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Short communication

An ultrasensitive squamous cell carcinoma antigen biosensing platform utilizing double-antibody single-channel amplification strategy

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ABSTRACT

A novel electrochemical immunosensor was developed for ultrasensitive detection of squamous cell carcinoma antigen (SCCA), which was based on the double-antibody single-channel amplification strategy. For the first time, human immunoglobulin antibody (anti-HIgG) was used as the supporting framework to amplify the loading quantity of SCCA antibody (anti-SCCA). In this strategy, SCCA can be detected without using mesoporous nanometers to amplify the signal. In addition, Pd icosahedrons were first used as the connecter to immobilize the antibodies and strengthen the sensitivity. Only one touch point exists under the limited condition between a sphere and another shape in geometry, thus the Pd icosahedron is an excellent candidate as the role of connecter. Gold nanoparticles (Au NPs) decorated with mercapto-functionalized graphene sheets (Au@GS) were synthesized as the transducing materials. The fabricated immunosensor exhibited an excellent detection limit of 2.8 pg/mL and wide linear range of 0.01–5 ng/mL. This kind of immunosensor would provide a potential application in clinical diagnosis.

1. Introduction

Squamous cell carcinoma antigen (SCCA) is a kind of tumor antigen TA-4 separated from a cervical squamous cell carcinoma (SCC) (Takeuchi et al., 2003). The morbidity of cervical SCC increases from 12% to more than 90% with elevated circulating levels of SCCA (Kato, 1992). It is regarded as a sensitive tumor marker in patients with cervical cancer. Until now, several techniques have been developed in its detection, such as common sandwich immunosensor (Wu et al., 2013), enzyme-linked immunosensor assay (Erickson et al., 2010), and chemiluminescence immunoassay (Li et al., 2014b). All of these methods employ mesoporous materials to amplify signals with complex processes. Thus, a new strategy for the marker detection is necessary in the clinical test and therapeutic evaluation.

Immunoassay is an effective method in tumor marker detection (Ren et al., 2014a, 2014b). But the amplification strategy is mainly based on using the mesoporous nanomaterials (Lei and Ju, 2012; Wu et al., 2012; Zhang et al., 2011a) to enlarge the capacity of biomolecules and enhance signals (Bi et al., 2013; Jeong et al., 2013; Qu et al., 2014).

In this research, the double-antibody single-channel immunosensor was fabricated to realize the marker detection, which employed human immunoglobulin antibody (anti-HIgG) instead of mesoporous nanomaterials acting as the supporting framework to amplify the loading quantity of SCCA antibody (anti-SCCA) for the first time. In this strategy, the immunosensor would be able to achieve excellent properties by the following reasons. First, the immuno-active materials own better recognition capability to ensure the sensor specific recognition (Kiefel et al., 1987). Second, biomolecules have prominent hydrophilcity to form homogeneous solution than nanomaterials (Cheng and Rossky, 1998). Third, the size of antibody or antigen is about 10–15 nm which is much smaller than that of 100 nm (the traditional size of nanomaterials applied in immunosensor), so the nanomaterials (large scale) may not obtain a satisfied result due to an inadequate adsorption. Several smaller sized nanomaterials have been applied in the immunosensor in recent years (Feng et al., 2012a). Thus, the double-antibody single-channel immunosensor is an excellent candidate method to detect tumor markers.

In this research, gold nanoparticles (Au NPs) decorated with mercapto-functionalized graphene sheets (Au@GS) were

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synthesized to increase the fixing quantity of anti-HIgG. Pd icosahedrons were first used in immunosensor to amplify the antigen detection quantity. In geometry, typically, only one touch point exists between a sphere and another shape under the limited condition. In the area of immunosensor, spherical materials (such as noble metal, mesoporous materials, quantum dots) have been applied in the sensor frequently (Feng et al., 2012b; Li et al., 2014a; Zhang et al., 2011b). However, the point-of-touching may exhibit lower binding force than the spot-of-touching. The Pd icosahedrons own twenty spots and every spot may contact the biomolecules more tightly than the point-of-touching of nanosphere.

2. Experimental

In this work, mercapto-functionalized graphene sheets were synthesized to capture more Au NPs. And the testing system was three-electrode configuration (ESI†). The glassy carbon electrode (GCE) was polished with a series of alumina powders (1, 0.3, 0.05 μm) and washed with water. Afterwards, the Au@GS modified electrode could adsorb more anti-HIgGs to protect and strengthen the framework and enlarge the capacity of HIgG. Once BSA blocked the non-specific binding sites (other binding sites other than HIgG), HIgG would be incubated on the anti-HIgG subsequently. Then, a certain proportion of antibodies (anti-HIgG: anti-SCCA=2:8) were incubated with Pd icosahedrons. The antibody incubation compound anti-HIgG@Pd@anti-SCCA (Ab_S@Pd@Ab_H) was blocked with BSA as well. And Ab_S@Pd@Ab_H was incubated on the former layer. After the SCCA was captured on the electrode, the sensor was fabricated well (Fig. 1) (detailed in ESI†). The signal could be achieved by the solution of $K_3Fe(CN)_6$ (4 mM) and KNO_3 (0.1 M).

3. Results and discussion

GS–SH is synthesized according to a reported method (Wang et al., 2014) with some modification for the first time in this strategy (ESI†). The Au NPs are connected on the GS–SH sufficiently. From Fig. 2A, it can be clearly seen that the GS is silk-like and the morphology is straticulate. Au@GS is shown in Fig. 2B. In the margin and middle of the GS, there exist some brilliant points

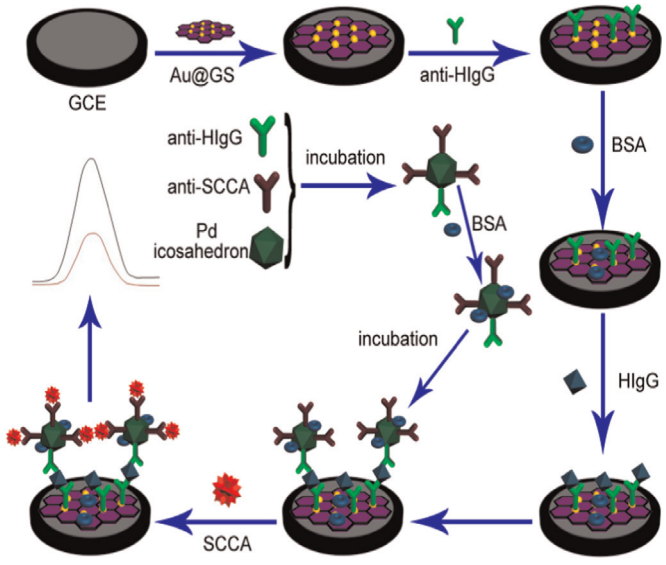

Fig. 1. Fabrication of the double-antibody single-channel immunosensor for SCCA detection.

(a, c) and relative tint points (b, d). These points are all Au NPs indicating the GS is straticulate and the Au NPs are connected on GS uniformly.

Pd icosahedrons are used as the connecter between anti-HIgG and anti-SCCA. And the preparation of Pd icosahedrons is shown in ESI†. Fig. 3A is the SEM image of Pd icosahedrons at low magnification. It displays that the Pd nanoparticles are dispersed well and the size was uniform. Fig. 3B is the TEM image of Pd icosahedrons, and it can be seen obviously that the icosahedron was synthesized well compared with the inset of Fig. 3B which is an icosahedron in 3D image.

The size of Pd icosahedrons is about 40 nm in diameter and the antibody is about 10–15 nm. This is a balanced and appropriate size, indicating the combination of Ab_S@Pd@Ab_H was effective. A Rubik's cube theory is proposed and that may be explained as follows. First, the connecter (Pd icosahedron) is in the same order of magnitude with the antibodies in size, and the incubation (Ab_S@Pd@Ab_H) can be prepared well like a 64 Rubik's cube (ESI†, Fig. S2). The inside middle box (8 cubic) is like the Pd icosahedron, and the outside 56 cubes are like the antibodies, which can realize many times amplification. Second, if the connecter is smaller than the size of antibody (the connecter and antibody are not in a comparative size), the antibodies cannot be combined well on the particles due to the limitation of size. Third, if the connecter is much larger, for example, over a hundred nanometers, the connecter is many times larger than antibody in size which may leave over many nonspecific active sites resulting in the inaccuracy of the results. In addition, much larger particles may not exhibit a better testing result due to the bad film-forming property. Thus, the Pd icosahedron is the better candidate as a connecter in the double-antibody single-channel amplification strategy.

In this research, a SCCA sensor was developed. $K_3[Fe(CN)_6]$ was used as the signal source. In order to realize better detecting effect, optimization of the experimental conditions was conducted.

The ratio of antibodies, pH values, concentration of base solution ($K_3[Fe(CN)_6]$) and concentration of base material (Au@GS) were investigated in this study (ESI†, Fig. S3). The obtained results are anti-HIgG: anti-SCCA=2:8, pH=7.4, $C_{K3[Fe(CN)6]}$=4 mg/mL, $C_{Au@GS}$=1.5 mg/mL respectively.

Under the optimum conditions, immunosensor was used to detect different concentrations of SCCA. The square wave voltammetry data were shown in Fig. S4 (ESI†). It was shown in Fig. 4 the analysis and detection of SCCA with prepared sensor were satisfactory. It worked well over a broad liner range of 0.01–5 ng/mL with a low detection limit of 2.8 pg/mL at a signal-to-noise ratio of 3σ (where σ is the standard deviation of a blank solution, n=11). The equation was ΔI=3.22 log C+7.96, r=0.9968.

The selectivity of the sensor plays an essential role in the analysis of biological samples. Several other tumor markers were detected in the selectivity research. As shown in Fig. S5 (ESI†), the SCCA-free immunosensors (20 ng/mL CEA, AFP and CA125 respectively) exhibited the similar current change which approached to zero. The other four sensors exhibited similar current change which indicated the sensor was not disturbed by other tumor markers. The selectivity of the immunosensor was acceptable.

Stability is another considered factor in potential practical application. Several fabricated immunosensors were stored in refrigerator at 4 °C. A week later, the signal strength decreased to 95% of the initial value, and half a month later, it decreased to 88% of initial value (Fig. S7), indicating the result was satisfactory.

Reproducibility was also studied in this immunosensor fabrication. 5 prepared sensors were tested under identical conditions (Fig. S6). The RSD of the measurement was within 2.7%, suggesting the precision of the biosensor was reasonably good for SCCA detection.

To evaluate the performance of the novel immunosensor,