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Research Paper

An electrode-separated piezoelectric immunosensor array with signal enhancement based on enzyme catalytic deposition of palladium nanoparticles and electroless deposition nickel-phosphorus

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ABSTRACT

A differential piezoelectric immunosensor array was reported with signal enhancement based on the biometallization of palladium nanoparticles (Pd NPs) and successive electroless deposition Ni-P layer. In this strategy, the primary antibody probes were immobilized on the aminated quartz surface in an electrode-separated piezoelectric sensor (ESPS). After bounding with the corresponding antigen and alkaline phosphatase (ALP) conjugated second antibody, the ALP in the sandwich-type immunocomplex can catalyze the substrate of *p*-aminophenyl phosphate to generate *p*-aminophenol, which reduces Pd(II) in solution to Pd NPs onto the quartz surface. A successive signal amplification was performed by Pd NPs-promoted catalytic electroless deposition of Ni-P layer. A differential measurement mode was employed to eliminate the influence of non-mass effects, including the changes in conductivity, viscosity, density and temperature of the solution, as well as the baselines draft of piezoelectric sensors. The responses of the differential ESPS in fundamental frequency, third and fifth overtones were compared. The proposed method was applied to the determination of human IgG with a detection limit of 1 pg/mL. Compared with biometallization of Pd NPs, the sensitivity is enhanced further by three orders of magnitude due to high activation energy in Ni-P deposition reaction and low baseline draft in differential measurement mode.

1. Introduction

The sensitive detection of protein biomarkers in real serum samples plays an important role in diagnosing diseases at an early state. Owing to its high sensitivity and specificity in the biomarker detection, electrochemical immunosensors have received much attention [1–8]. As an important branch in electrochemical sensors, the quartz crystal microbalance (QCM) is a mass sensing platform based on the piezoelectric properties of quartz crystals. Hence, QCM is a powerful acoustic tool enabling direct detection of binding events in real time and without labeling [9–19]. A non-viscoelastic small mass added to the surface of the QCM can be quantified using the linear Sauerbray equation [20], given as follows:

$$\Delta F = -2.26 \times 10^6 n f_0^2 \Delta m \quad (1)$$

where ΔF (Hz), f_0 (MHz) and n are the frequency shift, fundamental frequency and overtone of the piezoelectric crystal, respectively. Δm is the mass deposited on the pre area of electrode surface (g/cm^2).

According to Eq. (1), QCM could get benefit from bioaffinity reactions since the mass changes involving biomolecules bound to the crystal surface are expected to result in easily detectable frequency shifts in the QCM. However, the detection sensitivity of a QCM in liquid phase is usually limited to the level of around 1 ng/cm^2 [21]. To detect a smaller mass change is remained a challenge. Intensive efforts have been paid to enhance the sensitivity of QCM biosensors. It was demonstrated that a QCM resonator operating at higher fundamental frequency can increase the frequency shift signal and the sensitivity [22–24]. But such design and measurement are difficult due to the fragility of the ultrathin quartz wafer and much higher noise level in high-frequency region. Hence, a mass amplification strategy is an alternative approach to increase the sensitivity of QCM sensor [25]. Various mass amplification methods including with nanoparticles labeling [26–33], rolling circle amplification

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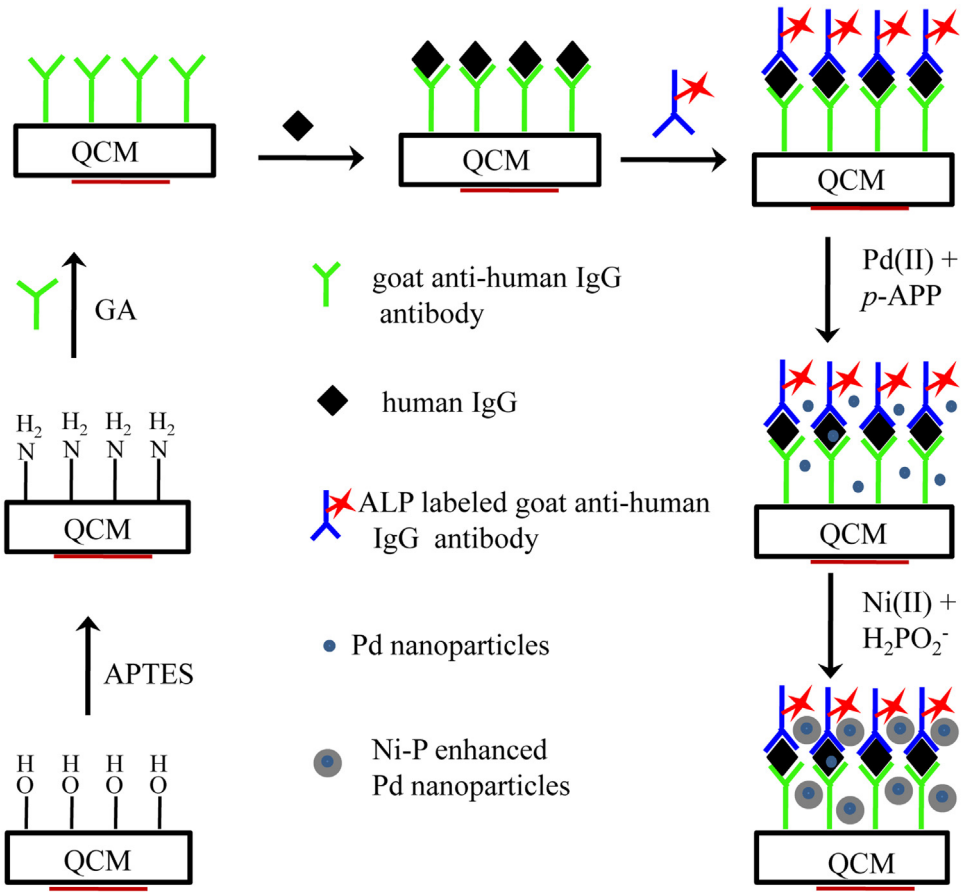

Fig. 1. Scheme of piezoelectric immunoassay procedure with signal amplification by biometallization of Pd NPs and electroless deposition of Ni-P.

[34], displacement-type competing [35], and biocatalytic deposition [36], etc., have been reported.

Biometallization is proved to be a promising strategy to improve the sensitivity of electrochemical biosensors [37]. In this strategy, an enzyme-labeled second antibody or DNA probe is used to form the sandwich-type immunocomplex or DNA hybrid, which can catalyze a substrate to produce the reducing agent for metallic deposition. Alkaline phosphatase (ALP) is one of the most used enzymatic labels for the development of immunosensors and DNA hybridization assays. The hydrolysis products of the ALP substrates such as *p*-aminophenyl phosphate (*p*-APP), 3-indoxyl phosphate and ascorbic acid phosphate are known to be versatile reducing agents, which can reduce silver cation to produce a silver deposition. Then the amount of silver deposited is quantified by the stripping or impedance measurements [38–45] and colorimetric detection [46].

The aim of this research is to exploit the sensitivity enhancement in the piezoelectric immunosensors based on the biometallization and successive mass amplification strategy. With human immunoglobulin G (hIgG) chosen as the model analyte, the detection protocol is outlined in Fig. 1. The primary antibody probes were immobilized on the aminated quartz surface in an electrode-separated piezoelectric sensor (ESPS). After bounding with the corresponding target antigen and ALP conjugated second antibody, Pd(II) in solution was reduced to palladium nanoparticles (Pd NPs) onto quartz surface by *p*-AP, which was generated from the substrate of *p*-APP in the presence of ALP in the sandwich-type immunocomplex. To enhance the sensitivity further, the signal tag of Pd NPs was served as a high efficiency catalyst in the electroless plating nickel-phosphorus (Ni-P). The Ni-P enhancement layer is chosen because the activation energy in the electroless deposition of nickel-phosphorus layer from the mixture of Ni(II) ions and hypophosphite is relatively high [47]. Without a catalyst such as Pd NPs, the deposition rate of Ni-P is very small, especially at low solution temperature. Thus, the Ni-P enhancement is superior to silver enhancement approach due to the much lower background level. But other metals (such as Pt, Au, Ag, Cu, Ni, Fe) also have some catalytic effect to electroless plating Ni-P. To eliminate the additional catalytic effect from Au or Ag electrodes in the QCM, the ESPS array with bare quartz surface in contact with liquid phase (Fig. 2) was employed in this work. However, the resonant frequency of an ESPS is more sensitive to the change in solution conductance than a normal QCM. Hence, a differential measurement model is used in the ESPS array to eliminate the influence of non-mass effect and to suppress the baseline draft of biosensors. On the other hand, the piezoelectric immunosensor array will significantly increase the efficiency of detection of target analytes [48–51]. The responses of the differential ESPS in three overtones (n = 1,3,5) were investigated. Under optimized experimental conditions, the proposed method was applied in the determination of hIgG with detection limit of 1 pg/mL. The successive signal-amplification by Ni-P deposition enhances the sensitivity by three orders magnitude.

2. Experimental

2.1. Chemicals and setup

Goat anti-human IgG antibody, human IgG, and ALP conjugated goat anti-human IgG antibody were purchased from Beijing Dingguo Biotechnology Development Center (Beijing, China). Bovine serum albumin (BSA), glutaraldehyde (GA), *p*-aminophenyl