Sensors and Actuators A 241 (2016) 203–211

Contents lists available at ScienceDirect

Sensors and Actuators A: Physical

journal homepage: www.elsevier.com/locate/sna

Development of an impedimetric immunobiosensor for measurement of carcinoembryonic antigen

Chia-Hsien Yeh, Kuan-Feng Su, Yu-Cheng Lin*

Department of Engineering Science, National Cheng Kung University, Tainan, Taiwan

ARTICLE INFO

Article history:
Received 13 May 2015
Received in revised form 9 December 2015
Accepted 29 January 2016
Available online 9 February 2016

Keywords:
Biosensor
Carcinoembryonic antigen
Tumor
Detection limit
Quantitative measurement
Impedance signals

ABSTRACT

This paper presents an impedimetric immunobiosensor for detecting the impedance signals of various concentrations of carcinoembryonic antigen (CEA), a type of protein molecule which is typically associated with certain tumors. The biosensor was developed by parallel electrodes and improved sandwich immunoassay, and used specific binding of antibodies to change the impedance signals of various CEA concentrations. We observed an obvious difference in impedance signals after 13 min, and a better linearity (a steep slope between impedance and CEA concentration) at 100 Hz. Finally, when the concentration of the AuNPs-anti-CEA antibody was diluted 30 for times, the detection limit for CEA concentration was 1 ng/mL with 1 μg/mL of anti-CEA antibody. Therefore, the developed biosensor has advantages including smaller sample volume (25 μL), rapid quantitative measurement (10 min), and smaller detectable concentrations (1 ng/mL).

1. Introduction

An immunoassay is a biochemical analysis according to a specific interaction between antibody and antigen for clinical diagnoses [1] of methamphetamine (MET), human chorionic gonadotropin (HCG), human immunodeficiency virus (HIV), immunoglobulin E, etc. Different labels are used in immunoassay, including enzyme-linked immunosorbent assay (ELISA) [1,2], a fluorescent immunoassay (FIA) [3,4], a chemiluminescence immunoassay (CIA) [5,6], and radioimmunoassay (RIA) [7].

ELISA is commonly used for detecting the concentration of an antibody or antigen by linking an enzyme (used as a marker) to an antibody or antigen. The antibody bound with the enzyme reacts with a colorless substrate to generate a colored product that can be detected by an ELISA reader [8,9]. Although ELISA has many detection models, such as direct test, indirect test, competitive test, and sandwich test, expensive costs and complex procedures have confined its application.

Other detection methods for measuring the specific antigen or antibody concentration have been reported, including the surface plasma resonance, atomic force microscopy, quartz crystal microbalance, and surface enhanced Raman spectroscopy. To enhance the level of sensitivity and discrimination, enzyme, fluorescent material, or other labels were conjugated with the specific targets (antibody or antigen) to amplify the detection signals and to reduce the background noise [10–14]. Recent studies have proved that the colloid metal nanoparticles have the advantages of diameter control, stability, biocompatibility with antibody, antigen, or DNA. For example, the detected signal was amplified by increasing the diameter of nanoparticles to enhance the measurement sensitivity of colloid metal nanoparticles [15]. Because the high electron density of the gold nanoparticles (AuNPs) created a dark brown spot, the result was a distinct image for labeling [16]. This image response could be further enhanced by immunogold silver staining (IGSS), and the silver particles were precipitated on the surfaces of AuNPs and AgNPs [17,18]. To improve the sensitivity of gold nanoparticle-based methods for detecting nanomolar or picomolar concentrations, researchers have proposed the electrochemical techniques [19–22], absorption spectrometry, surface enhanced Raman spectroscopy (SERS) [23–26], and surface plasma resonance (SPR) [27,28]. However, these methods require expensive instruments and complicated processes.

Another new method, electrical detection, is proposed for protein (antigen or antibody) analysis [29–35]. Laureyn et al. used interdigitated gold electrodes for an impedimetric immunosensor [29]. Porter et al. used the self-assembled monolayer and electrodes for an electro-active immunoassay system [30]. Park et al. used an electrical detection device coupled with an AuNP probe and the silver enhancement method for a DNA array, which yielded sensitivity similar to that of fluorescence detection [31]. Li et al. combined an

* Corresponding author at: Department of Engineering Science, National Cheng Kung University, 1 University Road, Tainan 701, Taiwan.
E-mail address: yuclin@mail.ncku.edu.tw (Y.-C. Lin).

http://dx.doi.org/10.1016/j.sna.2016.01.053
0924-4247/

electro-immunosensing (EIS) microchip with AuNPs for measurement of antibody-antigen recognition using immunoassay [32].

One significant application of immunoassay is detecting concentratin of Carcinoembryonic antigen (CEA), which is mainly used as a tumor marker for gastrointestinal cancers, especially colorectal cancer Preoperative concentrations of CEA may be included with the standard staging procedures for assessing prognosis [36,37], and the conventional quantitative method for detecting CEA concentration was liquid chromatograph-mass spectrometer (LC/MS) and gas chromatograph-mass spectrometer (GC/MS). Although the LC/MS and GC/MS methods can provide accurate data, the instruments required are expensive and their operation is more complicated. So, many immunoassay methods for detecting CEA concentration in serum have been reported, such as enzyme-linked immunosorbent assay [38], fluoroimmunoassay [39], and liposome immunoassay [40], and these methods have been applied to commercially available kits,. But, even with the CEA detection limit from 0.1 to 0.5 ng/mL, these methods are still time-consuming, sample intensive, and expensive. To overcome these disadvantages, some immunoassay-based biosensors have been proposed [41–43]. Sato et al. used a bead-bed immunoassay system on a microchip for CEA detection, and the detection limit was 0.03 ng/mL using a laser-induced thermal lens microscope (TLM). But, TLM is more expensive than the equipment required for ELISA [44]. Dai et al. used an electrochemical sensor based on thionine monolayer modified by gold electrode for determining CEA, but the analyzing procedure was time-consuming due to repetitive steps of cleaning. Although the detection limit for CEA was 0.2 ng/mL, the calibration of CEA determination from 0.6 to 200 ng/mL had two linear ranges between 0.6 and 17 and between 17 and 200 ng/mL [45]. Recently, Altintas et al. used the surface plasmon resonance (SPR) for detecting CEA. A self-assembled monolayer was formed on the gold chip, and the CEA antigen was incubated to increase the sensor signal. A CEA antigen detection limit of 3 ng/mL was obtained with a dynamic detection range of 3 –400 ng/mL [46]. Liu et al. used the paper-based colorimetric immunosensor for the detection of CEA. The immunosensor platform was prepared by depositing chitosan and porous gold onto filter paper, and a linear range from 0.005 ng/mL to 30 ng/mL was observed [47].

To enhance the detection sensitivity of CEA, we propose a biosensor integrated with a sandwich immunoassay model to detect immune-reaction signal by impedance measurement, which enables quantitative analysis. Section 2 describes the materials and methods, Section 4 presents the results and discussions, and Section 5 concludes this paper. With smaller sample volume (25 μL), rapid quantitative measurement (10 min), smaller detectable concentration (1 ng/mL), and linear calibration curve (R square = 0.98), the proposed biosensor and detection device are proved to be more efficient.

2. Materials and methods

2.1. Reagents

The CEA, anti-CEA antibody, and AuNPs-anti-CEA antibody were purchased from Arista Biologicals (Allentown, Pennsylvania, U.S.A) and stored at 4 °C. Toluene, 3-Glycidoxypropyltrimethoxysilane (GPTS), gelatin, and phosphate buffered saline (PBS) were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Tween 20 was obtained from Acros (Belgium, China).

2.2. Principle of immune colloidal gold technique

For the proposed sandwich-immunoassay method (Fig. 1), the surface of the glass slide was modified using the chemical method to immobilize the protein. Then, the modified glass slide was immobilized with the antibody. The gap size of the parallel ITO electrodes in the glass slide (76 mm × 26 mm) was 20 μm. After the sample solution (the various CEA concentrations in PBS) was mixed with the AuNPs-anti-CEA antibody, the anti-CEA antibody was immobilized on the glass slide and connected to the CEA bound with AuNPs-anti-CEA antibody. The interconnected AuNPs–AuNPs between the two ITO electrodes caused the electronic transport. When the CEA concentration increased, more AuNPs-anti-CEA antibodies were bound with the CEA sample to increase the centric conductance, while the impedance measurement decreased [48,49]. To increase the conductive signal and to improve the detection sensitivity, we used the CEA bound with AuNPs-anti-CEA antibody that bound to the anti-CEA antibody between the two electrodes of the biosensor. After the sandwich immunoassay reaction, the impedance variation could be easily measured using a commercial LCR meter, and the impedance data were then analyzed using a personal computer.

2.3. Design and fabrication of the biosensor

The biosensor consisted of two parts: a parallel ITO electrode in the glass slide and a circular reaction well. The mask of the ITO electrodes was designed using AutoCAD® 2010 software. The glass slide (76 mm × 26 mm) included eight parallel ITO electrodes, whose gap size and length were 20 μm and 1500 μm. A 3.0 mm thick layer of polydimethylsiloxane (PDMS) with a 2.5 mm diameter hole in it was bound to the glass surface with a parallel ITO electrode to form the fixed-volume reaction well (58 μL), as shown in Fig. 2. The parallel electrodes were fabricated on an ITO glass slide through standard MEMS technology, such as spin coating, exposure, developing, and wet etching. After the electrode pattern was transferred onto the ITO glass slide, the parallel electrodes were formed using chemical wet etching. Finally, an oxygen plasma machine (In-line plasma cleaner, NEWST-2002IL) was used to change the surface function groups of the PDMS reaction well from CH_3 groups to OH groups. The PDMS reaction well was tightly bound to the ITO glass substrate [50]. The parameters for oxygen plasma treatment were 75 mTorr of vacuum pressure and 70 W of RF power for 30 s.

2.4. Surface modification of the biosensor

Chemical surface modification to immobilize the anti-CEA antibody on the glass slide surface. The 3-glycidoxypropyltrimethoxysilane (GPTS) was used to change the function group of the glass surface to the epoxide functional group, so that the anti-CEA antibody (the functional group of the amine (-NH_2)) could be bound to the glass slide, as shown in Fig. 3. First, the glass slide was rinsed by acetone, isopropyl alcohol, and D.I. water, and then was dried with nitrogen gas. Second, an oxygen plasma machine was used to change the surface of the glass slide from the methyl group (-CH_3) to the hydroxyl functional group (-OH). The parameters for oxygen plasma treatment were 75 mTorr of vacuum pressure and 70 W of RF power for 35 s. Third, the glass slide was soaked in the GPTS-Toluene solution (5%) for 24 h, and the surface of the glass slide was bound with the epoxide functional group. After the glass slide was rinsed with the PBS solution (0.01 M) for several times, the modified glass slide was dried with nitrogen gas. When the protein solution was injected into the modified glass slide, the epoxide functional group was changed to aldehyde functional group (-CHO). The aldehyde functional group was bound with the amine functional group, and the surface of the glass slide was bound with the anti-CEA antibody. A contact angle machine (FTA-1000B, U.S.A) was used to measure the modified glass slide. Finally, the antibodies labeled with the fluorescent nanoparticles (Goat anti-Rabbit IgG, F(ab') 2, X-adsorbed (DTAF), 500 ng/mL) were injected into both the