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Impedance sensing of allergen–antibody interaction on glassy carbon electrode modified by gold electrodeposition

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Abstract

The interactions between the recombinant dust mite allergen Der f2 and murine monoclonal antibody were monitored by electrochemical impedance spectroscopy (EIS). Allergen Der f2 were immobilized through the nanogold formed by electrodeposition of gold on planar glassy carbon electrode. A 30-s gold electrodeposition provided a desirable substrate for the immobilization of allergen. Electrochemical deposition of gold on a glassy carbon electrode showed significant improvement in allergen immobilization. The impedance measurements were based on the charge-transfer kinetics of the $[Fe(CN)_6]^{3-/4-}$ redox pair. The interactions between allergen and antibody occurred on electrode surface altered the interfacial electron transfer resistance, R_{CT}, by preventing the redox species approaching the electrode. The results showed that R_{CT} increased with increasing concentration of monoclonal antibodies.

Keywords: Gold electrodeposition; Recombinant allergen; Antibody; Electron transfer resistance (R_{CT}); Electrochemical impedance spectroscopy (EIS)

1. Introduction

Type I allergic reaction, which is an immune disorder initiated by inhalant allergens, represents a health problem for approximately 30% of adults and up to 40% of children in western countries [1]. And with the development of industrialization, allergies are on the increase. This allergic reaction results in the release of biological mediators (as histamine and leukotrienes) due to the cross-linking of cell-bound immunoglobulin E (IgE) antibodies by allergen. Therefore, in recent years, allergen-specific IgE antibodies have received significant attention, both in industry and in academia. They may serve as probes in allergen identification and characterization based on the allergen-specific IgE interaction [2,3]. Besides the IgE antibodies, there are also productions of IgG antibodies during specific allergen immunotherapy or immunizing animals for monoclonal antibodies. These IgG antibodies are of high affinity to allergen, and may act as blocking antibodies interfering with the allergen-IgE interaction [4–6]. But the role of IgG antibodies in allergic reactions has remained controversial [7,8]. On the other hand, over the past 20 years, most important allergens from tree and grass pollens, mites, animal epithelia, insect venoms and food have been cloned, sequenced and expressed, and the development of recombinant DNA technology offers the possibility to produce recombinant allergic proteins [9–11]. Thus, it is highly significant to develop sensitive methods for monitoring the inadvertent presence of allergen, and further applied to diagnosis and therapy of allergic diseases.

The conventional methods for detection of allergen–antibody binding were mostly enzyme-linked immunosorbent assays (ELISAs), which required the use of enzymatic label [12,13]. This labeling process is time consuming and it may even alter the immunochemical activity of target biomolecules. Development of immunoassay methods that are capable of direct detection of allergen–antibody binding is most desirable. It has been proved that surface plasmon resonance (SPR) technique is a joice method for real-time observation of interacting biomolecules [14–16]. On the other hand, the development of direct (non-labeled) immunoassay method based on electrochemical techniques has attracted much attention [17]. The direct electrochemical immunoassay usually followed the measurement of potential, current, capacitance and conductivity changes due to the formation of immunocomplex (for example antigen–

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antibody complex) on the electrode surface [18–22]. Among these, electrochemical impedance spectroscopy (EIS) has been proved to be a sensitive and effective method to probe the interfacial properties of modified electrode [23–25]. Many research works have demonstrated that impedance spectroscopy has been widely used for monitoring immunological reactions [21,22,26–28].

A key issue in construction of an electrochemical immunosensor is the deposition of antigen or antibody molecules onto electrode surface in high amount and with the retention of their specific bioactivity. It has been reported that gold nanoparticle modification could largely increase the surface area of electrode and enhance the amount of immobilized antibodies [27–30]. However, in previous studies, gold nanoparticles were usually deposited on electrode surface through the bifunctional linkers. In our previous study, bifunctional linker (3-mercaptopropyl) trimethoxysilane (MPTS) was used for self-assembly of gold nanoparticles onto glassy carbon electrode surface [31]. Recently, nanogold electrodes obtained by directly electrochemical depositing gold nanoparticles onto the planar electrode surface were reported, and were applied for monitoring of biorecognition [32,33]. In this paper, we used nanogold electrodes formed by gold electrodeposition onto planar glassy carbon surface for sensing the interaction between recombinant dust mite allergen Der f2 and murine monoclonal antibody. The morphology of nanogold can be controlled to some extent by adjusting the concentration of deposition, deposition time or working potential. The immobilization of allergen and its binding to antibody were monitored by electrochemical impedance spectroscopy (EIS).

2. Experimental section

2.1. Reagents and materials

Recombinant dust mite allergen Der f2 and murine monoclonal antibody were kindly offered by Professor Liu (College of Life Science, Shenzhen University, China). $HAuCl_4 \cdot 3H_2O$ were purchased from Aldrich. $K_4[Fe(CN)_6]$, $K_3[Fe(CN)_6]$, phosphate buffer solution (PBS, pH 7.4) and other chemicals used were of analytical grade. All commercially obtained chemicals were used as received without further purification. All aqueous solutions were made using double distilled water, which was further purified to 18.2 MΩ with a 0.22 μm Millipore syringe filter.

2.2. Apparatus

Gold electrodeposition was performed with a CHI 832 (Shanghai, China). Electrochemical impedance measurements were carried out with an Autolab PG30 electrochemical analyzer system (Eco Chemie, Netherlands) with a FRA2 module. A three-electrode setup was employed throughout with an Ag/AgCl (saturated KCl) reference electrode, a pt flag as counter electrode, and a nanogold modified glassy carbon electrode as the working electrode. The field-emitted scanning electron microscopy (FE-SEM) was obtained from PHILIPS XL-30 ESEM with an accelerating voltage of 20 kV.

2.3. Gold electrodeposition

The planar glassy carbon electrodes were mechanically polished with 1.0, 0.3 and 0.05 μm α-Al_2O_3 powder successively, and washed ultrasonically in distilled water. The electrodes were dried under nitrogen flush, and then immersed into 0.1% $HAuCl_4$ solution containing 0.1 M KNO_3 as electrolyte, where electrochemical deposition was conducted at − 200 mV (vs. Ag/AgCl) by single potential mode.

2.4. Allergen immobilization and allergen–antibody reactions

Nanogold modified glassy carbon electrode was exposed to 0.2 ml 80 μg/ml recombinant dust mite allergen Der f2 solution in 20 mM pH 7.4 PBS. The electrode was incubated at 4 °C for overnight in allergen solution to ensure that the active sites of nanogold were all occupied by allergen molecules. The electrode surface loaded with allergen was thoroughly rinsed with phosphate buffer (pH 7.4) to remove the weakly absorbed allergen molecules. Then it was exposed to different concentration of murine monoclonal antibody solutions in 20 mM pH 7.4 PBS at room temperature for 60 min, rinsed with PBS and water to remove unbound antibodies before impedance measurements.

2.5. Electrochemical impedance measurement

The impedance spectra were recorded within the frequency range of 0.1 Hz to 100 kHz. The amplitude of the applied sine wave potential in each case was 5 mV, while the direct current (dc) potential was limited at the formal potential of the redox pair $[Fe(CN)_6]^{3-/4-}$ (0.23 V vs. Ag/AgCl). The electrolyte solution was 2.5 mM $K_4[Fe(CN)_6]$ + 2.5 mM $K_3[Fe(CN)_6]$ + 0.1 M KCl in 20 mM pH 7.4 PBS. Prior to each experiment, the electrolyte solution was bubbled with high-purity nitrogen for 20 min to remove dissolved oxygen.

3. Results and discussion

3.1. Morphology characterization of nanogold modified electrodes

Fig. 1 shows the FE-SEM images of nanogold modified glassy carbon electrodes obtained by electrodepositing at the potential of − 200 mV (vs. Ag/AgCl) in 0.1% $HAuCl_4$ for 30 s (A, B) and 150 s (C, D). The bare planar glassy carbon electrode surface was comparably smooth and no gold particles could be seen, which were not shown in Fig. 1. The formation of gold nanostructures on glassy carbon electrode surface could be adjusted by controlling the time of electrodeposition and choosing the concentration of deposition solution at certain applied potential [32,33]. Evidently, the SEM images shown in Fig. 1(A, B) revealed discrete spherical gold nanocrystals grown directly on the bare planar glassy carbon substrate. These spherical gold nanocrystals were mainly in a diameter range of 40 ± 8 nm. With extension of the electrodeposition time, a number of aggregated gold crystallites were observed. When the deposition time was reached to 150 s, as shown in Fig. 1(C, D), the discrete gold

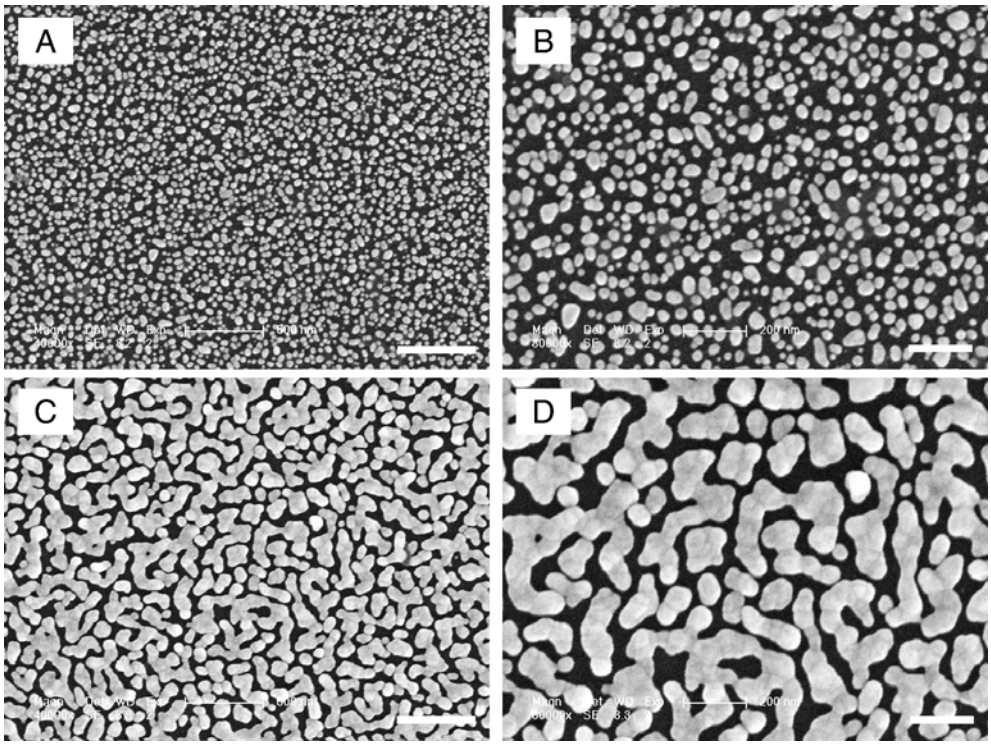

Fig. 1. FE-SEM images of nanogold aggregates electrodeposited onto glassy carbon electrode under 30 s (A, B) and 150 s (C, D) in 0.1% $HAuCl_4$. Electrodeposition potential: −0.2 V. The scale bar is 500 nm (A, C) and 200 nm (B, D) respectively.

nanocrystals were aggregated to larger continuous nanogold aggregates. Further increase of deposition time, we only observed a gradual augmentation in the size of these aggregates.

3.2. Immobilization of allergen Der f2 on modified electrodes

The resulted nanogold modified glassy carbon electrodes were immersed into the solution of recombinant dust mite allergen Der f2 to immobilize allergen molecules onto the electrode surface, and further used as the sensing bases for electrochemical impedance analysis of allergen–antibody binding. Of all the impedance studies using the electrodes modified by gold electrodeposition for various deposition time, we found that the electrode modified by 30-s gold electrodeposition, which led to better evenly dispersed gold nanocrystals with relatively uniform size are most desirable. This might be due to the fact that these discrete gold nanocrystals are capable of carrying more allergen molecules.

At the same time, we also investigated the effect of incubation time on the amount of immobilized allergen by EIS. Fig. 2 shows the effect of incubation time on impedance spectra during the immobilization of dust mite allergen Der f2 onto nanogold modified glassy carbon electrode. It could be seen that the electron transfer resistance (the diameter of semicircle as shown in the impedance spectrum [22,25,26]) gradually increased along with the incubation time, indicating that more and more allergen molecules were immobilized on the electrode. When incubation time was reached 11 h, further prolonged the incubation time, no obvious changes in impedance responses were observed. This indicated that allergen molecule immobilized on the electrode was almost saturated. Thus, in our present case, incubation time was selected for overnight (about 12 h) to make sure that all reachable active sites of nanogold were occupied by allergen molecules. On the other hand, this incubation time could also guarantee the reproduction of the results. Because, under a certain concentration of allergen solution, the surface coverage of allergen on nanogold modified electrode was controlled by the incubation time.

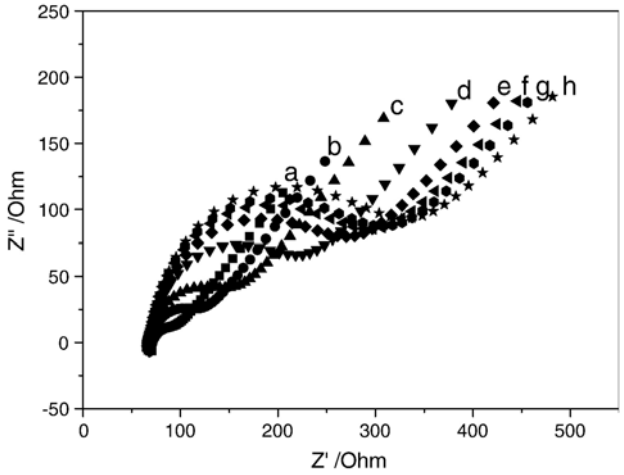

Fig. 2. Nyquist plots of the effect of the incubation time on the immobilization of dust mite allergen Der f2 onto nanogold modified glassy carbon electrode. Incubation time (h): (a) 1; (b) 2; (c) 3; (d) 5; (e) 7; (f) 9; (g) 11 and (h) 14. The impedance spectra were taken in 2.5 mM $K_4[Fe(CN)_6]$+2.5 mM $K_3[Fe(CN)_6]$+ 0.1 M KCl in 20 mM pH 7.4 PBS at 0.23 V (vs. Ag/AgCl) in the frequency range from 0.1 Hz to 100 kHz.

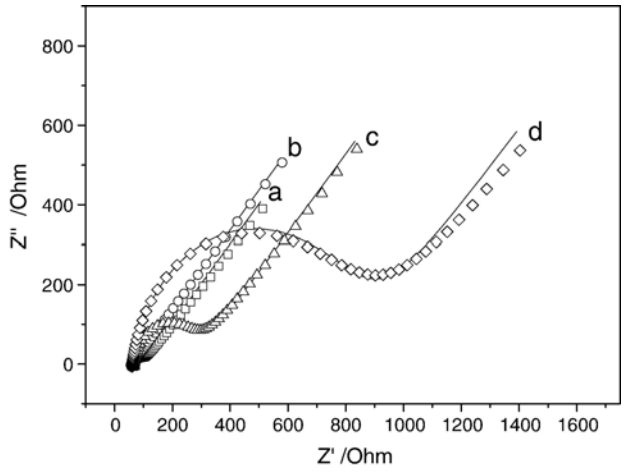

Fig. 3. Nyquist plots of electrochemical impedance spectra of modified glassy carbon electrode (GCE) recorded in a 20 mM PBS (containing 2.5 mM $K_4[Fe(CN)_6]$+2.5 mM $K_3[Fe(CN)_6]$+0.1 M KCl, pH 7.4) in the frequency range from 0.1 Hz to 100 kHz. (a) bare GCE; (b) nanogold/GCE; (c) allergen Der f2/nanogold/GCE; (d) monoclonal antibody/allergen Der f2/nanogold/GCE. Solid lines in the figure represent the fitted lines.

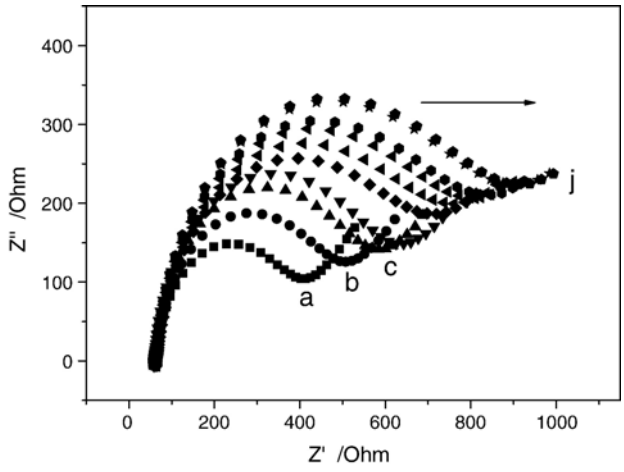

Fig. 5. Nyquist plots of electrochemical impedance spectra of allergen Der f2/nanogold/ glassy carbon electrode (GCE) after incubation with various concentrations of monoclonal antibody. The concentration of antibody (μg/ml): (a) 2; (b) 5; (c) 10; (d) 20; (e) 50; (f) 80; (g) 100; (h) 150; (i) 200; (j) 300. All the measurements were performed in 2.5 mM $K_4[Fe(CN)_6]$+2.5 mM $K_3[Fe(CN)_6]$+0.1 M KCl in 20 pH 7.4 mM PBS in the frequency range from 0.1 Hz to 100 kHz.

3.3. Impedance sensing allergen–antibody interaction

Compared to other electrochemical methods, impedance technique has the advantage that the system is investigated under stationary conditions, as opposed to the wide potential window used in CVs. It can provide more detailed information about the interfacial properties of surface-modified electrode. Fig. 3 shows the impedance responses of the $[Fe(CN)_6]^{3-/4-}$ redox probe in PBS on a bare glassy carbon electrode (curve a), the nanogold modified glassy carbon electrode (curve b), the recombinant dust mite allergen Der f2/nanogold/glassy carbon electrode (curve c) and the monoclonal antibody/allergen Der f2/nanogold/glassy carbon electrode (curve d) in the frequency range from 0.1 to 100 kHz. The impedance spectrum includes a semicircle portion at high frequencies corresponding to the electron transfer limited process and a linear part at the low frequencies resulting from the diffusion limited electrochemical process [22]. The diameter of the semicircle exhibited the electron transfer resistance of the modified layer, which showed its blocking behavior of the electrode. The increase or decrease in its value exactly characterized the modification of electrode surface. The modification of electrode surface with nanogold provided a biocompatible basis for the immobilization of allergen molecules onto electrode. Compared to bare glassy carbon electrode, significant differences in the impedance spectra were observed upon the immobilization of allergen and the binding of monoclonal antibody.

The impedance data were fitted with a commercial software Autolab data analysis (Eco Chemie, Netherlands). A modified Randles' equivalent circuit [34,35], shown in Fig. 4, was found to fit adequately the data over the entire measurement frequency range. The fitted curves were shown in Fig. 3 (solid lines), indicating the good agreement between the circuit model and the measurement system, especially in the higher frequency range. The circuit includes the following four elements: (1) the ohmic resistance of the electrolyte solution, R_{SOL}. (2) the Warburg impedance, Z_W; (3) $Q_{F/SOL}$, associated with the double layer, which reflects the interface between the assembled film and the electrolyte solution. This element can also be replaced by the

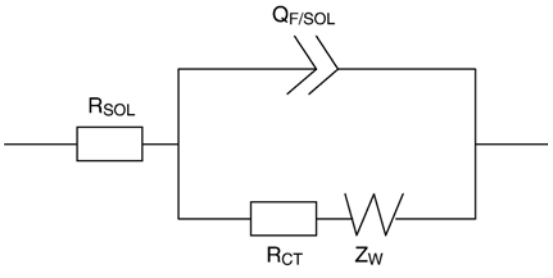

Fig. 4. Equivalent circuit applied to fit the impedance measurements in the presence of redox pair of $[Fe(CN)_6]^{3-/4-}$. R_{SOL}, the ohmic resistance of the electrolyte solution; $Q_{F/SOL}$, associated with the double layer capacitance; Z_W, the Warburg impedance; R_{CT}, the electron transfer resistance.

Table 1
Fitted impedance results to allergen–antibody interaction from Fig. 5

Concentration of antibody (μg/ml)	R_{CT} (ohm)	ΔR_{CT} (ohm)
2	217	94
5	406	183
10	480	257
20	528	305
50	604	381
80	644	421
100	687	464
150	715	492
200	773	550
300	782	559

R_{CT}, the electron transfer resistance associated with the interfacial electron transfer between modified layer and electrolyte solution, $\Delta R_{CT}=R_{CT(Allergen\text{-}Ab)}-R_{CT(Allergen)}$.

constant phase element impedance [36], Z_{CPE}. (4) the electron transfer resistance [37], R_{CT}. Ideally, Z_W and R_{SOL} represent the properties of the electrolyte solution and diffusion of the redox probe, thus they are not affected by modifications occurring on the electrode surface [38]. Negligible change in R_{SOL} was observed either during the electrochemical deposition of gold or the immobilization of allergen Der f2, or the subsequent coupling of monoclonal antibody, as shown in Fig. 3, which further demonstrated that the ohmic resistance of the solution was not affected by the modifications on the electrode surface. At the same time, as can be seen from Fig. 3, throughout the whole processes of electrode modification, the changes in R_{CT} were the most significant among other impedance components. Thus, R_{CT} was a suitable signal for sensing the interfacial properties of the modified glassy carbon electrode.

The increase or decrease in the value of R_{CT} was associated with the blocking behavior of the assembled layer on the electrode surface for the redox probe $[Fe(CN)_6]^{3-/4-}$, which reflected in the impedance spectroscopy as the increase or decrease in the diameter of the semicircle at high frequencies. Compared to bare glassy carbon electrode, the nanogold modified electrode not only provided a basis for immobilization of allergen, but also played a role similar to electron-conducting tunnel for electron transfer to the electrode surface. The value of R_{CT} increased after immobilization of allergen, this confirmed the success of immobilization of allergen Der f2 onto the electrode surface. A further increase in R_{CT} was obtained after exposing the electrode to the solution of monoclonal antibody. This increase was due to the reaction of allergen–antibody occurred on the electrode surface. The interaction of allergen and antibody resulted in the formation of allergen–antibody complex on electrode. This allergen–antibody complex acted as the inert electron transfer blocking layer, and hindered the diffusion of $[Fe(CN)_6]^{3-/4-}$ redox pair towards the electrode surface. As a result, the interfacial properties of the electrode were changed, thus the impedance response generated. The differences observed in the impedance responses for the monoclonal antibody/allergen Der f2/nanogold/GC electrode from its initial value were caused by the specific allergen–antibody interactions. This interaction between allergen and antibody is specific, because adding other monoclonal antibodies, which are not specific to the allergen, does not affect the impedance spectrum of the allergen Der f2/nanogold/GCE. During the process of immunizing animals for monoclonal antibodies, more than one type of monoclonal antibody was generally obtained. Some of these monoclonal antibodies were specific to allergen and could recognize different epitopes of the allergen [39]. When using these specific monoclonal antibodies, impedance changes similar to those reported herein were observed.

In order to evaluate the reaction between monoclonal antibody and allergen Der f2, we exposed the allergen Der f2/nanogold/GC electrode to various concentrations of monoclonal antibody. The corresponding Nyquist plots of impedance spectra were shown in Fig. 5, the fitting values of R_{CT} were presented in Table 1. The value of electron transfer resistance, R_{CT}, reflected as the diameter of semicircle in impedance spectrum, controlled the electron transfer kinetics of the redox probe at electrode interface, which relative to the concentration of antibody. From Fig. 5, one can see that the diameter of the Nyquist circle increased with increasing antibody concentration. This may due to the binding of more antibody molecules to immobilized allergen in higher concentration of antibody. Therefore, the interfacial electron transfer was retarded considerably, resulting in a corresponding increase in the electron transfer resistance.

In Table 1, the change of R_{CT} (ΔR_{CT}) is calculated by following equation:

$$\Delta R_{CT} = R_{CT(Allergen-Ab)} - R_{CT(Allergen)}$$

Where $R_{CT(Allergen-Ab)}$ is the value of the electron transfer resistance after monoclonal antibody coupled to allergen, while $R_{CT(Allergen)}$ is the value of the electron transfer resistance when allergen immobilized on the electrode. As can be seen, ΔR_{CT} increased with increasing antibody concentration within the detected concentrations of monoclonal antibody. While at higher concentrations of antibody, the increases of ΔR_{CT} were not obvious due to steric hindrance or saturation of coupled antibody molecules.

4. Conclusions

In this paper, nanogold modified glassy carbon electrodes were used as bases for sensing the interaction between recombinant dust mite allergen Der f2 and antibody by electrochemical impedance spectroscopy (EIS). Based on the present study, it proved that gold electrodeposition on a bio-inimicable substrate provided a friendly biocompatible surface for the immobilization of biomolecules in immunoassay. This gold electrodeposition method is more controllable, and no worry about the aggregation of nanoparticles. Furthermore, this study has led us to conclude that EIS is an effective method for sensing the reaction of antibody with allergen occurred on the electrode surface. The basic method used in this paper could also be applied to other immune systems.

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