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Novel single-wall carbon nanotube screen-printed electrode as an immunosensor for human chorionic gonadotropin

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ABSTRACT

Human chorionic gonadotropin (hCG) is a key diagnostic marker of pregnancy. A sensor device for detection of hCG has been fabricated using a CNT (carbon nanotube) screen printed electrode (SPE). The CNT working electrode was first electrochemically oxidised to yield a hydroxyl terminated surface, which was subsequently silanized to produce an amine terminated CNT. The aminated surface allowed oriented binding of an antibody (Ab) bioreceptor, targeted against hCG (anti-hCG), to the CNT-SPE. This was achieved by activating the –COOH group at the F_c terminal of the antibody and incubating the SPE-CNT-NH_2 electrode in the activated Ab solution.

Electrochemical Impedance Spectroscopy (EIS) and Raman Spectrometry with Confocal Microscopy studies were performed at each stage of the chemical modification process in order to confirm the resulting surface changes associated with each functionalization process.

The SPE-CNT-NH_2-Ab devices displayed linear responses to hCG in EIS assays in the concentration range from 0.01×10^{-9} g cm^{-3} to 100×10^{-9} g cm^{-3}. High specificity was observed with respect to hCG detected in solutions containing urine components–these components producing a negligible change in the sensor readout relative to changes induced by hCG. Successful hCG detection was also achieved using real urine samples from pregnant woman.

Overall, the immunosensor developed is a promising tool for detecting hCG in a point-of-care diagnostic (POC), due to the excellent detection capability, simplicity of fabrication, low cost, high sensitivity and selectivity.

1. Introduction

Immunosensors are widely used in clinical applications and consist of an Ab immobilized on a suitable support–which is capable of selective detection of a specific antigen. Their inherent selectivity and sensitivity are related to the chemical and physical arrangement of the biosensor.

In general, a gold support coupled with an electrochemical transduction detection method is the most common format presented in the literature for immunosensors [1]. Carbon nanostructured materials are also employed over the gold layer, mostly in the form of carbon nanotubes [2–10] that consist of single-wall (SWCNTs) or multiwall (MWCNTs) compositions [3,5,8,9]. The use of CNTs as electrode support is not however a common choice - with relatively few reports of CNT based biosensor electrodes [1,3,11,12]. Advantages in terms of electrical response would be expected however for CNT electrodes compared with gold-related devices due to the increased surface area of such electrodes.

In addition to the sensor electrode, the Ab is also a critical component of an immunosensor. It is natural protein, composed of four chains: two identical "heavy" and two identical "light" polypeptide chains, which together form a "Y-shaped" conformation. The arms of the Y-shaped molecule are the antigen binding fragments (F_{ab}), and the vertical portion is called the fragment-crystallizable (F_c) region. When an Ab is randomly attached to a conductive support, it may adopt four possible orientations: F_c attached to the support, F_{ab} attached to the support, one F_c and one F_{ab} attached to the support, or all three fragments attached to the support [13]. The actual orientation of the antibodies on the surface may indeed comprise a combination of these four positions. As the active sites

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of the antibody are on the F_{ab} segment, the ideal orientation of the immobilized antibody is when the F_c fragment binds to the adsorbent substrate surface and the F_{ab} is oriented so that it is exposed to the analyte solution [13]. Thus, this oriented Ab immobilization is expected to yield an analytical improvement of the final immunosensor.

There are few reported strategies related to the oriented immobilization of antibodies to solid supports. Feng, Luo, Ge, Wang, Huang, and Dai reported an affinity ligand fused to the N/C-terminus of chimeric antigen for site-oriented immobilization, which resulted in significant enhancement of analytic signal [14]. Rosales-Rivera, Acero-Sánchez, Lozano-Sánchez, Katakis, and O'Sullivan reported the oriented immobilization of antibodies on surfaces of gold, achieved using a carboxylic-terminated bipodal alkanethiol that is covalently linked with amino groups of the antibody's F_{ab} fragments [15]. A common approach includes attaching protein A or G to the biorecognition surface before binding the Ab. Proteins A/G have the ability to bind specifically to the Fc region, thereby allowing a suitable orientation of the biomaterial. Binding these proteins hinders however the nanostructural organization of the biosensing surface, considering that the proteins are typically randomly attached. Other more complex approaches reduce disulfide bonds present in the peptide chains of the Ab. The resulting thiol side chains are used after as reactive sites for conjugation to gold or maleimide functionalized surfaces [16]. The simplest process for Ab orientation modifies its F_c region in order to facilitate its chemical binding to an amine layer under mild conditions. This is achieved by carboxylic activation at the F_c terminal via carbodiimide chemistry [16].

But binding an activated Ab to a carbon support requires that the latter is modified to yield an amine terminated surface. Surface functionalization with free reactive nitrogen-containing groups, particularly primary amines has been implemented in several fields of material science, including adhesion of polymers, antibacterial surfaces, cell attachment [17–23]. The use of amine terminated organosilanes as amine-surface modifiers is among the most common approaches in this context [24–26]. In this, the silane group binds to silicon or glass substrates [27] where vicinal hydroxyl groups may be found. In practice, CNTs are not normally hydroxyled, but these hydroxyl groups may exist as defects in the carbon matrix or may be intentionally added by suitable chemical or electrochemical modification.

The 3-triethoxysilylpropylamine (APTES) is the most commonly used organosilane for producing amine surfaces and immobilizing different entities of biological interest on various substrates [7,28–34]. APTES has a terminal primary amine and three ethoxy groups *per* molecule bonded to the Si atom. These ethoxy groups can polymerize in the presence of water, which can give rise to different surface coverages: covalent attachment, two-dimensional self-assembly (horizontal polymerization), and multilayers (vertical polymerization). The resulting structure and coverage of the layers are highly dependent on several experimental parameters, including temperature [35] and humidity [33,36]. The simplest route yields a single aminopropylsilane layer having amine-terminal groups on the modified surface.

This work reports electrochemical oxidation procedures for CNTs, yielding hydroxyl functional groups, the subsequent attachment of APTES followed by oriented Ab attachment. This is applied herein to human chorionic gonadotrophin (hCG) detection, by using an anti-hCG antibody as a biological receptor. hCG is a 37 kDa glycoprotein hormone and is most well-known as an important diagnostic marker of pregnancy. Several immunosensing devices for hCG have been reported in the literature so far [37–41] but simple and low cost procedures are still needed for the routine POC determination of this biomarker. Considering that an immunosensor is not reusable and that the antibodies are expensive and need special storage conditions, it is very important to establish simple, low cost and rapid detection procedures, both at the stage of biosensor development and at its subsequent analytical application step.

The electrochemical analytical approach selected was EIS, because it enabled label-free, low cost and quick analytical procedures. The calibration curve for the CNT-SPE hCG sensor and the selectivity of the detection method have been determined and are presented. The sensor device has been used to successfully detect hCG in the urine of pregnant women.

2. Experimental Section

2.1. Materials and Reagents

All chemicals used were of analytical grade and water was de-ionized. Potassium hexacyanoferrate III ($K_3[Fe(CN)_6]$) and potassium hexacyanoferrate II ($K_4[Fe(CN)_6]$) trihydrate and magnesium chloride were obtained from Riedel Haen; 3-Triethoxysilylpropylamine (APTES) was obtained from VWR; N-Hydroxysuccinimide (NHS) and creatinine was obtained from Fluka; *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDAC) and Bovine serum albumin (BSA) were obtained from Sigma; Phosphate Buffered Saline (PBS) Tablets were obtained from Amresco. hCG protein was purchased from Abcam (UK). Anti-hCG antibody was supplied by Ig Innovations. Ammonium chloride and calcium chloride were obtained from Merck. Sodium dihydrogen phosphate was obtained from Scharlau. Potassium sulphate and sodium chloride were obtained from Panreac.

A solution of 0.5% APTES (0.021 mol dm^{-3}) prepared in water was employed. PBS solution was prepared by dilution of 1 tablet (1814.5-2005.5 mg/tab) of PBS in 200 cm^{-3} of D. I. water. EDAC, NHS and antibody solutions were prepared in this buffer. Synthetic urine was prepared with creatinine (5.3×10^{-6} mol dm^{-3}), magnesium chloride (9.8×10^{-7} mol dm^{-3}), calcium chloride (6.8×10^{-7} mol dm^{-3}), sodium dihydrogen phosphate (3.2×10^{-6} mol dm^{-3}), ammonium chloride (3.3×10^{-6} mol dm^{-3}), potassium sulphate (3.9×10^{-6} mol dm^{-3}) and sodium chloride (6.5×10^{-6} mol dm^{-3}).

2.2. Apparatus

Electrochemical measurements were conducted using a potentiostat/galvanostat from Metrohm (Autolab PGSTAT302 N), with Frequency Response Analysis (FRA) module, controlled by Nova software. CNT-SPEs were purchased from DropSens (DRP-110SWCNT), having a counter electrode of Platinum (Pt), a reference electrode of Ag/AgCl, and a 4 mm diameter CNT working electrode. The CNT-SPEs were interfaced with the potentiostat/galvanostat equipment by a switch box, also from DropSens (DRP-DSC). Raman measurements were performed using a Thermo Scientific DXR Raman microscope system with a 100 mW 532 nm excitation laser. Spectra were recorded using a 5 mW power and 50 μm pinhole aperture.

2.3. Surface modification

The surface of the CNT-SPE was modified first by electrochemical oxidation. The three-electrodes of the SPE were immersed in 0.5 mol dm^{-3} of H_2SO_4 and cyclic voltammetry (CV) performed by sweeping the potential from −0.2 to 1.5 V, at a sweep rate of 50 mV s^{-1}, for 20 cycles.

The CNT working electrode at the SPE was subsequently amine terminated, by reaction with 0.021 mol dm^{-3} of APTES in water.