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PREPARATION OF NANOGOLD LABELED GOAT-ANTI-RABBIT IgG AND THEIR APPLICATION IN AN IMMUNOASSAY

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Abstract

Gold nanoparticles labeled by goat-anti-rabbit IgG have been synthesized and characterized. The UV-vis characterization reveals that the attachment of antibody to the gold nanoparticles did not bring about significant change. Making use of nanogold labeled goat-anti-rabbit IgG, we identified the adsorption of rabbit IgG on copper in rabbit uterus by electron back scattering diffraction (EBSD) scans and X-ray Photoelectron Spectroscopy (XPS) analysis.
Key words: Gold nanoparticle, goat-anti-rabbit IgG, EBSD, XPS

Introduction

Among the nanomaterials, gold nanoparticles (AuNPs) have been attracted considerable interest in analytical and biomedical fields by virtue of their facile synthesis, the large specific surface area, high chemical stability, favorable biocompatibility, high surface free energy, good conductivity, optical properties, catalytic applications and high affinity for binding to amine/thiol-containing molecules [1].

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Recent progresses of the AuNP-labeled antibody or antigen for immunoassay involve either direct binding of antigen-AuNP bioconjugates to an antibody modified surface or the exposure of an antibody-derived surface to free antigen and then to a secondary antibody-AuNP conjugate. A great number of nanocompsites of AuNPs, have been designed and synthesized as bioprobes for high sensitive detection [2,3] and biocatalysis [4,5].

The immunoassays were carried out by evaluation of AuNP tags in a standard mode of antigen measurement such as UV-vis absorption spectroscopy[6], plasma mass spectrometry [7], surface-enhanced Raman scattering [8], atomic force microscopy (AFM) [9], and surface plasmon resonance (SPR) [10]. These results demonstrated that the use of gold nanoparticles was favorable for the immobilization of antibody and could enhance the sensitivity of immunoassay.

The detection of protein and its action is important for biomolecular research. In complex multi-protein environments similar to the environment found in vivo, the adsorption of proteins has been studied by various techniques including quartz crystal microbalance [11], radiolabeling [12], and immunological gold labels identification [13]. Spectroscopy methods including Fourier transform infrared spectroscopy (FTIR) [14], X-ray photoelectron spectroscopy (XPS) [15], and electron spectroscopy for chemical analysis (ESCA) [16] have also been applied.

In this work, we made the nanogold labeled goat-anti-rabbit IgG and studied its stability. Using nanogold labeled immunological identification, we identified rabbit IgG adsorption on Cu-IUD after implanted in rabbit uterus by EBSD scans and XPS analysis.

Materials and methods

Materials

Gold colloids (10±5nm in diameter), 10mg/mL rabbit IgG and goat-anti-rabbit IgG antibody were obtained from Biodee Biotechnology Co. Ltd. (Beijing, China). Bovine Serum Albumin (BSA) and Phosphate Balanced Solution (PBS) were purchased from Sigma (USA). The chemicals, unless mentioned otherwise, were of analytical reagent grade and used as received. Aqueous solutions were prepared in double distilled water.

The copper tube implanted in rabbit uterus is that of TCu220C IUD product (Tianjin Medical Instrument Factory, China). The purity of copper was 99.99%.

Preparation of gold nanoparticle bio-probes

Nanogold labeled goat-anti-rabbit IgG was prepared according to the previous literature with slight modification [17]. PH of 20mL gold colloids was adjusted to 9.0 using Na_2CO_3 solution. The gold colloids were centrifuged with the velocity of 3000 r/min for 20min to remove the pellet. 2mL goat-anti-rabbit IgG was purified and adjusted to pH9.0, then added to the colloids solution. The mixed solution was shaken for 30 min before 250μL PEG (1%,w/w)was quickly added. After 15min 10% NaCl solution was added, whereas 5 min later PEG2000 was added, and the solution was concentrated at 4%. After incubation for 2h, the unbound IgG antibody and PEG were removed by repeated centrifugation and redispersion of the pellet. The IgG-conjugated

particles were then stored at 4°C in a solution containing 5mL PEG (0.05%,w/w), phosphate buffer (pH 7.0) and sodium azide (0.05%,w/w).

UV/vis spectra

The UV/vis spectrum was recorded at room temperature on a UV/vis 3310 **s**pectrophotometer (Japan) equipped with quartz cells.

In vivo assessment

Surgery

Five adult female New Zealand rabbit approximately 2.5kg in weight were used in the in vivo study. All rabbits were implanted with the same weight of copper tubes. The animals were generally anesthetized for surgery and the lower abdomen was scrubbed with iodine. A cut was scissored and the copper tube was implanted into the uterus. Then the cut was sewed. The rabbits were sacrificed 10 min after surgery**.**

IgG adsorption detection

After implanted in rabbit uterus for 10 min, the copper tubes were taken out respectively, rinsed with PBS (Sigma, USA) and immersed in 20 mg/mL BSA (Sigma, USA) at 37.0±0.1□ for half an hour. After taken out from BSA, the copper tubes were rinsed with PBS and then immersed in nanogold labeled goat-anti-rabbit IgG for half an hour at 37.0±0.1 ^{o}C. After taken out, rinsed with PBS and dried at room temperature, the copper tubes were analysed with electron back scattering diffraction (EBSD) and X-ray Photoelectron Spectroscopy (XPS) methods. EBSD analyses were carried out with the SUPRA55 field-emission (FE) scanning electron microscope (SEM) and were obtained using a 20kV acceleration voltage.

The copper tubes taken from the pack were immersed in 10mg/mL rabbit IgG solution (Biodee Biotechnology Co., China) at 37.0±0.1 ^{o}C for 10 min. Then the copper tubes were taken out, rinsed with PBS and immersed in 20mg/mL BSA solution at 37.0±0.1 ^{o}C for half an hour. After taken out and then rinsed with PBS, the copper tubes were immersed in nanogold labeled goat-anti-rabbit IgG for half an hour at 37.0±0.1 ^{o}C. After taken out, rinsed with PBS and dried at room temperature, the copper tubes were analysed with EBSD and XPS methods as positive control. The copper tubes after taken out from pack were analysed directly with EBSD and XPS methods as negative control.

Results and discussions

UV-vis characterization

The gold nanoparticles were treated by goat-anti-rabbit IgG solution, and the protein could easily adsorb onto the nano particles *via* electrostatic interaction. The UV-vis spectra of the gold nanoparticles were shown in Fig. 1. The unmodified gold nanoparticles in aqueous solution showed absorption maxima around 510 nm, which is attributed to the typical plasmon band of the nanoscale gold. After goat-anti-rabbit IgG modification, only a slight shift in the surface plasmon band, from 512 to 520 nm, was observed. The position of plasmon band is very sensitive to the size and surrounding environment of the gold nanoparticles. Fig. 1 reveals that the attachment of antibody to

the gold nanoparticles did not bring about significant change to either the position or the shape of the plasmon bands, indicating no obvious change of the nanoparticle was introduced.

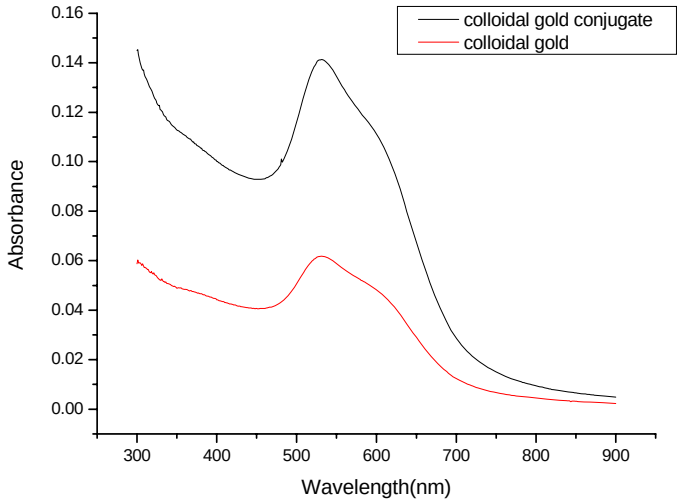


Fig.1. UV-vis spectra of unmodified gold nanoparticles and nanogold labeled goat-anti-rabbit IgG.

In vivo study

3.2.1 IgG adsorption identification

In vivo study, the adsorption of IgG on Cu-IUD implanted in rabbit uterus was identified by nanogold labeled immunological identification by EBSD scans and XPS analysis.

The nanogold conjugate could be functionalized with sulfo-Nhydroxysuccinimide ester, because the ester has reactivity with primary amines and therefore could be covalently coupled to the antibodies by a simple protocol. In this study, we utilized the properties of the gold label to visualize binding of the antibody to the protein antigen of interest. Nanogold labeled goat-anti-rabbit IgG would be bound to surface-adsorbed rabbit IgG and be identified by the presence of nanogold particles. Because gold has higher electron density than copper, nanogold particles appear as bright spots in the phase image. Fig. 2a,b show that the region containing IgG and conjugated nanogold particles shows bright spots, while in the BSA coated region bright spots could not be detected. By comparing Fig. 2a,b, it can be concluded that IgG protein exists in rabbit uterine solution and the protein can be adsorbed on the surface of implanted copper.

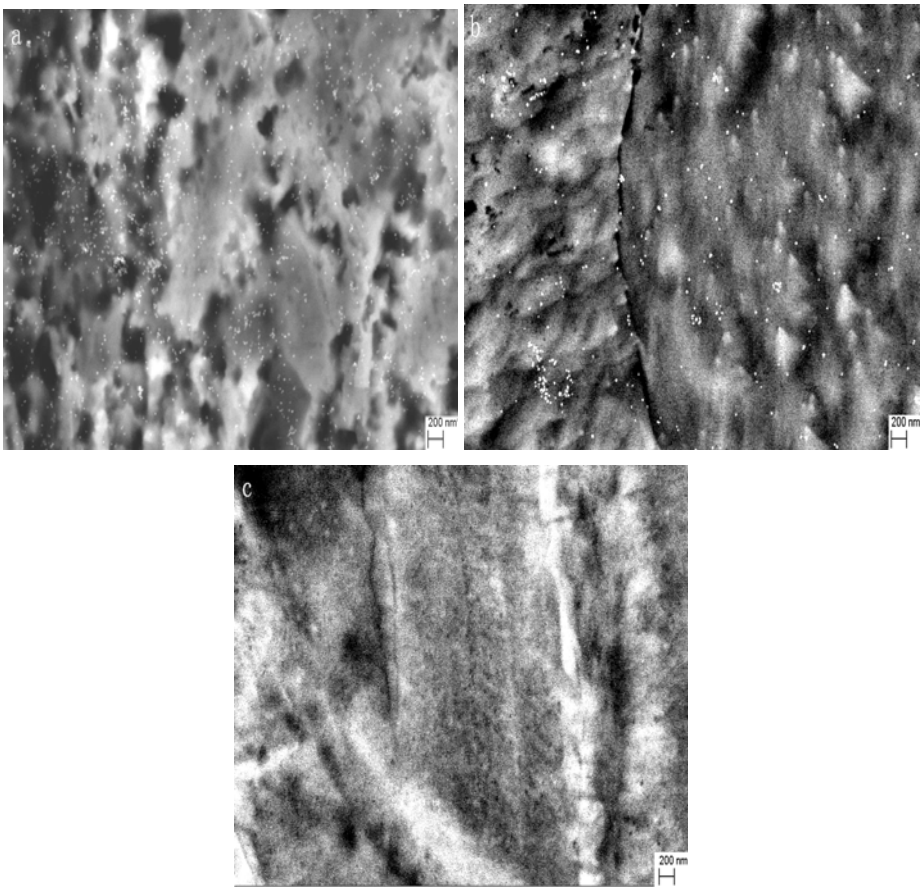
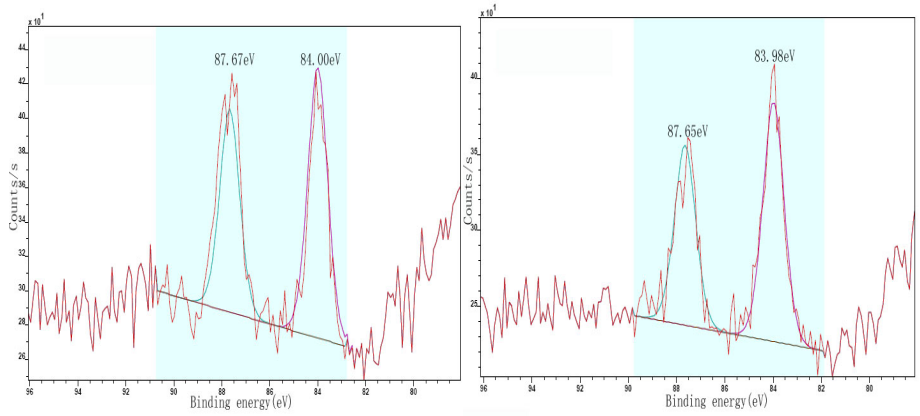


Fig. 2. EBSD scans of the copper tube（a）immersed in rabbit IgG solution for 10min; (b) implanted in rabbit uterus for 10min; (c) before immersion.

To further analyze the surface composition of copper surfaces, XPS characterization was employed. Fig. 3a,b show the Au4f core level spectrum on copper surface immersed in rabbit IgG solution and copper implanted in rabbit uterus for 10 min respectively. The XPS signature of the Au4f doublet (84.0 ± 0.03 eV and 87.7 ± 0.05 eV for the 4f7/2 and 4f5/2) results from the metallic gold Au0, which suggested the formation of nanogold labeled goat-anti-rabbit IgG. The spectrum intensity in Fig. 3a was higher than that in Fig. 3b. The different spectrum intensity between Fig. 3a and Fig. 3b indicated that the packing of nanogold particles on copper tube immersed in IgG solution was denser than that on copper tube implanted in rabbit uterus for 10 min.



(a) *(b)*

Fig. 3. XPS analysis for Au nanoparticles (a) for copper immersed in rabbit IgG solution for 10min; (b) for copper implanted in rabbit uterus for 10min

Conclusions

We prepared nanogold labeled goat-anti-rabbit IgG and compared them with the unmodified gold nanoparticle. It was found that the attachment of antibody to the gold nanoparticles did not introduce obvious change of the nanoparticle. Besides, we identified rabbit IgG adsorption on copper using nanogold labeled goat-anti-rabbit IgG by EBSD and XPS analysis and compared adsorption amount. The application of nanogold labeled technology in biomedical fields needs a further research.

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