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Chemiluminescence excited photoelectrochemical competitive immunosensing lab-on-paper device using an integrated paper supercapacitor for signal amplication

Yanhu Wang[a], Haiyun Liu[a], Panpan Wang[a], Jinghua Yu[a], Shenguang Ge[b], Mei Yan[a,*]

[a] Key Laboratory of Chemical Sensing & Analysis in Universities of Shandong, School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, PR China
[b] Shandong Provincial Key Laboratory of Preparation and Measurement of Building Materials, University of Jinan, Jinan 250022, PR China

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ABSTRACT

A chemiluminescent (CL) excited photoelectrochemical (PEC) immunosensor for detection of carcinoembryonic antigen (CEA) was introduced into microfluidic paper-based analytical devices (μ-PADs) integrated with a paper supercapacitor (PS) amplifier, and a terminal digital multi-meter (DMM) detector, based on CdS/TiO_2 hybrid modified porous Au-paper electrode. The effective matching of energy levels between the conduction bands of CdS and TiO_2 allowed for fast electron injection from excited CdS to TiO_2 upon irradiation, which inhibited the recombination process of electron–hole pairs and prompted PEC performance. Using CEA/ABEI-AuNPs-GOx bioconjugates as signal labels which featured CEA, N-(aminobutyl)-N-(ethylisoluminol) (ABEI) and glucose oxidase (GOx) linked to Au nanoparticles for signal amplification could greatly enhance the sensitivity. GOx could catalyze glucose to produce H_2O_2, which acted as a co-reactant in the ABEI-AuNPs-H_2O_2-*p*-iodophenol (PIP) CL system as well as sacrificial electron donor to scavenge the photogenerated holes in the valence band of CdS QDs, further causing an enhanced photocurrent. The quantification mechanism of this strategy is based on the charging of this PS by the photocurrent. The generated photocurrent could be stored by the PS and released instantaneously through a low cost, portable, and simple DMM to obtain an amplified current for the quantification of CEA. Under the optimal conditions, this analytical platform could detect CEA at concentrations at picomole level. This work offers a new route to highly selective and sensitive detection of biologically important small molecules.

1. Introduction

The increasing demands of disease diagnostics and therapeutic analysis require the development of sensitive and accurate detection of disease-related proteins. Immunoassays based on specific molecular recognition of an antigen by its antibody have been widely used for quantitative analysis of protein biomarkers in biological samples for clinical purposes [1,2]. Conventional immunoassays for the detection of biomarkers include enzyme-linked immunosorbent assay (ELISA) [3], electrochemistry [4], electrochemiluminescence (ECL) [5], mass spectrometry [6], quartz crystal microbalance (QCM) [7] and surface plasmon resonance (SPR) immunoassays [8]. Although good sensitivity has been achieved, some of these techniques involve deficiency, such as relatively sophisticated instruments, significant sample volume, limited sensitivity, and clinically unrealistic expense and time. Therefore, there is a real need to develop operationally simple, highly sensitive, and inexpensive methods to detect levels of the biomarkers in both normal and cancer patient sera [9].

As a newly emerging but dynamically developing analytical technique for the sensing platform, photoelectrochemical (PEC) sensors have attracted considerable attention owing to their high sensitivity, rapid measurement speed, and inexpensive instrumentation [10]. Such PEC sensors combine the advantages of optical methods and electrochemical sensors, and thus show great promise for analytical applications. Besides the excitation light sources and photovoltaic materials employed, PEC biosensor possess better sensitivity due to the difference of input and output responses [11]. Thus, this technique shows promising analytical applications and has attracted considerable research interest. To date, many semiconductor nanoparticles (NPs), such as CdS, CdSe, ZnO, and TiO_2 [12–15], have been used in the construction of PEC sensors.

* Corresponding author. Tel.: +86 531 82767161; fax: +86 531 82765969.
E-mail address: chm_yanm@126.com (M. Yan).

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As an important semiconductor nanomaterial, TiO_2 has attracted increasing attention in the field of PEC sensing due to its high photosensitivity, good chemical stability and environmental safety [16–18]. Despite of the unique size- and shape-dependent optic and electronic properties of TiO_2, the wide band gap of TiO_2 limits its direct applications in PEC biosensing since it can only absorb ultraviolet (UV) light which limits the utilization of solar light. Meanwhile, the strong oxidizing power of the photogenerated holes of TiO_2 upon illumination may cause destructive effects for biomolecules [19,20].

Hence, many efforts have been made toward the development of TiO_2-based composites in visible-light activated PEC biosensing, which could largely increase the solar light utilization efficiency and reduce the destructive effect of UV light and the photogenerated holes of illuminated TiO_2 to biomolecules [21,22]. CdS quantum dots (QDs) is a well-known narrow band gap semiconductor material, which is photoelectrochemically active in the visible range and has been successfully used in PEC immunosensors [23]. In this work, we fabricated a sensitive PEC immunosensor based on CdS/TiO_2 hybrid modified electrode which could greatly improve the photocurrent intensity of CdS QDs due to the enhanced charge separation [24–26].

Currently, much effort has been directed to develop cost effective, simple, and miniaturized analysis tools. And microfluidic paper-based analytical devices (μPADs) which are particularly well-suited as a platform for the development of simple and cost-effective molecular diagnostic assays have attracted more and more attention during the past five years [27]. In this work, a novel microfluidic paper-based PEC device (μ-PPECD) was designed and fabricated based on the principles of origami. Wax was used as the paper hydrophobization and insulation agent to construct a hydrophobic barrier on paper. To further enhance the sensitivity of PEC assay, gold nanoparticles (AuNPs) modified paper working electrode (Au-PWE) with high conductivity and surface area was employed as the working electrode to obtain a high photocurrent through the further acceleration of electron transfer in the paper sample zone [28].

In all the conventional PEC methods, to measure the weak photocurrents sensitively, an electrochemical workstation [29] or lock-in amplifier [30] is required. However, the expensive and sophisticated workstation or lock-in amplifier makes the instrument complicated and departs from the portable and low-cost trend for μ-PADs. Hence, a newly developed strategy for substitution of the electrochemical workstation or lock-in amplifier is highly deserved [31]. The paper supercapacitor (PS) [32], an all state-of-the-art circuit component that can temporarily store a large amount of electrical energy and release it when needed, has attracted much attention due to its high electrical energy storage, long life cycle, and fast charging–discharging rate. Therefore, a novel all-solid-state PS was constructed and integrated into the prepared μ-PPECD as an effective electrical energy storage unit to collect and store the photocurrents. The stored electrical energy could be released instantaneously through a low-cost, portable, and simple digital multi-meter (DMM) to obtain an amplified current, allowing the expensive and sophisticated electrochemical workstation or lock-in amplifier to be abandoned in the PEC assay.

To further develop a simple, low-cost, and portable PEC immunoassays on μ-PAD, a chemiluminescent (CL) excited PEC assay was adopted in this work, based on the N-(aminobutyl)-N-(ethylisoluminol) functionalized gold nanoparticles (ABEI-AuNPs)–H_2O_2-PIP CL system, using *p*-iodophenol (PIP) as CL enhancer [28,31,33]. Additionally, when the proposed PEC immunosensor was exposed to a solution containing glucose and PIP, the glucose oxidase (GOx) which featured with analyte could catalyze the oxidation of glucose to produce gluconic acid and H_2O_2. The latter product acts as the co-reactant in the CL system as well as a sacrificial electron donor to scavenge photogenerated holes in the valence band of CdS QDs, thereby further improving the accumulation of electrons to lead to an enhanced photocurrent.

In this work, a novel paper-based CL-excited competitive PEC immunosensor for carcinoembryonie antigen (CEA) detection was constructed on μ-PADs using CdS/TiO_2 hybrid modified Au-PWE as the working electrode, a PS as the current-amplifier, and a DMM as the terminal current detector. AuNPs modified with CEA, ABEI and GOx to form CEA/ABEI-AuNPs-GOx bioconjugate, were used to enhance detection sensitivity by dual signal amplification via the competitive immunoreaction of CEA/ABEI-AuNPs-GOx and CEA with the antibodies immobilized on CdS/TiO_2 hybrid modified Au-PWE. An effective matching of energy levels between the conduction bands of CdS QDs and TiO_2 allowed for fast electron injection from excited CdS QDs to TiO_2 upon irradiation, which reduced the recombination process of electron–hole pairs and prompted PEC performance. Meanwhile, H_2O_2 the oxidation product of glucose acted as co-reactant in the CL system and a sacrificial electron donor to scavenge the photogenerated holes in the valence band of CdS QDs, further causing an enhanced photocurrent. The proposed methods presented high sensitivity, reproducibility, specificity and stability for CEA detection. Moreover, the established PEC biosensor would also provide a generic approach to analyze numerous substances in bioanalysis, as well as for simple, rapid, low-cost point-of-care testing in remote regions, developing or developed countries.

2. Experimental

2.1. Materials and reagents

All reagents were of analytical-reagent grade and directly used for the following experiments as supplied. Ultrapure water obtained from a Millipore water purification system (≥18.2 MΩ, Milli-Q, Millipore) was used in all assays and solutions. CEA and capture antibody (Ab) were purchased from Shanghai Linc-Bio Science Co. Ltd. (Shanghai, China). Blocking buffer was phosphate buffer solution (PBS) containing 0.5% bovine serum albumin (BSA) and 0.5% casein. Cadmium chloride ($CdCl_2{\cdot}2.5H_2O$), NaOH, ethylene glycol (EG), ascorbic acid (AA), sodium sulfide (Na_2S) and potassium ferricyanide were purchased from Shanghai Chemical Reagent Co. (China). Poly(vinyl alcohol) (PVA) (98–99% hydrolyzed, medium molecular weight), poly(dimethyldiallylammonium chloride) (PDDA) (20%, w/w in water, molecular weight = 200,000–350,000), and PIP were purchased from Alfa Aesar. Titanium butoxide ($Ti(OBu)_4$), thioglycolic acid (TGA), glucose oxidase (GOx, EC 1.1.3.4, 158.9 units/mg, from *Aspergillus niger*), glucose were all obtained from Sigma-Aldrich. Tetrachloroauric acid ($HAuCl_4$) as the precursor for the formation of AuNPs was purchased from Shanghai Sangon Biological Engineering Technology & Services Co. Whatman chromatography paper #1 was obtained from GE Healthcare Worldwide (Pudong Shanghai, China) and used with further adjustment of size.

2.2. Synthesis of TiO_2 Spheres, CdS QDs and CdS/TiO_2 hybrid

The TiO_2 sphere was prepared according to the previous work [34]. In a typical procedure, 1 mL of $Ti(OBu)_4$ was added to 22.5 mL of EG. This mixture was kept under vigorous stirring at room temperature for 8 h. This solution was then quickly poured into 100 mL of acetone containing 1.25 mL of deionized water and 3.0 mL of acetic acid. This mixture was kept under vigorous stirring at room temperature for another 3 h, yielding a white precipitate that was harvested by centrifugation. The solid, comprised of titanium glycolate microspheres, was washed several times with ethanol by