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A dye-sensitized solar cell acting as the electrical reading box of an immunosensor: Application to CEA determination

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ABSTRACT

Monitoring cancer biomarkers in biological fluids has become a key tool for disease diagnosis, which should be of easy access anywhere in the world. The possibility of reducing basic requirements in the field of electrochemical biosensing may open doors in this direction. This work proposes for this purpose an innovative electrochemical immunosensing system using a photovoltaic cell as an electrical reading box. Immunosensing ensures accuracy, the electrochemical-ground of the device ensures sensitivity and detectability, and the photovoltaic cell drives the system towards electrical autonomy. As proof-of-concept, Carcinoembryonic antigen (CEA) was used herein, a cancer biomarker of clinical relevance.

In brief, a conductive glass with a fluorine doped tin oxide film was used as conductive support and modified with anti-CEA by means of a bottom-up approach. All stages involved in the biochemical modification of the FTO surface were followed by electrochemical techniques, namely electrochemical impedance spectroscopy and cyclic voltammetry. This electrode acted as counter electrode of a dye-sensitized solar cells, and the electrical output of this cell was monitored for the different concentrations of CEA. Under optimized conditions, the device displayed a linear behaviour against CEA concentration, from 5 pg/mL to 15 ng/mL. The immunosensor was applied to the analysis of CEA in urine from healthy individual and spiked with the antigen.

Overall, the presented approach demonstrates that photovoltaic cells may be employed as an electrical reading box of electrochemical biosensors, yielding a new direction towards autonomous electrochemical biosensing.

1. Introduction

Biosensors are analytical devices that convert molecular recognition of a specific analyte into a measurable signal via a transducer, allowing quick responses, low cost and little requirements (Florescu et al., 2007; Sin et al., 2014; Turner, 2013). Among the different approaches in clinical analysis, electrochemical immunoassays hold a great impact in the literature (Turner, 2013). Immunoassays involve antigen/antibody reactions, inspired in the immune response (Turner, 2013; Emon, 2007). The use of antibody ensures appropriate selectivity towards a target compound, even when this compound is in very low concentrations. Electrochemical immunosensors are of special interest herein, due to their simplicity, easy miniaturization, specificity, selectivity, high sensitivity and rapid analysis (Teixeira et al., 2014). Although using an enzymatic reaction, glucose meters are a public icon of electrochemical biosensors. In general, the combination of antibodies and electrochemical biosensors has been proven successful in many applications (Turner, 2013).

This works emerges a novel biosensor technology that consists on integrating an electrochemical immunosensor inside a photovoltaic (PV) cell. PV cells generate current from light with reasonable efficiency (Wei et al., 2010) and may therefore lead electrochemical-based biosensors towards an electrical-independent approach. The first step in this direction consists in evaluating the impact of an immunosensor on the PV and understanding if the electrical performance of such new system depends on the concentration of a given target compound. To pursue this target, two key elements are identified next: the type of the PV and the target compound to be tested in this proof-of-concept design.

There are different types of PV cells. Dye-sensitized solar cells (DSSCs) have attracted much attention due to their low cost, easy manufacture and high energy conversion efficiency (Grätzel, 2001; O'Regan and Grätzel, 1991). DSSCs contain (a) a photoanode electrode, (b) a redox system electrolyte and (c) a cathode or counter electrode (CE) (Shirke et al., 2011; Hagfeldt et al., 2010). Merging a biosensor and a DSSC could be as easy as using one of these two electrodes

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(photoanode or cathode) as the biosensor, provided that the biosensor itself would be constructed on a conductive glass support that could be interfaced within the final DSSC. Eventually, one could try to replace any of these two electrodes. From a practical perspective, the photoanode is more sensitive to water-content components, hindering its interaction with water-based samples. Replacing the CE would be simpler, if the sensing element displayed electrocatalytic properties towards the redox probe. In alternative to the typical platinum-based CEs (Hagfeldt et al., 2010), carbon-based materials may be employed. These include graphite (Yen et al., 2009), graphene (Bon et al., 2010), carbon nanotubes (Yen et al., 2009); conducting polymers (Gholamkhass and Holdcroft, 2011; Yen et al., 2009); or conductive inks (Dang et al., 2015; Yang et al., 2012) containing metal nanoparticles. The most widely used materials are silver nanoparticles, due to their simple preparation, high-yielding, low viscosity and thus easy to flow under the conductive substrate surface, low resistivity and good electrical properties (Gliga et al., 2014; Yang et al., 2012). Thus, the CE is prepared herein with a conducting carbon-silver ink, which may be interfaced with antibodies.

As proof-of-concept, Carcinoembryonic antigen (CEA) was selected herein as target compound. CEA is an important tumour marker employed in clinical diagnosis of colorectal cancer (Lech et al., 2016). Many immunoassay-based analytical approaches have been described in the literature for CEA detection (Casey and Kofinas, 2008; Hammarström, 1999; Kemmegne-Mbouguen et al., 2014; Liu et al., 2010; Lv et al., 2010; Miao et al., 2014; Shi and Ma, 2011; Tan et al., 2006; Emon, 2007), which are typically expensive, complex and time consuming, also requirying specially equipped laboratories and highly qualified personnel. Electrical-based techniques such as potentiometry (Wang et al., 2010), and amperometry (Kemmegne-Mbouguen et al., 2014; Lv et al., 2010) have also been described. Overall, all these methods would benefit from the new approach proposed herein, which is simple in terms of device set-up and expects to yield suitable analytical features, while targeting electrical independency in a near future.

Thus, this work proposes (1) a different and simple immunosensor design for CEA, employing simple chemistry strategies that allow suitable orientation of the Ab-CEA on top of the modified conductive support; and (2) merging this biosensor on a DSSC. The electrode design is optimized and the biosensor is tested in both formats: conventional modified glass and working as the CE of the DSSC. The system is further successfully calibrated in real urine samples spiked with the protein.

2. Experimental section

2.1. Reagents and solutions

All chemicals were of analytical grade and water was deionized or ultrapure Milli-Q laboratory grade. Ethanol absolute from Panreac (Spain); acetonitrile from Scharlau (Spain); acetic acid glacial (100% p.a.) and ethylene glycol (analytical reagent) from Analar Normapur (USA); phosphate buffered saline (PBS) pellets, E404, biotechnology grade, from Amresco (USA); ethylenediamine and silver nitrate ($AgNO_3$) from Merck (Germany); potassium hexacyanoferrate III ($K_3[Fe(CN)_6]$), potassium hexacyanoferrate II ($K_4[Fe(CN)_6]$) trihydrate and iodine (I_2) from Riedel-de-Häen; bovine serum albumin (BSA), CEA from human fluids (CEA); *cis*-bis(isothiocyanato)bis(2,2´-bipyridyl)−4,4´-dicarboxilate) ruthenium(II) (dye N3), *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimidehydrochloride (EDAC), 1-hexyl-3-methylimidazolium iodide (HMII), *N*-hydroxysuccinimide (NHS), lithium iodide, monoclonal anti-carcinoembryonic antigen antibody produced in mouse (Ab-CEA), 4-tert-butylpyridine (TBP) and titanium (IV) oxide (TiO_2) (> 99.7%, anatase, < 25 nm) from Sigma-Aldrich (USA). The Ab-CEA solution was used after a 10 × dilution of the commercial product in PBS buffer (pH 7.4), and was used throughout this work.

2.2. Equipment

The electrochemical measurements were made in a potentiostat/galvanostat, Autolab PGSTAT302N, from Methrom (United Kingdom), equipped with a Frequency Response Analysis (FRA) module, interfaced to computer and controlled by NOVA 1.9 software. DSSC measurements were made in the same equipment, coupled to a LED driver accessory, also from Methrom (United Kingdom). Electrochemical impedance analysis (EIS) was performed in a frequency range 0.01–10,000 Hz. The photocurrent density-photovoltage (J-V) response of the solar cell was obtained using PV measurement system, under 77.7 mW/cm^2, having a LED driver operating with 450 mA output warm white LED. The cell area was fixed at 3.6 cm^2. Sintering was made in a Nabertherm GmbH P330 oven (Germany).

Solid materials were characterized by Fourier Transform Infrared Spectrometry (FTIR), using a Nicolet iS10 FTIR spectrometer, from Thermo Scientific (USA), couple to the Attenuated Total Reflectance (ATR) sampling accessory of diamond contact crystal, also from Nicolet. The spectra were collected under room temperature/humidity control, after background correction. The number of scans for sample and background was set to 100. The *x*-axis ranged from 650 to 4000 cm^{-1} and *y*-axis expressed in % transmittance. The resolution was 4000.

Solid materials were also analyzed by Raman spectrometry, using a DXR Raman spectroscope from Thermo Scientific (USA), equipped with a confocal microscope. The Raman spectra was collected with a 532 nm excitation laser, in the Raman shift from 200 to 3500 cm^{-1}, for 0.3 mW power and exposures of 90 s, in combination with a 50 × objective magnification for focus and collection of Raman-scattered light. The confocal aperture was set to 25 μm pinhole.

2.3. FTO electrode modification

A fluoride-doped tin oxide (FTO, sheet resistance 13.0 Ω/sq) glass was obtained from Sigma-Aldrich (USA), and was cut into 2.0 × 1.5 cm size slices. The slices were ultrasonically cleaned, first for 15 min in acetone and after for 15 min in deionized water, and then dried in nitrogen atmosphere.

The cleaned FTO electrodes were modified with an organic silver conductive ink (OSC ink). For the synthesis of the OSC ink, silver nitrate (0.08 g/mL) was first dissolved completely with a reducing agent (1.11 g/mL, ethyleneglycol), under vigorous stirring, at room temperature. Then, a complexing agent (0.90 g/mL, ethylenediamine) was added dropwise, to the previous solution, over a few min. (~ 60 s), followed by 500 μL of deionised water (Chang et al., 2012; Dang et al., 2015; Tao et al., 2013; Yang et al., 2012). The chemical reaction occurred slowly, changing from the pale yellow to a dark brown solution, revealing the formation of silver particles (Dang et al., 2015). The synthesized OSC ink was deposited on the surface of the pre-treated FTO coated glass and thermally sintered, set to a temperature of 200 °C, along 10 h. Thus, a silver film formed onto the FTO layer (FTO/Ag-NH_2) and acted as support of the immunosensing layer.

2.4. Assembly of the immunosensing layer

The immunosensor (FTO/Ag-NH_2/Ab-CEA) was assembled as represented in Fig. 1A. A solution of ethylenediamine (30 mg/mL) was placed first on the previous silver film for 2 h, at room temperature. Different conditions were tested to improve the efficiency of amine binding, in terms of time (during 30 min, and 1 and 2 h) and temperature (4, 25 and 60 °C).

In parallel, a solution of Ab-CEA was activated to bind the amine support. This was done by reacting the Ab-CEA solution with NHS (50 mg/mL) and EDAC (10 mg/mL), prepared in PBS buffer (pH 7.4), for 10 and 15 min, respectively. Next, the activated Ab-CEA was added to the Ag-NH_2 layer, at 4 °C, for 1 h. The resulting surface was then loaded with BSA solution (33.2 mg/mL), for 2 h, at 4 °C, in order to