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A disposable two-throughput electrochemical immunosensor chip for simultaneous multianalyte determination of tumor markers

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Abstract

A disposable two-throughput immunosensor array was proposed for simultaneous electrochemical determination of tumor markers. The low-cost immunosensor array was fabricated simply using cellulose acetate membrane to co-immobilize thionine as a mediator and two kinds of antigens on two carbon electrodes of a screen-printed chip, respectively. With two simultaneous competitive immunoreactions the corresponding horseradish peroxidase (HRP) labeled antibodies were captured on the membranes, respectively, on which the immobilized thionine shuttled electrons between HRP and the electrodes for enzymatic reduction of H_2O_2 to produce detectable signals. The electrochemical and electronic cross-talks between the electrodes could be avoided, which was beneficial to the miniaturization of the array without considering the distance between immunosensors. Under optimal conditions the immunosensor array could be used for fast simultaneous electrochemical detection of CA 19-9 and CA 125 with the limits of detection of 0.2 and 0.4 U/ml, respectively. The serum samples from clinic were assayed with the proposed method and the results were in acceptable agreement with the reference values. The proposed method for preparation of immunosensor array could be conveniently used for fabrication of disposable electrochemical biochip with high throughput and possessed the potential of mass production and commercialization.

Keywords: Bioanalysis; Biosensors; Immunosensor; Electrochemistry; Multianalyte determination; Biochip; Tumor marker; CA 19-9; CA 125

1. Introduction

In recent years, biochip technology has attracted considerable interest due to the need of massive parallel detection for both clinical diagnostics and study of chemical biology and/or life science. The fabrication of biochips based on microarrays has also made great progress. However, they have not been extensively applied in clinic due to the high cost for biochip preparation. On the other hand, the diagnostic value of a single tumor marker measurement is limited because most markers are not specific. To improve the diagnostic value the accurate multianalyte test of combinations of tumor markers has become more and more important for the screening and diagnosis of a certain type of tumors, which makes the multianalyte assays and sensor arrays for tumor markers be becoming an interesting and promising research field. Thus, tremendous efforts are focusing on the development of low-cost multianalyte immunoassay methods and convenient preparation of immunosensor arrays. Using a general screen-printed technique and simple immobilization method this work proposed a low-cost immunosensor array for fast multianalyte electrochemical immunoassay of tumor markers.

Simultaneous multianalyte immunoassays (SMIAs) are a promising analytical method in protein analysis with the advantages of short analysis time, simplified analytical procedure, decreased sampling volume, improved test efficiency and reduced cost as compared to parallel single-analyte assays (Brecht and Abuknesha, 1995; Diaz-Gonzalez et al., 2005). Usually SMIAs include multiple label assays (Kricka, 1992; Masseyeff et al., 1997; Liu et al., 2004), spatially resolved methods (Ding et al., 1999; Christodoulides et al., 2002; Mastichiadis et al., 2002; Taitt et al., 2002), separation based techniques (Chen and Evangelista, 1994; Haake et al., 2000) and sequential detection (Gonzalez-Martinez et al., 2001). Multi-label methods and spatially resolved assay systems have been well developed (Brecht and Abuknesha, 1995). However, the multi-label assays need a compromise in assay conditions for different labels, thus the assay formats based on spatially separated test zones,

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particularly optical SMIAs relying on fluorescence emission and optical reflectance (Cho and Bright, 2002; Cho et al., 2002; Joos et al., 2002), have gained more considerations recently. Although optical SMIAs are highly sensitive, they need expensive array detector, for example, charge-coupled device (CCD) camera for optical detection (Rowe et al., 1999; Delehanty and Ligler, 2002; Barry and Soloviev, 2004; Knecht et al., 2004). Thus, assays based on alternative detection strategies have also been developed. One of the developing spatially resolved SMIAs is electrochemical immunoassay. It is an effective analytical technique with a most peculiar feature in miniaturization for high-throughput applications and low cost of the entire assay system (Yao et al., 1995; Yu et al., 2004).

Many attempts have been made towards development of electrochemical sensor array for SMIAs (Meyerhoff et al., 1995; Bordes et al., 1997; Ronkainen-Matsuno et al., 2002; Kojima et al., 2003; Seidel and Gauglitz, 2003; de Prada et al., 2004; Dill et al., 2004; Liang et al., 2004; Paitan et al., 2004; Wilson, 2005; Wilson and Nie, 2006a,b). Although the miniaturization of electrochemical sensor array can reduce both the incubation time and the cost for an assay because of the low volume of the immunoassay reaction chamber and the use of a disposable sample carrier (Ronkainen-Matsuno et al., 2002; Seidel and Gauglitz, 2003), cross-talk is the main trouble in such system (Ding et al., 1999). For example, at a pioneer electrochemical immunosensor array prepared with a plasma-ploymerized siloxane film to immobilize capture antibodies the cross-talk caused by the diffusion of enzymatic product, hydrogen peroxide, to surrounding working electrodes depends upon the distance between electrodes and the time required for the measurements (Kojima et al., 2003). Wilson (2005) and Wilson and Nie (2006a,b) excluded the electrochemical cross-talk by controlling the distance between adjacent electrodes larger than the diffusion distance of enzymatic product. This work suggested one method to avoid completely the electrochemical cross-talk by immobilizing simply an electron-transfer mediator on individual immunosensor to shuttle electrons, which produced detectable signals without interference resulted from the diffusion of enzymatic product, thus was advantageous to the further miniaturization of electrochemical sensor array.

Compared with the fabrication of immunosensor array reported previously (Kojima et al., 2003; Dill et al., 2004; Wilson, 2005; Wilson and Nie, 2006a,b), this work used a screen-printed technique to prepare the substrate electrode array. This technique was much simpler than the methods of microelectronics and photolithography for preparation of electrochemical immunosensor array (Kojima et al., 2003; Dill et al., 2004; Wilson, 2005; Wilson and Nie, 2006a,b) and decreased the cost of the array fabrication. It enabled easy production of very flexible configurations of electrode array devices with low cost and good disposability and portability. Using two important tumor markers, carbohydrate antigen 19-9 (CA 19-9) and carcinoma antigen 125 (CA 125) as models a novel immunosensor array for SMIAs was developed. The consideration in interval of detecting two analytes and the separate distance between neighboring electrodes was unnecessary, which was beneficial to rapid detection and the miniaturization of multianalyte detection device. The proposed method could be conveniently used for low-cost fabrication of electrochemical biochips with mass production and high analyte-throughput.

2. Materials and methods

2.1. Reagents

CA 19-9 and CA 125 enzyme-linked immunoabsorbent assay (ELISA) kits were all purchased from CanAg Diagnostics AB (Sweden). The CA 19-9 ELISA kits consisted of a series of CA 19-9 standard solutions with different concentrations from 0 to 240 U/ml and a stock solution of horseradish peroxidase (HRP)-labeled CA 19-9 monoclonal antibody. The CA 125 ELISA kits consisted of a series of CA 125 standard solutions with different concentrations from 0 to 500 U/ml and a stock solution of HRP-labeled CA 125 monoclonal antibody. Bovine serum albumin (BSA) was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). The dilution solution for the enzyme conjugate contained 0.04% BSA and 1.0 mM ethylenediamine tetraacetic acid (EDTA) and 0.1 M phosphate buffered saline (PBS). Thionine, cellulose acetate (CA, approximately 40% of acetate) and H_2O_2 (analytical reagent grade) were from Shanghai Biochemical Reagent Company (China). All other reagents were of analytical reagent grade and used without further purification. 0.1 M PBS of various pHs were prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4, and then adjusting the pH with 0.1 M NaOH and H_3PO_4. Doubly distilled water was used throughout the experiments. Serum specimens provided by Jiangsu Institute of Cancer Prevention and Cure were stored at 4 °C.

2.2. Instrumentation

Electrochemical measurements were both performed on a CHI 730 electrochemical analyzer (CHI Co., USA) in a three-electrode configuration and an eDAQ manufactures e-corder system with a QuadStat (eDAQ Co., Australia) in a four-electrode configuration. For simultaneous dual-analyte detection on the CHI 730, the two working electrodes were connected to a single potentiostat with a switch. Scanning electron micrographs of bare screen-printed carbon electrode (SPCE), CA membrane modified SPCE and the immunosensors were obtained with a JSM-5610LV scanning electron microscope (JEOL, Japan). The reference values of CA 19-9 and CA 125 in sera were obtained with an automation electrochemiluminescent analyzer (Elecsys 2010, Roche, Switzerland).

2.3. Preparation of immunosensors array

The dual-analyte immunosensor array was composed of two thionine/antigen modified graphite working electrodes, W1 and W2 (2 mm in diameter, 2 mm edge-to-edge separation) (Fig. 1A), one graphite auxiliary electrode, and one Ag/AgCl reference electrode. Both working electrodes shared the same reference and auxiliary electrodes. The four-electrode array was fabricated according to the steps reported previously (Yu et al.,