

SENSITIVE LABELLES IMPEDANCE IMMUNOSENSOR USING GOLD NANOPARTICLES-MODIFIED OF SCREEN-PRINTED CARBON INK ELECTRODE FOR ACT-PROSTATE SPECIFIC ANTIGEN DETECTION

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ABSTRACT

Screen-printing (thick-film) technology is well identified as a reliable technique for fabrication of electrodes which can be used as transducer in biosensor with several advantages including low cost, design flexibility, good reproducibility and a wide choice of materials. Besides, the electrochemical impedance spectroscopy (EIS) recently has been chosen as a main detection method because it is labelless and less destructive to the activities of biomolecule. Therefore, this work demonstrates a new approach to develop a sensitive and label-free impedimetric immunosensor based on screen-printed electrode for applications in prostate cancer diagnostics. The result shows that detection limit of this sensor for ACT-PSA antigen was determined to be 0.5 fg/mL with the sensor area of 2.64 mm².

KEYWORDS: Screen-printed electrode, label-free detection, electrochemical impedance spectroscopy, self-assembled monolayers, prostate cancer diagnostics, ACT-PSA detection.

INTRODUCTION

According to the 2011 report of the World Health Organization, there was about 10.1 million people with cancer around the world, in which the mortality of patients have up to 6.2 million. By the next 25 years, there will be 30 million people with cancer worldwide including 20 million deaths, in which the rate of cancer mortality is the highest concentration for developing countries. Furthermore, patients only go to hospitals in the late-stage, 70% of patients with cancer detected at the hospital had distant metastasis to multiple organs in the body. Meanwhile, the early detection of cancer can be treated and help prolong life. As recommended by the World Health Organization, 1/3 of people with cancer can be detected early, 1/3 can be prevented and 1/3 can be prolonged life. Therefore, research tasks in order to go to the fabrication technology prototype biosensor capable of early and accurate diagnosis of cancer is extremely necessary and urgent in the current period. Biosensor not only helps last the patient's life but also has accurate prognosis in the successful treatment of the disease.

Depend on the method of signal transduction, the biosensors could be divided into four basic groups including optical, mass, thermal and electrochemical sensors. Among these transducers, electrochemical transducers are potential sensing method thanks to their high sensitivity, able to miniaturization, and easy to integrate into circuit to perform automatic sensing. Furthermore, the biosensor employs inexpensive commercial screen-printed carbon ink electrode (namely DEP chip) as the basis which will allow for simple disposable and portable instrumentation with low cost [1]. In the previous works of our group [2, 3], we described the development and characterization of label-free impedimetric immunosensor for α -hCG antigen detection using DEP chip as basis. The experimental results exposed that the designed impedimetric immunosensor is more sensitive than the other previously reported immunosensor, in the case of detection limit and linear range for antigen detection. On the other hand, the immobilization of antibody molecules is a decisive factor for successful fabrication of immunosensor. Therefore, in this work, we developed novel biosensor based on both nanostructure material and electrochemical transducer with the purpose to increase the sensitivity as well as reduce the size of the device.

EXPERIMENTAL

All reagents used were of the analytical grade or the highest commercially available purity and used as supplied without further purification. All solutions were prepared with deionized water of resistivity no less than 18 M Ω .cm. The commercial disposable electrochemical printed (DEP) chip were obtained from BioDevice Technology Ltd., Japan. The chips were fabricated by screen-printing technology and designed as system with three electrodes containing carbon ink working,

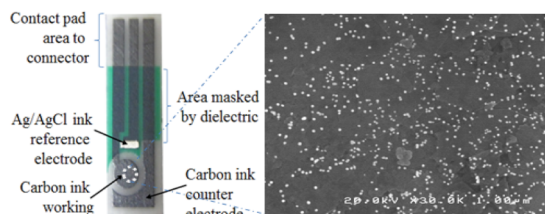


Figure 1. The structure of DEP chip used within this work and SEM micrograph of carbon ink working electrode surface deposited by gold nanoparticles.

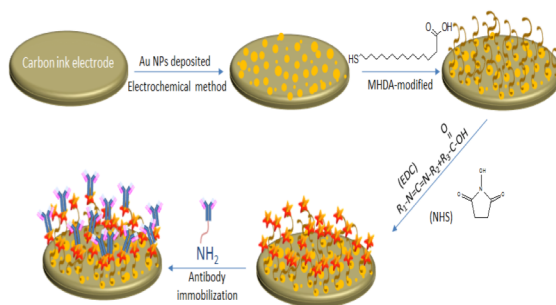


Figure 2. Schematic diagram represents the fabrication of impedimetric ACT-PSA immunosensor based on DEP chip.

carbon ink counter and Ag/AgCl ink reference electrodes. Surface area of the working electrode is 2.64 mm^2 . An Autolab PGSTAT 30 system was used to perform EIS measurements.

The carbon ink electrode of DEP chip is modified first by deposition of gold nanoparticles (AuNPs) on working electrode using cyclic voltammetry method. The structure of DEP chip and morphology of carbon ink working electrode deposited by AuNPs are shown in figure 1. After that, polyclonal antibody (Pab) of prostate specific antigen (PSA) was immobilized onto AuNPs-modified electrodes via COOH group of self-assembled monolayer (SAM) of 16-mercaptohexadecanoic acid (MHDA), which can serve as a linker for covalent biomolecular immobilization. The figure 2 shows the whole activation process.

RESULTS AND DISCUSSION

In electrochemical impedance sensor, the detection is based on the principle that any substance attached on its electrode will change the measured impedance. In this case, the binding of PSA antibody and antigen can be considered as a coating film which is expected to affect the impedance signal. The method was described more detail in the previous works of our group [2, 3].

Figure 3 shows the impedance responses for each step in the stepwise modification of electrodes. The results show that the conductance of carbon ink working electrode increased after the electrode was modified by AuNPs. This confirms that the AuNPs was successfully fabricated on carbon ink electrode via using cyclic voltammetry method. Additionally, a significant difference in the impedance spectra of Pab PSA immobilization-modified electrode as well as binding of ACT-PSA compared to AuNPs-modified electrode was observed. The R_{CT} value of AuNPs-modified electrode is $(1.21 \pm 0.06) \text{ k}\Omega$. However, after immobilizing Pab PSA on the electrode surface, the diameter of semicircle in impedance spectrum drastically increases with an increase in R_{CT} value to $(2.16 \pm 0.15) \text{ k}\Omega$. A remarkable increase in R_{CT} value to $(7.13 \pm 0.53) \text{ k}\Omega$ was observed in the step of ACT-PSA antigen binding. So Pab PSA was successfully immobilized onto AuNPs-modified carbon ink electrode surface via SAM layer.

To investigate the interaction between ACT-PSA antibody and antigen, the Pab PSA antibody-AuNPs modified electrodes are exposed to various concentration of ACT-PSA antigen (from 0.1 pg/mL to 10 ng/mL). The corresponding Nyquistplots of impedance spectra are shown in figure 4A. The diameter of the Nyquist semicircle increases with increasing of ACT-PSA antigen concentration was observed. This could be due to the binding of more antigen molecules to immobilized PSA antibody in higher concentration of antigen. Therefore, the interfacial charge transfer was hindered significantly, resulting in a corresponding increase in the charge transfer resistance. The calibration curve obtained by plotting the relative R_{CT} versus antigen concentration is illustrated in the figure 4B. Based on the linear range was attained from 0.1 pg/mL to 10 ng/mL with the linear equation of $R_{CT} (\text{k}\Omega) = 7.04 + 1.34 \cdot \log C (\text{pg/mL})$ ($R^2 = 0.998$), the detection limit for ACT-PSA of the sensor was determined to be 0.5 fg/mL with the sensor area of 2.64 mm^2 . Sensitivity comparison of different techniques for PSA antigen detection is shown on Table 1. Obviously, our sensor has simple design and more sensitive than them in both cases of detection limit and linear range.

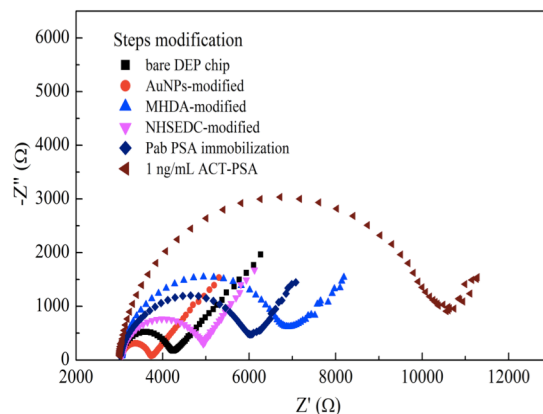


Figure 3. The impedance changes during stepwise modification of the carbon ink working electrode.

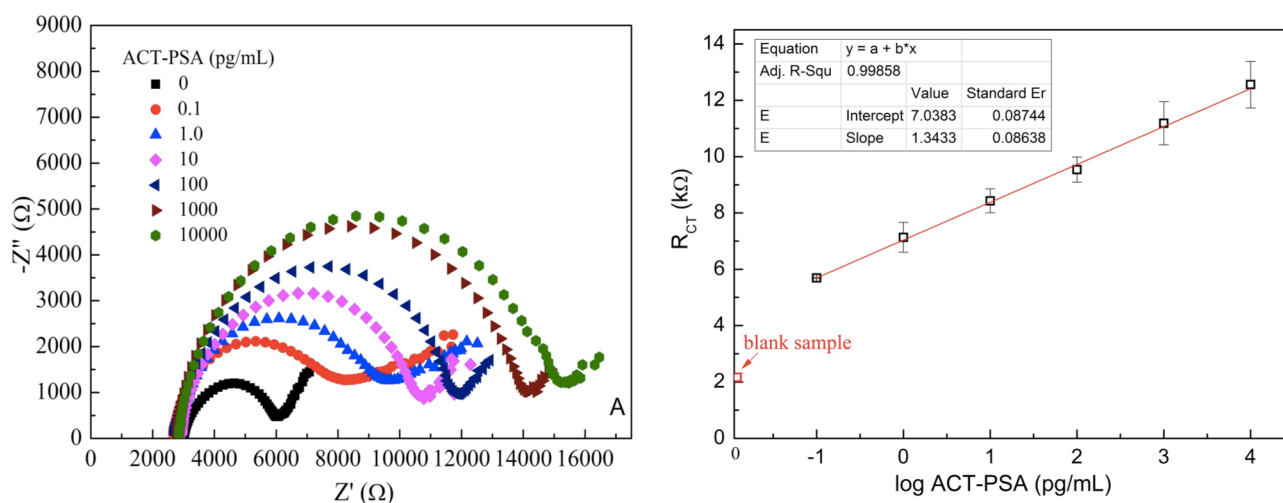


Figure 4. A) Impedance spectra of Pab PSA-AuNPs modified electrodes exposed to difference concentration of PSA-ACT antigen and B) the calibration curve, which using R_{CT} as function of PSA-ACT concentration C. All data points are average values for responses of three electrodes. The error bars give a measure of the reproducibility of the system.

Table 1. Sensitivity comparison of different techniques for PSA detection.

Detection method	Detection limit (fg/mL)	Description	Ref.
Electrochemical immunoassay	3000	Quantum dot (QD) functionalized graphene sheets (GS) were prepared and used as labels	[6]
Electrochemical transistor immunoassay	1000	The polystyrene doped PEDOT based OECT with AuNPs used as labels	[7]
Nanowire sensor arrays	50 ÷ 100	Ab immobilized silicon nanowire field effect devices were used	[8]
Nanomechanical resonator	50	Harmonic oscillators used to measure the reduction in resonance frequency after antigen binding	[9]
AuNPs - based bio-barcode	0.09	AuNPs functionalized with hybridized oligonucleotide barcodes were used	[10]

CONCLUSION

The work concerns successful implementation of a simple and specific approach for ACT-PSA antibody immobilization onto AuNPs-modified carbon electrode surface of DEP chip via COOH group of SAM, which can serve as a linker for covalent biomolecular immobilization. The results indicated that AuNPs film deposited on the carbon ink working electrode of DEP chip provide a highly active surface for the immobilization of PSA antibody molecules with maintained immunoactivity. In addition, the used inexpensive DEP chip with carbon ink working electrode as basis for the sensor will allow simple, disposable and portable instrumentation with low cost. Moreover, it was found that EIS is an impressive method and more simple than the other method for monitoring the interaction of antigen with antibody that occurred on the electrode surface.

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