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Detection and discrimination of alpha-fetoprotein with a label-free electrochemical impedance spectroscopy biosensor array based on lectin functionalized carbon nanotubes

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ABSTRACT

A label-free electrochemical impedance spectroscopy (EIS) biosensor for the sensitive determination and discrimination of alpha-fetoprotein (AFP) was developed by employing wheat-germ agglutinin (WGA) lectin as molecular recognition element. The EIS biosensor was fabricated by adsorbing carboxyl-functionalized single-wall carbon nanotubes (SWNTs) onto a screen-printed carbon electrode (SPCE) and subsequently covalently coupling WGA onto the surface of the SWNTs-modified electrode. Upon binding of AFP to the biosensor, the electron transfer resistance was increased and the increase in the electron transfer resistance was linearly proportional to the logarithm of the concentration of AFP in the range from 1 to 100 ng/L with a detection limit of 0.1 ng/L. It was found that the employment of SWNTs as immobilization platform could reduce the background and enhance the EIS response. Moreover, the lectin-based biosensor array fabricated with different lectins was used to evaluate the glycan expression of AFP N-glycan and discriminate AFP between healthy and cancer patients serum samples. This work demonstrates that the employment of carbon nanotubes as immobilization platform and lectin as molecular recognition element in biosensor array is a promising approach for the determination and discrimination of glycoproteins for cancer diagnosis. The strategy proposed in this work could further be used for high-throughput, label-free profiling of the glycan expression of cancer-related glycoproteins and to develop methods for cancer diagnosis in the early stages.

1. Introduction

The development of the highly sensitive and high-throughout method for the detection of tumor markers is one of the most rapidly growing research areas in clinical tests for early discovery of cancer [1,2]. Alpha-fetoprotein (AFP), with a molecular weight of 70 kDa, has been known as a reliable biomarker for hepatocellular carcinoma [3]. A variety of methods have been developed to determine AFP, such as fluorescence immunoassay [4], electrogenerated chemiluminescence (ECL) immunoassay [5,6] and electrochemical immunoassay [7–13]. Despite the extensive development of these methods, each design has its own advantages and disadvantages. Among them, electrochemical immunoassay has received much attention due to its unique advantages, such as a low detection limit, small analyte volume, simple instrumentation, and minimal manipulation [14].

Choice of molecular recognition elements is of great importance for the fabrication of electrochemical bioassay because molecular recognition element onto electrode not only produces a functionalized sensing interface but also determines the sensitivity and selectivity of the bioassay [15]. Lectins, a group of proteins extracted from plants or animals, can strongly bind to specific carbohydrate moieties on the surface of glycoproteins [16] and thus are particularly interesting candidates as molecular recognition elements because of their ease of production and intrinsic stability. Compared with antibody-based immunoassay, the lectin-based bioassay could detect not only the content but also the aberrant glycosylation of the tumor markers, and could increase the specificity for cancer diagnosis because aberrant glycosylation is a fundamental characteristic of progression of cancer [17,18]. For example, the detection of the sialylated AFP, associated with hepatocellular carcinoma and other benign liver diseases, could be used to increase the specificity for cancer diagnosis.

Extensive efforts have been devoted to the development of electrochemical lectin-based immunoassays for the determination of the proteins and for studying the interactions between lectins and

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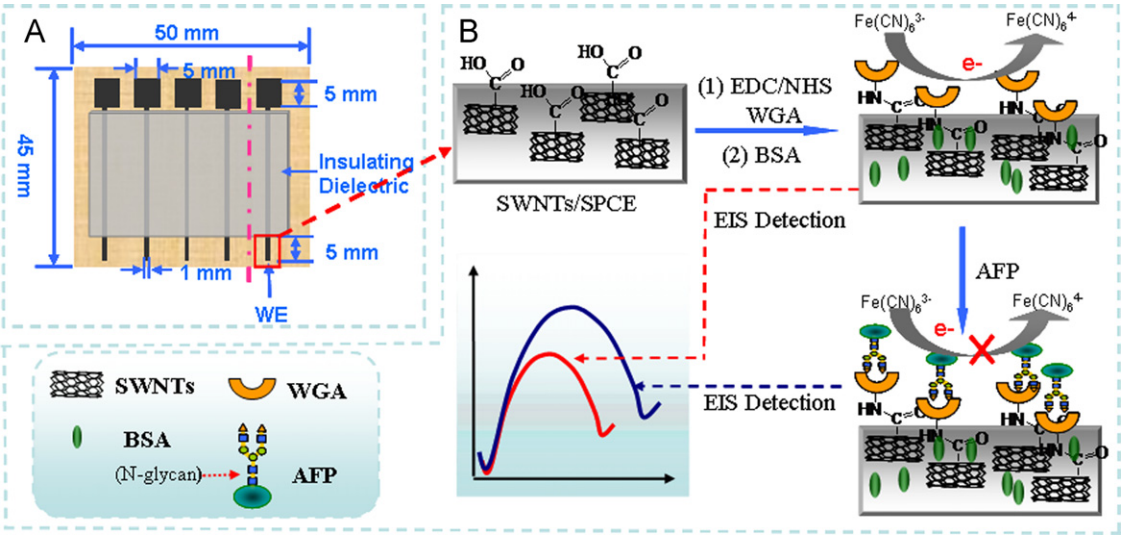

Scheme 1. Schematic representation of the base electrode array (A), fabrication of WGA-based biosensor and detection of AFP (B). (For interpretation of the references to color in this scheme, the reader is referred to the web version of this article.)

carbohydrates on tumor markers [17–20]. However, most reported methods are based on a labeling (analytes or recognition elements) strategy, which not only requires a complicated labeling procedure but also reduces the bioaffinity of the recognition elements. Electrochemical impedance spectroscopy (EIS) technique offers several advantages such as simplicity, high sensitivity and serving as an elegant way to interface biorecognition events and signal transduction [21,22]. Label-free electrochemical lectin-based immunoassays for the detection of glycoproteins have also been reported [23–25]. Oliveira et al. reported a biosystem to analyze the interactions between CramoLL lectin and fetuin for the detection of glycoprotein in the serum of patients contaminated with dengue serotypes 1, 2 and 3 [23]. Belle et al. developed an EIS label-free, rapid method for the detection of glycan-lectin interactions by immobilizing lectins of *Sambucus nigra* agglutinin and peanut agglutinin on layered Cu/Ni/Au printed circuit board electrodes [24]. However, the sensitivity of these methods is limited.

The aim of this work is to develop a simple and sensitive biosensor array for the detection of AFP and evaluation of AFP N-glycan. Firstly, a label-free EIS biosensor was designed by employing wheat-germ agglutinin (WGA) lectin as molecular recognition element and carboxyl-functionalized SWNTs as amplification platform. Single-walled carbon nanotubes (SWNTs) have emerged as a very promising new class in designing novel biosensing devices due to their high conductance, tensile strength, and chemical stability [26,27]. As shown in Scheme 1, the WGA-based biosensor was fabricated by adsorbing carboxyl-functionalised SWNTs onto a screen-printed carbon electrode (SPCE) and further covalently coupling WGA onto the surface of the SWNTs-modified electrode. Upon binding of AFP, the WGA-based biosensor produces an increased EIS response that is directly proportional to the concentration of AFP. The characteristics and analytical performance of the WGA-based biosensor for the detection of AFP are reported. Moreover, a lectin-based biosensors array fabricated with different lectins preliminarily evaluate the glycan expression of AFP N-glycan and discriminate AFP between healthy and cancer patients serum samples.

2. Experimental

2.1. Reagents and apparatus

Lectins including wheat-germ agglutinin (WGA) from *Triticum vulgaris* (wheat), *Lens culinaris* agglutinin (LCA), concanavalin A (Con A), type IV from *Canvalia ensiformis* seeds, *S. nigra* agglutinin (SNA), and *Datura stramonium* agglutinin (*jimson weed, thorn apple*) (DSA), in addition to N-acetyl-glucosamine (GlcNAc), glucose, rhamnose, N-(3-dimethylamino-propy)-N′-ethylcarbodimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) and N,N-dimethylformamide (DMF) were purchased from Sigma-Aldrich (USA). Human alpha-fetoprotein (AFP, M803209) was obtained from Fitzgerald Industries International, Inc. (USA). Bovine serum albumin (BSA) and human serum albumin (HSA) were obtained from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (China). carboxyl-functionalized SWNTs (–COOH content 2.73 wt%) were obtained from Shenzhen Nanotech Port Co. Ltd. (China). Electrodag 423SS (carbon ink) and Electrodag 452SS (insulating dielectric oil) were obtained from Acheson Henkel Corporation (USA). An epoxy substrate (0.5 mm thickness) was used as a base substrate of the printed electrode.

Lectin solutions were prepared in 10 mM phosphate buffer saline (PBS, pH 7.4, 10 mM Na_2HPO_4/NaH_2PO_4 and 100 mM NaCl). AFP and GlcNAc solutions were prepared in 10 mM PBS containing 1 mM $CaCl_2$ and 1 mM $MnCl_2$. Ultra-pure water (18.2 MΩ cm) from a water production device (Milli-Q, Millipore) was used in all experiments. All other reagents were of analytical grade. Clinical serum samples were a gift from Shaanxi Cancer Hospital.

A CHI-660 electrochemical workstation (Chenhua Instruments Co., Shanghai, China) was used for the electrochemical measurements. All electrochemical experiments were performed using a conventional three-electrode system with a fabricated biosensor array or an SPCE as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl (sat. KCl) as the reference electrode. All potentials are reported with respect to the reference electrode.

An atomic force microscope (AFM, SPM-9500J3, SHIMADZU Corporation, Japan) was used in contact mode to monitor the topography of the lectin-modified surfaces. The data were analyzed by IP (Thermomicroscope proscan image processing software version 2.1) and SPMLab NT 6.0.2 (Veeco, USA).

2.2. Fabrication of the biosensor

A base electrode array consisting of five working SPCE was fabricated by screen-printing technology according to Ref. [28] (Scheme 1A) [28]. An individual electrode was exactly cut from the epoxy substrate to get SPCE (as shown in Scheme 1, red dashed