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Rechargeable battery-based constant-potential electroluminescence multiplexed immunoassay on single working electrode for sequentially anodic and cathodic detection through a self-assembly toggle switch

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ABSTRACT

A common rechargeable battery with an output voltage of 1.20 V was used to develop a constant potential-triggered electroluminescence (ECL) protocol, and a low-cost toggle switch was self-assembled to convert the anodic or cathodic applied potential on working electrode by simply rotating. Based on the potential-resolution strategy, a facile ECL multiplexed immunoassay was constructed on single indium tin oxide working electrode for sequential detection of carcinoma antigen 153 and carcinoma antigen 199 (one working electrode for two analytes) by employing an anodic ECL label ($Ru(bpy)_3^{2+}$-conjugated silica nanoparticles, +1.20 V) and a cathodic one (carbon nanocrystals dotted silica nanoparticles, −1.20 V). Under the optimal condition, the proposed multiplexed immunoassay displayed wide linear range with low detection limit, and the assay results for two tumor markers in human serum samples obtained by this system were in acceptable agreements with the reference values from commercially used method in Cancer Research Center of Shandong Tumor Hospital. The rechargeable battery-based ECL system and potential-resolution strategy equipped with a self-assembly toggle switch provided a low-cost, promising multiplexed immunoassay for point-of-care diagnosis.

1. Introduction

Though cancer is one of the most devastating diseases worldwide, most metastatic cancer deaths can be avoided by early, sensitive and accurate detecting [1–3]. In many cases, the detection of single biomarker is not sufficient to diagnose cancer, and it cannot represent the various mechanisms caused by multiple genetic changes, resulting in low diagnosis rate. Compared with single-analyte immunoassay, multiplexed immunoassay (MIA) is more efficient in clinical application since it can detect more than one analyte in a single run; meanwhile, it can shorten analytical time and decrease sampling volume and detection cost. More importantly, MIA can improve the accuracy of immunoassay in a wide range of cancer types by detecting several components in one complex sample [4,5].

For MIA, multi-label mode is widely adopted using different labels, including enzymes [6], radioisotopes [7], fluorescence dyes [8], metal ion chelates [9], and nanoparticals [10] to tag antibodies or antigens corresponding to the analytes (one label per analyte), whose signals are distinguished by some parameters such as wavelength, decay times, stripping potential, or melting temperature. However, when the multi-label mode MIA was performed on signal sensing zone, the corresponding enzyme, radio or fluorescence-based MIA inevitably suffered the cross-talk from signal overlapping of the multiple labels [11,12]. Another mode is performed using one universal label to detect all analytes on different spatial areas, but this spatial-resolved mode MIA suffers from the cross-talk due to the diffusion of the universal label [13–16]. Though this drawback can be partly overcome by rendering the distance between the adjacent sensing zones to be large enough, this strategy still encounters a difficulty in arranging more equal sensing zones in limited detection space [17]. In this paper, one of the main purposes is to develop a MIA realized on single sensing zone without the cross-talk and signal overlapping.

So far, the commercially available clinical immunoassays such as enzyme-linked immunosorbent assay [18–21] and mass spectrometric immunoassay [9,22] have been well carried out in developed economies, but they are often not applicable in developing countries, because these analytical systems are too expensive, large, complicated, and dependent on specific infrastructures. Therefore, it is of considerable interest to the further research for sensitive, accurate, rapid and simple alternative methodology. Electroluminescence (ECL) [23–26], which combines the

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advantages of chemiluminescence and electrochemistry, shows high sensitivity and wide dynamic concentration response range. More importantly, ECL is potential- and spatial-controlled. Recently, we proposed a constant-potential mode ECL immunoassay on microfluidic paper-based analytical devices (μ-PADs) [27,28], and it showed good practical applications. However, there are still pressing needs for more simple, low-cost and durable methods: (1) though the wax-patterned μ-PADs are low-cost, the commercially wax printer (FUJIXEROX Phaser 8560DN, Japan) is expensive; (2) the durability of μ-PADs was not as good as that of indium tin oxide (ITO)-, ceramic- or polyethylene terephthalate (PET)-based chips; (3) when the anodic or cathodic applied potential on working zone is altered, it must be manually done through opening the luminescence detector and reversing the connection mode of power supply, therefore, a simple power supply change-over device (named toggle switch) arranged outside of the luminescence detector would be helpful.

In this paper, a rechargeable battery-triggered constant-potential mode ECL system was designed and further applied on an ITO two-electrode device to develop a simple, low-cost and durable ECL chip. In addition, to exactly control the anodic or cathodic applied potential on working zone, a corresponding low-cost, simple toggle switch was designed and fabricated for the first time. Based on these components, we constructed an ECL MIA on two electrodes (one working electrode and one auxiliary electrode) using two kinds of ECL labels ($Ru(bpy)_3^{2+}$-conjugated silica nanoparticles ($RuSi@Ru(bpy)_3^{2+}$ NPs) as anodic ECL reagent, carbon nanocrystals dotted silica nanoparticles (CNCs@Si NPs) as cathodic ECL reagent), and realized sequential detection under different mode of applied potential. Gold-coated magnetic iron nanoparticles (Fe@Au MNPs) were used as matrix to immobilize the primary monoclonal antibodies, which can be easily adsorbed on or removed from the working electrode surface with external magnetic field. Moreover, the magnetism-controlled matrix can simplify the washing procedure and provide a separation and enrichment effect to increase the accuracy. Using carcinoma antigen 153 and carcinoma antigen 199 as model analytes, the performance of the MIA was investigated. This work could not only make contribution to the ECL, but also provide potential applications for the simple, low-cost MIA detection in remote regions and developing countries for point-of-care diagnostics.

2. Materials and methods

2.1. Reagents

All reagents were of analytical-reagent grade and directly used for the following experiments without purification, and all solutions were prepared using Millipore (model milli-Q) purified water. ITO glass (a thickness of ITO layer of 150 nm and resistance of <15 Ω/square; thickness of glass: 1.1 mm) was obtained from Xiamen ITO Photoelectricity Industry (Xiamen, China). Antigens, primary monoclonal antibodies ($McAb_1$) and the secondary antibodies ($McAb_2$) for carcinoma antigen 153 (CA 153) and carcinoma antigen 199 (CA 199) were purchased from Shanghai Linc-Bio Science Co. Ltd. Ethanol, Triton X-100, cyclohexane, 1-hexanol, acetone, tetraethoxysilane (TEOS), vaseline, hydrochloric acid (HCl), nitric acid (HNO_3), $NH_3{\cdot}H_2O$, sodium citrate, potassium peroxydisulfate ($K_2S_2O_8$), $FeCl_3$ and $FeCl_2{\cdot}4H_2O$ were purchased from Tianjin Damao Chemical Reagent Factory. $HAuCl_4$, vaseline, tripropylamine (TPrA), 3-aminopropyltriethoxysilane (APTES), glutaraldehyde (GA), 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were obtained from Alfa Aesar China Ltd. L-Cysteine (L-Cys) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

2.2. Apparatus

The ECL signal was obtained on a commercialized luminescence detector (Xi'an Remex Analytical Instrument Ltd. Co.,) equipped with a photomultiplier tube (PMT, 200–800 nm) biased at 800 V. Transmission electron microscope (TEM) images were obtained from a Hitachi H-800 microscope. Scanning electron microscopy (SEM) images were recorded on a JEOL JSM-5510 scanning electron microscope.

2.3. Fabrication of rechargeable battery-based power supply equipped with a self-assembly toggle switch

The rechargeable battery-based power supply (Scheme 1A), fabricated according to a simple circuit theory (inset of Scheme 1A), consisted of a small rechargeable battery (a, price: ~$2; size: 11 mm in diameter, 44 mm in height; output voltage: 1.20 V), a battery jar (b, made of a discarded centrifuge tube), copper wires (L_1–L_4), and a self-assembly toggle switch (K, Scheme 1A). L_1–L_4 were used to link the self-assembly toggle switch "K" with the battery (L_1 and L_2) or the ITO two-electrode device (L_3 and L_4). The core component of the anodic/cathodic constant potential device is the toggle switch "K", and the diagrammatic sketch was shown in Scheme 1B–D. As shown in Scheme 1B, the toggle switch was fabricated on two cylindroid poly (methyl methacrylate) (PMMA) bases ("a" and "b", 20 mm in diameter), and two copper universal sockets "c" (red for "+", black for "−") were fixed on the base "a". A longer screw "d" was used as the supporting rod for base "b" on which two screws were fixed as contactors "e" (red for "+", black for "−"). With the help of a blind nut "f" and a returning spring across "g", base "b" was fastened and could be rotated manually. When the "+" universal socket contacted the "+" contactor and the "−" universal socket contacted the "−" contactor shown in Scheme 1C, the rechargeable battery-based power supply produced and output an anodic constant potential (+1.20 V) to trigger the anodic ECL (named mode I). Similarly, when the toggle switch was under mode II (Scheme 1D), the cathodic constant potential (−1.20 V) was provided to trigger the cathodic ECL. By simply rotating, the simple process to change mode can be performed manually within 1 s.

2.4. Fabrication of the ITO two-electrode device

Briefly, a piece of ITO glass (1.5 cm × 3.0 cm) was successively washed with ethanol and millipore purified water, and then dried in an oven at 90 °C. Then, the PMMA stencil contained two hollowed-out sections for screen-printing vaseline, which was already generated by traditional photolithography technique, was placed onto the ITO glass, and vaseline was smeared on the ITO surface. The two covered regions were respectively used as working electrode and auxiliary electrode. After the slice was immersed in HCl solution (6.0 M) for 20 min, the slice was washed with purified water. A multimeter was used to examine whether the ITO layer, except the covered zones, had been resolved completely. The acid etch process would be repeated until the ITO layer was resolved completely. Finally, the vaseline was removed by ethanol and purified water, and insulator ink was applied around the two electrodes to form a round area as the reaction cell (Scheme S1).

2.5. Preparation of this single working electrode-based MIA

Scheme 2 shows the preparation of this single working electrode-based MIA. In brief, L-Cys (0.1 mol/L, 0.5 mL), EDC/NHS (v/v = 1:1, 20 μL, 20 mg mL^{-1}) and PBS (pH ~ 7.4, 20 μL) were added