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L-Proline bio-inspired synthesis of AuPt nanocalliandras as sensing platform for label-free electrochemical immunoassay of carbohydrate antigen 19-9

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ABSTRACT

In this study, an ultra-sensitive and selective label-free immunosensor based on monodispersed AuPt nanocalliandras (AuPt NCs) was constructed for the determination of carbohydrate antigen 19-9 (CA 19-9). AuPt NCs were synthesized by a one-step eco-friendly wet-chemical method, using *L*-proline as the green growth-director and stable agent. The architectures were mainly characterized by transmission electron microscopy (TEM), high-angle annular dark-field scanning TEM (HAADF-STEM), X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS). The results revealed that AuPt NCs provided desired microenvironment to immobilize CA 19-9, amplify the currents of CA 19-9 by using $K_3[Fe(CN)_6]$ as the probe, and improve the electrical conductivity of the immunosensor. The immunoassay showed wide linear range from 0.05 to 50 U mL^{-1} and low detection limit of 0.03 U mL^{-1}, which was explored to detect CA 19-9 in human serum samples. The as-developed approach can be extended for ultrasensitive detection of biomolecules in clinical analysis.

1. Introduction

As one of the most lethal human malignancy, pancreatic cancer is a major urgent health problem [1], owing to the environmental pollution and unhealthy lifestyle [2]. However, it is still extremely poor for its treatment, although about 15%–20% of the patients have the potential to cure. It is pity that only 1/5 of them survive to 5 years [3]. Hence, an effective early detection is significantly essential for patients, due to the high risk of pancreatic cancer and poor prognosis [4].

Carbohydrate antigen 19-9 (CA 19-9) is an isolated Lewis antigen of mucin 1 (MUC1) protein with an average molecular weight of 1000 KDa [5], which is commonly utilized as tumor marker for early-staged detection and estimation of cancer risk [6]. Hence, the detection technology of CA19-9 plays a key role in the prognosis of pancreatic cancer.

Up to date, plenty of detection diagnostic approaches have been developed, including chemiluminescence immunoassay [7,8], radioimmunoassay [9,10], and enzyme-linked immunoabsorbent assay [11,12]. However, most of them are somewhat suffered from time-consuming, sophisticated processes, skillful technology and/or specially equipped laboratories [13]. Thus, it is desired to develop highly sensitive, selective and user-friendly immunoassays.

Taken together, great attention is paid for the development of electrochemical immunoassays [14–16] with the advantages such as short analytical time, simple pretreatment, high sensitivity and inexpensive instruments [17]. Among all kinds of electrochemical immunoassays [18], label-free immunoassay is attracted increasing interest because of its low cost, easy fabrication and time-saving [19]. Sensing nanomaterials is the key step for the construction of label-free immunosensor [20,21], which provide eco-friendly microenvironment for the immobilization of biomolecules and promote the electron transfer between the target molecules with the electrode [22].

Notably, bimetallic nanomaterials are widely used as sensing substrates to construct label-free immunosensors, thanks to their good biocompatibility and high electrocatalytic activity [23]. Among them, AuPt alloy is very popular, because the incorporation of Au can improve the electrical conductivity, superior biological stability and enhance the biocompatibility to protein [24]. Also, alloying Au with Pt has remarkable enhancement on the stability of the resulted materials [25]. Additionally, AuPt nanocrystals

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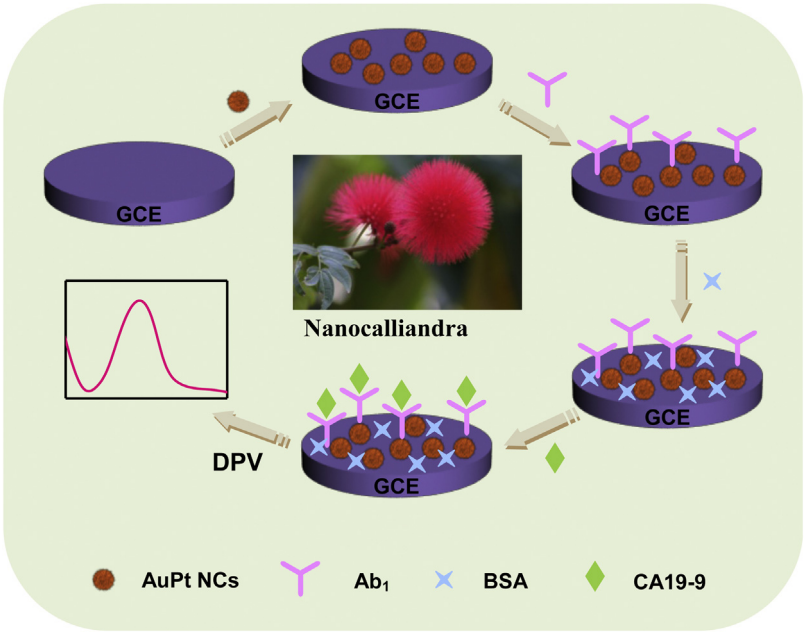

Fig. 1. Illustration of the fabrication scheme of AuPt NCs based immunosensor.

with porous structures have enlarged surface, which exhibit an enhanced electrocatalytic performance [26].

L-Proline, one of important amino acids, is acted as a weak agonist of the glycine receptor and of both N-methyl-D-aspartate receptor (NMDA) and non-NMDA ionotropic glutamate receptors [27]. It has been widely used in catalysis, pharmaceuticals, cosmetics, food industry, and biosynthesis of proteins [28,29]. Herein, alloyed AuPt nanocalliandras (AuPt NCs) were synthesized in the presence of *L*-proline by one-step wet-chemical method. Based on AuPt NCs, a label-free CA19-9 immunosensor was fabricated accordingly for the electrochemical determination of CA 19-9 and explored for the assay of CA 19-9 in human serum samples.

2. Experimental

2.1. Preparation of AuPt NCs

The synthetic procedure of AuPt NCs was carried out as follows: firstly, 617 μL of $HAuCl_4$ (24.3 mM), 389 μL of H_2PtCl_6 (38.62 mM) and 0.0303 g of *L*-proline were subsequently put into water under stirring with the final volume of 9.0 mL. After adjusting the pH to 11.0, 1.0 mL of the freshly-prepared ascorbic acid (AA) solution (100 mM) was dropwise added to the mixture at room temperature and stirred for 10 min. Then, the final products were separated by centrifugation at 7000 rpm for several times and washed thoroughly with water and ethanol, followed by drying at 60 °C in a vacuum for further use.

2.2. Fabrication of the CA19-9 immunosensor

As illustrated in Fig. 1, the freshly-cleaned glassy carbon electrode (GCE) was initially covered by 6 μL of the as-prepared AuPt NCs suspension. After drying in air, anti-CA19-9 was immobilized onto the electrode surface by specific interactions between Au and amine/mercapto groups available of antibody [30], followed by incubating the electrode into the bovine serum albumin (BSA) solution (1 *wt.*%) for 1 h at 37 °C. Next, the modified electrode was further casted with 6 μL of the CA19-9 solution with different concentrations, and stored at 4 °C for further use.

In this study, the construction processes of CA19-9 immunosensor was investigated using $K_3[Fe(CN)_6]$ as the probe [31].

3. Results and discussion

3.1. Characteristics of AuPt NCs

The structural features of the typical AuPt NCs were investigated by transmission electron microscopy (TEM). As seen in Fig. 2A and B, the synthesized product contains numerous well-dispersed calliandra-like particles with roughly porous surfaces. Each calliandra has an average particle size of 46.1 nm (inset in Fig. 2A). The particle size is appropriate and matches well with the antibody with the value of 10–15 nm, indicating the effective combination [31].

As described by high-resolution TEM (HR-TEM) images (Fig. 2C), there are many clear lattice fringes observed, with the lattice spacing distances of 0.234, 0.236 and 0.235 nm from the marked positions. These values are matched well with the inter-planar d-spacing distance of the (111) crystal planes of AuPt alloy [32]. The selected area electron diffraction (SAED) pattern (Fig. 2D) reveals that AuPt NCs have the face-centered cubic (*fcc*) structures corresponding to the (111), (200), (220), and (311) crystalline facets [33].

High angle annular dark-field scanning TEM (HAADF-STEM) mapping images show the distinct morphology of the nanocalliandra (Fig. 3A–D), which reveal the formation of AuPt alloy [34]. Meanwhile, as described by the cross-section compositional line scanning profiles (Fig. 3E), the homogeneous distribution of Pt and Au elements in each nanocalliandra reflects the bimetallic alloyed feature [35].

As displayed by the energy-dispersive X-ray spectroscopy (EDS) pattern (Fig. 3F), C, Pt, Au and Cu elements coexist in AuPt NCs. Specifically, the atomic ratio of Pt to Au is estimated to be 51:49, which is in good accordance with the initial ratio of the metal precursors ($PtCl_6^{2-}$:$AuCl_4^-$). Additionally, C and Cu elements are originated from the copper grid [36].

Herein, *L*-proline was used as the capping agent for the formation of AuPt NCs. Fig. S1 (Supporting Information, SI) shows the effects of the *L*-proline concentrations on the AuPt products. The