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Use of quantum dot beads-labeled monoclonal antibody to improve the sensitivity of a quantitative and simultaneous immunochromatographic assay for neuron specific enolase and carcinoembryonic antigen

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ABSTRACT

Detection of multiplex tumor markers was of great importance for cancer diagnosis. Immunochromatographic test strip (ICTS) was the most frequently-used point-of-care detection means. Herein, a convenient and fast method for simultaneous quantitative detection of neuron specific enolase (NSE) and carcinoembryonic antigen (CEA) was developed based on ICTS using quantum dot beads (QBs) as marking material. Good monodispersity, high colloidal stability and carboxyl-modified (COOH–) QBs were used. For this method, two test lines were applied to the NC membrane for simultaneous analysis of CEA and NSE respectively. The ideal limit of CEA and NSE detection was 0.0378 ng/mL and 0.0426 ng/mL with scarcely any cross-reactivity. Moreover, the fluorescent signal intensity of the nitrocellulose membrane could be easily read out in the cooperation of the "Handing" system without professional operators. The possible clinical utilization of this platform was demonstrated by detecting 100 clinic human serums. The result showed that the platform had sensitivity of 99% and 97% for CEA and NSE, while the specificity was 97% and 100% respectively. Our results indicated that the QBs based ICTS not only owning the ability of sensitive and specific simultaneous detection of CEA and NSE, but also showing the potential in developing this ICTS into a routine part of early lung cancer diagnosis.

1. Introduction

Cancer constitutes an enormous burden on society in most countries. An estimated 1.8 million new lung cancer cases occurred in 2012, accounting for about 13% of total cancer diagnoses and about 25% of all cancer deaths [1]. Usually the measurement of a single tumor marker is not sufficient to diagnose a particular cancer [2]. Thus, simultaneous detection of multiple biomarkers at low concentrations facilitates diagnosis of lung cancer and treatment monitoring, further significantly improves the treatment efficiency and survival rates [3]. Compared with the single index assay, the multiplexed assay is more convenient and requiring less sample, shorter analysis time, and lower cost per assay [4].

Small cell lung cancer (SCLC) accounts for 13% of newly diagnosed cases of lung cancer. Highly malignant, short doubling time, early metastasis and easy to relapse make it be a notable health-care issue [5]. Neuron specific enolase (NSE) is a putative serum marker of SCLC. Moreover, it could act as a prognostic factor and disease activity monitor in SCLC [6] and a person is suspected of suffering from SCLC when the NSE level is higher than 24 ng/mL in serum [7].

Abbreviations: ICTS, immunochromatographic test strip; CEA, carcinoembryonic antigen; NSE, neuron specific enolase; CA72-4, carbohydrate antigen 72-4; PSA, prostate specific antigen; AFP, alpha-fetoprotein; QBs, quantum dot beads; QDs, quantum dots; SCLC, small cell lung cancer; EDC, 1-ethyl-3-(3- dimethyllaminopropyl)-carbodiimide hydrochloride; BSA, bovine serum albumin; NHS, N-hydroxy-succinimide; PBS, phosphate buffer saline; PVC, polyvinyl chloride; NC, nitrocellulose; T1, test line 1; T2, test line 2; C line, control line

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Carcinoembryonic antigen (CEA) is a broad spectrum of tumor markers which level indicates the progression of disease, curative effect and the prognosis estimate for colorectal cancer, breast cancer and lung cancer [8,9]. The normal level of CEA in a healthy person ranges from 2.5 to 5 ng/mL. Thus simultaneous analysis of NSE and CEA can in significant measure improving the sensitivity and specificity for lung cancer diagnosis.

Various methods have been reported to realize simultaneous analysis of multiple tumor markers in the past few years, such as ELISA [10], electrochemiluminescence immunoassay [2], electrochemical immunoassay [11,12], fluorescence detection [13,14], label-free methods [15,16] and so on. However, some methods are time consuming and need well-equipped facilities, complex operations, well-trained technicians and long analysis time. These restricted conditions limit their point-of-care testing application of tumor marker detection.

Immunochromatography test strip (ICTS) which combines immunolabel with chromatography, offer huge advantages of easy operation, low cost, short analysis time, minimized analyte volume as well as the capability of easily implementing point-of-care testing. For the purpose of improving detection sensitivity and further realizing accurate quantitative detection, numerous materials have been employed, such as colloidal gold nanoparticles [17–20], magnetic nanoparticles [21–23], organic fluorophores [24], heavy metal nanoparticles [25], upconverting nanomaterials [26] and quantum dots (QDs) [27–31]. Gold nanoparticles are the most common used label materials for ICTS. However, it has some shortcomings such as qualitative or semi-quantitative, low signal intensity, poor sensitivity, false positives or false negatives, background interference [32–35]. QDs are novel fluorescent nanoparticle label materials that have been greatly impressed by researchers due to the unique optical characteristics [36] including broad absorption and narrow emission wavelengths, high photostability and brightness. Specially, it has the ability to improve the limit of detection for ICTS. However, further ameliorating of sensitivity of ICTS based on QDs still face numerous challenges. The chemical and colloidal stability of QDs in biological environments would decrease after combination with some particular antibodies [37]. Furthermore, only 2–5 proteins around 100 kDa could be attached to a 5 nm QD for steric reasons [38] and the shape of the protein would influence the coupling rate [39]. To resolve these issues, we introduced macromolecule-coated QDs nanobeads (QBs) as label material which own better chemical and colloidal stability. In addition, by integrating more QDs into a single nanoparticle, the increasing quantity of QDs in each binding event will amplify the fluorescence signal [40,41].

Although there have already some approaches to realize simultaneous analysis of CEA and NSE, but so far, ICTS-based detection hasn't been reported. In this study, we developed a QBs based ICTS which allows quantitative and simultaneous detection of NSE and CEA. In addition, we also fabricated QDs-based ICTS to compare the differences in performance between the two ICTS. It turned out that the quantification limit of QDs and QBs for CEA were the same (1 ng/mL), and QBs (5 ng/mL) perform much better than QDs (100 ng/mL) for NSE detection. Meanwhile, the QBs maintained better specificity than QDs. Thus, we successfully fabricated an easy operation, rapid and accurate point-of-care platform that have great potential application in early lung cancer diagnosis in hospital, communities and homes.

2. Materials and methods

2.1. Materials and chemicals

Water-soluble QDs (620 nm) and QD nanobeads (610 ± 10 nm) were bought from WuHan JiaYuan Quantum Dots Corporation., Ltd. Goat anti-mouse IgG were obtained from Shanghai JieYi Biotechnology Co., Ltd. (China). The mouse anti-CEA(coating) McAb, mouse anti-CEA(labeling) McAb, CEA antigen (Ag) and NSE antigen purchased from Shanghai Linc-Bio Science Co., LTD. Mouse anti-NSE (9601) McAb and mouse anti-NSE (9602) McAb were bought from Medix Biochemica(Finland). 1-ethyl-3-(3- dimethyllaminopropyl)-carbodiimide hydrochloride (EDC), N-hydroxy-succinimide (NHS), Tween-20 and bovine serum albumin (BSA) were obtained from Aladdin (China). NC membrane M135 was provided by Millipore (USA). Conjugate pad, sample pad, adsorbent pad, semirigid polyvinyl chloride (PVC) sheets were supplied by Shanghai JieYi Biotechnology Co., Ltd. (China). XYZ Dispensing System (BioDot, XYZ3060, USA) were purchased from BioDot Inc. (Irvine, CA, USA).

2.2. Preparation of the two probes based on QBs

The conjugation of QBs with anti-NSE McAb (9601) and anti-CEA McAb (labeling) was the same. In this paper, we call them Ab1 and the anti-CEA (coating) and anti-NSE (9602) dispensed in the NC membrane were called Ab2. Water-soluble QBs were used to label antibodies. The protein conjugated QBs were prepared by using EDC and NHS as cross linkers. 1 mg QBs mixed with 2 mg EDC and 1 mg NHS in and phosphate buffer saline (PBS) (10 mM, pH 6.8) were placed in a variable speed rotator at 25 °C. After 30 min of incubation, EDC and NHS solutions were removed at 16,000 rpm for 5 min and were resuspended in 1 mL of 10 mM PBS buffer (pH 7.2). Purified Ab1(5 mg/mL) was immediately added to the solution with thorough mixing and placed in a rotator at 25 °C for 4 h. The QBs-Ab1 conjugates were collected by centrifugal filtration for at least three times at 16,000 rpm for 5 min, the concentrated conjugation mixture in the tubes was dissolved by 1 mL PBS (10 mM, pH 7.2) containing 2% BSA (m/v) and blocked by 1 h. Then, washed the conjugations for three times with PBS (10 mM, pH 7.2) and resuspended at PBS (10 mM, pH 7.4) containing 0.1% of BSA. Furthermore, we also prepared QDs-anti-CEA McAb and QDs-anti-NSE McAb conjugates at the same condition for comparison.

To optimize the ratio between Ab1 and QBs, we conjugated 20–70 μg of Ab1 with QBs respectively according to the coupling method above to get the most appropriate ratio.

2.3. Preparation of QBs based ICTS

The QBs based ICTS consists of four components: sample pad, conjugate pad, nitrocellulose (NC) membrane and absorbent pad, all parts were pasted sequentially on a PVC backing card with 2 mm overlap of each component. The sample pad was first saturated with BS (containing 2% NaCl, 0.5% BSA and 0.1% Tween-20), then dried at 37 °C and stored in a drying oven at room temperature. The prepared QBs-anti-NSE McAb and QBs-anti-CEA McAb solutions were mixed at a ratio of 1:1 and was sprayed onto the conjugate pad, then dried at 4 °C for 1 h. We dispensed 2 mg/mL of anti-CEA (coating) and anti-NSE (9602) and 1 mg/mL of goat anti-mouse IgG on the NC membrane with a width of 1 mm to serve as test line 1 (T1), test line 2 (T2) and control line (C line) respectively using XYZ Dispensing System. Finally, the whole assembled plate was cut into 3 mm strips and were stored at room temperature until use.

2.4. Portable test strip reader

Fluorescent signals from QBs captured by T1, T2 line and C line were measured using a portable test strip reader called "Handing". Handing was the third generation our lab developed to analyze ICTS [42–44]. As shown in Fig. 1, the Handing system has three components: a hand-held test terminal, a lateral flow test strip cartridge and a data server. The hand-held testing terminal was the crucial part for the quantitative determination which used an array of LED lamps as the excitation light source and a highly sensitive complementary metal oxide semiconductor (CMOS) camera as the detector for signal. The LED lamps provided 365 nm ultraviolet light to excite the QBs probes