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Disposable Electrochemical Immunosensor Diagnosis Device Based on Nanoparticle Probe and Immunochromatographic Strip

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We describe a disposable electrochemical immunosensor diagnosis device that integrates the immunochromatographic strip technique with an electrochemical immunoassay and exploits quantum dot (QD, CdS@ZnS) as labels for amplifying signal output. The device takes advantage of the speed and low cost of the conventional immunochromatographic strip test and the high sensitivity of the nanoparticle-based electrochemical immunoassay. A sandwich immunoreaction was performed on the immunochromatographic strip, and the captured QD labels in the test zone were determined by highly sensitive stripping voltammetric measurement of the dissolved metallic component (cadmium) with a disposable screen-printed electrode, which is embedded underneath the membrane on the test zone. The new device coupled with a portable electrochemical analyzer shows great promise for in-field and point-of-care quantitative testing of disease-related protein biomarkers. The parameters (e.g., voltammetric measurement of QD labels, antibody immobilization, the loading amount of QD−antibody, and the immunoreaction time) that govern the sensitivity and reproducibility of the device were optimized with IgG model analyte. The voltammetric response of the optimized device is highly linear over the range of 0.1−10 ng mL^{-1} IgG, and the limit of detection is estimated to be 30 pg mL^{-1} in association with a 7-min immunoreaction time. The detection limit was improved to 10 pg mL^{-1} using a 20-min immunoreaction time. The device has been successfully applied for the detection of prostate-specific antigen (PSA) in human serum sample with a detection limit of 20 pg mL^{-1}. The results were validated by using the commercial PSA enzyme-linked immunosorbent assay kit and showed high consistency. The new disposable electrochemical diagnosis device thus provides a rapid, clinically accurate, and quantitative tool for protein biomarker detection.

As research moves into the era of proteomics, scientists are faced with the challenge of developing effective methods and tools for identifying and quantitating proteins.[1] Such new techniques are essential for diagnosing various disease states, for defense against biological threats, and for improving drugs. Immunoassay is one of the most popular approaches for protein analysis.[2] The traditional immunoassay approach is the enzyme-linked immunosorbent assay (ELISA) in connection with optical readers.[3] The sensitivity is enhanced by multiple-enzyme turnover events. The major drawbacks of ELISA are the relatively long analysis time and complexity due to the multiple-step processes involving antibody−antigen complexation, washing, and the addition of reagents.[4] The immunosensor is an alternative tool to replace the traditional ELISA.[4] On the basis of a specific reaction of the antibody and antigen, immunosensors provide a sensitive and selective tool for determining immunoreagents. Although most of the reported immunosensors have simplified the operations, shortened the assay time, and provided a good sensitivity, practical applications of the developed immunosensors stay in the laboratory-development level and have not been widely used for in-field or point-of-care (POC) diagnosis.

Immunochromatographic strip tests (ISTs) combine chromatography with conventional immunoassay to offer a new analytical tool for protein analysis and clinical diagnosis.[5−7] It has been widely used as an in-field and POC diagnosis tool to detect and identify infectious disease, cancer, cardiovascular problems, and biological warfare agents.[2,8−10] The benefits of such ISTs include (1) their user-friendly format, (2) the very short time (generally less than 10 min) to obtain test results, (3) less interference due to chromatographic separation, (4) long-term stability over a wide range of climates, and (5) a relatively low cost. The early stage of ISTs is to use visible judgment (colorimetric) for the qualitative or semiquantitative analysis of the analytes. However, some of these tests are still not sufficiently sensitive or specific for accurate POC use. The visual readout of the strip is usually limited to a yes/no answer; this is not adequate when the level of an analyte is important.[9] Therefore, much effort has been directed to the development of quantitative IST, which can offer accurate concentration information for the targets of interest.

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Such a quantitative IST has been achieved by adapting a signal transducer. Different transducers, such as optical readers (fluorescence[11−13] and color scanner[14]), electrical readers (conductivity meter,[15,16] electrochemical detectors[17,18]), and magnetic readers,[19,20] have been used to convert the captured particle labels to digital signals. Brooks and co-workers[11,12] have reported the fluorescence immunochromatographic assay for quantifying analytes in liquid samples. Quantitative ISTs based on gold nanoparticles have been developed to detect human chorionic gonadotropin (HCG),[21−23] prostate-specific antigens (PSAs),[21,22] aflatoxin B_1,[24] and β-adrenergic agonist clenbuterol residues.[25] Such immunochromatographic assays based on optical detectors may have applications in analyzing and diagnosing diseases. However, a significant limitation of this assay is that the results may suffer from optical interference (e.g., photobleaching) and the effect of liquid in the chromatographic test strip.[18] Fluorescence immunochromatographic assays are often complicated by the requirement of an elaborate excitation and detection scheme and by the broad emission bands. Some of the optical detectors are expensive and may need complex software for imaging and analysis, which limits their in-field and POC applications.

Electrochemical immunoassays and immunosensors have evolved dramatically over the past two decades and are ideally suited for meeting the portability requirements of decentralized POC testing or field detection of bioagents.[26] Combining immunochromatographic technology and electrochemical immunoassay presents a new avenue to develop in-field and POC diagnosis devices. For example, Lee et al. first developed a liposome immunosensor for theophylline, combining an immunochromatographic membrane and a thick-film electrode.[17] Lu et al. developed immunochromatographic electrochemical biosensor-based metal ion labels to quantify the concentration of HCG.[18] Although these ISTs based on electrochemical detectors overcome long analysis times and the complex operations of conventional electrochemical immunoassays and immunosensors, the sensitivities are low. Recently, nanomaterial-based electrochemical immunoassays and immunosensors have attracted considerable interest, and they provide high sensitivity because of their signal amplification.[27,28] Gold nanoparticles,[29] silica nanoparticles,[30] semiconductor crystals (CdS, PbS, ZnS),[31] and apoferritin nanoparticles[28,32] are used as labels to enhance the sensitivities of the electrochemical immunoassay. Most of these electrochemical immunoassays based on nanoparticle labels were performed with functionalized magnetic beads under batch conditions.[28,31,32] Although this technology provided good sensitivity for detecting proteins, it cannot be used for in-field or POC applications.

In this paper, we present a disposable electrochemical immunosensor diagnosis device (DEIDD), which combines conventional ISTs, an electrochemical immunoassay, and highly sensitive nanoparticle labels. Quantum dots (QDs, CdS@ZnS) were used as labels during immunoreaction events, and IgG was used as a model analyte to optimize the fabrication of device and experimental parameters. This new device is a rapid, clinically accurate, and less expensive tool for detecting protein biomarkers. It opens a new door to apply highly sensitive electrochemical immunosensors and immunoassays for in-field and POC applications.

EXPERIMENTAL SECTION

Reagents. Glutaraldehyde was purchased from Polyscience, Inc. (Warrington, PA). Polyester backing materials, glass fibers, and absorbent materials were purchased from Millipore Corp. (Bedford, MA), and nitrocellulose membrane (AE 98) was purchased from Schleicher & Schuell BioScience, Inc. (Keen, NH). Anti-mouse IgG and mouse IgG were purchased from Sigma and Aldrich. Mouse monoclonal PSA antibody pairs (MO-T40081A and MO-T40081 B), standard serum PSA, and the control (serum without PSA) were obtained from Anogen (Mississauga, Canada). Human serum samples were obtained from Golden West Biologicals (Temecula, CA). Human PSA ELISA kit (Catalog No. 1500) was purchased from Alpha Diagnostic International (San Antonio, TX). The Qdot 655 antibody conjugation kit was purchased from Molecular Probes, Inc. (Eugene, OR). The reagent components in the kit include QD nanocrystals, succinimidyl *trans*-4-(*N*-maleimidylmethyl)cyclohexane-1-carboxylate (SMCC) stock solution, dithiothreitol (DTT) stock solution, dye-labeled marker for antibody elution, β-mercaptoethanol, separation media, and exchange buffer. Phosphate buffer saline (PBS; 0.01 M), bovin serum albumin (BSA), and Tween-20 were purchased from Sigma-Aldrich. All chemicals used in this study were analytical reagent grade. All stock solutions were prepared using deionized water purified with the Nanopure System (Barnstead, Kirkland, WA) or autoclaved water.

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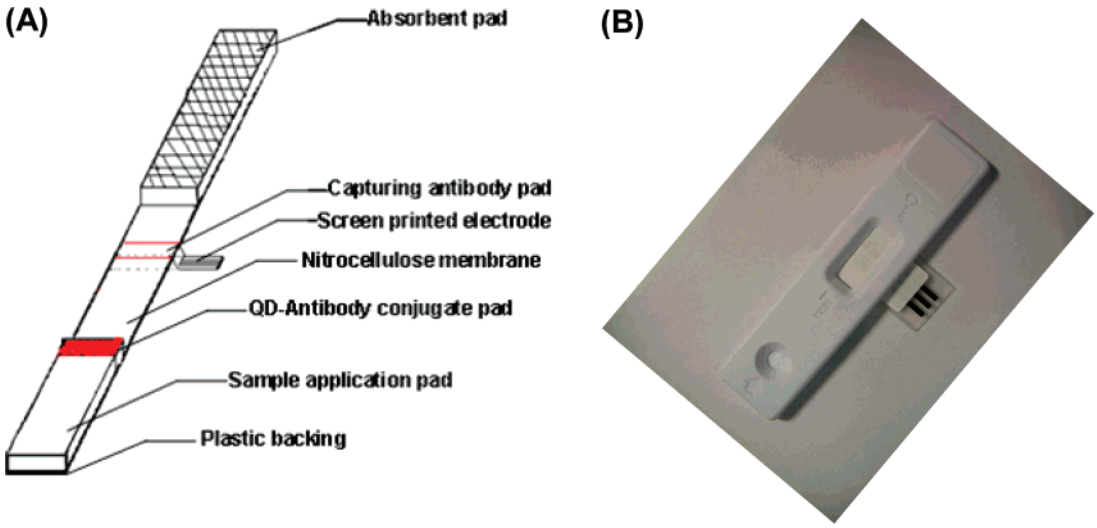

Figure 1. (A) Schematic illustration of DEIDD; (B) photograph of DEIDD.

Instruments. Square-wave voltammetric (SWV) measurements were performed with an electrochemical analyzer CHI 660A (CH Instruments, Austin, TX) or a portable electrochemical analyzer (CH Instruments) connected to a personal computer. Disposable screen-printed electrodes (SPE) consisting of a carbon working electrode, a carbon counter electrode, and an Ag/AgCl reference electrode (Alderon, Durham, NC) were used for electrochemical measurements. A sensor connector (Alderon Biosciences, Inc.) allowed for connecting the DEIDD or SPE to the CHI electrochemical analyzer. ELISA measurements were carried out with a microplate reader, and microplates (Alpha Diagnostic International, San Antonio, TX) were used for immunoreactions.

Preparation of QD/Anti-IgG Conjugate. QD/anti-IgG conjugate was prepared following the QD conjugation kit protocol from the manufacturer. Briefly, QD nanocrystals in dimethyl sulfoxide solution were first activated with 10 mM SMCC at room temperature for 1 h. Then the resulting mixture was subjected to a NAP-5 desalting column with the exchange buffer provided with the conjugation kit as the elution solvent. The colored eluate with ~500 μL was collected. At room temperature, 300 μL of anti-IgG with a concentration of 1 mg/mL was reduced with 20 mM DTT for 0.5 h. The resulted mixture was labeled with a dye-labeled marker and was purified with NAP-5 desalting column. The colored fraction of ~500−600 μL was collected. The above two purified collections were mixed and allowed to react at room temperature for 1 h to form the conjugation complex. Following the addition of β-mercaptoethanol, the conjugation reaction was quenched. The quenched reaction mixture was then concentrated to 20 μL using the 0.5-mL concentrator supplied in the kit. Ultrafiltration was run at 7000 rpm for ~10−15 min on a benchtop Eppendorf centrifuge. Following the instructions from the kit, Superdex 200 gel was slowly packed in the column. After the conditioning with water and PBS, the size-exclusion column was used to purify the conjugated biocomplex. When the dark-brown color appeared in the dead space of the column, ~10 drops of colored eluent was collected. Therefore, ~200 μL of purified QD/anti-IgG conjugate was achieved.

Preparation of DEIDD. A schematic diagram of the DEIDD is shown in Figure 1A. It is composed of an immunochromatographic strip (IS) and a disposable SPE. The IS consists of five components: sample application pad, QD−antibody (QD−Ab) conjugate pad, nitrocellulose membrane, capturing antibody pad, and absorbent pad. The disposable SPE (f), consisting of a carbon working electrode, a carbon counter electrode, and an Ag/AgCl reference electrode, was placed underneath the capturing antibody pad at the test zone. To accommodate multiple functional components within the integral device of the DEIDD, all of the components were mounted on a common backing layer (typically an inert plastic, e.g., polyester).

Preparation of Sample Application Pad. The sample application pad was made from glass fiber. The pad was cut out to 6 × 20 mm and 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 in a desiccator at room temperature.

Preparation of the Conjugate Pad. The conjugate solution was prepared by diluting 10 times the QD-labeled anti-IgG with PBS buffer containing 0.5% (w/v) BSA. The conjugate pad was prepared by adding a desired volume of QD−Ab conjugate onto the glass fiber pad and then drying it under nitrogen. The pad was stored in a desiccator at 4 °C.

Preparation of Nitrocellulose Membrane for Lateral Flow. The lateral-flow nitrocellulose membrane was treated with blocking reagent (a solution of 3% w/v BSA and 0.1% Tween-20 in 0.01 M PBS buffer) for 2 h, and this was followed by three 5-min washings with 0.1% Tween-20 in 0.01 M PBS buffer. The membrane was then dried in a nitrogen box for 1 h and stored at 4 °C in a dry state.

Preparation of Capturing Antibody Pad. The capturing antibody pad was prepared by the covalent binding of anti-IgG to a glutaraldehyde-activated nitrocellulose membrane following the protocol of Masson.[33] The nitrocellulose membrane (4 cm × 4 cm) was first treated with 2.5% diaminoheptane solution for 1 h with constant agitation. This treatment introduced amino groups on the nitrocellulose membrane. The membrane was washed with 0.01 M PBS buffer and water for 12 h on a shaking incubator at room temperature with several new replacement washing solu-

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tions. This was followed by a short rinse with PBS buffer. The diaminoheptane-modified nitrocellulose membrane was activated with 1% glutaraldehyde in 0.01 M PBS buffer for 4.5 h with constant shaking at room temperature followed by 3 h of washing with water to remove all noncovalently bound glutaraldehyde. The membrane was then dried in a nitrogen box for 1 h and stored at 4 °C in a dry state. The glutaraldehyde-activated nitrocellulose membrane was cut into pieces with a homemade punch. Each membrane was accurately cut to 0.2 cm in width and 0.6 cm in length. The prepared membrane was directly immersed into 1000 μg mL^{-1} anti-IgG solution followed by drying at 4 °C. This procedure was repeated twice to maximize the amount of anti-IgG attached to the surface of the membrane. The antibody-immobilized membrane was finally blocked with PBS containing 1% BSA and then dried in the nitrogen box. The membrane was stored at 4 °C before use.

For comparing experiments, the capturing-antibody pad was also prepared by physical adsorption of anti-IgG on the untreated nitrocellulose membrane pad. The pad was finally blocked with PBS-containing 1% BSA and then dried in the nitrogen box.

Cleaning SPE. The SPE was pretreated electrochemically by cyclic voltammetric scanning 10 times at the potential window of 0−1.5 V. The treated SPE was washed with distilled water and dried in air.

Assembling. The sample application pad, conjugate pad, capturing-antibody pad, absorption pad, nitrocellulose membrane, and treated SPE were laminated on a plastic backing plate (Figure 1A) and mounted on a master card (Figure 1B).

Sample Assay Procedure. One-hundred microliters of sample solution containing a desired concentration of IgG was applied to the sample application zone. After waiting for a desired time (for example, 7 min), two insulator lines were drawn at the test zone with a liquid blocker super PAP pen (Daido Sangyo Co., Ltd., Tokyo, Japan). The distance between the two lines is ~10 mm. The DEIDD was then inserted into a sensor connector for electrochemical measurements. Before the measurement, 10 μL of 1 M HCl was dropped into the test zone to release Cd^{2+} ions from the captured QD labels. Fifty microliters of detection solution consisting of 10 μg mL^{-1} Hg and 500 ng mL^{-1} Bi in 0.2 M acetate buffer (pH 4.6) was then added for electrochemical measurement. SWV measurements were performed using an in situ plated Hg/Bi film on the SPE by a 2-min accumulation at −1.4 V. Subsequent stripping was performed after a 2-s rest period from −1.0 to −0.5 V with a step potential of 4 mV, amplitude of 25 mV, and frequency of 15 Hz.

PSA Assay with DEIDD. The preparation of DEIDD for PSA assay is similar with the above procedure. Primary anti-PSA antibody was immobilized on capturing pad, and the secondary anti-PSA antibody was conjugated with QD and immobilized on the conjugate pad. PSA sample solutions with different concentrations were prepared by diluting standard serum PSA with PBS. Control solutions were prepared by diluting control serum (without PSA) with PBS. A calibration curve of PSA was obtained by measuring a series of different concentrations of PSA. PSA assay with the DEIDD in human serum was performed by spiking standard serum PSA in a diluted human serum (20 times with PBS). The results were validated with commercial ELISA PSA kit (Alpha Diagnostic International).

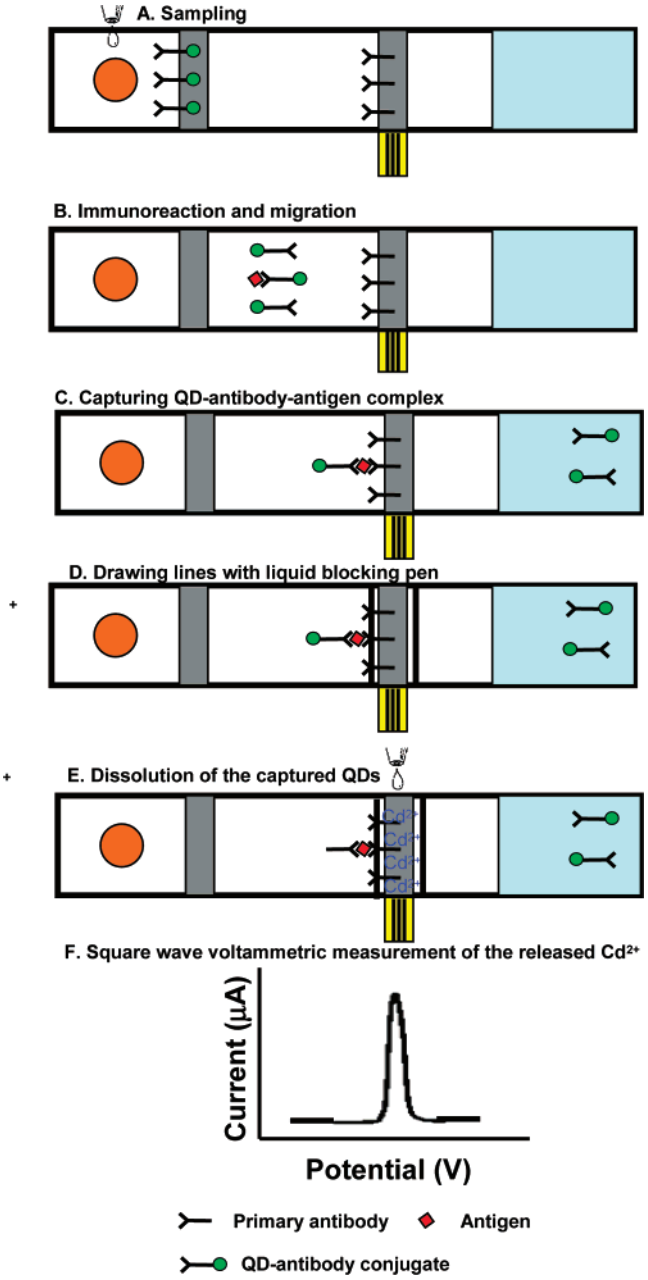

Figure 2. Measurement principle of the DEIDD.

RESULTS AND DISCUSSION

Principle. In this paper, IgG was used as a model analyte, and QDs (CdS@ZnS) were used as the labels with which to tag anti-IgG. Figure 2 illustrates the protocol of the DEIDD for measuring the IgG model target. The DEIDD is composed of an IS and a disposable SPE. The IS has three discrete zones that allow fluid to flow across them. The first zone is a sample-loading zone, and the second is a contact zone with a glassy-fiber pad loaded with nanoparticle-labeled anti-IgG antibodies by adsorption. The third zone is a test zone, which has the immobilized secondary anti-IgG antibodies (Ab) to selectively capture the QD−Ab−IgG immunocomplexes. An absorbent pad is embedded at the end of the IS that forces migration of the sample solution. In this design, the SPE is located under the membrane of the test zone. During the assay, a liquid sample (~100 μL) will be first applied to the sample loading zone (Figure 2A). Then capillary

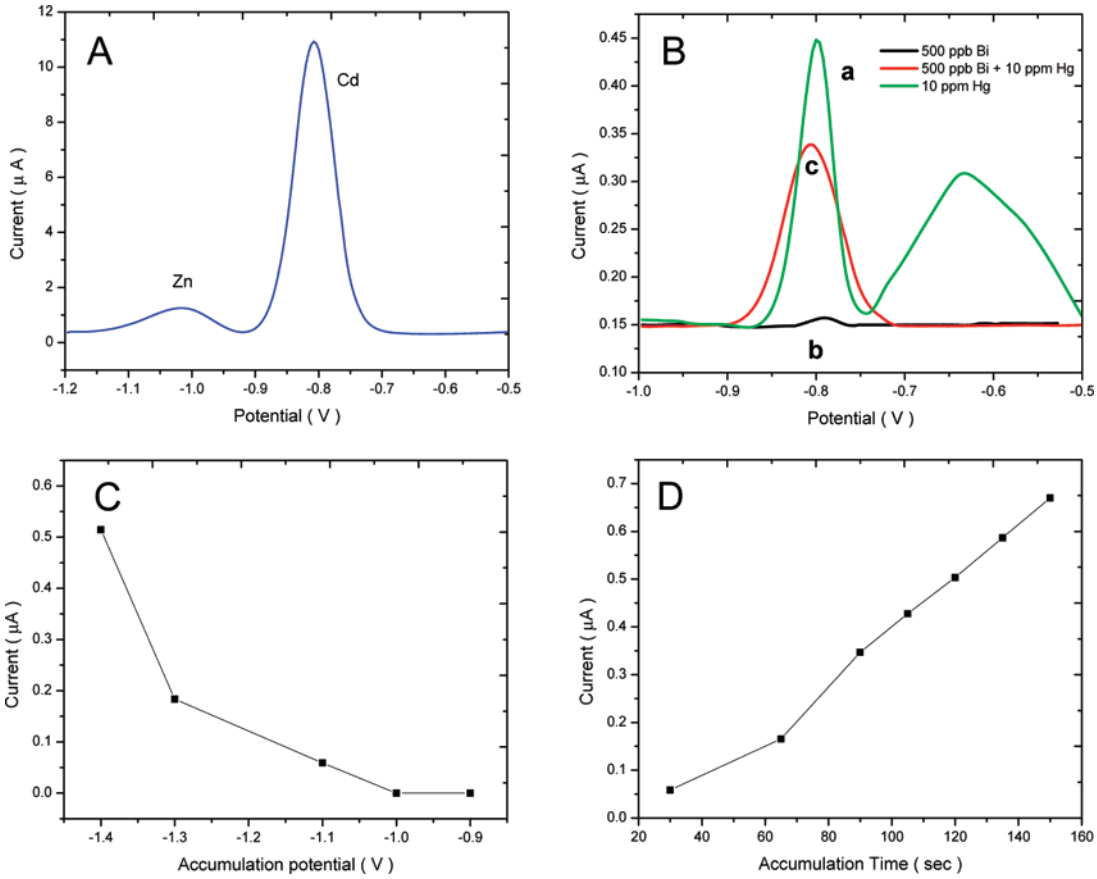

Figure 3. (A) Typical square wave voltammograms of a dissolved QD−antibody conjugate**.** A total of 2.5 μL of 50 times diluted QD was mixed with 10 μL of 1 M HCl. After 1-min dissolution, 50 μL of 0.2 M acetate buffer containing 500 ng mL^{-1} bismuth was added. The resulting mixture was transferred to a screen-printed electrode for SWV measuring. The released metal ions were measured with SWV using an in situ plated bismuth film on the SPE, following a 1-min pretreatment at 0.6 V and using a 2-min accumulation at −1.4 V. Subsequent square wave stripping was performed after a 2-s rest period from −1.4 to −0.5 V with a step potential of 4 mV, an amplitude of 20 mV, and a frequency of 25 Hz. (B) SWV of 20 ng mL^{-1} standard cadmium solution with in situ-plated bismuth film (a), mercury film (b), and bismuth/mercury hybrid film (c). Bismuth concentration, 500 ng mL^{-1}; mercury concentration, 10 μg mL^{-1}; other conditions, same as in (A). (C) Accumulation potential effect on SWV signal of 20 ng mL^{-1} cadmium with in situ-plated bismuth/mercury film; accumulation time, 2 min. (D) Accumulation time effect on SWV signal of 20 ng mL^{-1} cadmium with in situ-plated bismuth/mercury film; accumulation potential, −1.4 V.

action causes liquid sample to migrate toward the other end of the strip. As the liquid sample migrates into the contact zone, IgG (analytes) in the sample will react with the QD−Ab conjugates, which are adsorbed in this zone, forming QD−Ab−IgG complexes. The complexes continue to migrate along the strip by capillary action (Figure 2B). When these complexes reach the test zone, the secondary anti-IgG antibodies, which are immobilized on the test zone, capture the QD−Ab−IgG to form a sandwich type of QD−Ab−IgG−Ab complex. These complexes remain in the test zone while the unbound constituents include the excess of QD−Ab, and the fluid fraction continues to flow into the absorbent medium at the end of the DEIDD (Figure 2C). After a complete assay, two blocking lines were drawn with a liquid block pen along the test zone (Figure 2D). The function of the lines was to prevent the diffusion of electrolyte during dissolution of the captured QDs and electrochemical detection. Then the DEIDD was inserted into a portable electrochemical analyzer (for the in-field test) or connected with a sensor connector (Bench operation) for electrochemical measurements. Before the electrochemical measurement, the captured QD labels at the test zone were dissolved with 10 μL of 1 M HCl solution to release metal ions (cadmium, Figure 2E), and this was followed by adding 50 μL of supporting electrolyte (0.2 M acetate buffer containing 10 μg mL^{-1} mercury and 500 ng mL^{-1} bismuth). The released metal ions were quantified by SWV (Figure 2F**)**. The concentration of the IgG analyte can be obtained because the IgG concentration in the sample is proportional to the amount of QD labels in the sandwich complex within the testing zone.

Electrochemical Measurements of QDs on the SPE. In the current study, QDs were used as labels for electrochemical immunosensing. QDs had a core/shell structure with CdS as the core and ZnS as the shell. The electrochemical signal of the QD−Ab conjugate was first studied under bench conditions by SWV, measuring its metal components after a dissolution step. Figure 3A displaces a typical voltammogram of the QD−Ab conjugate after dissolution with 1 M HCl. Two well-defined peaks were observed with peak potentials at −1.1 and −0.82 V. Comparing this with the standard stripping peak potentials of metal,[34] one can conclude that the striping peak at −1.1 V corresponds to zinc, and the stripping peak at −0.82 V corresponds to cadmium. The current intensity of the cadmium stripping peak is five times more than that of zinc, indicating that the amount of the cadmium

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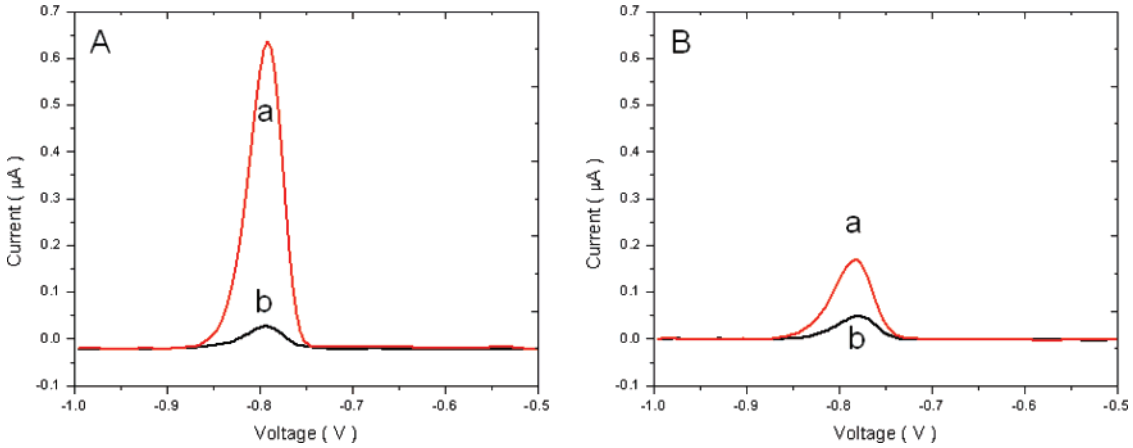

Figure 4. Electrochemical responses of 50 (curve a) and 0 ng mL^{-1} (curve b, control) IgG on DEIDD fabricated with a covalent binding antibody pad (A) and a physical adsorption antibody pad (B) at the test zone. Immunoreaction time on immunochromatographic strip, 7 min; SWV measurements were performed using an in situ-plated Hg/Bi film on the SPE by a 2-min accumulation at −1.4 V. Subsequent stripping was performed after a 2-s rest period from −1.0 to −0.5 V with a step potential of 4 mV, amplitude of 25 mV, and frequency of 15 Hz. For the sample assay procedure, see details in the experimental section.

component is much higher than that of the zinc component. This is consistent with the component and structure of QD. Considering the sensitivity of the immunoassay, the cadmium stripping peak current was used for quantification.

Traditional SWV measurements of metal ions are performed with an in situ or preplated mercury film or a bismuth film-coated electrochemical transducer, such as a glassy carbon electrode and a thick-film carbon electrode. To obtain the best sensitivity of the voltammetric measurement, we compared the voltammetric responses of the QD−Ab conjugates after dissolution with different metal films coated with SPE. Figure 3B presents the SWV responses of the dissolved QD−Ab conjugate with an in situ-plated mercury film (curve a) and a bismuth film (curve b)-coated SPE. One can see that the SWV signal of cadmium on an in situ-plated mercury film is much higher than that of an in situ-plated bismuth film. However, the SPE that is coated with a mercury film presents a high background current, which limits the electrode to measuring low concentrations of cadmium ions. In contrast, the SPE that is coated with a bismuth film exhibits a low background current. We thus investigated the SWV signal of cadmium on an SPE coated with an in situ-plated bismuth/mercury hybrid film (curve c, Figure 3B). It was found that the SWV signal was slightly smaller than that of the mercury film/SPE, but the background current is significantly decreased. Therefore, an SPE coated with an in situ-plated mercury/bismuth hybrid film was used for SWV measurements in the following experiments.

In the current study, the SPE was put underneath a nitrocellulose membrane of the DEIDD, and electrochemical measurements of the dissolved cadmium ions from QDs were performed in a silent solution. It is different with the classical stripping voltammetry, which is performed by applying a potential to accumulate the metal ions on the sensor surface in a stirred solution and then stripping out by a potential scanning in an unstirred condition.[34] So it is necessary to investigate the stripping voltammetric conditions of the metallic component with the in situ-plated bismuth/mercury film at current conditions. The accumulation potential and the accumulation time effects were studied with 20 ng mL^{-1} cadmium standard solution on SPE. It was found that the stripping-peak current of cadmium decreased as the accumulation potential increased at the potential range of −1.4 to −0.9 V (Figure 3C). Therefore, −1.4 V was selected as the accumulation potential. Figure 3D also presents the relationship plot between accumulation time and SWV signal (peak current of cadmium). It can be seen that the SWV signal increases with the increase of accumulation time; the signal does not saturate even after a 160-s accumulation time. Considering the whole assay time, a 120-s accumulation time was used in the following experiments if the accumulation time is not mentioned.

Optimization of Antibody Immobilization. The principle of the proposed DEIDD is based on a sandwich immunoreaction on an IS. Capturing antibodies were immobilized on the test zone to capture the formed antigen−antibody−QD complex. Effective antibody immobilization is very important to produce reproducible results and reduce nonspecific bindings, which produce false signals. In the current study, two approaches, including physical adsorption and covalent binding, were compared during the antibody immobilization. Physical adsorption of the antibody was performed by dipping the untreated nitrocellulose membrane pad into the antibody solution and drying it with nitrogen. The antibody-adsorbed membrane was blocked with 1% BSA and dried with nitrogen. Covalent binding of the antibody to the nitrocellulose membrane was performed by first introducing amino groups on the membrane and then following a typical Schiff base reaction (see details in the Experimental Section).[33] Figure 4 presents the electrochemical responses of 10 ng mL^{-1} IgG with the DEIDD, which were prepared with the above antibody pads at the test zones. One can see that the SWV response of the DEIDD with the antibody pad prepared by covalent binding (Figure 4A, curve a) is significantly higher than that of the antibody pad prepared by physical adsorption (Figure 4B. curve a). Control experiments (in the absence of IgG) were also performed with the DEIDD. Electrochemical responses were also observed with both the covalent-binding antibody pad and the physical adsorption antibody pad, indicating nonspecific adsorption of the QD−Ab conjugate on the capturing antibody pad. It was found that the covalent-binding antibody pad presented a better reproducibility (relative standard deviation (RSD), 6.8%, $n = 6$) than that of the physical adsorption antibody pad (RSD, 20%, $n = 6$). The above

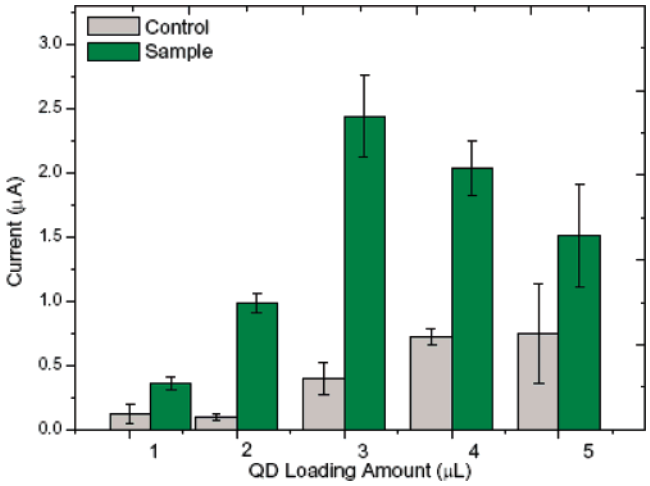

Figure 5. Effect of the loading of QD−Ab conjugate on the electrochemical response generated in DEIDD for 10 and 0 ng mL^{-1}(control) IgG. A covalent binding antibody pad at the test zone was used. The other conditions are the same as in Figure 4.

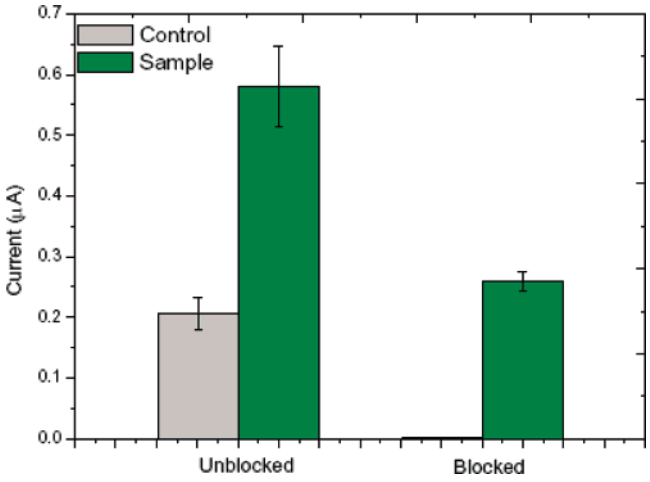

Figure 6. BSA blocking effect at test zone (antibody pad) on the electrochemical response generated in DEIDD for 10 and 0 ng mL^{-1} (control) IgG. Two microliters−

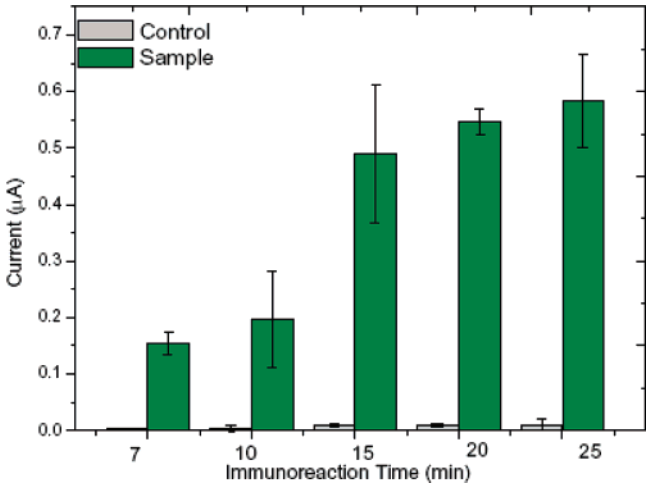

Figure 7. Immunoreaction time effect on the electrochemical response generated in DEIDD for 10 and 0 ng mL^{-1} (control) IgG.

results indicate that using the covalent-binding antibody pad to fabricate a DEIDD increases the immobilization amount of the antibody (thus the electrochemical response) and improves its reproducibility. Therefore, a covalent-binding antibody pad was used to prepare a DEIDD for the following experiments.

In the current study, the QD−Ab conjugate was immobilized on the glassy fiber by physical adsorption, namely, the conjugate pad. The electrochemical response of the DEIDD depends on the amount of QD−Ab conjugate captured on the test zone, which in turn corresponds to the amount of conjugate in the conjugate pad. The excess of the QD−Ab conjugate cause an increasingly nonspecific adsorption. To obtain a maximum response and a minimum nonspecific adsorption, the optimal amount of QD−Ab conjugate was estimated by measuring 50 (sample) and 0 ng mL^{-1} (control) IgG with increasing amount of QD−Ab on the conjugate pad. Figure 5 presents the histogram of the electrochemical responses of DEIDDs. The electrochemical response of the sample (50 ng mL^{-1} IgG, green bars) increased linearly up to 3 μL of QD−Ab on the conjugate pad and then tended to decrease. The decrease of the signal at a high loading amount of QD−Ab may be caused by the formation of QD−Ab−Ag−Ab−QD complexes during the immigration of solution between the conjugate pad zone and the test zone, leading to the decrease of captured QDs at the test zone (Ab−Ag−Ab−QD). The electrochemical responses of controls (0 ng mL^{-1} IgG, gray bars) increased with the increase of the loading amount and then tended to saturate at 4 μL. The saturation of nonspecific adsorption at the higher loading amount is attributed to the limited area of the capture-antibody pad at the test zone. The maximum ratio between sample signal and control signal was obtained by using 2 μL of QD−Ab conjugate, which was used to prepare the conjugate pad in the following experiment.

Nonspecific adsorption is one of main issues in the development of nanoparticle label-based immunoassay. In the current study, there was an electrochemical response obtained from the control sample (in the absence of IgG). It came from the nonspecific adsorption of QD−Ab conjugate on the capture-antibody pad (test zone). To minimize such nonspecific adsorption, we found that adding 0.5% BSA in the QD−Ab conjugate solution and blocking the capturing antibody pad (after antibody immobilization) with 1% BSA significantly reduced the nonspecific adsorption. Figure 6 presents the electrochemical responses generated in the DEIDD with 10 (sample) and 0 ng mL^{-1} (control) IgG in the absence and presence of BSA. It can be seen that the electrochemical signal of the sample decreased after using BSA as a blocking reagent; no signal was generated in the device with the control sample. The delimitation of such nonspecific adsorption may be attributed to the shield effect of BSA, which was adsorbed on the surface of the QD and the capturing antibody pad.

Optimization of Analytical Parameters. Parameters of the assay affected the response of the DEIDD. To optimize the experimental parameters, several factors were considered: immunoreaction time on the immunochromatogram strip, the volume of the sample solution, and the release time of the captured QD labels at the test zone. First, we studied the effect of the immunoreaction time on the electrochemical response generated in the DEIDD. Figure 7 presents the electrochemical responses generated in the DEIDD with 10 and 0 ng mL^{-1} IgG at different immunoreaction times. It can be seen that the electrochemical response of the sample increased with the increase of the immunoreaction time and saturated at 20 min. For the control

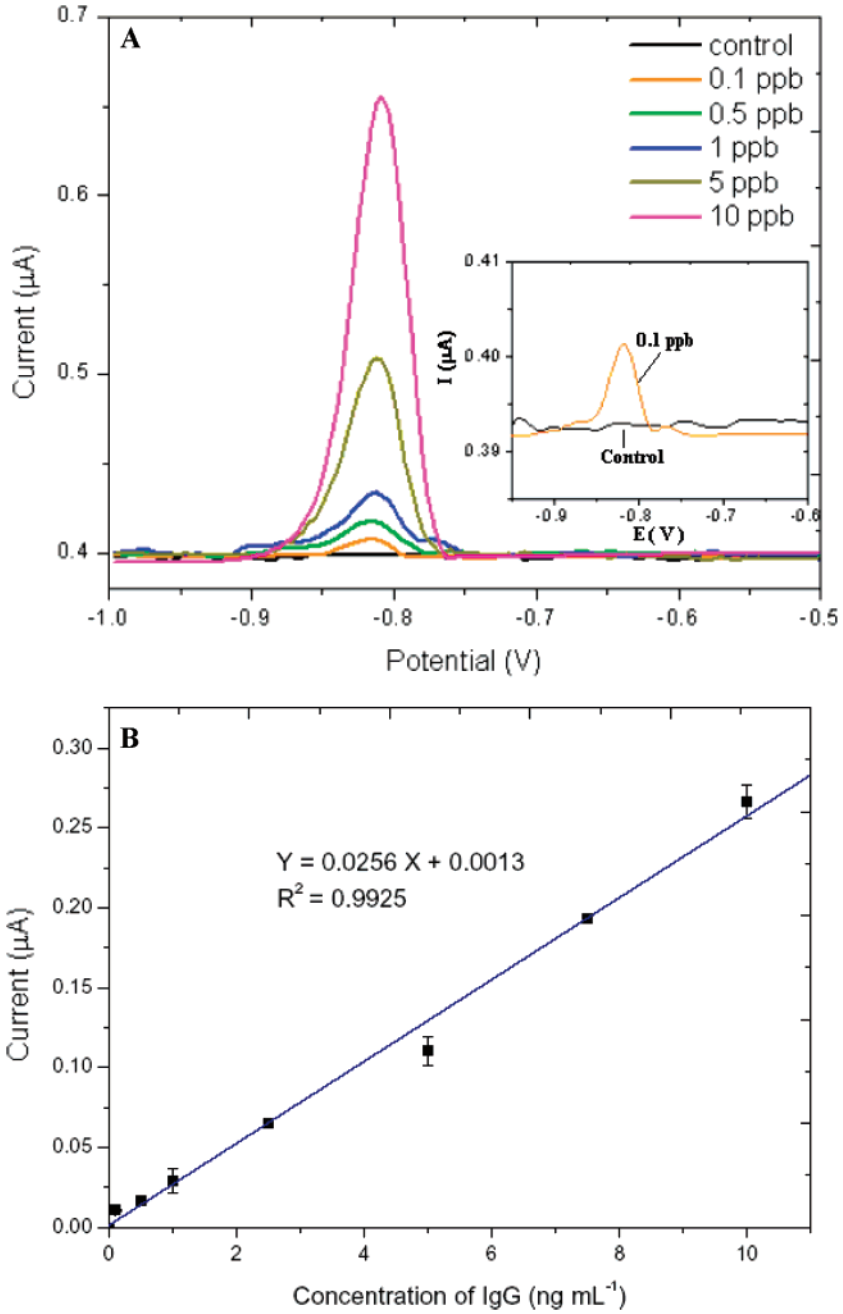

Figure 8. (A) Typical SWV responses of the optimized DEIDD with increasing IgG concentration (0.1−10 ng mL^{-1}). The inset shows the electrochemical responses of 0.1 and 0 ng mL^{-1} IgG. (B) The resulting calibration plot. The immunoreaction time was 7 min; 2 μL of QD−Ab conjugate was loaded on the glass fiber (conjugate pad). Electrochemical measurement conditions were the same as in Figure 4.

experiments, no signal was observed at 7 min; small responses appeared at 10 min and then increased slightly with the increase of immunoreaction time. From the above results, a long immunoreaction time would improve the sensitivity of the DEIDD. Considering the whole assay speed and time, a 7-min period of immunoreaction time was employed. We also studied the effect of sample volume and the release time of the captured QD label at the test zone. It was found 100 μL of sample and 1 min of release time with 10 μL of 1 M HCl were appropriate for sampling and electrochemical measurement, respectively (results not shown).

Performance of DEIDD. Under optimal experimental conditions, we examined the performance of the DEIDD with different concentrations of IgG. Figure 8A displays typical electrochemical responses of the DEIDD with increasing concentrations of IgG (0.1−10 ng mL^{-1}, from bottom to top). Well-defined voltammetric peaks (cadmium) are observed with low concentrations of IgG. The peak current intensities increased with the increase of IgG concentrations. The resulting calibration plot of the currents versus [IgG] (Figure 8B) is linear over the 0.1−10 ng mL^{-1} range and is suitable for quantitative work. Figure 8A (inset) shows the SWV signal for a 0.1 and 0 ng mL^{-1} (control) IgG solution. A negligible signal was observed in the control experiment (in the absence of IgG). Such behavior is ascribed to the blocking step with 1% BSA. The response of 0.1 ng mL^{-1} IgG indicates a detection limit of 30 pg mL^{-1} (~0.19 pM, based on S/N = 3) in connection with the 7-min immunoreaction time. The detection

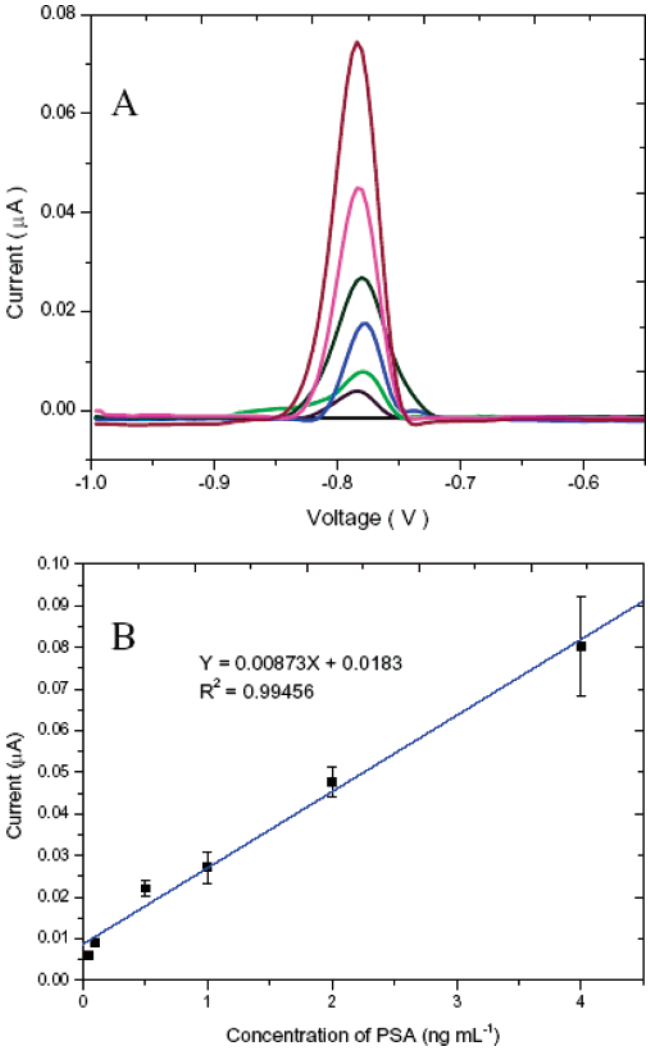

Figure 9. (A) Typical SWV responses of the optimized DEIDD with increasing PSA concentration (0.05−4 ng mL^{-1}). (B) The resulting calibration plot. The immunoreaction time was 10 min; 2 μL of QD−PSA−Ab conjugate was loaded on the glass fiber (conjugate pad). Electrochemical measurement conditions were the same as in Figure 4.

limit was improved to 10 pg mL^{-1} (65 fM) in connection with a 20-min immunoreaction time. This detection limit corresponds to 6.5 amol in the 100-μL sample solution, which is comparable to that of the fluorescence immunochromatographic assay (47.23 pg mL^{-1} microcystins)[13] and the benchtop electrochemical immunoassay.[27,28,30−32] It is better than the other reported quantitative immunochromatographic assay.[21−25] The sensitive and specific response was coupled with high reproducibility. A series of measurements of 5 ng mL^{-1} IgG with six DEIDDs yielded reproducible cadmium signals with an RSD of 6.3% (data not shown).

PSA Assay with the DEIDD. To demonstrate the feasibility of the DEIDD for the detection of protein biomarkers, the device was then applied to detect PSA biomarker. Serum PSA within 4−10 ng mL^{-1} is the "diagnostic gray zone", which predicts that the patient has significant probability of prostate carcinoma.[35] Mouse monoclonal PSA antibody pairs were used for the preparation of QD−antibody conjugates and the immobilization of capturing antibody on the strip, respectively. Tests were started with PBS buffer solution spiked with standard serum PSA. Figure 9A shows SWVs of the DEIDD with different concentrations of PSA (0.05−4 ng mL^{-1}, from bottom to top). Well-defined voltammetric peaks (from cadmium oxidation) are observed with all concentrations of PSA. It can also be seen from this figure that peak current increases with the increase of the concentration of PSA. Figure 9B shows the plot of the peak current versus the concentrations of PSA obtained from Figure 9A. This calibration curve is linear over the 0.05−4 ng mL^{-1} range with $R^2 = 0.994$. It can be seen that the electrochemical response from the control serum sample (absence of PSA) was negligible. The detection limit of the DEIDD for PSA can be as low as 0.02 ng mL^{-1} (0.61 pM), which is much lower than current clinical recommendations for a qualitative test of a PSA marker with a cutoff point at 4.0 ng mL^{-1}.[36]

Table 1. Comparison of PSA Determinations on Human Serum Samples by DEIDD and ELISA

		DEIDD		ELISA	
human serum sample	spiked PSA conc (ng mL^{-1})	cal conc (ng mL^{-1})	recovery (%)	cal conc (ng mL^{-1})	recovery (%)
1	0	nd[a]		nd	
2	0.5	0.53 ± 0.13	105.86	0.56 ± 0.04	111.47
3	2	1.98 ± 0.05	98.91	2.32 ± 0.19	116.00

[a] nd, not detectable.

To explore its clinical application, this DEIDD device was examined with a human serum sample (Golden West Biologicals, Inc.) spiked with PSA and the results were validated by commercial ELISA PSA kit (Alpha Diagnostic International). The sample solutions with final concentrations of 0, 0.5, and 2 ng mL^{-1} PSA were prepared by spiking standard PSA into the diluted human serum (20 times with PBS). The serum sample without addition of standard PSA served as a control. These samples were detected at the DEIDD, and electrochemical signals were recorded. PSA concentrations of the spiked samples were quantified based on the electrochemical signal from these samples and the calibration curve. PSA assay with ELISA was performed by following the manufacturer's instruction in the ELISA kit, and a microplate reader was used to record the absorbance from standard PSA samples and the human serum samples spiked with standard PSA. Table 1 summarizes the results of these spiked serum samples with the DEIDD and ELISA. As shown in this table, the recoveries for these spiked samples obtained from DEIDD are in the range of 85−110%, which indicates that the detection of PSA at the DEIDD in biological samples is accurate. ELISA data from Table 1 validate that DEIDD data for PSA measurements are accurate and reliable. Successfully detecting the spiked human serum samples with the DEIDD demonstrates the promise for various clinical applications.

CONCLUSION

We have successfully developed a DEIDD based on an immunochromatographic strip and nanoparticle probe-based elec-

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trochemical immunoassay for rapid, sensitive, and quantitative detection of proteins. The device takes advantage of the speed and low cost of the conventional immunochramatographic strip tests and the high sensitivity of the nanoparticle-based electrochemical immunoassay. Under optimal conditions, the device was capable of detecting a minimum 30 pg mL^{-1} IgG model analyte in 7 min. The device has been successfully applied for the detection of PSA in human serum sample. The results were validated by using the conventional ELISA and showed high consistency. We intend to use Biodot system (Irvine, CA) and new metallic phosphate nanoparticle tags[32] to simplify the preparation of DEIDD and the electrochemical detection, respectively. The new device coupled with a portable electrochemical analyzer shows great promise for in-field and POC quantitative testing for disease-related protein biomarkers. The developed device thus provides a rapid, clinically accurate, and quantitative tool for protein detection.

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