One-Step Quantitative Detection of Human Chorionic Gonadotropin by Integrating Immunochromatography Test Strip with Fluorescence Detection of Quantum Qots

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Abstract— **The Immunochromatography test strip (ICTS) is a one step and the commonest commercial point-of-care diagnostic format. Their advantages of convenient and fast testing enable the rapid decisions on diseases. However, the fundamental limitation of quantitative and sensitive analysis severely hampers their application in the field of early detection. Herein, we overcame these limitations by integrating of quantum dots with distinct optical and electronic properties, which were served as the signal reporters for ICTS. Human chorionic gonadotropin (HCG), which has been recognized as a clinic marker of pregnancy, was used as a model analyte to demonstrate the performance of the QDs-based ICTS platform. Under optimized conditions, the detection limit was about 0.24 IU/L. Meanwhile, the concentrations of HCG could be determined within 20 min with high specificity, using only 40 μL of sample. Our data suggests that the QDs-based ICTS platform is a rapid, low-cost, highly sensitive and specific test platform for quantitative point-of-care diagnostics, which holds promise to become part of routine medical testing for other protein markers detections.**

Keywords— **Quantum dots, one step detection, immunochromatography test strip, quantitative detection.**

I. INTRODUCTION

During the past decade, a number of methods have been developed for protein markers detection. Although methodological advances have been achieved, detection methods are still severely restricted [1][2]. For example, conventional methods, such as enzyme-linked immunosorbent assays (ELISA) and chemiluminesc ence immunoassay (CLIA), suffer from the disadvantages of being time-consuming, having low sensitivity and a laborious process. Besides that, these detection methods also require expensive instruments, highly skilled personnel to perform the procedures. Recently, the Immunochromatography test strip (ICTS) has been becoming a powerful tool for detection in a majority of fields for their convenient analysis [3]. However, the limitation of sensitive and quantitative analysis severely hampers their application as a reliable medical testing in early detection of disease. In order to overcome the limitations, more quantitative ICTS has been developed by using various signal reporters, such as colloidal gold [4], up-converting phosphors [5], colored latex particles [6], magnetic nanoparticles[7] and organic fluorophores [8].Quantum dots (QDs) as nano-scaled fluorescent labels, has wildly used in biomedical applications [9]. Their high level of brightness and extraordinary photostability allow ultrasensitive detection, which also provide new opportunities for ICTS.

We herein report on the development of a QDs-based ICTS platform designed to provide fast and portable detection of protein biomarker, using HCG as a model analyte. HCG has been proved as a valuable protein marker for pregnancy. Then the effective reaction time, sensitivity, specificity and capability of quantitative detection of these QD s based ICTS were investigated to display its performance. As expected, our data show the QDs-based ICTS could provide a one step quantitative detection for HCG in a short time with high specificity and sensitivity. Thus the QDs-based ICTS may be widely used for protein markers detection in hospital, community, and even at home, as a first-response point-of-care device.

II. MATERIALS AND CHEMICALS

A. 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), poly(tert-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) (ABC triblock copolymer) (Aldrich).

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1-ethyl-3-(3-dimethyl- laminopropyl)-carbodiimide hydrochloride (EDC, GL Biochem(Shanghai) Ltd.). N-hydroxysuccinimide (NHS, GL Biochem (Shanghai) Ltd.). Bovine serum albumin (BSA) and fetal bovine serum (FBS) were supplied by Beijing Dingguo Biotechnology Co., Ltd (China). Mouse monoclonal antibody, goat anti-mouse IgG, nitrocellulose membranes, glass fibers and absorbent pad were obtained from Bioscience (Tianjin) diagnostic technology Co., Ltd. All chemicals were used without further purification.

B. Synthesis of CdSe/CdS/CdxZn$_{1-x}$S/ZnS Nanocrystals

The core/shell QDs CdSe/CdS/CdxZn$_{1-x}$S/ZnS were synthesized based on successive ion layer adsorption and reaction (SILAR) method. First, CdSe nanocrystals were made by a typical synthetic procedure, 0.0386 g of CdO, 0.4 mL of OA, and 4 mL of DOE were added to a three-necked flask and heated to 260°C under argon until complete dissolution of CdO. After cooling to room temperature, 2.5 g of ODA and 0.5 g of TOPO were added into the flask. The solution was heated to 300°C. The solution containing 0.14 g of selenium, 2 mL of TOP was injected into the solution quickly. Then the growth temperature was set to 250 °C for 10 min. After that time, the heating mantle was removed and the products were isolated using at least three times hexane-methanol extractions.

Next, for shell growth, a tipical procedure reported previously was slightly modified. The solution containing the CdSe were mixed with 1.5 g of ODA and 5.0 g of ODE in a new three-neck flask, and the system was kept at 100 °C under argon flow for 15 min to remove the undesired materials. Subsequently, the system was heated to 240 °C for shells growing. The amount of the injection solution for each monolayer was determined by calculating the number of the surface atoms of a given size of a nanocrystal, and the Cd precursor (0.4 M) solutions, Zn precursor (0.4 M) solutions, sulfur precursor (0.4 M) solution were previous prepared, The final product was diluted by hexane after a methanol extraction at least three times.

C. Preparation of Biocompatible QDs and QD-Antibody Conjugats

To prepare biocompatible QDs, the QDs and 2 mg of octadecylamine (ODA) grafted poly(tert-butyl acrylate-co-ethyl acrylate-co-methacrylic acid) (ABC-g-ODA) was mixed with 960 μL of dichlormethane to form the organic phase. Then 10mL of de-ionized (DI) water was slowly added into the organic phase under an ultrasonicator at 200 W for 5 min. Then the mixture was stirred magnetically until the organic phase was totally removal. Then the products were washed 3 times with DI water and centrifuged to collect.

The QD-antibody conjugates were prepared as followed. The biocompatibie QDs, mouse monoclonal antibody and EDC were mixed in PBS buffer solution (0.01 M, pH=7.4) at a QDs/antibody/EDC molar ratio of 1:8:4000. After reacting for 2 h, the final products were purified by centrifugation and stored in PBS buffer solution (0.01 M, pH 7.4, 0.5% BSA) overnight at 4°C.

D. Test Strip Preparation and Fluorescence Assay Procedure

The test strip consists of sample pad, conjugate release pad, reaction membrane, absorbent pad, and backing card. QD-antibody conjugates were diluted 20 times with blocking buffer (pH 7.4), which containing 0.01 M PBS, 3% (w/v) BSA, 5% (w/v) sucrose, 2% (w/v) PEG4000, 0.1% (w/v) Tween-20). After that, the solutions were dispensed onto the conjugate pads. The pads were dried at 37°C for 2 h. The T zone and C zone of the strips were prepared by dispensing a certain volume of 2 mg/mL mouse monoclonal antibody solution and goat anti-mouse IgG respectively with the dispenser XYZ-3050 BioJet Quanti 3000 onto a reaction membrane. After 2 h of drying at 37 °C, the membrane was blocked with 1% BSA at room temperature for 1 h, dried under vacuum for 30 min, and then stored at 4 °C. Then the parts were assembled on a plastic adhesive backing card. Each part overlapped 2 mm to ensure the solution migrating through the strip during the assay. Then the whole assembled plate was cut into 4mm strips and stored at room temperature.

40 μL of samples containing a desired concentration of HCG in Fetal bovine serum (FBS) was dropped onto the sample pad and flowed through the reaction membrane under capillary action. Control experiment was performed by FBS samples without any analyte. After the completion of immunoreactions, the strip in a cassette was inserted into laboratory-built test strip reader [10] to read fluorescence intensity of the test zone and the control zone to quantify the analytes. The results were got by reading the fluorescent response with the strip reader after appropriate time.

III. RESULTS AND DISCUSSION

A. Principle of the Method

As schematically illustrated in Fig.1, a strip usually realized with five components, a sample pad, a conjugate pad, a reaction membrane, an absorbent pad and a backing card [11]. The principle of protein biomarkers detection was

based on the sandwich assays. Sample was added on the sample pad and migrated to the conjugate pad driven by the capillary action, then the analyte in the sample interacted with the QD-antibody conjugates and form the immune complex, after that, the immune complex migrated to the reaction membrane, where the immune complex was recognized by capture antibodies immobilized onto reaction membrane surfaces to form test zone and the control zone. Then, excess reagents moved past the capture lines and were entrapped in the absorbent pad. Results were interpreted on the reaction membrane as the presence or absence of test zone, read either by naked eye with excited by a handheld ultraviolet lamp or using a strip reader. Fluorescence intensity of the T zone and C zone were related to the amount of analyte in the sample and reflected validity of the strip.

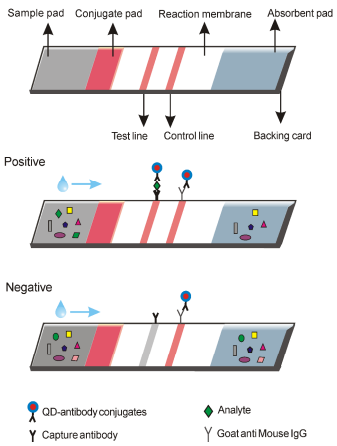

Fig. 1 Schematic illustration of ICTS detection of protein markers.

Herein, quantitative analysis was carried out by recording fluorescence intensity in the T zone and the C zone respectively by the test strip reader. Fluorescence intensity ratio between the T zone and the C zone was recorded as fluorescence signal (T/C). The signal to noise ratio (S/N) was defined as fluorescence peak area ratio between the target and the blank control samples. Detection results of the strips were analyzed by test strip reader for three times and the average values were plotted as a function of analyte's concentration.

B. Effective Reaction Time of the QDs-Based ICTS

Figure2A displays the signal response (T/C) of the ICTS under different reaction time ranging from 5min to 30min using 50 IU/L HCG, and the responses of FBS were set as the blank control group. Fig. 2B revealed the corresponding signal to noise ratio (S/N). As shown in the figure, the detectable signal emerged at the first 5 min after samples were applied, and either the T/C or S/N reached a constant at 20 min. The standard error was based on three duplicated measurements of analytes. These results indicated the short immunoreaction time of this biosensor. Thus, signals for quantitative analysis were measured at 20 min after the sample addition in all the succeeding studies.

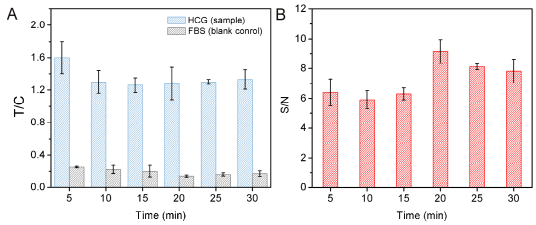

Fig. 2 Effect of immunoreactions time on the fluorescent responses of ICTS

C. Specificity of the QDs-Based ICTS

To recognize the ability of targets selectively, we prepared another 4 type of proteins marker including CA199, CEA, HCG and AFP, FBS was set as the blank control group to further test the strip specificity. When the non-specific binding existed, the non-target proteins marker would be captured by capture antibody on test zone. Consequently, strong signal response of the T zone would be detected with CA199, CEA, HCG, AFP and FBS. As a result, a clear distinction could be observed between the strips added HCG and the strip added FBS, CA199, CEA, AFP and PSA (Figure 3). Both the T/C and the S/N of the strips added HCG antigen were obvious higher than the strips added non-targeted antigens and FBS. We did not observe any crosstalk or interference with either CA199, CEA, AFP, PSA or FBS. From these measurements, we conclude that the test strips featured very high specificity.

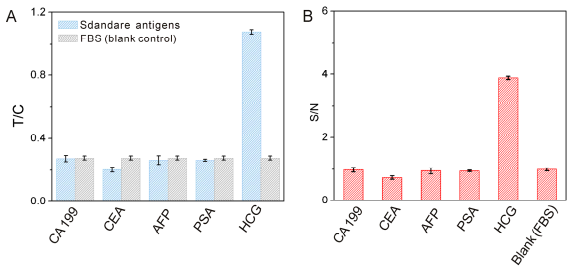

Fig. 3 Characterization of ICTS specificity.

D. Sensitivity and quantitative analysis of the QDs-based ICTS

To explore the feasibility for clinical application, the ability of quantitative detection of the QDs-based ICTS was characterized by analyzing standard HCG samples at a concentration gradient of 0~1000 IU/ml diluted in FBS. Fig. 2 revealed the fluorescence imaging of the ICTS excited by the ultraviolet lamp. As seen in the Fig. 2 the brightness of the fluorescence band could be easily observed by naked eyes. This result indicated that we can easily judge the existence or not of target proteins by observing the fluorescence band directly. For quantitative detection, there was a significant linear relationship between HCG concentration and fluorescence signal in the log-log plot with R^2=0.994, (y=0.427x-1.051). The detection limit was 0.24 IU/L (S/N=3), which was calculated as the concentration corresponding to 3 times the standard deviation of the blank control. Error bars are based on three duplicated measurements of HCG at different concentrations.

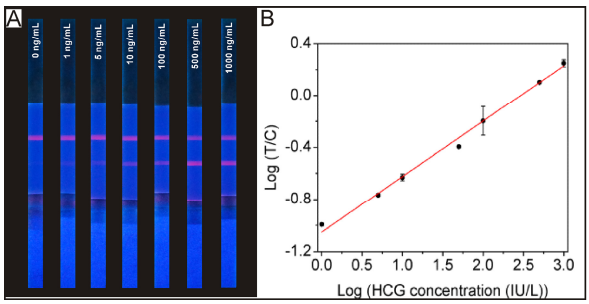

Fig. 4 Fluorescence imaging of the QDs-based ICTS added different concentrations HCG (A) and calibration curve of the quantitative detection (B)

IV. Conclusions

A well-performing biosensor was designed by combining the ICTS with QDs for the quantitative detection of protein biomarkers. The integration enabled the ICTS to sensitively and specifically determine the concentration of HCG in 20 min. Under the optimized conditions, the detection limit was about 0.24IU/L. There was a significant linear relationship between HCG concentration and fluorescence signal in the log-log plot with R^2=0.994. These results demonstrated the QDs-based ICTS was a rapid, sensitive, specific and low cost point-of-care diagnostic format. In view of its advantages, the new biosensors may be a nascent sensing technology that opens up new opportunities for the early detection of diseases at the site of patient care.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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