Biosensors and Bioelectronics 30 (2011) 145–150

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Quantum dot-based immunochromatography test strip for rapid, quantitative and sensitive detection of alpha fetoprotein

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ARTICLE INFO

Article history:
Received 26 May 2011
Received in revised form 12 August 2011
Accepted 9 September 2011
Available online 16 September 2011

Keywords:
Quantum dot
Immunochromatography test strip
Alpha fetoprotein
Rapid detection

ABSTRACT

Rapid, quantitative detection of tumor markers with high sensitivity and specificity is critical to clinical diagnosis and treatment of cancer. We describe here a novel portable fluorescent biosensor that integrates quantum dot (QD) with an immunochromatography test strip (ICTS) and a home-made test strip reader for detection of tumor markers in human serum. Alpha fetoprotein (AFP), which is valuable for diagnosis of primary hepatic carcinoma, is used as a model tumor marker to demonstrate the performance of the proposed immunosensor. The principle of this sensor is on the basis of a sandwich immunoreaction that was performed on an ICTS. The fluorescence intensity of captured QD labels on the test line and control line served as signals was determined by the home-made test strip reader. The strong luminescence and robust photostability of QDs combined with the promising advantages of an ICTS and sensitive detection with the test strip reader result in good performance. Under optimal conditions, this biosensor is capable of detecting as low as 1 ng/mL AFP standard analyte in 10 min with only 50 μL sample volume. Furthermore, 1000 clinical human serum samples were tested by both the QD-based ICTS and a commercial electrochemiluminescence immunoassay AFP kit simultaneously to estimate the sensitivity, specificity and concordance of the assays. Results showed high consistency except for 24 false positive cases (false positive rate 3.92%) and 17 false negative cases (false negative rate 4.38%); the error rate was 4.10% in all. This demonstrates that the QD-based ICTS is capable of rapid, sensitive, and quantitative detection of AFP and shows a great promise for point-of-care testing of other tumor markers.

1. Introduction

Advances in technologies have driven a new era in bioassay in the last decade. As a result of the highly complementary stereo chemistry, electrostatic interactions, hydrogen bond, Van der Waals force and integrative action of hydrophobic regions between antigens and antibodies, immunoassays possess higher selectivity and sensitivity than any kind of physical and chemical analysis alone can achieve in the field of biological analysis, and thus have become critical in modern life science research (Golden et al., 2009; Thangawng et al., 2009; Rieger et al., 2009; Wolter et al., 2008; Tang et al., 2010). It has been used in medical test, food quality monitor, poison detection, environment supervision, and may become early diagnosis of pathogens, malignant tumors and cardiovascular diseases. Since Yalow and Berson combined radioactive isotopes with the immune response to establish radioimmunoassay in 1959 (Yalow and Berson, 1959), a series of immunoassays have sprung out and developed, such as enzyme-linked immunosorbent assay (Hansen et al., 2008; Kurita et al., 2010; Rissin et al., 2010; Xia et al., 2009), fluorescence immunoassay (Boldt et al., 2006; Bruchez et al., 1998), chemiluminescence immunoassay (Old et al., 2009), and so on. Although they have the advantage of excellent specificity, high sensitivity and quantification, there are still some inconvenience and shortcomings. They are tedious, time-consuming, expensive and need complex operations. ICTS, appeared in the early 1980s, is a new detection technology which combines immunolabel with chromatography. It is fast, intuitive, user-friendly and inexpensive, and can truly realize point-of-care testing (Wang et al., 2011; Shim and Eremin, 2009; Yager et al., 2006; Zhou et al., 2010; Hua et al., 2010). Since Beggs et al. employed gold nanoparticles as reporters for the detection of human chorionic gonadotropin (HCG) in 1990, gold nanoparticles have been the most

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doi:10.1016/j.bios.2011.09.002

widely used reporters of ICTS (Beggs et al., 1990; Pyo et al., 2006; Gas et al., 2010; Li et al., 2009; Zhou et al., 2009; Gandhi et al., 2009). However, this method was either qualitative or semiquantitative and generally used for analyzing high concentrations of analytes. The above limitations with traditional ICTS have resulted in the increased use of various reporters, such as colored latex particles (Laitinen and Vuento, 1996), magnetic nanoparticles (Lönnberg et al., 2008; Peck et al., 2006; Workman et al., 2009), up-converting phosphor (Corstjens et al., 2001) and organic fluorophores (Bruchez et al., 1998; Ow et al., 2005; Santra et al., 2005; Ahn et al., 2003; Khreich et al., 2008; Oh et al., 2009). Detection systems relying on optical signal transduction are advantageous because they are sensitive, fast and have the potential for multiplex detection of analytes of interest. As a result, fluorescence-based ICTS have attracted more attention. However, the most used fluorophores may suffer from photo bleaching and low emission intensity in the test strip membrane, which seriously compromises the sensitivity and stability of the assay.

Semiconductor QDs have attracted intensive attention during the last two decades. Particularly, QDs are regarded as promising fluorescent probes due to their unique properties (e.g. size-tunable emission, broad adsorption, narrow and symmetric photoluminescence spectra, strong luminescence, and robust photostability) (Carion et al., 2007; Liu et al., 2008; Smith et al., 2008; So et al., 2006; Wu et al., 2010; Zrazhevskiy et al., 2010). On the basis of its excellent optical properties, QD is predicted to be an ideal reporter for fluorescence-based ICTS. Currently, the investigations of QD-based ICTS are remaining at an early stage and few reports about the application have appeared. Lin et al. developed a QD-based lateral flow test to detect a protein marker nitrated ceruloplasmin and for the first time made quantitative analysis on protein by recording only the fluorescence intensity of QDs captured on the test line (Li et al., 2010). However, different batches of ICTS may be heterogeneous, which will make two different strips hold inconsistent fluorescence intensities on test lines with the same sample due to differences in chromatography and immunoreactions. What is more, serum samples differ in pH value, ionic strength and protein content from person to person, which will influence the release of QDs-antibody conjugates on the conjugation pad. Thus, the test results determined only by the intensity of test line will be inaccurate in clinical patient serum testing.

In this study, fluorescence intensity of test zone and control zone on a single strip are both measured for offsetting the inherent factors of test strips and human serum themselves. Based on this judgmatic detection concept, QDs as fluorescence reporters are introduced into a portable biosensor for rapid, sensitive, selective and quantitative analysis of AFP. AFP, a kind of tumor marker was used here as a model target analyte to verify the reliability of the prepared QD-based ICTS. Quantitative detection of AFP was realized by recording the fluorescence intensity of QDs both captured on the test line and the control line. Then the results were determined by the ratio of the fluorescence intensities of test line and control line (T/C ratio). This kind of detection method makes the result credible. Furthermore, we have employed the QD-based ICTS to test 1000 clinical samples. Experimental results demonstrate that QD-based ICTS hold good capability for rapid, quantitative and clinically accurate analysis of trace amount of AFP. Thus, this sensor platform may break a new path for rapid point-of-care screening and clinical diagnosis.

2. Materials and methods

2.1. Materials and chemicals

Selenium powder (99.99%, Aldrich), cadmium oxide (CdO, 99.5%, Aldrich), tri-n-octylphophine (TOP, 90%, Aldrich), tri-n-octylphosphine oxide (TOPO, 90%, Aldrich), octadecylamine (ODA, 90%, ACROS), 1-octadecene (ODE, 90%, ACROS), oleic acid (OA, 90%, Aldrich), sulphur (Aldrich), triblock copolymer from sigma consisting of a polybutylacrylate segment, a polyethylacrylate segment and a polymethacrylic acid segment. At a molecular weight of ca.100 kDa, for better encapsulation of QDs, about 25% of the free carboxylic acid groups were derivatized with octylamine (a hydrophobic side chain). 1-ethyl-3-(3-dimethyllaminopropyl)-carbodiimide hydrochloride (EDC, GL Biochem (Shanghai) Ltd.). Goat anti-mouse IgG, bovine serum albumin (BSA) and fetal calf serum (FCS) were supplied by Beijing Dingguo Biotechnology Co., Ltd (China). Mouse monoclonal AFP antibody was obtained from Bioscience (Tianjin) diagnostic technology Co., Ltd. All chemicals were used without further purification.

2.2. Preparation of QD- anti-AFP monoclonal antibody conjugates

QDs and QD-antibody conjugates were prepared according to a previously protocol published by our research group (Zhang et al., 2008). Briefly, 0.3 mmol of CdO, 0.4 mL of OA, 4.0 mL of ODE were loaded into a 50 mL flask. First, the mixture was heated to 300 °C under an argon flow, and CdO was dissolved to generate a colorless homogeneous solution. Next, the solution was cooled to room temperature, and then 2.50 g ODA and 0.50 g TOPO were added into the flask. Then the system was heated again to 280 °C under an Ar flow. After that, a selenium solution (1.8 mmol of Se powder dissolved in 2 mL of TOP) was injected quickly. Following the injection of selenium, nanocrystals were grown at 250 °C for 10 min. The TOPO-capped QDs were precipitated by adding ethanol, collected by centrifugation, washed with methanol several times and dissolved in chloroform. Then they were mixed with 1.5 g of ODA and 5.0 g of ODE in a 25 mL three-neck flask. The flask was pumped down to remove any chloroform from the system. Subsequently, the system was switched to argon flow and the reaction mixture was further heated to 240 °C to grow shells. The final product was diluted by hexane after a methanol extraction.

The water soluble QDs were prepared by the ultrasonic emulsification method. QDs and amphiphilic polymer at a molar ratio of 1:10 were dissolved in dichloromethane. Next, 6.0 mg of pluronic F-68 (F-68) was dissolved in 2.0 mL of DI water in a 5 mL beaker with fast stirring for several minutes to obtain a homogeneous emulsion. Then the emulsion was ultrasonificated at 200 W. Meanwhile, the QDs-polymer mixed solution was slowly injected into the solution within 4 min. After injection, the sample solution was quickly stirred under the reduced pressure for 30 min to remove the remains of organic solvents. Finally, the resulting aqueous solution was centrifuged and washed twice with DI water to remove residual F-68 and excess polymer.

To prepare the QD-antibody conjugates, carbodiimine chemistry was applied for conjugation using EDC as cross-linkers. Carboxylic acid groups of amphiphilic polymer displayed on the QDs surface bound with the amine groups of proteins. The polymer-coated QDs were reacted with anti-AFP monoclonal antibody at a QDs/antibody/EDC molar ratio of 1:8:4000 in phosphate buffer saline (PBS) (0.01 M, pH 7.2) for 2 h at room temperature. The final QD-antibody bioconjugates were purified by centrifugation and washed with DI water.

2.3. Preparation of QD-based ICTS

The test strip consists of four components: sample pad, conjugate pad, nitrocellulose (NC) membrane and absorbent pad. All of these parts were pasted on a backing card. The sample pad (6 mm × 20 mm) was made of glass fiber and fist saturated with a buffer (pH 8.0) containing 20 mM sodium borate, 2.0% (w/v) sucrose, 2.0% BSA, and 0.1% (w/v) NaN_3. Then it was dried and stored

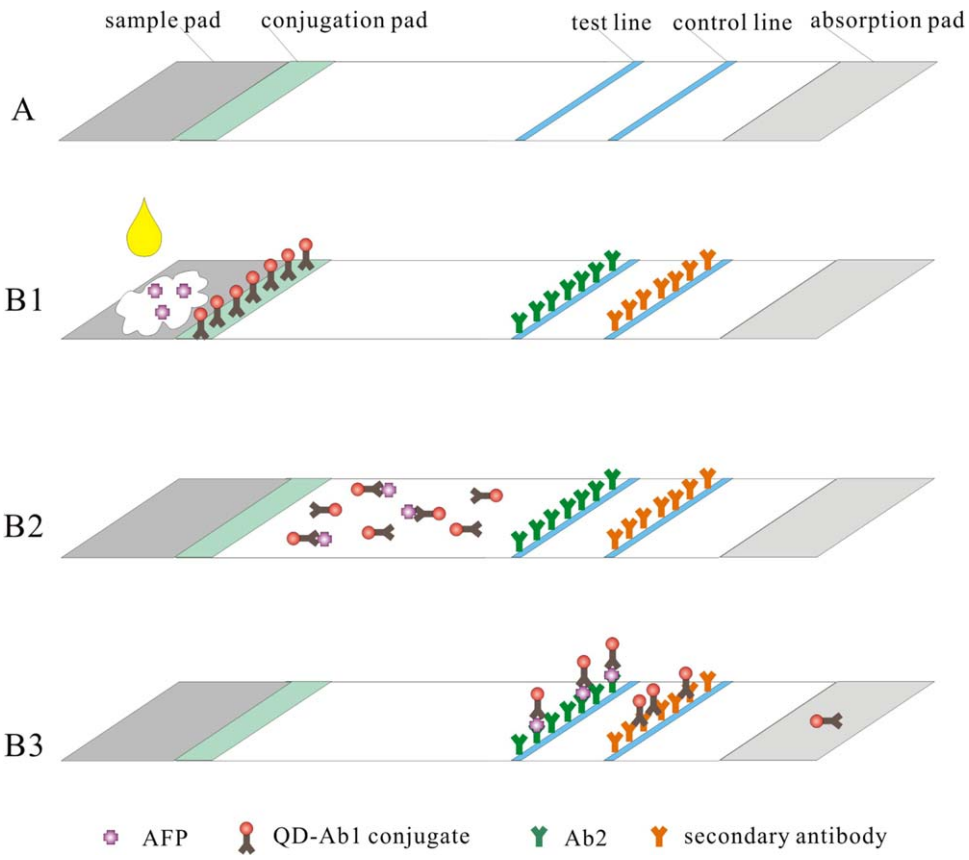


Fig. 1. (A) Schematic illustration of the test strip and (B1-B3) the detection of AFP using QD-based ICTS. (B1) sample containing AFP is applied to sample pad. (B2) AFP combines with QD-anti-AFP monoclonal antibody conjugates and migrates along the porous membrane by capillary action. (B3) The formed complexes continue to migrate along the membrane and are captured by the other kind of anti-AFP monoclonal antibodies (Ab2) to form (QD) Ab1–Ag–Ab2 complexes on the test line. As the liquid sample continues migrating, the excess (QD) Ab1–Ag complexes are captured by the secondary antibody resulting in the accumulation of QD on the control line. The excess QD conjugates continue to migrate toward the absorption pad.

in a drying oven at room temperature. The conjugate solution was prepared by diluting 10 times the QD-anti-AFP monoclonal antibody conjugates ((QD) Ab1) with PBS buffer containing 0.5% (w/v) BSA. The conjugate pad was obtained by adding a desired volume of (QD) Ab1 onto the glass fiber pad and then drying it at 4 °C. The lateral-flow NC membrane was treated with blocking solution (a solution of 3% w/v BSA and 0.1% Tween-20 in 0.01 M PBS) for 2 h, and this was followed by three 5-min washings with 0.1% Tween-20 in 0.01 M PBS. The membrane was then dried in a nitrogen box for 1 h and stored at 4 °C. Using an automatic dispenser (Biodot XYZ3000, USA), we dispensed the protein solution onto the NC membrane with a 1 mm width and 1 μL/cm of volume as test line and control line. The NC membrane was pasted on the center of the backing plate. The conjugating pad (glass fiber) on which the diluted (QD) Ab1 was dispensed previously was pasted on the plate by overlapping 2 mm with the NC membrane. The sample pad was pasted on the same end by its margin justified to the conjugating pad. The absorbent pad was pasted on the other end of the NC membrane by the same overlapping of 2 mm. Then the whole assembled plate was cut into 4 mm strips and stored at room temperature.

2.4. Designing and preparation of test strip reader

A tablet computer (Hanwang Technology Co., Ltd) equipped with a mini printer was coupled with an optic fiber spectrometer (Avantes). A 405 nm laser diode was used to excite the QDs, and a stepper motor controller was used to drive the strip at a constant speed. All the detection procedure was monitored by the computer, and the test results could be printed immediately after screening.

2.5. Fluorescence assay procedure

Certain volume of sample solution containing a desired concentration of AFP was added onto the sample pad. Fetal calf serum (FCS) was used as control. Both the sample and control solution were migrated toward the absorption pad by capillary action. After appropriate time, the cassette containing the test strip was inserted into our designed fluorescence reader, followed by recording fluorescence intensity of the test line and the control line to quantify the analytes. For clinical experiments, certain volume of human serum was added onto the sample pad. The results were obtained by reading the optical response with the strip reader after appropriate time.

3. Results and discussion

3.1. Principle of the method

In this study, QDs were chosen as labeling materials to tag AFP antibodies. The analysis of QDs (Supplement Figs. 1 and 2) reveals that the as prepared QDs are uniform in size distribution, and have good optical properties. The principle of the QD-based ICTS is based on the sandwich immunoassay, as illustrated in Fig. 1. The ICTS is composed of sample loading pad, conjugation pad, absorption pad, and NC membrane (Fig. 1A). All the components were assembled onto a plastic adhesive backing card. Sample containing AFP antigen (Ag) was added to the sample loading pad as illustrated in Fig. 1B1. Then sample solution migrated into the conjugation pad and targets in sample solution bounded with (QD)

Ab1 based on antibody–antigen interaction (Fig. 1B2). The formed complexes continued to migrate along the membrane and were captured by another anti-AFP monoclonal antibodies (Ab2) immobilized beforehand on the NC membrane to form (QD) Ab1–Ag–Ab2 complexes, which resulted in the accumulation of QDs on the test line. As the liquid sample continues migrating, the excess (QD) Ab1–Ag complexes were captured by the secondary antibody, which resulted in the accumulation of QD on the control line. After several minutes, there were QDs both on the test line and the control line (Fig. 1B3). The excess QD conjugates continued to flow into the absorption pad at the end of the strip. If there were no QDs accumulating on the control line, the test strip was invalidated and should be discarded. The fast immunoreaction and washing-free on ICTS detection made it rapid and easy-to-use.

Quantitative analysis was realized by reading the fluorescence intensities of the test line and the control line respectively by the test strip reader we designed (Supplement Fig. 3A1). The results were determined by the *T*/*C* ratio and printed out immediately after detection (Supplement Fig. 3A2). The more targeting antigens in the sample, the more QD conjugates would be captured on the test line, which leads to the increase of the *T*/*C* ratio. According to the principle described above, the *T*/*C* ratio would be proportional to the concentration of AFP in the samples, and the inherent heterogeneity of ICTS can be ignored based on *T*/*C* ratio measurement. Compared to the barely detection of test line, this quantitative approach is much more credible and applicable. Structures and principles of the instrument are shown in Supplement Fig. 3B. As can be seen from the scheme, a 405 nm laser diode was used as the excitation light source, and fiber optic spectrometer was used to detect the fluorescence intensity of QDs. The movement of the test strip was controlled by the stepper motor controller. After the detection, the test results could be printed out automatically by a mini printer. Compared with other devices such as flow cytometer and chemiluminescent analyzer, single excitation light source and optical fiber transmission system were used in our home-made test strip reader. This device is easy to operate and convenient to adjustment, with high automation.

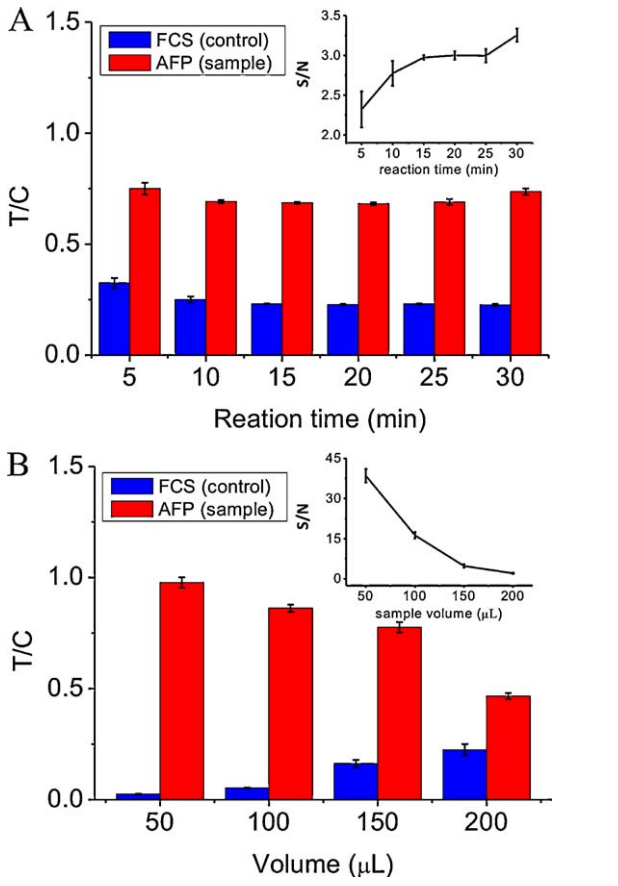


Fig. 2. Optimization of parameters of the immunosensor. (A) Effect of immunoreaction time on the fluorescent responses of QD-based ICTS. The QD-based ICTS was performed by applying 200 μL of FCS (control) or 200 ng/mL AFP to the sample pad. Fluorescent signals were recorded at different immunoreaction time. The inset shows the *S*/*N* under different immunoreaction time. (B) Effect of sample volume on the fluorescent responses of QD-based ICTS. Fluorescent signals were recorded after 10 min of immunoreaction. Other conditions were the same as those in (A). The inset shows the *S*/*N* under different sample volume.

3.2. Parameter optimization

The typical responses of QD-based ICTS to sample with and without AFP were illustrated in Supplement Fig. 4. Here, FCS served as the control. As shown in the Supplement Fig. 4A1 and A2, the fluorescent band occurred clearly when excited by a hand-held ultraviolet lamp. And it can be obviously seen both the test line and the control line in the presence of AFP, while only the control line in the absence of AFP. Supplement Fig. 4B1 and B2 shows the corresponding fluorescence response when detected by the device we designed. As can be seen from Fig. 4B1, the first peak would be attributed to the immuno-complexes ((QD) Ab1–Ag–Ab2) formed in the test zone. And the second peak came from the (QD) Ab1–Ag complexes captured by the secondary antibody (control zone). However, FCS also contributed weak signal on test line and exhibited a background (Supplement Fig. 4B2). Such response was resulted from the nonspecific adsorption. In order to decrease the nonspecific binding and increase the sensitivity, certain parameters were optimized, such as immunoreaction time on the test strip and the volume of added sample solution on sample pad.

First, the effect of immunoreaction time on the fluorescence response of the biosensor was investigated. Fig. 2A displays the fluorescent responses of QD-based ICTS to 200 ng/mL AFP under different immunoreactions time, where the responses to FCS were determined in parallel as the control. The inset shows the signal-to-noise ratio (*S*/*N*) under different immunoreaction time. The *S*/*N* refers the ratio of *T*/*C* of AFP and *T*/*C* of FCS. This ratio value relates to both the specific binding (*T*/*C* of AFP) and nonspecific adsorption (*T*/*C* of FCS). As can be seen from Fig. 2, the *S*/*N* increases sharply with the increase of immunoreactions time within the first 15 min and then tend to a slow growth. Error bar are based on three duplicated measurements of analytes. The result indicates that the sandwich immunoreactions on the test zone are fast. In order to get a rapid detection and a high *S*/*N*, we chose 10 min as the optimal immunoreaction time throughout the entire study. Although 10 min reaction time generates a higher *S*/*N*, nonspecific binding still exists. To eliminate the nonspecific adsorption of QD conjugates, the amount of sample volume for the assay was optimized. Fluorescence signals with different sample volume were obtained using 200 ng/mL AFP and FCS (a control) in parallel (Fig. 2B). As shown in Fig. 2B, with the increase of sample volume, the *T*/*C* ratio decreases in the AFP group and increases in the FCS control group. *S*/*N* result is shown in the inset of Fig. 2B. Interestingly, sample volume affects *S*/*N* remarkably in our study. As shown in the inset of Fig. 2B, a clear decrease in *S*/*N* is observed with the increase of sample volume. The experimental results indicate the correlation between *T*/*C* value and sample volume of AFP group is totally different from that of FCS group. It may be caused by the physical and chemical properties of QDs complex are different in AFP and FCS groups. In AFP group, (QD) Ab1 conjugates will capture the targeting analytes from the added sample solution to form large (QD) Ab1–Ag biocomplex, while in FCS group, (QD) Ab1 conjugates have no targeting analytes to be captured. This means the

geometry of QD complex in AFP and FCS groups are different. The varying geometry can make the chromatographic properties of QD complex different. Besides this, (QD) Ab1–Ag and (QD) Ab1 biocomplex may have different nonspecific binding behaviors on ICTS. This will also result in different correlations between T/C value and sample volume. The S/N was highest when the sample volume was 50 μL. Error bars are based on three duplicated measurements of analytes. In addition, we investigated the performance of 30 μL sample volume. On the basis of the experimental results, 30 μL was too little to bring QD conjugate to flow into the absorption pad. As a result, 50 μL was employed for the following experiments.

3.3. Analytical performance of the QD-based ICTS

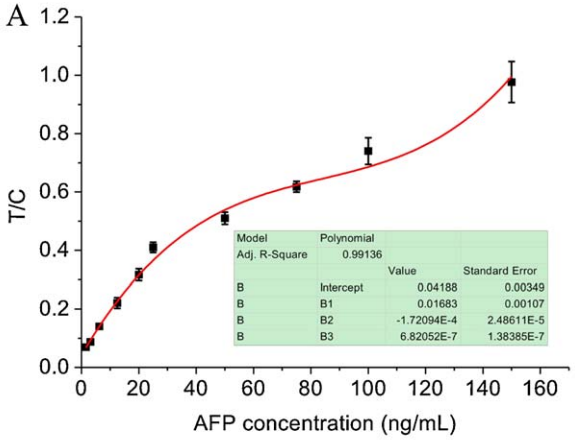
Under optimal experiment conditions, the analytical performance of the biosensor was further evaluated with standard AFP samples. As shown in Supplement Fig. 5A, fluorescence imaging of the test and control lines on the test strip after assay could be observed with naked eyes when excited by an ultraviolet lamp owing to the efficient fluorescence and photostability of QDs. The test line and the control line both occurred clearly in the presence of AFP. By naked eyes, we can easily give a yes/no answer. And we observed proportional changes in the brightness of test line associated with the concentration of AFP. Such result is working out as we expected, because more QD conjugates were captured on the test line with a higher AFP concentration. Furthermore, in the absence of AFP, the control line was easily observed while the test line was unable to be observed.

In order to realize the quantitative detection, the results were recorded by the home-made portable strip reader. Supplement Fig. 5B demonstrates the ratio between the fluorescence intensity on the test line and the control line with increasing AFP concentrations. It is found that the T/C ratio increased along with the increasing of AFP concentration. Meanwhile, trace amount of AFP as low as 1 ng/mL could be detected by this portable biosensor with 10 min of immunoreaction time. ICTS are usually used for rapid and point-of-care screening, and this can be done by the common-used gold-based ICTS in most cases. However, when the concentration of the analyte is low, colloidal gold-based ICTS are not competent because the captured gold on the test line is too fuzy to be observed. In addition, as for colloidal gold-based rapid test strips, another problem is the interference of hemolytic serum. The coloring is difficult to distinguish in some cases when hemolytic occurs. On the basis of fluorescence labels, QD-based ICTS could solve the problems easily. In some cases, we can immediately give the yes/no answer when the intensity of the test line is strong. Besides, the home-made test strip reader can be used to make quantitative analysis, which makes the biosensor much more sensitive than gold-based ICTS.

3.4. Clinical test of AFP in human serum

The prepared QD-based ICTS and home-made device were both used to explore the feasibility of detection for clinical application. The standard serum samples with different AFP concentrations, such as 150 ng/mL, 100 ng/mL, 75 ng/mL, 50 ng/mL, 25 ng/mL, 20 ng/mL, 12.5 ng/mL, 6.25 ng/mL, 3.125 ng/mL, 1.56 ng/mL and 0 ng/mL, were prepared. These samples were applied to QD-based ICTS, and the results were recorded by the test strip reader under the optimal conditions. Fig. 3A demonstrates the calibration curve obtained which is a polynomial curve with $R^2 = 0.991$. The data were linear in small range ($y = 0.01415x + 0.04614$ ($0 < x < 25$), $R^2 = 0.9956$). But in a wide dynamic range, the data could be fit to a (three order) polynomial. This could be ascribed to the immunoreaction was heterogeneous and the reaction time was short, which makes the reaction inadequate. The detection limit was 3.6 ng/mL

A



B

	Sample Size	Error Number	Error Rate
Positive Specimen	388	17	4.38%
Negative Specimen	612	24	3.92%
In All	1000	41	4.10%

Fig. 3. (A) QD-based ICTS polynomial response for 150 ng/mL, 100 ng/mL, 75 ng/mL, 50 ng/mL, 25 ng/mL, 20 ng/mL, 12.5 ng/mL, 6.25 ng/mL, 3.125 ng/mL, and 1.56 ng/mL AFP in human serum. (B) Clinical test of AFP results obtained by QD-based ICTS compared with that obtained by commercial electrochemiluminescence immunoassay AFP kit.

determined by 3 times the blank sample (FCS) standard deviation ($S/N = 3$), which is comparable to the value indicated by other techniques for AFP detection (the sensitivity of electrochemiluminescence is on the level of pmol). Error bars were based on three duplicated measurements of analytes at different concentrations. The biosensor was used to test 1000 human serum samples gathered from three hospitals (400 from Tianjin Medical University General Hospital, 300 from First Hospital of Lanzhou University and 300 from People's Hospital of Gansu Province) with the cut-off threshold of 25 ng/mL. This threshold value was determined on QDs-based ICTS by sixty-one serum samples from healthy people. And we defined the results tested by commercial AFP kit as true positive and true negative. If one sample which was positive detected by commercial AFP kit was negative detected by our home-made biosensor, the result was false negative, and vice versa. As can be seen from the table in Fig. 3B, the results were satisfactory with the exception of 24 false positive case (false positive rate 3.92%) and 17 false negative case (false negative rate 4.38%); the error rate was 4.10% in all. It indicates that the data for AFP measurement are accurate and reliable. Therefore, QDs-based ICTS and home-made strip reader demonstrates promise for sensitive detection of AFP cancer biomarker and open up a new avenue for various clinical applications.

4. Conclusions

A QD-based ICTS was developed to detect AFP. The assay is rapid (10 min), convenient and easy to use and needs only 50 μL of sample. It provides not only qualitative result for rapid screening, but also quantitative result which could be used to determine the AFP concentration in the sample. Under optimal conditions, the biosensor could be used to detect as low as 1 ng/mL AFP. The sensitivity was comparative with that of electrochemiluminescence immunoassay. 1000 clinical test results demonstrated that the immunosensor showed high consistency with that of commercial electrochemiluminescence immunoassay AFP kit. Considering their rapid detection, high sensitivity, accurate quantification and low cost, the QD-based ICTS will show promise for quantitative detection of AFP and other tumor markers in hospitals and rapid screening in rural areas.

Acknowledgement

The authors gratefully acknowledge the National High Technology Program of China (2007AA021808), the Tianjin Science and Technology Program (09ZCGYSF00900), the National Natural Science Foundation of China (51003078), the Opening Project of MOE Key Laboratory of Analytical Chemistry for Life Science, Nanjing University (KLACLS1001), and the Opening Project of National Engineering Laboratory for Modern Silk, Soochow University.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.09.002.

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