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Simultaneous quantitative detection of multiple tumor markers with a rapid and sensitive multicolor quantum dots based immunochromatographic test strip

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ABSTRACT

A novel multicolor quantum dots (QDs) based immunochromatographic test strip (ICTS) was developed for simultaneous quantitative detection of multiple tumor markers, by utilizing alpha fetoprotein (AFP) and carcinoembryonic antigen (CEA) as models. The immunosensor could realize simultaneous quantitative detection of tumor markers with only one test line and one control line on the nitrocellulose membrane (NC membrane) due to the introduction of multicolor QDs. In this method, a mixture of mouse anti-AFP McAb and mouse anti-CEA McAb was coated on NC membrane as test line and goat anti-mouse IgG antibody was coated as control line. Anti-AFP McAb-QDs_{546} conjugates and anti-CEA McAb-QDs_{620} conjugates were mixed and applied to the conjugate pad. Simultaneous quantitative detection of multiple tumor markers was achieved by detecting the fluorescence intensity of captured QDs labels on test line and control line using a test strip reader. Under the optimum conditions, AFP and CEA could be detected as low as 3 ng/mL and 2 ng/mL in 15 min with a sample volume of 80 μL, and no obvious cross-reactivity was observed. The immunosensor was validated with 130 clinical samples and in which it exhibited high sensitivity (93% for AFP and 87% for CEA) and specificity (94% for AFP and 97% for CEA). The immunosensor also demonstrated high recoveries (87.5–113% for AFP and 90–97.3% for CEA) and low relative standard deviations (RSDs) (2.8–6.2% for AFP and 4.9–9.6% for CEA) when testing spiked human serum. This novel multicolor QDs based ICTS provides an easy and rapid, simultaneous quantitative detecting strategy for point-of-care testing of tumor markers.

1. Introduction

Cancers are leading causes of death worldwide, accounting for 8.2 million deaths in 2012 according to World Cancer Report 2014 published by WHO and International Agency for Research on Cancer (IARC) on July 3rd 2014. Detecting multiple tumor markers s at early age facilitates diagnosis of cancer and treatment monitoring (Nagler et al., 1999; Duffy et al., 2003), and significantly improves the treatment efficiency and survival rates (McMahon et al., 2000; Pantel et al., 2008). However, detecting only a single tumor maker is usually inadequate for an accurate diagnosis of cancer since most are not specific to a particular tumor. To improve the accuracy of cancer diagnosis, it is necessary to combine testing of multiple tumor markers as it could improve the diagnostic specificity obviously (Visintin et al., 2008; Rusling, 2013). Various approaches have been proposed to perform simultaneous detection of multiple tumor markers in the past few years. For example, electrochemical immunoassay (Wilson, 2007; Xu et al., 2014), label-free methods (Lin et al., 2011; Kong et al., 2013), dielectrophoresi assays (Lee et al., 2010; Ramón–Azcón et al., 2011), chemiluminescence alalysis (Zong et al., 2012; Guo et al., 2013), biobarcoded Scanometric assay (Stoeva et al., 2006), spectrometry (Chon et al., 2011; Giesen et al., 2011), fluorescence detection (Tian et al., 2012; Akinfieva et al., 2013), photonic suspension array (Zhao et al., 2009). But all these techniques are not suitable for point-of-care testing because they require large and sophisticated instruments, complex operations, long analysis time and highly skilled personnel to manipulate. Limited equipments and staff without sufficient training may restrict early screening of tumor in remote and rural areas. Therefore, the demand for a easy, rapid and low cost analytical method that can simultaneous detect multiple tumor markers without use of a laboratory or trained personnel is urgent.

The porous membrane-based immunochromatographic test strip (ICTS), which combines immunolabel with chromatography has

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gained increasingly attention in recent years because it has advantages of minimal manipulation, small analyte volume, short analysis time, low cost, multianalyte analysis promising, no need of professional and as well as the capability of realizing point-of-care testing (Chaivisuthangkura et al., 2013; Zhou et al., 2013). A series of multianalyte ICTS has sprung out and been well developed in many fields (Gao et al., 2014; Castro et al., 2010; Guo et al., 2009; Ching et al., 2012). However, these methods are either colloidal gold-based or enzyme-linked, both these two approaches are not the optimal choice for developing simultaneous quantitative detection ICTS owing to their inherent limitations. The colloidal gold-based ICTS could quantify analyte (Lee et al., 2013) but it requires two or more test lines when working with multianalyte, so does the enzyme-linked ICTS. Therefore the material used for signal generation plays a decisive role in the ICTS, which determines the performance of the ICTS. Fluorescence materials are obvious choice because optical signals are sensitive and fluorescence-tagged ICTS have the potential to quantify multianalyte (Khreich et al., 2010). QDs, a class of semiconductor nanomaterials, have been used as fluorescent labels in ICTS (Berlina et al., 2013; Chen et al. 2014) due to their excellent optical and electronic properties. The high level of brightness and exceptional photostability of QDs enable it to be detected and quantified sensitively. The emission spectra of QDs are size-tunable, narrow, symmetrical while the absorption spectra are broad and continuous, which allows exciting all colors of QDs simultaneously with only a single excitation light source without substantial spectral overlap (Klimov et al., 2000; Chan et al., 2002; Larson et al., 2003; Hildebrandt, 2011; Jin and Hildebrandt, 2012). Water-soluble QDs with ample carboxyl or amine groups coated in their surface are easily to be conjugated to proteins. These fascinating properties make QDs a robust reporter to develop sensitive ICTS with capabilities to simultaneous quantify mutianalyte. Recently, Dzantiev et al. achieved simultaneous quantitative detection of three analytes by using multicolor QDs based ICTS with three test lines on NC membrane (Taranova et al., 2015). However, multicolor QDs based ICTS to with only one test line and one control line on the NC membrane has not been previously reported.

Here, we described the development of a novel multicolor QDs based ICTS which allows the simultaneous detection and quantification of multiple tumor markers with trace sample volume. AFP and CEA were used as models to verify the reliability of the immunosensor. The fluorescence intensity of test line and control line on a single strip were both measured and the ratio of the fluorescence intensity of test line and control line (*T*/*C* ratio) was used to offset the background factors (Yang et al., 2011). The novel multianalyte quantitative detection approach developed in this study combined the advantages of the ICTS and the excellent properties of QDs and resulted in a significant improvement in the performance of ICTS. An economical, easy, rapid, and sensitive detection strategy was innovated. The low cost but high performance multicolor QDs based multianalyte ICTS may open up a new avenue for rapid point-of-care cancer diagnostic.

2. Materials and methods

2.1. Chemicals and materials

Water-soluble quantum dots (546 nm and 620 nm) were obtained from Ocean Nanotech (USA), the QDs were CdSe/ZnS core/shell QDs with carboxyl groups displayed on their surface. The goat anti-mouse IgG, mouse anti-AFP (coating) McAb, mouse anti-AFP (labeling) McAb, mouse anti-CEA (coating) McAb, mouse anti-CEA (labeling) McAb, standard solution of AFP, CEA, PSA and CA125 were purchased from National Institutes for Food and Drug Control (China). Tween-20, bovine serum albumin (BSA), 1-ethyl-3-(3-dimethyllaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxy-succinimide (NHS) were obtained from Sigma (USA). Phosphate buffer powders were provided by Beijing Com-Win Biotech Co. Ltd. (China). All stock solutions were prepared using deionized water, with a electric resistivity of 18.25 MΩ at 25 °C. All chemicals were used without further purification.

NC membrane (Sartorius CN 140) was provided by Sartorius (Germany). Conjugate pad, sample pad, adsorbent pad, semirigid polyvinyl chloride (PVC) sheets and the manual dispenser were supplied by Shanghai JieYi Biotechnology Co., Ltd. (China). Fluorescence spectrophotometer was purchased from shanghai Lengguang Tech. (China). High-speed centrifuge and vortex mixer were common equipments in laboratory.

2.2. Conjugation of McAb with QDs (anti-AFP McAb-QDs$_{546}$ and anti-CEA McAb-QDs$_{620}$)

Multicolor QDs were chosen as fluorescent materials to develop the immunosensor. In our research, we found that when the multicolor QDs were excited simultaneously, the emission peaks between two kinds of QDs will not overlap only when their emission wavelengths with a difference of at least 40 nm. In this study, 546 nm QDs and 620 nm QDs were used to label antibodies. The protein conjugated QDs were prepared by using EDC and NHS as cross linkers. The surface carboxyl groups of the QDs bound with the amino groups of McAb under catalyzing of EDC and NHS. To prepare anti-AFP McAb-QDs$_{546}$ and anti-CEA McAb-QDs$_{620}$, 35 μL of 8 μM 546 nm QDs and 35 μL of 8 μM 620 nm QDs were pipetted into two centrifuge tubes, respectively, activated by adding EDC and NHS into each tube. The mixture solution was dissolved in PBS buffer to yield a final concentration of 0.4 mg/mL of EDC, 0.2 mg/mL of NHS, mixed by vortex for 30 min at room temperature. The mouse anti-AFP McAb and mouse anti-CEA McAb solutions were diluted into gradient concentration, added into the tubes containing activated 546 nm QDS and activated 620 nm QDs solutions, respectively, the resultant mixture were incubated at room temperature for 60 min by gentle shaking. The McAb-QDs conjugates were collected by centrifugal filtration for three times at 12,000 rpm for 30 min, the concentrated conjugation mixture in the tubes was dissolved by 1 mL PBS containing 1% BSA (m/v). The reaction were performed with constant mixing for 30 min at room temperature, then the prepared conjugates solution were stored at 4 °C.

2.3. Fabrication of multicolor QDs based ICTS

The multicolor QDs based multianalyte ICTS was composed of sample pad (glass fiber, 25 mm × 30 cm), conjugate pad (glass fiber, 8 mm × 30 cm), NC membrane (25 mm × 30 cm), absorption pad (30 mm × 30 cm), all parts were pasted on a PVC baking card. The sample pad was saturated with PBS containing 0.1% Tween-20 (v/v), then dried at room temperature and stored in seal bag. The prepared anti-AFP McAb-QDs$_{546}$ and anti-CEA McAb-QDs$_{620}$ solutions were mixed at a desired ratio and was applied onto the conjugate pad, dried at 37 °C overnight and stored at 4 °C. To prepare test line and control line, the relative humidity of laboratory was controlled at approximately 45% to 60%. A solution of mouse anti-AFP and a solution of mouse anti-CEA were mixed to yielded a final concentration of 1 mg/ml of each McAb, the mixture antibodies were immobilized on NC membrane as test line. The goat anti-mouse IgG was immobilized at a concentration of 0.5 mg/ml as control line. After immobilization, the NC membrane was dried at 37 °C for 1 h and then blocked with PBS containing 1% BSA (w/v). Absorbent pad was not treated. The sample pad, conjugate pad, coated membrane and absorbent pad were sequentially laminated and pasted to a PVC backing pad with proper