Sensors and Actuators B 242 (2017) 87–94

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Paper-based laser induced fluorescence immunodevice combining with CdTe embedded silica nanoparticles signal enhancement strategy

Mei Zhao, Huifang Li, Wei Liu*, Weiru Chu, Ying Chen

Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an, 710062, PR China

ARTICLE INFO

Article history:
Received 29 August 2016
Received in revised form 28 October 2016
Accepted 2 November 2016
Available online 5 November 2016

Keywords:
Laser induced fluorescence
Paper-based immunodevice
Silica nanoparticle
CdTe quantum dot
Signal enhancement

ABSTRACT

Paper-based laser induced fluorescence immunodevice for alpha fetoprotein (AFP) detection was developed in this work by using CdTe quantum dots (QDs) embedded silica nanoparticles as the signal enhancement label. A homemade laser induced fluorescence device with two optical fibres was used here for the fluorescence detection. Laser issued by a semiconductor laser light source (405 nm, 1 mW) was exposed to the paper-based chip with very lower background through an optical fiber and the fluorescence (542 nm) induced by laser was collected to a photomultiplier tube (PMT) through a glass fiber. The paper-based chip was fabricated by the wax-printing method and the antibody can be directly immobilized on the plasma treated paper surface. Then CdTe QDs embedded silica nanoparticles with the signal enhancement function was labeled on the signal antibody. After a sandwich-type immunoreaction, AFP was linearly detected in the range from 0.001 ng/mL to 20.0 ng/mL with a detection limit of 0.4 pg/mL. Laser induced fluorescence with the homemade device was for the first time applied on the paper-based chip with very higher sensitivity. What's more, the proposed strategy opened a promising platform for sensitive monitoring of cancer biomarkers in biological analysis and medical diagnostics.

1. Introduction

As paper has the advantages of low cost, highly abundant and biodegradable, the microfluidic paper-based analytical devices (μPADs) have developed to be a very promising platform for point-of-care (POC) diagnosis, public health and environmental fields in recent years [1,2]. μPADs was first proposed by Whitesides [3] who used the patterned paper for glucose and protein detection. Thereafter, increasing attention has been paid to μPADs in the field of POC diagnosis [2,4,5] because μPADs hold great potential to deliver POC diagnostics to developing countries. Immunoassay is one of the widely used and standard diagnostic techniques [6] and has been implemented in μPADs by several research groups [7–9]. Moreover, the paper-based immunodevice has the function of ease to use, rapid detection and possible analysis of complex samples without extensive pretreatment.

The first paper-based enzyme-linked immunosorbent assay (ELISA) which was regarded as a pioneering work in μPADs was still reported by Whitesides' group [7]. It was based on the colorimetric assays on paper microzone plate by using HIV-1 envelope antigens as a model for immunoassay. From then on, colorimetric detection method is the most widely used method in μPADs [10,11]. However, colorimetric assay is not sufficiently sensitive and the visual readout always cannot indicate a very lower level of the analytes. Recently, for improving the sensitivity, the detection methods including electrochemistry [12,13], electrochemiluminescence (ECL) [14,15] and chemiluminescence (CL) have been used more and more frequently on the paper-based immunodevice [16,17]. While, for ECL and electrochemical detection method, electrodes should be integrated on the paper surface firstly and only the electrochemically active species could be detected on the μPADs. For CL detection method, luminol system is the common used CL system [16] but the best CL signal can always get in the basic medium which is not suitable for the immunoassay.

Fluorescence (FL) [18–20] is another kind of optical detection method which can be used on the paper-based device with higher sensitivity. However, two flaws which are the needs for additional instrumentation and the paper itself [21] has a higher background signal will forbid to perform fluorometric measurements on the paper-based immunodevice. As the cellulose fibres can scatter the light and the strong light source on the instrument can induce the index of refraction between cellulose and air [22], laser was chosen here as the light source with the stable and lower power. By using a lower power of 1 mW, the laser light source was stabler

* Corresponding author.
E-mail addresses: weiliu@126.com, liuwei2@hotmail.com (W. Liu).

http://dx.doi.org/10.1016/j.snb.2016.11.004
0925-4005/

than the common fluorescence spectrophotometers and the lower background can be got on the paper surface with good repeatability. Combined with two optical fibres and a photomultiplier tube (PMT), a home-made laser induced fluorescence (LIF) device was assembled here. This home-made LIF device had been used in our group for immunoassay of bovine serum albumin (BSA) [23] and staphylococcal enterotoxin B [24]. However, this homemade device was for the first time used on the paper-based immunodevice to detect alpha-fetal protein (AFP) with higher sensitivity.

Recently, for the fluorescence detection, quantum dots (QDs) [25,26] have been used as a novel kind of fluorescence probe. With the quantum effect and size effect [26], QDs [25] have strong photoluminescence and good photostability. However, QD also had the disadvantages of cytotoxicity [27] as a result of the release of heavy metal ions and the susceptible surface to external environment. Then QDs was hybrided with some other materials such as silica [28,29] to overcome the above drawbacks. Along with providing chemical and physical shielding from the environment, silica nanoparticles still possess [30–32] the advantages of good water dispersibility, biocompatibility and surface functionality. Silica nanoparticles [33–36] can enhance the chemical stability of QDs, impede the diffusion of heavy metal ions into external environment, and reduce the toxicity of QD. For the covalently functionalization can be easily happened on the silica nanoparticles [34], functional group such as amino group [35,36] can be bonded on the silica nanoparticles. Then the silica nanoparticles can be acted as the label for the biomacromolecule. However, by encapsulating enzyme or coating with quantum dots, silica nanoparticles also can be adopted as a label with the signal enhancement function [31,37,38]. In this work, by using the CdTe QDs embedded silica nanoparticles as the signal antibody label, an amount of signal molecules of CdTe QDs were bio-conjugated to a single antibody. Then the enhanced signal can be achieved from the conjugated signal molecules. With this signal enhancement strategy on the paper-based immunodevice, AFP was detected with very higher sensitivity.

In this work, we described the paper-based LIF immunodevice combining with CdTe QDs embedded silica nanoparticles signal enhancement method for sensitive determination of AFP. A self-assembly LIF device which had a lower stable power light source was for the first time adopted on the paper-based device. CdTe QDs embedded silica nanoparticles was used as the effective signal enhancement fluorescence antibody label and the detection sensitivity was increased 4.7-fold compared to antibody-QD (Ab-QD) conjugation. With the low FL background and signal enhancement strategy, this paper-based LIF immunodevice was successfully performed for AFP detection in human serum with a detection limit of 0.4 pg/mL. The proposed paper-based LIF immunodevice opened a new era for sensitive detection of biomolecules and the established strategy was transferable for the detection of other disease-related biomolecules at the lowest level at their earliest incidence.

2. Experimental

2.1. Reagents and materials

AFP antibodies and ELISA kits for AFP were purchased from Zhengzhou Biocell Biotechnology Co. Ltd. (China). Bovine serum albumin (BSA), NaH_2PO_4, Na_2HPO_4, glutaraldehyde, ammonium hydroxide and absolute ethanol were purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Coupling buffer for antibody immobilization was 0.01 M phosphate buffer solution (PBS) with pH value of 7.4. Different concentration of AFP standard solution was prepared in PBS. Blocking buffer for the residual reactive sites was PBS containing 0.5% BSA. Whatman chromatography paper #1 (WCP#1) (200.0 mm × 200.0 mm), sodium borohydride ($NaBH_4$), cadmium chloride hydrate ($CdCl_2{\cdot}2{\cdot}5H_2O$), sodium tellurite ($NaTeO_3$), trisodium citrate dihydrate, glutathione (reduced form), tetraethylorthosilicate (TEOS), 3-aminopropyltriethoxysilane (APTES) were purchased from Sigma (U.S.A.). The clinical serum samples were provided by healthy adult volunteers from Shaanxi Normal University Hospital. The deionized water was used in all assays. All chemicals and reagents were of analytical grade and used without further purification.

2.2. Apparatus

The paper-based chip was created by ColorQube 8570 wax printer (Wlisonville, OR. U.S.A). The paper surface was treated to create the aldehyde group by oxygen plasma of the PDC-32G device (Mycro Technology Co. Ltd., U. S. A). The FT-IR spectra of paper-based chips were taken by fourier transform infrared spectroscopy (Bruker, Germany). The transmission electron microscopy (TEM) images of CdTe QDs and CdTe QDs embedded silica nanoparticles were taken by a Hitachi H-600 TEM (Tokyo, Japan). Images of energy dispersive x-ray (EDX) were performed on a Quanta 200 environmental scanning electron microscopy (ESEM) (Hitachi, Japan).

2.3. Fabrication of paper microzone plates

The paper microzone plates were designed and fabricated by the wax printing method as the reported work [39,40]. A design was created by using the drawing software (CorelDRAWX5) and then printed onto paper using a ColorQube 8570 wax printer. The paper zone with a diameter of 6 mm was prepared for later use.

2.4. Synthesis of CdTe QDs

The CdTe QDs were synthesized in the aqueous phase by a previous method [41]. 4 mL of 0.04 M cadmium chloride was diluted to 50 mL in a three-necked flask. Then trisodium citrate dihydrate (0.1 g), glutathione (0.05 g), Na_2TeO_3 (0.01 M, 4 mL) and $NaBH_4$ (0.05 g) were added into the three-necked flask with stirring for 2 h. The mixture was reacted at 90 °C in oil bath for a certain period of time. The obtained QDs were precipitated with ethanol and the precipitates were separated by centrifugation and were redissolved in 50 mM phosphate buffer solution (pH 7.4). The precipitation process was repeated for three times in order to eliminate the free glutathione ligands and salts in the crude CdTe QDs colloids. Then the obtained CdTe QDs solution was stored in the refrigerator (4 °C) for later use.

2.5. Synthesis of CdTe QDs embedded silica nanoparticles and surface modification

The CdTe QDs ($\lambda em = 530$ nm) embedded silica nanoparticles were prepared by a modification process based on the StÖber method [34]. Typically, 20 mL of ethanol, 4 mL of 9.0 μM QDs solution were mixed and stirred for 30 min at room temperature. Then, 0.1 mL of TEOS was added dropwise into the solution, followed by adding 0.2 mL of ammonium hydroxide, and finally the mixture was left to react for 12 h. After that, 50 μL of APTES was added into the above mixture under vigorous stirring to modify the silica surface with amino groups. After another 12 h of reaction, the products were centrifuged at 9000 rpm for 10 min and the precipitate was washed with ethanol and ultrapure water for several times. Finally, the amino functionalized CdTe QDs embedded silica nanoparticles were redispersed in 4 mL of ultrapure water and it was stored at 4 °C for further use.