously, different sizes gold nanoparticles sol was dropped on the ITO glasses. And the reflectance spectra (figure 3(b)) peak about 600nm has a red shift compared with the absorption spectrum. We obtained the reflectance spectra (figure 3(c)) of TiO$_2$ inverse opals (red line) and TiO$_2$ inverse opals loaded gold nanoparticles (black line) by FDTD simulation calculation. The peaks in the reflectance spectra are near 655nm, and TiO$_2$ inverse opals loaded gold nanoparticles is not very different compared with TiO$_2$ inverse opals. Simultaneously, we obtained the reflectance spectra (figure 3(d)) of TiO$_2$ inverse opals (red line) and TiO$_2$ inverse opals loaded gold nanoparticles (pink line) via experiment. And we can see the AuNPs LSPR peak near 606nm, and TiO$_2$ inverse opals photonic band gap is near 655nm. From the reflection spectrum of experiment and simulation, it can be seen that the reflection peak position basically matched.
Figure 3. (a) The absorption spectra of different size AuNPs sol. (b) The reflectance spectra of different size Au NPs. (c) The reflectance spectra of the samples were obtained via FDTD simulation. (d) The reflectance spectra of the samples were obtained via experiment.

3.3. Biomolecules Modification to AuNPs

The position of the sample for observation under the SEM was shown in figure 4(a). From the elemental analysis spectra figure 4(b), we can see the characteristic elements in need for spectra analysis. The content of sulfur element of L-cysteine biomolecules can be observed from the EDS map figure 4(c), which confirmed that the L-cysteine molecules were linked to the surface of gold nanoparticles by experiment.

Figure 4. (a) The SEM image of the sample for observation. (b) Element energy spectrum. (c) Element content spectrum
Figure 5. (a) The reflectance spectra of TiO$_2$ inverse opals loaded AuNPs in the H$_2$O (FDTD Simulation) or PBS (experiment). (b) The reflectance spectra of samples changes with L-cysteine solution concentration. (c) The reflectance spectra of the sample changes with Avidin solution concentration. (d) The shift of the characteristic peak position has a linear relationship with the changes of Avidin concentration.

Figure 5 shows the reflectance spectra of different molecules modified to the sample in turn. Through the FDTD simulation, we found that the reflection peak of TiO$_2$ inverse opals loaded with gold nanoparticles was 820 nm when it is placed in water. However, the reflection peak of TiO$_2$ inverse opals loaded with gold nanoparticles was 720 nm via experiment when it is placed in PBS solution. And the reflected peaks in figure 5(a) have a red shift compared to figure 3(d). When the samples were placed in the medium environment of the solution, we found that the local plasmon peak in the spectra of the gold nanoparticles is not obvious. And the activity of gold nanoparticles will gradually decrease due to biomolecules modified to the surface of gold nanoparticles [10]. We believe that the gold nanoparticles and the TiO$_2$ inverse opals are coupled to form a wide reflection peak. And the reflected characteristic peak changes with biomolecules concentration. The reflectance spectra of different concentration L-cysteine modified to AuNPs via vertical self-assembly was shown in figure 5(b). The binding of different concentrations L-cysteine causes the spectrum to move weakly, but the reflectance increases with concentration increasing. Then the reflectance spectra of the prepared samples immersed into different Avidin concentration was shown in figure 5(c). And a red shift in the characteristic peak wavelength changes with Avidin solution concentration. Simultaneously, the reflectivity increases with Avidin concentration increasing. And we found that the minimum limit of detection can reach 200nM. The sensitivity of the detection is characterized by taking the logarithm of the concentration of biomolecules as the abscissa and the wavelength of the reflection peak as the ordinate. Changes in peak position with concentration are shown in figure 5(d). So that we can achieve the purpose of biomolecular detection as a sensor.

4. Conclusion
In summary, we have fabricated a large area uniformly arranged three-dimensional porous structure TiO$_2$ inverse opals loaded the AuNPs. L-cysteine and Avidin biomolecules were successfully modified to the AuNPs by forming a chemical bond in turn. And a red shift in the characteristic peak wavelength changed with Avidin solution concentration. Au nanoparticles supported on TiO$_2$ inverse opals as a kind of biosensor can efficiently and rapidly be used to detect biomolecules.

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6. References

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