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Short communication

Digital multimeter-based immunosensing strategy for sensitive monitoring of biomarker by coupling an external capacitor with an enzymatic catalysis

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ABSTRACT

A new digital multimeter (DMM)-based immunosensing system was designed for quantitative monitoring of biomarker (prostate-specific antigen, PSA used in this case) by coupling with an external capacitor and an enzymatic catalytic reaction. The system consisted of a salt bridge-linked reaction cell and a capacitor/DMM-joined electronic circuit. A sandwich-type immunoreaction with target PSA between the immobilized primary antibody and glucose oxidase (GO*x*)-labeled detection antibody was initially carried out in one of the two half-cells. Accompanying the sandwiched immunocomplex, the conjugated GO*x* could catalyze the oxidation of glucose, simultaneously resulting in the conversion of $[Fe(CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$. The difference in the concentrations of $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ in two half-cells automatically produced a voltage that was utilized to charge an external capacitor. With the closing circuit switch, the capacitor discharged through the DMM, which could provide a high instantaneous current. Under the optimal conditions, the resulting currents was indirectly proportional to the concentration of target PSA in the dynamic range of 0.05–7 ng mL^{-1} with a detection limit (LOD) of 6 pg mL^{-1}. The reproducibility, precision, and selectivity were acceptable. In addition, the methodology was validated by analyzing 12 clinical serum specimens, receiving a good accordance with the referenced values for the detection of PSA.

1. Introduction

Enzyme immunoassay based on specific antigen–antibody interaction is a very powerful technique for the detection of clinically important analytes in a variety of biological matrixes (Akhavan-Tafti et al., 2013; Gordon and Michel, 2012). Nowadays, ongoing efforts have been made worldwide to develop new immunoassays with the aim of manufacturing portable and affordable diagnostic devices based on various signal-generation principles, such as fluorescence (Yu et al., 2013; Zhao et al., 2013), surface-enhanced Raman scattering (MacLaughlin et al., 2013), electrochemistry (Zhang et al., 2013a), electrochemiluminescence (Akhavan-Tafti et al., 2013), and colorimetric assay (Gao et al., 2013). Despite many advances in this field, there are still demands for application of new schemes and strategies to development of advanced immunoassay.

Electrochemistry holds great potential as the next-generation detection strategy because of its high sensitivity, simple instrumentation, and excellent compatibility with miniaturization technologies (Tang et al., 2010). For successful development of new immunoassay methods, two basic aspects for obtaining acceptable degree of sensitivity and reproducibility exist (Date et al., 2013). The first key point is to exploit highly efficient signal-transduction labels (tags) (Makaraviciute and Ramanavicience, 2013). Another important issue is to adapt a simple and sensitive signal-transduction method (Vidal et al., 2013). Undoubtedly, enzyme labels are used more widely than any other type of labels. This is because a single molecule of enzyme, *e.g.*, horseradish peroxidase, may cause the conversion of 10^7 molecules of substrate per minute (Zhang et al., 2013b).

The digital multimeter (DMM) is one of the most common items of test equipment used in the electronics industry today (Chun et al., 2013). DMM displays a measured value, and may also display a bar of a length proportional to the quantity being measured (Cravanzola et al., 2013). While there are many other items of test equipments that are available, the multimeter is able to provide excellent readings of the basic measurements of amps, volts and ohms. Recently, the DMM was also applied to the area of

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analysis due to its capability of instantaneous release of stored electrical energy. Liu et al. (2012) developed an origami paper analytical device that utilized aptamers as the sensing probe and very simple electrochemical readout with a DMM. Ge et al. (2013) and Wang et al. (2013) also demonstrated a microfluidic paper-based analytical device combining microfluidic origami device with an internal chemiluminescence light source and an external DMM for the detection of biomolecules. Inspired by these schemes, our motivation in this work is to explore a new electrochemical immunoassay protocol for sensitive determination of low-abundance proteins by coupling the merits of the conventional enzyme immunoassays with a portable and inexpensive DMM.

Prostate-specific antigen (PSA), as a glycoprotein enzyme encoded in humans by the *KLK3* gene, is present in small quantities (<4 ng mL^{-1}) in the serum of men with healthy prostates, but is often elevated in the presence of prostate cancer or other prostate disorders (Wu et al., 2007). Herein, we report the proof-of-concept of a novel and powerful DMM-based immunosensing platform for sensitive monitoring of PSA (as a model) by combining with an enzyme immunoassay format. The assay system comprises of salt bridge-linked two half-cells and DMM/capacitor/switch-joined electronic circuit. In the presence of target PSA, a sandwiched immunocomplex is formed in the reaction cell using glucose oxidase (GO*x*)-labeled polyclonal anti-PSA antibody as detection antibody. Upon introduction of $[Fe(CN)_6]^{3-}$, the labeled GO*x* can trigger the conversion of $[Fe(CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$, and causes the change in the concentrations of $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$ between two half-cells, thus producing a voltage to charge the capacitor. With the switch closed, the capacitor discharges through the DMM to produce an instantaneous current. By monitoring the change in the current, we can quantitatively determine the concentration of target PSA in the sample.

2. Experimental

2.1. Materials and reagents

Mouse anti-human monoclonal prostate-specific antibody (designated as mAb_1) and polyclonal anti-human PSA antibody (designated as pAb_2) were purchased from Amyjet Sci. Inc. (Wuhan, China). PSA standards with various concentrations were obtained from Biocell Biotechnol. Co., Ltd. (Zhengzhou, China). Glucose oxidase (GO*x*) and bovine serum albumin (BSA) were obtained from Dingguo Biotechnol. Inc. (Beijing, China). A pH 9.6 carbonate buffer (1.59 g Na_2CO_3, 2.93 g $NaHCO_3$ and 0.2 g NaN_3) and a pH 7.4 phosphate-buffered saline (PBS, 0.01 M) (2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.24 g KH_2PO_4, 0.2 g KCl and 8.0 g NaCl) were prepared by adding the corresponding chemicals into 1000 mL distilled water. The blocking buffer and washing buffer were obtained by adding 1.0 wt% BSA and 0.05% Tween 20 (v/v) in PBS, respectively. All other reagents were of analytical grade and were used without further purification. Ultrapure water obtained from a Millipore water purification system (Milli-Q, Millipore) was used in all runs. Clinical serum samples were made available by Fujian Provincial Hospital, China.

2.2. Preparation of glucose oxidase–pAb_2 (GO*x*–pAb_2) conjugates

GO*x*–pAb_2 conjugates were prepared according to Hermanson's method (Hermanson, 2008). Initially, a solution containing 1.0 mg mL^{-1} pAb_2 and 2.5 mg mL^{-1} GO*x* was prepared by mixing them in pH 7.4 PBS (0.01 M) at 4 °C. Then, 10 μL of 25% glutaraldehyde (v/v) was added into 200 μL of the above-prepared mixture in a fume hood, and incubated for 2 h at 4 °C. Following that, sodium borohydride at a final concentration of 10 mg mL^{-1} was injected in the mixture, and the reaction was carried out for 1 h under the same conditions to reduce the resultant Schiff bases and any excess aldehydes. To remove any insoluble polymers that might have been formed, the conjugates were filtered through a 0.45 μm filter. Finally, the obtained GO*x*–pAb_2 conjugates were purified by dialysis using pH 7.4 PBS, and dispersed in 500 μL pH 7.4 PBS for further use.

2.3. Preparation of salt bridge-linked immunoassay system

Prior to experiment, a U-shaped glass tube-based salt bridge was prepared using agarose and KCl electrolyte. Briefly, 30 mg of agarose was initially added into 970 μL distilled water, and heated until the agarose was completely dissolved. Following that, 300 mg of KCl powder was added to the resulting solution. After adequate stirring, the mixture was injected into the U-shaped glass tube. Finally, the salt bridge was formed until the agarose congealed, which was immersed into KCl saturated solution when not in use.

The immunoreaction system consisted of one immunoreaction cell and one control cell, as shown in Fig. 1. A high-binding polypropylene 96-well microtiter plate (Ref. 655061, Greiner, Frickenhausen, Germany) was coated overnight at 4 °C with 50 μL per well of mAb_1 at a concentration of 10 μg mL^{-1} in 0.05 M sodium carbonate buffer (pH 9.6). The microplate was covered with adhesive plastic plate sealing film to prevent evaporation. On the following day, the plate was washed three times with pH 7.4 PBS, and then incubated with 300 μL per well of blocking buffer for 1 h at room temperature (RT) with shaking. The plate was then washed as before, which was used for the antigen–antibody reaction.

2.4. Immunoassay protocol and DMM measurement

The immunoassay protocol and DMM measurement are schematically illustrated in Fig. 1. Initially, 50 μL of PSA standards or samples with various concentrations in pH 7.4 PBS were added to the immunoreaction cell, and incubated for 1 h at 37 °C under shaking on an end-over-end shaker (MS, IKA GmbH, Staufen, Germany). After washing, 50 μL of excess GO*x*–pAb_2 was added into the well and incubated for 1 h at 37 °C with shaking. The plates were washed again. Following that, the immunoreaction cell and another control cell without target PSA were connected using the above-prepared salt bridge. Two platinum wire electrodes linked with the

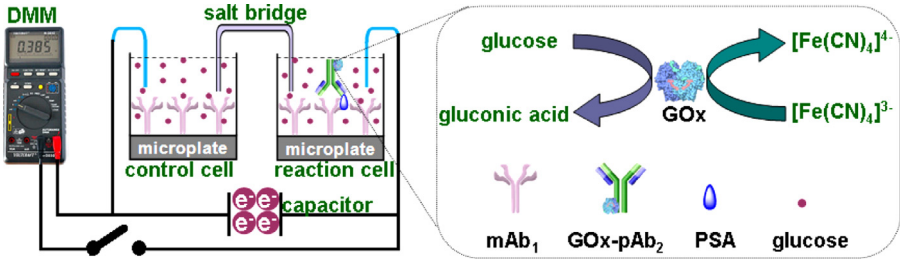

Fig. 1. Schematic illustration of the DMM-based immunoassay protocol.