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Simple and sensitive aptasensor based on quantum dot-coated silica nanospheres and the gold screen-printed electrode

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ABSTRACT

A novel electrochemical aptasensor involving quantum dots-coated silica nanospheres (QDs/Si) and the screen-printed gold electrodes (SPGE) was developed for the detection of thrombin. The screen-printed electrodes with several advantages, including low cost, versatility, miniaturization, and mechanical regeneration after each measurement cycle, were employed. On the other hand, the gold nanoparticles (AuNPs) were electrodeposited on the surface of SPGE to obtain AuNPs/SPGE. And this sandwich format (Apt/thrombin/Apt–QDs/Si) was fixed on the AuNPs/SPGE to fabricate the electrochemical aptasensor. The bound CdTe QDs were dissolved in an acid-dissolution step and were detected by electrochemical stripping analysis. The proposed aptasensor has excellent performance such as high sensitivity, good selectivity and analytical application in real samples. The combination of nanoparticles with the screen-printed electrode is favorable for amplifying electrochemical signals, and useful for large-scale fabrication of the electrochemical aptasensors, which would lay a potential foundation for the development of the electrochemical aptasensor.

1. Introduction

Aptamers are synthetic, single-stranded DNA or RNA molecules, which possess various merits such as remarkable target diversity, high binding affinity, convenient automated-synthesis, ease of labeling, and high stability. Since its discovery in the early 1990s, aptamer technology has progressed tremendously [1,2]. Aptamers have been generated against a wide variety of targets ranging from small molecules [3], peptides [4], amino acids [5], and proteins, including cell membrane proteins [6]. Recently, aptasensors for proteins have been developed based on different techniques including, quartz crystal microbalance [7], surface plasma resonance (SPR) [8], fluorescence [9–11], electrochemistry [12,13], and colorimetry [14,15]. Among them, electrochemical assays have been widely used in this field of electrochemical aptasensors because electrochemical methods posses inherent properties of high sensitivity, simplicity, fast response and portability [16–19].

In order to improve the performance of the electrochemical aptasensor, many research efforts have been made. On one hand, signal enlargement based on nanoparticles (Au or Pt nanoparticles and quantum dots (QDs)), has attracted considerable attention to improve the sensitivity of the aptasensor [20,21]. For example, Willner and coworkers reported on the application of nucleic acid (aptamer) functionalized Pt nanoparticles as catalytic labels for the amplified electrochemical detection of biomolecules [12]. Zhang et al. reported electrochemical biosensor for detection of adenosine based on amplification with reporter probe DNA modified Au nanoparticles [21]. Liu et al. reported an aptamer-based sandwich type sensor by using CdSe QDs as electrochemical labels for the detection of thrombin in human serum [22]. Zhu and coworkers successfully fabricated a sensitive electrochemical aptasensor for the detection of adenosine triphosphate (ATP) by combining three-dimensionally ordered macroporous (3DOM) gold film and quantum dots (QDs) [23].

On the other hand, to broaden the practical application of the electrochemical aptasensor in routine analysis, the mechanical and chemical regeneration of the electrode surface after each measurement cycle is also essential [24,13]. To date, such a problem has been only partially solved with the use of disposable printed electrodes [25], which have been widely used for large-scale fabrication of electrochemical sensors and biosensors (e.g. glucose test strip) [26]. Therefore, it can be expected that the screen-printed electrodes enabled the production of modern aptasensors for direct detection of targets with versatility, low cost, and portability.

In this work, a novel and sensitive electrochemical aptasensor was fabricated based on quantum dots-coated silica nanospheres and the gold nanoparticles modified screen printed electrode. Herein, the AuNP-modified screen-printed gold electrode was

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fabricated, which may be significant for more thrombin–aptamer molecules immobilizing onto the surface of the screen-printed gold electrode, also favorable for large-fabrication of the electrochemical aptasensor. Additionally, thrombin–aptamer was covalently bound to CdTe QDs on the surface of silica nanoparticles. Enhanced sensitivity was also achieved by an increase of CdTe QDs loading aptamer [27]. Therefore, this proposed strategy based on nanoparticles and the screen-printed electrode would have potential for sensitively aptasensing targets with miniaturization, and mechanical regeneration after each measurement cycle.

2. Experimental

2.1. Chemicals and materials

Human thrombin, 6-mercaptohexanol (MCH), and $HAuCl_4 \cdot 3H_2O$ were from Sigma (St. Louis, MO). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), Tris(hydroxymethyl)aminomethane, and (3-aminopropyl)-triethoxy-silane (APTS) were purchased from Sangon Co., Ltd. (Shanghai, China). Thiolated thrombin aptamer (TBA) (5′-HS-$(CH_2)_6$-GGTTGGTGTGGTTGG-3′) was obtained from Sangon Co., Ltd. (Shanghai, China). TBA was diluted to 1 μM in 34 mM Tris–HCl buffer (pH 7.4, 233 mM NaCl, 8.5 mM KCl, 1.7 mM $MgCl_2$, 1.7 mM $CaCl_2$) for use. The TBA solution was stored at 4 °C before use. All other chemicals were of analytical grade. All samples and buffer solutions were prepared using ultrapure water obtained from a Milli-Q water purification system.

2.2. Instruments

All electrochemical experiments were performed on a CHI650D electrochemical workstation (Chenhua Instrument Company of Shanghai, China) connected to a personal computer. The screen-printed gold electrode (SPGE) consisting of gold working electrode, carbon counter electrode, and Ag/AgCl reference electrode was bought from eDAQ Technology Corporation for electrochemical measurements. The sensor connector allows connecting the SPGE to the electrochemical analyzer.

2.3. Preparation of CdTe QDs coated silica nanospheres (QDs/si) and bioconjugation of CdTe QDs/Si with aptamers

CdTe QDs coated silica nanoparticles (QDs/Si) were prepared according to the reported literature [28]. In brief, 0.02 g silica nanoparticles were added with 0.2 mL of ATPS and 2 mL of ethanol, and then stirred for 6 h. The liquor was suspended and centrifuged. The amino-functionalized silica nanoparticles were washed with ethanol repeatedly for five times. Secondly, amino-functionalized silica nanoparticles disperse in a mixed solution of 2.5 mg mL^{-1} CdTe QDs and EDC (20 mg mL^{-1}). The mixed suspension was stirred at 4 °C for 12 h. Unbound QDs were removed by successive centrifugation and washed with water several times. Finally, the as-prepared QDs/Si nanospheres were obtained and dispersed in water. To generate QD coated silica nanosphere labels, CdTe QDs coated silica nanoparticles were exposed to the aqueous thiolated thrombin aptamers, and was stirred overnight at room temperature to prepare Apt–QDs/Si bioconjugations. Apt–QDs/Si bioconjugations were collected by centrifugation at 14,000 rpm for 45 min to remove the supernatant. Afterwards, the resulting Apt–QDs/Si bioconjugations were dispersed in Tris–HCl buffer and stored at 4 °C for later use.

2.4. Fabrication of the sensing interface

Firstly, the screen-printed gold electrode (SPGE) was washed throughout with distilled water and dried under nitrogen stream. And then, Au nanoparticles (AuNPs) were electrochemically deposited onto the surface of SPGE to prepare AuNPs–SPGE according to reports in literature [21]. In brief, the pretreated electrodes were immersed into $HAuCl_4$ (6.0 mM) solution containing 0.1 M KNO_3, where electrochemical deposition was conducted at 400 mV by single-potential mode to obtain the AuNPs modified SPGE.

For immobilization of the aptamer, 10 μL of 1 μM thiolated TBA solution was placed on the AuNPs/SPGE for 16 h to produce a self-assembled monolayer (SAM) of aptamer. After treatment with 1 mM 6-mercaptohexanol (MCH) for 1 h, the unmodified region of the electrode was blocked and then the resulting MCH/TBA/AuNPs/SPGE electrode was incubated for 120 min with various concentrations of thrombin. Subsequently, the Apt–QDs/Si solution was coated on the thrombin/MCH/TBA/AuNPs/SPGE, and the interaction was kept at room temperature for 120 min to obtain a sandwich sensing system, the Apt–QDs/Si/thrombin/MCH/TBA/AuNPs/SPGE. After each step, the electrode was rinsed thoroughly with ultrapure water and then dried with a stream of nitrogen.

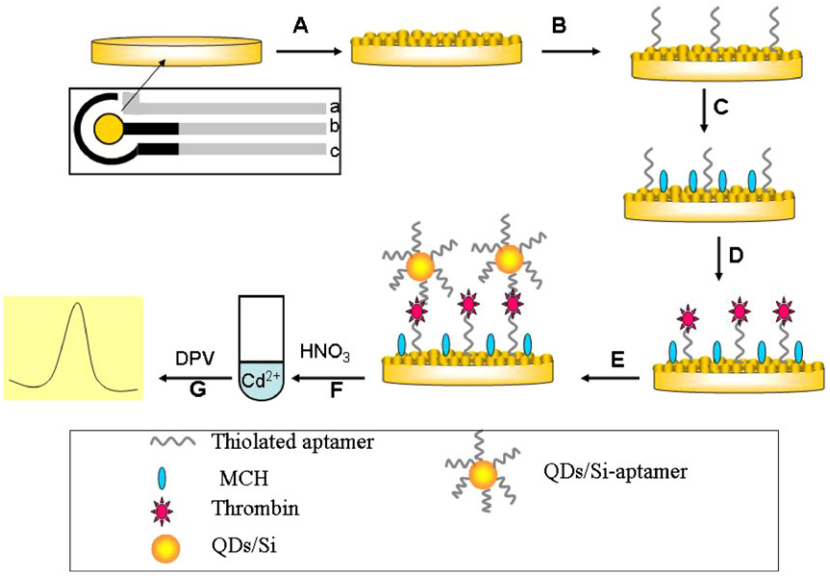
2.5. Electrochemical measurements

Nitric acid (0.1 M) was used for the dissolution step since it can efficiently oxidize the CdTe quantum dots after carefully optimizing reaction time and concentration. Preliminary experiments of dissolving CdTe quantum dots with 500 μL HNO_3 and potentiometer detection of the released Cd^{2+} showed that it was fully oxidized after 1 h. The detection was performed in 0.1 M phosphate buffer solution. The analytical procedure contained 60 s of electrodeposition at 0.6 V, 600 s electrodeposition at −1.00 V, and stripping from −0.95 to −0.45 V using a differential pulse voltammertry (DPV) with 4 mV potential steps, 25 Hz frequency, and 25 mV amplitude. Raw voltammograms were treated with the automatic baseline-correction method offered by computer-controlled instruments [29].

3. Results and discussion

3.1. Design strategy of the aptasensor

The design strategy of the aptasensor is illustrated in Scheme 1. Firstly, the bare SPGE was washed throughout with deionized water and blown dry with nitrogen. AuNPs were electrochemically deposited onto the surface of the treated SPGE to obtain the AuNPs/SPGE, as shown in Scheme 1A. The thiolated thrombin–aptamer (TBA) was immobilized on the AuNPs/SPGE surface via the self-assembly (Scheme 1B) and then blocked by MCH to fabricate the MCH/TBA/AuNPs/SPGE (Scheme 1C). Afterwards, the MCH/TBA/AuNPs/SPGE was used to capture the analyte thrombin in sample solution, obtaining the thrombin/MCH/TBA/AuNPs/SPGE (Scheme 1D). The Apt/QDs–Si was subsequently bound to the resulting thrombin/MCH/TBA/AuNPs/SPGE, forming a sandwich system, the Apt/QDs–Si/thrombin/MCH/TBA/AuNPs/SPGE (Scheme 1E). By adding HNO_3, the QDs from the complex were dissolved and cadmium ions were released (Scheme 1F). These cadmium ions were measured with DPV using a glassy carbon (GC) electrode (Scheme 1G). The concentration of cadmium ions was directly proportional to the amount of thrombin.



Scheme 1. Schematic representation of the electrochemical aptasensor for the detection of thrombin based on the screen-printed gold electrode and quantum dot-coated silica nanosphere.

3.2. Characterization of SPGE and AuNPs/SPGE

The bare screen-printed gold electrode (SPGE) and the AuNPs–SPGE were characterized by cyclic voltammetry in the presence of equimolar $[Fe(CN)_6]^{3-/4-}$, and the corresponding results are shown in Fig. 1A. The peak currents of $[Fe(CN)_6]^{3-/4-}$ at the SPGE (curve a in Fig. 1A) almost cannot be observed. This demonstrates that the conductivity of the SPGE is low, which results from polymeric ink and the low gold proportion of the screen-printed gold electrode. However, in case of the AuNPs/SPGE, the redox peaks of $[Fe(CN)_6]^{3-/4-}$ appeared obviously and peak currents increased greatly (curve b in Fig. 1A). It can be due to more electroactive sites introduced by electrodeposition of AuNPs, which may be useful for the immobilization of thiolated thrombin aptamer. Additionally, the electrochemical voltammograms of the bare SPGE (a) and the AuNPs/SPGE (b) in 0.5 M H_2SO_4 were investigated, and the corresponding results are shown in Fig. 1B. It is observed that the electrochemical behavior of the AuNPs/SPGE is similar to that of the bare gold electrode, which further proves that AuNPs have been electrochemically deposited onto the surface of the SPGE.

3.3. Electrochemical impedance measurements.

It was reported that impedance spectroscopy as one of the most popular strategies may be useful to track the surface features [30]. On the basis of the charge transfer kinetics of the $[Fe(CN)_6]^{3-/4-}$ redox probe, faradaic impedance spectra with an $[Fe(CN)_6]^{3-/4-}$ redox probe were modeled using the equivalent circuit approach of Radi et al. [13], as shown in the inset of Fig. 2. The circuit includes the commonly existing electrolyte resistance (R_s) and Warburg impedance (Z_w) resulting from the diffusion of ions from the bulk of the electrolyte to the interface, double-layer capacitance (C_d), and the electron-transfer resistance (R_{et}). Among them, R_{et} can directly and sensitively respond to changes of the electrode interface [18,31]. Whereas a linear section characteristic at the lower frequency is attributable to a diffusion-limited process, a squeezed semicircle portion observed at higher frequencies corresponds to the electron-transfer limited process.

Herein, impedance spectroscopy was employed to monitor the fabrication of the modified electrodes. Fig. 2 shows the Nyquist

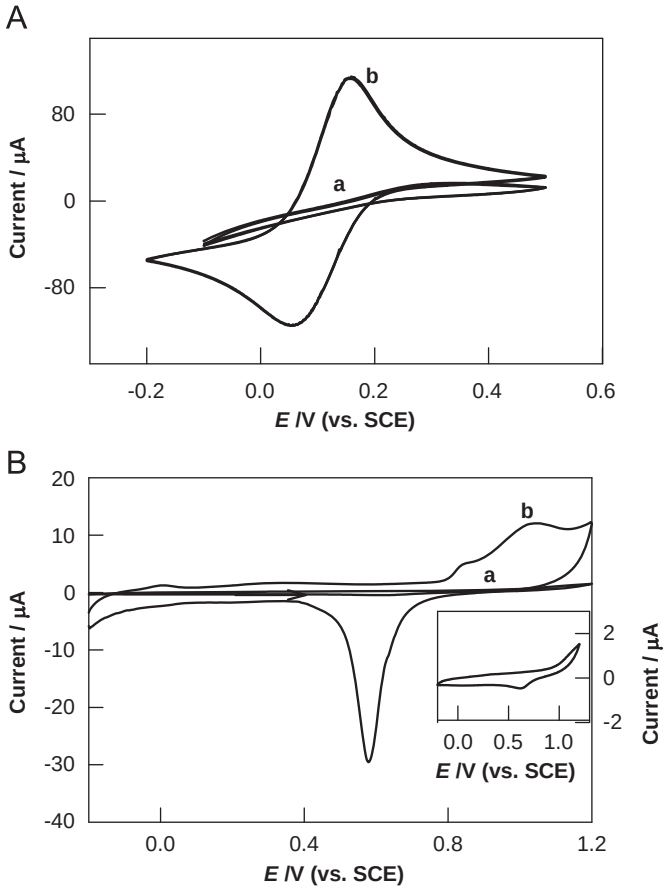


Fig. 1. (A) Cyclic voltammograms of (a) the bare screen-printed gold electrode (SPGE) and (b) the Au nanoparticles modified SPGE (AuNPs/SPGE) in aqueous solution containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ (1:1). (B) Cyclic voltammograms of (a) the bare screen-printed gold electrode (SPGE) and (b) the Au nanoparticles modified SPGE (AuNPs/SPGE) in aqueous solution containing 0.5 M H_2SO_4.

plots of EIS for the AuNPs/SPGE (a), TBA/AuNPs/SPGE (b), MCH/TBA/AuNPs/SPGE (c), thrombin/MCH/TBA/AuNPs/SPGE (d), and Apt–QDs/thrombin/MCH/TBA/AuNPs/SPGE (e). All modified

electrodes show obvious increase in diameter compared to that of the AuNPs/SPGE, indicating much higher R_{et} values. This may be because the aptamer is not only nonconductive, but also a negatively charged TBA on the Au electrode surface effectively repels the $[Fe(CN)_6]^{3-/4-}$ anions [16]. This demonstrates that TBA has been self-assembled successfully on the AuNPs/SPGE. In comparison with the TBA/AuNPs/SPGE, it was found that the assembly of MCH onto the TBA/AuNPs/SPGE led to a significant increase in R_{et} (curve c in Fig. 2), which is consistent with that reported in the literature [32]. Then, the MCH/TBA/AuNPs/SPGE was incubated in 50 ng mL^{-1} thrombin for 2 h, and there was a large increase in R_{et}, which may originate from the bulky thrombin molecules blocking the electrode surface. This implies that thrombin has been bound to the surface of the modified electrode by the aptamer–protein interaction. Afterward, the SPGE/AuNPs/TBA/MCH/thrombin was treated with Apt–QDs to form a sandwich of Apt–QDs/thrombin/MCH/TBA/AuNPs/SPGE, and a large extent increase in diameter was observed. This may be because the Apt–QDs layer formed another barrier which prevents $[Fe(CN)_6]^{3-/4-}$ from transferring to the electrode surface, which would be useful to improve the sensitivity of the electrochemical aptasensor.

On the other hand, in order to further improve the sensitivity of the electrochemical aptasensor, CdTe quantum dots coated silica nanosphere (CdTe QDs/Si) were used to form Apt–QDs/Si conjugations. Herein, the Apt–QDs/thrombin/MCH/TBA/AuNPs/SPGE and Apt–QDs/Si/thrombin/MCH/TBA/AuNPs/SPGE were investigated by electrochemical impedance spectroscopy (EIS), as shown in Fig. 3. Just as expected, the Apt–QDs/Si bioconjunctions caused dramatic increase in the electron-transfer resistance. This is reflected by the enhancement of R_{et} of the Apt–QDs/Si/thrombin/MCH/TBA/AuNPs/SPGE (curve b in Fig. 3), ca. 3 times higher than that of the Apt–QDs/thrombin/MCH/TBA/AuNPs/SPGE (curve a in Fig. 3). This experimental result may result from the increase of CdTe QDs loading on the surface of silica nanospheres, leading to more aptamer molecules immobilizing onto the modified SPGE [27]. Meanwhile, this demonstrates that signal amplification by the QDs/Si label has been realized. Thus, it can be believed that the Apt–QDs/Si/thrombin/MCH/TBA/AuNPs/SPGE would be favorable for electrochemical aptasensing thrombin with high sensitivity.

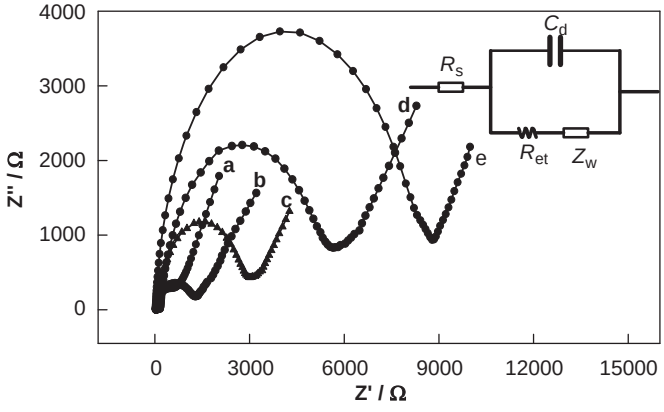


Fig. 2. Electrochemical impedance spectra of (a) the bare SPGE, (b) the AuNPs/SPGE, (c) the MCH/AuNPs/SPGE, (d) the TBA/MCH/AuNPs/SPGE, and (e) thrombin/TBA/MCH/AuNPs/SPGE in aqueous solution containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) as the redox probe. The concentration of thrombin was 50 ng mL^{-1}. The impedance spectra were recorded within the range 100 kHz–10 mHz at the formal potential of $[Fe(CN)_6]^{3-/4-}$. The amplitude of the alternate voltage was 5 mV. Inset: equivalent circuit model for electrochemical impedance measurement system. R_s, solution resistance; C_d, double-layer capacitance; R_{et}, electron-transfer resistance; Z_w, Warburg impedance.

3.4. Electrochemical detection of thrombin

In this work, the target protein thrombin on the modified electrodes was detected with electrochemical measurement

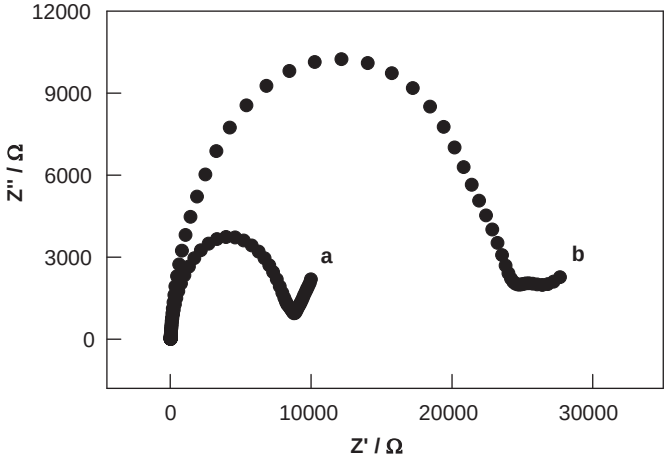


Fig. 3. Electrochemical impedance spectra of (a) Apt–QDs/thrombin/MCH/TBA/AuNPs/SPGE and (b) the Apt–QDs/Si/thrombin/MCH/TBA/AuNPs/SPGE. Other conditions were the same as those in Fig. 2.

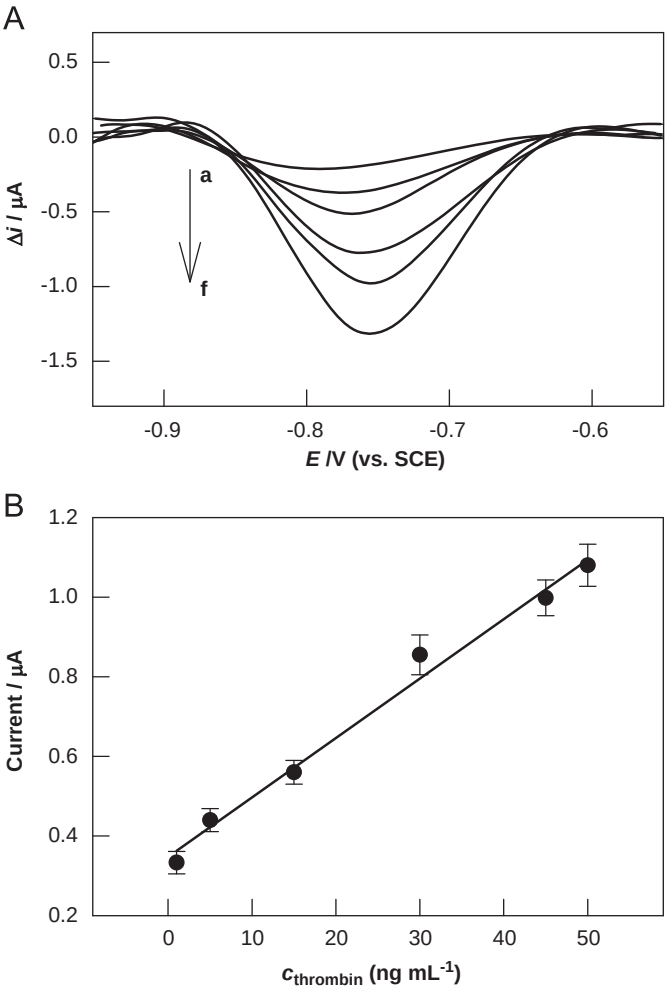


Fig. 4. (A) Differential pulse voltammograms acquired in 1 ng mL^{-1}(a), 5 ng mL^{-1}(b), 15 ng mL^{-1}(c), 30 ng mL^{-1}(d), 50 ng mL^{-1}(e), and 100 ng mL^{-1}(f) of thrombin using the assay method under the optimal conditions, and (B) the linear current–concentration curve of the aptasensor for thrombin detection.

Table 1
Comparsion of the present detected thrombin sensor.

Electrode	Mechanism	Reporter	Detection limit
MB–aptamer/thrombin [34]	Target binding-inhibited electron transfer	MB	6.4 nM
Aptamer/thrombin/aptamer–PtNP [35]	Sandwich formation and PtNP electrocatalyzed reduction of H_2O_2	PtNPs	1 nM
Aptamer/thrombin aptamer–AuNP [36]	Target binding-induced network-like of TCA/AuNPs formation	AuNP	7.82 aM
Aptamer/thrombin [37]	Target/aptamer quadruplex-induced polymer conformational change	Fluorescence cationic polythiophene	10 μM/ 0.01 nM
aptamer/thrombin [38]	Target/aptamer quadruplex-induced AuNPs aggregation	AuNPs	83 nM/0.83 nM
Aptamer/thrombin/aptamer–AuNP [39]	Sandwich formation and catalytic enlargement of Au	AuNPs	2 nM

based on the CdTe QDs/Si labels. The CdTe quantum dots were oxidized with HNO_3 [33], and the dissolved cadmium ions were determined by DPV. Fig. 4A shows the typical differential pulse voltammertry of cadmium ions with increasing thrombin concentration. As illustrated in Fig. 4A, the DPV response increased with the increase of the thrombin concentration. And the corresponding calibration plot of response versus [thrombin] is linear from 1 to 50 ng mL^{-1}, as shown in Fig. 4B. The correlation coefficient is 0.9942 and the lowest detectable concentration of thrombin is 0.1 ng mL^{-1}. And comparing the aptasensor with these presented thrombin sensors in literature, as shown in (Table 1). It was obtained that the sensitivity of the aptasensor is high or comparable. It can be attributed to the following reasons: (a) the AuNPs deposited SPGE may offer a congenial microenvironment and stereo-attaching sites suitable for more aptamer molecules immobilizing on the modified electrode; (b) silica nanoparticles with good monodispersion and similar surface can load more QDs and aptamer molecules on each nanosphere [28]; and (c) stripping analysis is a powerful technique for trace metal measurements.

3.5. Selectivity

In order to illustrate the selectivity of the aptasensor, control experiments were performed using three different proteins (BSA, fibrinogen, and IgG antibody) to replace thrombin. The MCH/TBA/AuNPs/SPGE was exposed to a large excess of different proteins for ca. 120 min without the presence of thrombin. It was found that the nonspecific adsorption of the foreign proteins (BSA, fibrinogen, and IgG) to the base aptamer does not happen obviously, and the effects of these foreign proteins on thrombin detection are almost negligible. These control experiments indicate that the aptasensor is specific to thrombin and possesses high selectivity.

3.6. Analytical application of the aptasensor

The analytical applicability of the electrochemical aptasensor has been evaluated by the standard addition method. A series of samples were prepared by adding thrombin of different concentrations to human blood serum (obtained from Cancer Hospital of Hunan Province, China). From the analytical results, it can be concluded that the recovery and relative standard deviation values are acceptable. This implies that the aptasensor has a promising feature for the analytical application in complex biological samples. And also, the use of the screen-printed electrode would be more favorable for the practical application of the electrochemical aptasensor with portability in routine analysis.

4. Conclusion

Based on quantum dot-coated silica nanospheres (QDs/Si) and the Au nanopartilces (AuNPs) modified screen-printed gold electrodes (SPGE), a simple and sensitive method for aptasensing protein has been developed. The screen-printed gold electrode was useful for large-scale fabrication of the electrochemical aptasensors and can be incorporated in portable systems. The combination of AuNPs with QDs/Si as labels may be favorable for amplifying electrochemical signals. On the basis of these, the aptasensor showed a good precision and high sensitivity for thrombin concentrations. Therefore, it can be concluded that this proposed protocol provides a potential method for the development of the electrochemical aptasensor or other biological assays for routine analysis.

Acknowledgments

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References

[1] C. Tuerk, L. Gold, Science 249 (1990) 505 – 510.
[2] A.D. Ellington, J.W. Szostak, Nature 346 (1990) 818–822.
[3] C. Manironi, A. Di Narbo, P. Fruscoloni, G.P. Tocchini-Valentini, Biochemistry 36 (1997) 9726–9734.
[4] D. Nieuwlandt, M. Wecker, L. Gold, Biochemistry 24 (1995) 5651–5659.
[5] A. Geiger, P. Burgstaller, H. von der Eltz, A. Roeder, M. Famulok, Nucleic Acids Res. 24 (1996) 1029–1036.
[6] S.E. Lupold, B.J. Hicke, Y. Lin, D.S. Coffey, Cancer Res. 62 (2002) 4029–4033.
[7] L. Michael, B. Petersen, H. Wolf, E. Prohaska, Anal. Chem. 74 (2002) 4488–4495.
[8] S.J. Lee, B.S. Youn, J.W. Park, J.H. Niazi, Y.S. Kim, M.B. Gu, Anal. Chem. 80 (2008) 2867–2873.
[9] V. Pavlov, B. Shlyahovsky, I. Willner, J. Am. Chem. Soc. 127 (2005) 6522–6523.
[10] Y. Jiang, X. Fang, C. Bai, Anal. Chem. 76 (2004) 5230–5235.
[11] R. Nutiu, Y. Li, Angew. Chem., Int. Ed. 44 (2005) 1061–1065.
[12] R. Polsky, R. Gill, L.K. aganovsky, I. Willner, Anal. Chem. 78 (2006) 2268–2271.
[13] A.E. Radi, J.L.A. Sánchez, E. Baldrich, C.K. O'Sullivan, Anal. Chem. 77 (2005) 6320–6323.
[14] C.C. Huang, Y.F. Huang, Z. Cao, W. Tan, H.T. Chang, Anal. Chem. 77 (2005) 5735–5741.
[15] J.W. Liu, Y. Lu, Angew. Chem. Int. Ed. 45 (2006) 90–94.
[16] J.A. Hansen, J. Wang, A.N. Kawde, Y. Xiang, K.V. Gothelf, G. Collins, J. Am. Chem. Soc. 128 (2006) 2228–2229.
[17] L. Shen, Z. Chen, Y. Li, P. Jing, S. Xie, S. He, P. He, Y. Shao, Chem. Commun. 21 (2007) 2169–2171.
[18] D. Xu, X. Yu, Z. Liu, W. He, Z. Ma, Anal. Chem. 77 (2005) 5107–5113.
[19] X. Zuo, S. Song, J. Zhang, D. Pan, L. Wang, C. Fan, J. Am. Chem. Soc. 129 (2007) 1042–1043.
[20] J. Zhang, S.P. Song, L.Y. Zhang, L.H. Wang, H.P. Wu, D. Pan, C.H. Fan, J. Am. Chem. Soc. 128 (2006) 8575–8680.
[21] S.S. Zhang, J.P. Xia, X.M. Li, Anal. Chem. 80 (2008) 8382–8388.
[22] H. Yang, J. Ji, Y. Liu, J.L. Kong, B.H. Liu, Electrochem. Commun. 25 (2009) 38–40.
[23] J.J. Zhou, H.P. Huang, J. Xuan, J.R. Zhang, J.J. Zhu, Biosens. Bioelectron. 26 (2010) 834–840.

[24] Z.S. Wu, M.M. Guo, S.B. Zhang, C.R. Chen, J.H. Jiang, G.L. Shen, R.Q. Yu, Anal. Chem. 79 (2007) 2933–2939.
[25] R.M. Umek, S.W. Lin, J. Vielmetter, R.H. Terbrueggen, B. Irvine, C.J. Yu, J.F. Kayyem, H. Yowanto, G.F. Blackburn, D.H. Farkas, Y.P. Chen, J. Mol. Diagn. 3 (2001) 74–84.
[26] M. Tudorache, C. Bala, Anal. Bioanal. Chem. 384 (2006) 601–619.
[27] L.Y. Chen, C.L. Chen, R.N. Li, Y. Li, S.Q. Liu, Chem. Commun. 19 (2009) 2670–2672.
[28] J. Qian, C.Y. Zhang, X.D. Cao, S.Q. Liu, Anal. Chem. 82 (2010) 6422–6429.
[29] J. Wang, S. Bollo, J.L.L. Paz, E. Sahlin, B. Mukherjee, Anal. Chem. 71 (1999) 1910–1913.
[30] Z. Zhelev, R. Bakalova, H. Ohba, R. Jose, Y. Imai, Y. Baba, Anal. Chem. 78 (2006) 321–330.
[31] C.Z. Li, Y.L. Liu, J.H.T. Luong, Anal. Chem. 77 (2005) 478–485.
[32] B. Li, Y. Wang, H. Wei, S. Dong, Biosens. Bioelectron. 23 (2007) 965–970.
[33] W. Zhao, J.J. Xu, H.Y. Chen, Front. Biosci. 10 (2005) 1060–1069.
[34] Y. Xiao, A.A. Lubin, A.J. Heeger, K.W. Plaxco, Angew. Chem., Int. Ed. 44 (2005) 5456–5459.
[35] R. Polsky, R. Gill, L. Kaganovsky, I. Willner, Anal. Chem. 78 (2006) 2268–2271.
[36] J. Zheng, W. Feng, L. Lin, F. Zhang, G. Cheng, P. He, Y. Fang, Biosens Bioelectron. 23 (2007) 341–347.
[37] H.A. Ho, M.J. Leclerc, Am. Chem. Soc. 126 (2004) 1384–1387.
[38] H. Wei, B.L. Li, J. Li, E.K. Wang, S.J. Dong, Chem. Commun. (2007) 3735–3737.
[39] V. Pavlov, Y. Xiao, B. Shlyahovsky, I.J. Willner, Am. Chem. Soc. 126 (2004) 11768–11769.