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A reagentless amperometric immunosensor for α-1-fetoprotein based on gold nanowires and ZnO nanorods modified electrode

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ABSTRACT

A novel strategy for the preparation of reagentless immunosensor for rapid determination of α-1-fetoprotein (AFP) in human serum has been developed. The immunosensor was prepared by immobilizing α-1-fetoprotein antibody (AFP Ab) onto the glassy carbon electrode modified by gold nanowires (Au NWs) and ZnO nanorods (ZnO NRs) composite film. Gold nanowires and ZnO nanorods were produced by an electrodeposition strategy using nanopore polycarbonate (PC) membrane. A sandwich immunoassay format was employed to detect AFP with horseradish peroxidase (HRP)-labeled AFP as tracer. The morphology of the Au NWs and ZnO NRs composite film has been investigated by scanning electron microscopy (SEM) and energy disperse spectroscopy (EDS) analysis. The resulting immunosensor offered an excellent amperometric response for AFP ranging from 0.5 to 160.0 ng mL^{-1} with a detection limit of 0.1 ng mL^{-1}. Because of the combination of the biocompatibility of ZnO NRs and the direct electron-transfer of Au NWs between HRP and electrode, the proposed immunosensor displayed a direct electrochemical response of HRP to the reduction of H_2O_2 with high sensitivity, quick response, good repeatability and long-term stability.

1. Introduction

α-1-Fetoprotein (AFP), an oncofetal glycoprotein with a molecular weight of approximately 70,000 Da, is well known as a tumor marker. AFP is produced mainly by the embryonic yolk sac during fetal life, and at lower levels by the developing gastrointestinal tract and production is nearly completely switched off in normal adults [1–3]. It is known that the average concentration of AFP is typically less than 25 ng mL^{-1} in healthy human serum [4] and elevated AFP concentration in plasma may be an early indication of hepatocellular carcinoma and teratoblastoma, while high concentrations in amniotic fluid may indicate severe congenital fetal defects such as spina bifida and anencephaly [5,6]. Thus, it is necessary to measure AFP for the clinical diagnosis and even early detection of liver carcinoma.

Conventional methods for determination of AFP are immunoradiometric assay (IRMA) and enzyme-linked immunosorbent assay (ELISA). IRMA is extremely sensitive, but it also has the obvious disadvantages of short shelf life of ^{125}I-labeled antibody, radiation hazards, complicated wash procedure and expensive reagents [7]. ELISA is less sensitive, time-consuming, so is not a desirable method when rapid feedback is required [8].

Electrochemical immunosensors are of great interest because of their potential utility as specific, simple and

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direct detection techniques and reduction in size, cost, and time of analysis compared with conventional immunoassay techniques [9,10]. Numerous immunosensors based on potentiometric [11], amperometric [12,13] and piezoelectric [14] transducers for determination of AFP have been developed. However, most of immunosensors need some mediator molecules to achieve the electron-transfer [13–16], which leads to a more complex immunoassay system and increases operation time and analytical expense. Therefore, to eliminate the addition of mediator or other reagents has become a highly desirable project [17].

Nanostructured materials have received broad attention due to their novel optical, electrical, catalytic, magnetic properties, excellent biocompatibility, strong adsorption ability and potential applications in nanoelectronic devices, nanosensors, and catalysts [18,19]. One-dimensional (1D) nanomaterials, especially metallic nanowires, show improved signal to noise ratios, high faradaic current density, fast electron-transfer rate, enhanced sensitivities, better detection limit and the large surface area of nanowires with increasing the number of electroactive sites [20,21]. Gold nanowires (Au NWs) were confirmed to have high surface area with efficient mass transport characteristics and favorable biocompatibility. It was also shown that Au NWs can improve the sensitivities and decrease the detection limit [22–24]. In addition, Au NWs have numerous of electroactive sites, which show high catalytic activities to many reactions and achieve direct electron-transfer between proteins and electrodes. Zinc oxide (ZnO) nanostructured materials have been one of the most promising oxide semiconductor materials because of their high surface area, biocompatibility, nontoxicity, ease of fabrication, chemical and photochemical stability and electrochemical activity at potentials above their conduction band edge [25]. Therefore, ZnO nanostructured materials are used as the substrates for biomolecule immobilization. One-dimensional ZnO are considered to be one of the most important nanomaterials for fabricating nanodevices with applications in optics, electronics, mechanics, and biomedical sciences [26].

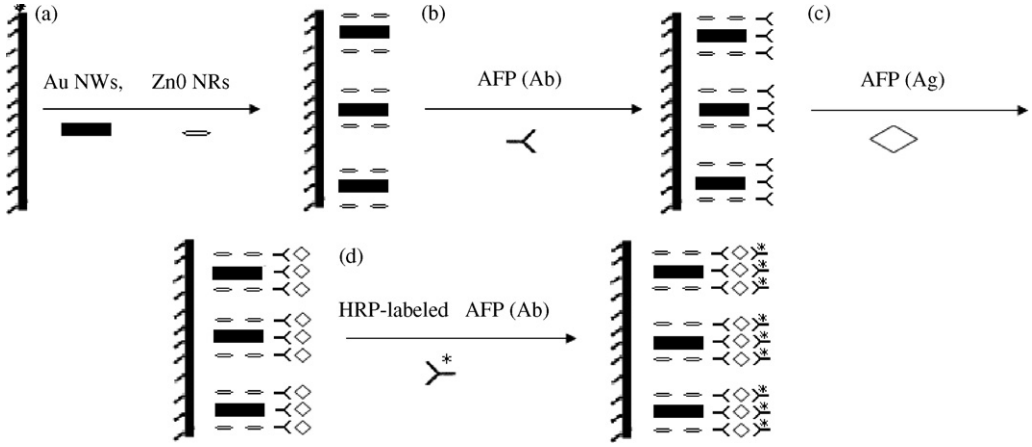
This paper reports the first attempt to develop a reagentless immunosensor based on the combination of the direct electron-transfer of Au NWs and the biocompatibility of ZnO NRs. ZnO nanorods and gold nanowires have synactic effect to improve the sensitivity of immunosensor. The immunosensor was prepared by immobilizing α-1-fetoprotein antibody (AFP Ab) onto the glassy carbon electrode modified by gold nanowires (Au NWs) and ZnO nanorods (ZnO NRs) composite film. A sandwich immunoassay format was employed to detect AFP with horseradish peroxidase (HRP)-labeled AFP as tracer and hydrogen peroxide as enzyme substrate. The determination of AFP was established by chronoamperometry to record the reduction current response of H_2O_2 catalysed HRP by means of the direct electron-transfer of Au NWs between HRP and electrode without addition of mediators or nonimmunoreagents. This immunosensor displayed high sensitivity, quick response, long-term stability and better detection limit. This strategy significantly simplifies the immunoassay procedure, shortens the analytical time, and thus provides a new promising platform for clinical immunoassay.

2. Experimental

2.1. Apparatus and reagents

Cyclic voltammetric and amperometric measurements were carried out on XJP-821(C) polarograph (Jiangsu, China). Scanning electron microscopy (SEM) analysis was performed using XL30ESEM-TMP microscope (Philips, Holland). Energy disperse spectroscopy (EDS) analysis was conducted using an energy dispersive analysis system of X-ray (EDAX, USA). A three-electrode cell (10 mL) with the immunosensor as the working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum foil electrode as counter electrode was used. All potentials were measured and reported versus the SCE. The measurements were carried out at $25 \pm 1\,^{\circ}C$.

Diagnostic kit for α-1-fetoprotein (AFP) was produced by Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Track-etched porous polycarbonate (PC, Anodisc 47, 0.2 μm) membrane was provided by Whatman. Chitosan (CHIT, MW $\sim 1 \times 10^6$; 75–80% deacetylation), bovine serum albumin (BSA, 96–99%) were supplied by Sigma (St. Louis, MO, USA). All other chemicals used, such as chloroauric acid tetrahy-



Scheme 1 – Schematic diagram of the stepwise immunosensor fabrication process: (a) coating of Au NWs and ZnO NRs; (b) AFP (Ab) loading; (c) AFP (Ag) loading; (d) HRP-labeled AFP (Ab) loading.