Boronic Acid Functionalized Magnetic Composites with Sandwich-like Nanostructures as a Novel Matrix for PDGF Detection

Jing Zheng, Min Zhang, Xiaohui Guo, Jianping Wang, Jingli Xu**

Abstract

In this paper, we present a novel multifunctional magnetic composite with sandwich-like nanostructure, which is composed of a yolk-egg-like magnetic silica core and a continuous polydopamine(PDA)-Au coating. Firstly, yolk-egg-like magnetic composite($\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}$) was prepared by a combined high temperature decomposition method with mussel chemistry. Then, Au nanoparticles(NPs) were in-situ generated on the surface of the $\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}$ by reaction between the PDA shell and HAuCl_4 , forming a multilayer sandwich-like nanostructure. Benefiting from the good affinity of the PDA shell and Au NPs with thiol group, the 4-mercaptophenylboronic acid was successfully assembled on the surface of Au NPs and PDA layer. The hybrid magnetic composite functionalized with boronic acid was used as affinity probes to selectively capture PDGF from solution. Meanwhile, methylene blue(MB)-doped silica NPs ($\text{SiO}_2\text{-MB}$) were prepared to immobilize aptamer. In the presence of PDGF, the aptamer labeled $\text{SiO}_2\text{-MB}$ ($\text{SiO}_2\text{-MB-aptamer}$) specifically bound with the PDGF protein. By monitoring the change of the electrochemical signal of MB, we were able to detect the binding events between the aptamer and PDGF in homogeneous solutions. The sensor was highly selective and sensitive with a detection limit of 0.22 fM for PDGF. The developed method showed wide potential applications in protein monitoring and cancer diagnosis.

Key Words: Magnetic Composites, Boronic Acid, Glycoprotein, PDGF, electrochemical detection

1. Introduction

Glycoproteins, which occupy more than 50% of the total proteins in mammalian systems, play vital roles in many biological processes. As the expression of many glycoproteins are associated with the occurrence of diverse diseases, glycoproteins are of significant importance in fundamental research and practical applications such as clinical diagnosis, prevention^[1], and treatment^[1,2]. The recognition and quantification of glycoproteins are expected to not only provide insight into the molecular basis of cancer, but also discover new biomarkers for tumor visualization and early cancer diagnosis. Human platelet-derived growth factor BB (PDGF-BB), as a glycoprotein, is an important cytokine in serum that serves as an indicator for tumor angiogenesis^[3], which plays a key role in cell transformation processes, tumor growth and progression^[4]. Therefore, cognition and quantification of PDGF-BB are particularly significant in biomedical fields. The development of accurate, highly selective, sensitive, and facile detection of cancer-related proteins PDGF is of great importance. To date, various commonly available methods, such as traditional antibody-based radioisotopic methods and ELISA techniques have been developed for the detection of PDGF^[5-7]. However, the antibody-based methods and ELISA techniques are all limited to require large sample volumes, high cost, or complicated process etc. Therefore, the development of novel detection methods for PDGF has been urgent desired. Recently, aptamers are emerging as a new class of molecules that rival commonly used antibodies in protein recognition and profiling^[8], which have been widely used as molecular recognition elements in various biosensing system, based on chemiluminescence^[9], fluorescence^[10], electrochemistry^[11], colorimetry^[12], and surface-enhanced Raman spectroscopy(SERS) ^[13]. Compared to other detection methods, aptamer-based electrochemical biosensors have attracted more attention because of their remarkable sensitivity, low cost, simplicity, reliability, and miniaturization^[14]. Meanwhile, the construction of nanomaterial-based aptasensors has offered new opportunities to improve the performance of protein detection^[15,16]. Especially, multifunctional platforms with different nanoscale objects have aroused great interest^[17]. Zhang et al^[18] has developed a carbon-based nanocomposites with aptamer-templated silver nanoclusters for the highly sensitive and selective detection of PDGF. Jun et al^[19] used the aptamer-functionalized hybrid carbon nanofiber FET-type electrode for the fabrication of a highly

sensitive and selective PDGF Biosensor. Jang et al^[20] demonstrated the aptamer-functionalized multidimensional hybrid conducting-polymer plate based field-effect transistor sensor for detecting PDGF. Recently, Huang et al^[21] has developed an electrochemical aptasensor for PDGF detection based on layered MoSe₂-Gr composites and exonuclease III-aided signal amplification. However, for the above sensors, these direct immobilization of the nanocomposites on the electrode may facilitate the sensitive and selective detection of PDGF, but the process for the modification is time consuming and inconvenient. Therefore, the development of direct detection method in the solution without the modification of the electrode will serve as a basis for building innovative electrochemical biosensors.

Herein, magnetic nanocomposites can be used as the matrix to realize the direct electrochemical detection in the solution. For example, magnetic carbon nanotubes was applied for the direct electrochemical detection of dopamine in both artificial saline solutions and real sample matrixes^[22]. Fe₃O₄@Au magnetic nanocomposites have been used as the selective electrochemical probes for the simultaneous detection of Ag⁺ and Hg²⁺ detection^[23]. Combined with the above mentioned, a simple method for fabricating yolk-egg-like magnetic composites (SiO₂@Fe₃O₄@PDA@Au) supported electrochemical sensor for PDGF is proposed. Compared with previous reports, the current strategy presents several advantages. (1) the SiO₂ cores in the composite not only improve their good aqueous dispersion and colloid stability^[24], but also can be used as the support for Fe₃O₄ NPs. (2) the Fe₃O₄ NPs have high specific surface area, stability and magnetic properties, which give rise to enrichment of magnetic targets^[25]. Moreover, the immobilized Fe₃O₄ NPs on the surface of SiO₂ cores facilitate the coating of PDA in view of its hydrophilic property. (3) The outer PDA shell protects Fe₃O₄ from corrosion and oxidation, and can be both carrier and reductant for the Au NPs. Moreover, the PDA is used for the immobilization of 4-mercaptophenylboronic acid, because PDA can react with thiols and amines *via* Michael addition or Schiff base reaction^[26]. (4) Au NPs act as excellent scaffolds for the fabrication of aptasensors owing to the good biocompatibility, low-toxicity, and excellent photostability^[27-28]. In addition to large active sites and high electrical conductivity^[29], the 4-mercaptophenylboronic acid functionalized Au NPs can greatly increase the density of immobilized glycoprotein because of the strong affinity between cis-diol residues and boronic acid^[30-31].

Therefore, multifunctional magnetic nanocomposite ($\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}@\text{Au}$) was prepared. Then, 4-mercaptophenylboronic acid was assembled onto the surface of the composite for the immobilization of PDGF. The as-prepared composite gathered excellent characteristics of magnetic responsiveness of magnetic nanoparticle, biocompatibility of gold, and enhanced affinity properties of boronic acid. Meanwhile, PDGF-binding aptamer^[32], with high affinity for PDGF-BB proteins was immobilized on methylene blue(MB)-doped silica(denoted as $\text{SiO}_2\text{-MB}$) NPs. Herein, MB was dotted on silica spheres, which not only prevented the leakage of entrapped MB from the particles, but also amplified the detection signals^[33,34]. Moreover, the resulting $\text{SiO}_2\text{-MB}$ NPs exhibited high biocompatibility and high electron-transfer efficiency as a mediator. Subsequently, the aptamer labeled $\text{SiO}_2\text{-MB}$ (denoted as aptamer- $\text{SiO}_2\text{-MB}$) could be used to specifically bind with PDGF. The fabricating procedure of PDGF aptasensor by a sandwich format of $\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}@\text{Au}$, PDGF and aptamer- $\text{SiO}_2\text{-MB}$ has been shown in Fig. 1. Firstly, the $\text{SiO}_2@\text{Fe}_3\text{O}_4$ composite was synthesized by sol-gel method and high temperature decomposition process. Secondly, PDA was deposited onto the surface of $\text{SiO}_2@\text{Fe}_3\text{O}_4$ composite in tris-buffer solution(pH 8.5). The PDA shell on the $\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}$ composite could react with HAuCl_4 to *in-situ* generate Au NPs on its surface. Then, 4-mercaptophenylboronic acid was assembled onto the surface of the $\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}@\text{Au}$ composite, which could be applied to selectively enrich glycoproteins(PDGF). Meanwhile, in the presence of the target protein, aptamer- $\text{SiO}_2\text{-MB}$ was easily conjugated onto the surface of the $\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}@\text{Au}$ composite *via* the specific binding between aptamer and PDGF. After the PDGF was captured by $\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}@\text{Au}$ composite, the $\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}@\text{Au}/\text{PDGF}/\text{aptamer-}\text{SiO}_2\text{-MB}$ conjugate gathered to the surface of the electrode with an external magnetic field behind the electrode. Thus, the concentration of MB near the electrode surface was increased, which led to the improvement on the electrochemical responses and the sensitivity of the measurement. Using this strategy, we were able to detect the binding events between the aptamer and the protein in homogeneous solutions without separation. Moreover, the process of magnetic separating greatly improved the efficiency of the assays. This work greatly simplified the measurement process, and achieved a higher sensitivity as a result of less dilution and lower probability for contamination. Furthermore, this solution-based method was much simpler without need for labeling or immobilization the protein. This aptamer based sensor was highly selective and ultrasensitive.

Satisfactory results were achieved in human serum samples using this proposed sensor. To the best of our knowledge, this is the first report regarding the synthesis of boronic acid functionalized magnetic composite for the immobilization and detection of PDGF. The method for protein detection is simple and the detection limit is 0.22 fM.

Fig. 1 Schematic representation for the configuration of the biosensor for PDGF

2. Experimental

2.1 Chemicals

Analytical reagents such as iron(III) acetylacetonate ($\text{Fe}(\text{acac})_3$), ethanol, tetraethoxysilane (TEOS), 4-mercaptophenylboronic acid, diethylenetriamine (DETA), dopamine hydrochloride, glutaric dialdehyde, triethylene glycol (TREG), tritonX-100(OP), HAuCl_4 , methylene blue (MB) were purchased from Aladdin Reagent Co. Ltd. (Shanghai, China).

The aptamer oligonucleotides was obtained from Sangon Biotechnology Inc. (Shanghai, China). The sequence is 5-NH₂-CAGGC TACGGCACGT AGAGC ATCAC CATGA TCCTG-3. Platelet-derived growth factor (PDGF)-BB, human IgG, hemoglobin (Hb), lysozyme (Lys), bovine serum albumin (BSA) and thrombin (THR) were obtained from Dingguo Biochemical Reagents Company (Beijing, China). PBS buffer (0.1 M, pH=7.0) and other reagents were commercially available. Analytical reagents were purchased from Aladdin Reagent Co. Ltd. (Shanghai, China).

They were all of analytical reagents grade and used without further purification. All other chemicals involved were of analytical-reagent grade. Deionized and sterilized water (resistivity > 18 M Ω · cm) was used throughout.

2.2. Instruments and characterization

Differential pulse voltammetry (DPV) was performed using a CHI Instruments model 660E Electrochemical Analyzer (CHI Instrument Inc., USA). The electrochemical system comprised of a working electrode of glassy carbon electrode, an Ag/AgCl reference electrode, and a platinum wire counter electrode. Fourier transform infrared (FT-IR) spectra was obtained on a Nicolet AVATAR 380 FT-IR spectrophotometer (Thermo Fisher Nicolet, USA). The morphology of the as prepared composites were observed using a scanning electron microscope (SEM, Hitachi S-8000, Japan) in a secondary electron scattering mode at 5 kV and a transmission electron microscope (TEM, JEOL 2100F, Japan). Powder X-ray diffraction (XRD) patterns were recorded on a Bruker D4 X-ray diffractometer with Ni-filtered Cu K α radiation (40 kV, 40 mA).

2.3 Preparation of SiO₂ spheres

SiO₂ spheres were prepared via a modified stöber method. In a typical reaction, 75 mL of ethanol, 10 mL of water, 3.2 mL of ammonia (27%, aqueous solution) were mixed. Then 6 mL of TEOS was added in the solution under vigorous stirring. After that, the reaction mixture was stirred for 12 h at room temperature. Then, the SiO₂ spheres were collected by centrifugation, washed with water and ethanol several times, then redispersed in ethanol solution with concentration of 70 mg/mL.

2.4 Preparation of SiO₂@Fe₃O₄ spheres

3 mL SiO₂ spheres (70 mg/mL) ethanol solution and 40 mL triethylene glycol were added into the flask, and the mixture was then heated to 95 °C to remove ethanol. After that, 320 mg of the iron precursor Fe(acac)₃ was added into the flask. The solution was sonicated by using an ultrasound bath for 5 min, and then heated to 278 °C with vigorous stirring under the protection of nitrogen for refluxing 30 min. After cooling to room temperature, 40 mL ethanol was added to dilute the solution. The composite was separated by an external magnet, and washed with ethanol several times. The obtained composite was dried in vacuum.

2.5 Preparation of SiO₂@Fe₃O₄@PDA composite

SiO₂@Fe₃O₄@PDA composite was prepared as follows. Briefly, 200 mg as-synthesized SiO₂@Fe₃O₄ and 100 mg dopamine hydrochloride were dissolved in 100 mL tris-buffer (pH 8.5) solution, and allowed to proceed for 36 h at room temperature under stirring. The products were washed with ethanol and water for several times and dried for further use.

2.6 Preparation of SiO₂@Fe₃O₄@PDA@Au composite

100 mg SiO₂@Fe₃O₄@PDA composite was added to 50 mL 0.6 mM HAuCl₄ solution and allowed to proceed for 12 h at room temperature under stirring. The product was collected and washed by the water and ethanol for several times and dried for further use.

2.7 Boronic acid modified SiO₂@Fe₃O₄@PDA@Au composite

Boronic acid modified SiO₂@Fe₃O₄@PDA@Au composite was prepared according to following processes^[35]. Firstly, 0.0020 g of SiO₂@Fe₃O₄@PDA@Au composite, 0.0040 g of 4-mercaptophenylboronic acid and 30.0 mL of ethanol were mixed and sonicated. The resulting mixture was stirred for 24 h at room temperature and then continued to centrifuge for 30 min at 5500 rpm to remove the excessive boronic acid. The precipitate was washed with ethanol four times, and stored in the refrigerator for further use.

2.8 Preparation of MB-doped silica spheres

The MB-doped silica spheres (SiO₂-MB) were prepared in a nonionic water in-oil microemulsion of OP/hexanol/cyclohexane aqueous solution (pH 1.5). The water-in-oil microemulsion was prepared by mixing 4.0 mL of cyclohexane, 1.0 mL of *n*-hexanol, 1.0 mL of OP. After stirred for 50 minutes, 250 μ L of 1.04×10^{-2} M MB solution was added and continued to stir. 30 μ L of NH₃·H₂O and 50 μ L of TEOS were added and the reaction was allowed to stir for 9 h followed by addition of 15 mL of ethanol to break the microemulsion system and recover the SiO₂-MB spheres. The particles were centrifugated and washed a couple of times with ethanol and final washing with water to remove the surfactant molecules and physically adsorbed MB molecules. The as-prepared particles were stored in PBS buffer for further use.

2.9 Synthesis of SiO₂-MB labeled aptamer

Firstly, amino-functionalized SiO₂-MB spheres were prepared by adding 1% of DETA and 1.0×10^{-3} M of acetic acid in SiO₂-MB solution. The mixture was stirred for 120 min for the formation of amino-functionalized SiO₂-MB, and the excessive acetic acid was added to remove

the DETA.

The SiO₂-MB labeled aptamer was further prepared as following procedure. Briefly, amino-functionalized SiO₂-MB spheres were conjugated with amino-modified aptamer through glutaraldehyde coupling. By adding 29 μ L PBS (0.1000 M, pH 7.0) into centrifugal tube with 1OD DNA, aptamer with the concentration of 1.02×10^{-4} M was obtained. The SiO₂-MB spheres were immersed in 5% glutaraldehyde solution under shaking at room temperature for 2 h. SiO₂-MB labeled aptamer was prepared by adding 1OD of DNA aptamer solution into 1mL of MB-SiO₂ spheres solution and the mixture was shaken for 2 h at room temperature, then rinsed with distilled water. The resulting probe solution was stored in the refrigerator for further use.

2.10 Enrichment of PDGF by boronic acid modified SiO₂@Fe₃O₄@PDA@Au composite

For PDGF enrichment in standard solutions, 0.0513 g of boronic acid modified SiO₂@Fe₃O₄@PDA@Au composite was dispersed in 3.0 mL of PBS buffer (0.1M, pH 7.0) and sonicated for 5 min. The target PDGF was prepared by adding PBS into centrifugal tube to the concentration of 9.67×10^{-6} M solution, and then diluted to the concentration we wanted. 200 μ L aliquots of PDGF with different concentrations were equilibrated with 200 μ L of SiO₂@Fe₃O₄@PDA@Au suspension in PBS under vigorous shaking for 30min. After that, 150 μ L of the aptamer-SiO₂-MB was added and the final volume was adjusted to 1000 μ L by 0.1 M PBS buffer containing 1.0 mM of Mg²⁺(pH=7.0). Then the mixed solution was vibrated at 37 °C for 30 minutes. After the incubation, the SiO₂@Fe₃O₄@PDA@Au/PDGF/aptamer-SiO₂-MB conjugates were washed with 200 μ L of PBS buffer for three times, and the supernatant fluid was discarded through the magnetic separation and the nonspecifically bound SiO₂-MB was eliminated.

2.11 Electrochemical detection

All electrochemical experiments were performed in the self-designed electrochemical cell, with a glassy carbon working electrode (diameter = 5.0 mm) at the bottom, an Ag/AgCl (saturated KCl) reference electrode and a platinum auxiliary electrode. The final conjugates were resuspended in 0.1 M PBS solution. By applying magnetic field, the target protein PDGF in the sample captured by magnetic SiO₂@Fe₃O₄@PDA@Au composite could be gathered on the surface of the electrode. SiO₂-MB composite was accumulated onto the magnetic composite coated electrode surface by immersing the electrode into the stirred 0.1 M PBS (pH 7.0) for 5 min.

After accumulation, cyclic voltammetry was used to investigate the electrochemical performance of the composite covered electrode at 100 mV/s from -0.6 to 0.2 V. Differential pulse voltammetry (DPV) was performed with the scan range from 0.40 to -0.80 V (Incr E 0.004 V, amplitude 0.05 V, pulse width 0.05 s, pulse period 0.5 s), resulting in an analytical signal due to the reduction of MB, which relates to the amount of the MB nanoparticles for the sandwich format.

3. Results and discussion

3.1 Characterization of SiO₂@Fe₃O₄@PDA@Au composite

The morphologies and structures of the composite at different synthetic steps were observed by SEM and TEM. Firstly, silica spheres were prepared by the hydrolysis of TEOS in alcohol-ammonia mixture solution and the diameter of the spheres were about 200 nm with very narrow size distribution (Fig. 2A(a)). And the size of silica could be precisely controlled by tuning the TEOS concentration and the ratio of the ethanol/water. As shown in Fig. 2A(a,b), well dispersed SiO₂ spheres could be observed clearly, and the surface was very smooth. After reacted with Fe(acac)₃ in TREG, the SiO₂@Fe₃O₄ composite with rough surface was obtained. From the SEM and TEM imaging (Fig. 2A(c,d)), the Fe₃O₄ with an average size of about 10 nm were uniformly distributed on the surface of the SiO₂ spheres. After the functionalization of the Fe₃O₄ NPs with a layer of hydrophilic polyol molecules, these SiO₂@Fe₃O₄ composite were highly water-dispersed, which was beneficial for PDA coating. As shown in Fig. 2A(e,f), uniform PDA layer of ~18 nm was coated on the surface of SiO₂@Fe₃O₄ composites, resulting in core-shell SiO₂@Fe₃O₄@PDA composites. Moreover, the thickness of PDA shell could be modified by adjusting the reaction time, reactant concentrations, and other factors^[36]. Due to the metal-binding ability of catechol present in the PDA layer, the SiO₂@Fe₃O₄@PDA@Au composite was synthesized in the presence of HAuCl₄ solution. SEM and TEM images (Fig. 2A(g,h)) showed successful adhesion of numerous Au NPs on the surface of SiO₂@Fe₃O₄@PDA composite. In the case of synthesis of Au NPs, the PDA and its derivatives could act as reactive templates, which will facilitate the synthesis of polymer-supported Au NPs within one pot step. Due to the hydroxyl-rich groups in PDA, AuCl₄⁻ can be reduced into Au NPs on the PDA shell without the use of other reducing agents. This greatly fabricates the synthetic procedures of the SiO₂@Fe₃O₄@PDA@Au composite.

Fig. 2 (A) SEM and TEM images of the as-prepared SiO₂(a,b), SiO₂@Fe₃O₄(c,d), SiO₂@Fe₃O₄@PDA(e,f), SiO₂@Fe₃O₄@PDA@Au(g,h); (B) XRD diffraction patterns of the as-prepared SiO₂(a), SiO₂@Fe₃O₄(b), SiO₂@Fe₃O₄@PDA(c), SiO₂@Fe₃O₄@PDA@Au(d); (C) XPS survey spectrum of boronic acid functionalized with SiO₂@Fe₃O₄@PDA@Au.

The XRD patterns of the as-synthesized SiO₂, SiO₂@Fe₃O₄, SiO₂@Fe₃O₄@PDA, SiO₂@Fe₃O₄@PDA@Au composite are shown in Fig. 2B. Due to the amorphous crystallinity of the SiO₂, the diffraction peak at 28° for SiO₂ is observed for all samples. Six characteristic peaks are observed for SiO₂@Fe₃O₄ value index as 30.1°, 35.5°, 43.1°, 53.4°, 57.0°, and 62.6°, the peak positions with corresponding 2θ value are indexed as (220), (311), (400), (422), (511) and (440), respectively, which match well with the database of face center-cubic phase of Fe₃O₄ (JCPDS Card No.19-629). The major peaks for SiO₂@Fe₃O₄@PDA composite (Fig. 2B(c)), are similar to the pristine Fe₃O₄ particles (Fig. 2B(b)), revealing that the as-prepared core-shell composite consist of the Fe₃O₄ component. Moreover, the diffraction peak at 28° is increased due to the coating of amorphous PDA shell. As shown in Fig. 2B(d), four additional peaks at 38°, 43°, 65° and 78° represent the Bragg reflections from (111), (200), (220) and (311) planes of Au (JCPDS card No. 04-0784), indicating the existence of Au NPs.

In order to confirm the successful modification of boronic acid on $\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}@\text{Au}$ composites, the X-ray photoelectron survey spectrum(XPS) is employed. It is shown in Fig. 2(C) that the C1s peak at 284.0 eV, O1s at 531.6 eV, N1s at 399.6 V, Si 2p at 100.7 eV, and B1s at 189.2 eV are observed, respectively. Additionally, there are two peaks located at the binding energies of 84.2 and 87.8 eV, which is consistent with the emission of 4f photoelectrons from Au^0 , suggesting the successful formation of Au NPs. The PDA shell with above 10 nm thickness makes the characteristic peak of the Fe element indistinct. It can be seen that an obvious B1s peak was observed in the inset of Fig. 2(C). The presence of Au, B, S in the XPS spectrum demonstrates boronic acid has been successfully functionalized on the surface of the composites.

3.2 Characterization of $\text{SiO}_2\text{-MB}$ spheres

Methylene Blue (MB) is a cationic dye whose electrochemical properties are well known in the electrochemical detection. Herein, MB doped silica spheres($\text{SiO}_2\text{-MB}$) were applied as an electrochemical indicator to investigate the hybridization with the target protein. These uniform $\text{SiO}_2\text{-MB}$ spheres were prepared by the water-in-oil(W/O) microemulsion method. The obtained $\text{SiO}_2\text{-MB}$ spheres were highly dispersible in water. The UV-Vis(Fig. S1) and FT-IR((Fig. S2) were employed to investigate the optical characteristics of the $\text{SiO}_2\text{-MB}$ spheres. Electrochemical characterization of $\text{SiO}_2\text{-MB}$ spheres were examined by cyclic voltammetry (Fig. S3). The results showed that the $\text{SiO}_2\text{-MB}$ spheres retained their high electron-transfer efficiency.

The chemical stability of the incorporated dye and reproducibility of its electrochemical behavior was also investigated. The anodic peak currents (ipa) and cathodic peak currents (ipc) as a function of the number of cycles were measured over the range of $-0.60\text{-}0.20$ V in PBS solution. No decrease on the peak currents was observed after 100 cycles, indicating that MB was well protected inside the silica network and did not leak from the matrix under these conditions. Moreover, these $\text{SiO}_2\text{-MB}$ spheres could act as tiny conduction centers and facilitate the electron transfer. In the present work, electro-active $\text{SiO}_2\text{-MB}$ spheres were further used as efficient and uniform electron transfer mediators in the PDGF biosensor.

3.3 Principle for PDGF recognition

In this work, boronic acid modified $\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}@\text{Au}$ composite was used to enrich PDGF, and $\text{SiO}_2\text{-MB}$ sphere was used as mediator material in the sensor. Based on the specific

interaction between aptamer and PDGF, the functionalized magnetic composite decorated with PDGF on the surface bound with SiO₂-MB-aptamer in the sample solution. Owing to the magnetic property of the electrode, the SiO₂@Fe₃O₄@PDA@Au/PDGF/ aptamer-SiO₂-MB could be easily immobilized on the electrode surface. Afterward, the electrochemical responses of MB could be detected for representing corresponding target PDGF. This method exhibited many advantages compared with other assay methods. First, SiO₂@Fe₃O₄@PDA@Au possessed enormously large surface area than a flat solid phase for molecule immobilization and reaction. Second, benefitting from the magnetic property, electrochemical species were easily brought to the magnetic electrode for direct electrochemical measurement, which eliminated tedious electrode modification procedure and accelerated the analysis rate. The interference from sample matrix could also be significantly minimized. Third, the method did not require any sophisticated modification or amplification processes, which was suitable for potential applications.

The reduction signal of MB was observed as a result of the specific binding between the aptamer and their targets. To understand the binding between the protein and the aptamer, the electrochemical responses of MB were investigated by CV measurements. The CV responses of the sensor were shown in Fig. 3(A). It was almost the same as the signal of the SiO₂-MB probe alone(Fig. S3). The obvious electrochemical response indicated that SiO₂-MB was bound tightly on the electrode surface. As shown in Fig. 3(A) (curve b), in the presence of PDGF, an apparent electrochemical response was detected. This behavior was due to the fact that PDGF was bound to the aptamer and the SiO₂-MB reporter was immobilized on the surface of the electrode. Control experiment in the absence of PDGF revealed no electrochemical change, confirming that the electrochemical response was originated from the protein-aptamer interaction.

Fig. 3(A). Cyclic voltammograms of the sensor in the absence of PDGF (a) and in the presence of PDGF ($c = 6.11 \times 10^{-15}$ M) (b). The incubation was performed in 0.1 M PBS (pH 7.0) containing 1.0 mM Mg^{2+} by adding 200 μL of $\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}@\text{Au}$ suspension, 200 μL aliquots of PDGF solution and 150 μL of the aptamer- $\text{SiO}_2\text{-MB}$, at 37 $^\circ\text{C}$. The electrochemical detection was performed in 0.1 M PBS solution. Cyclic voltammograms at the glassy carbon electrode was performed over the range of -0.6 to 0.2 V with scan rate of 100 mV/s. (B) The effect of temperature and time on the electrochemical responses. The incubation was performed in 0.1 M PBS containing 1.0 mM Mg^{2+} by adding 200 μL of $\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}@\text{Au}$ suspension, 200 μL aliquots of 1.61×10^{-14} M PDGF solution, 150 μL of the aptamer- $\text{SiO}_2\text{-MB}$, at different temperatures (a: 27 $^\circ\text{C}$ b: 30 $^\circ\text{C}$ c: 37 $^\circ\text{C}$ d: 42 $^\circ\text{C}$ e: 47 $^\circ\text{C}$) with the hybridization time varied from 10 to 35 min. DPV was performed with the scan from 0.40 to -0.80 V. Error bars show the standard deviations of measurements from five independent experiments.

The effect of temperature and incubation time on the electrochemical response was evaluated. As shown in Fig. 3(B), the DPV response reached its maximum as the temperature progressively increased to 37 $^\circ\text{C}$. Further increase of temperature led to a decrease of the sensitivity of the biosensor, which was due to the partial denaturation of the protein and the possible dissolution of the sensor. Thus, the 37 $^\circ\text{C}$ was selected for experimental temperature. Fig. 3(B) also displayed the effect of the hybridization time upon the DPV response. The response increased with the hybridization time up to 30 min, and then leveled off. Apparently, 30 min was sufficient for hybridization. In addition, some other factors that affected protein/DNA interaction, such as the pH and ionic strength of the buffer, were also investigated. It was found that a change of pH in the range of 6-9 had little effect on the sensitivity of PDGF detection.

As for the effect of ionic strength on the PDGF-binding assay, the electrochemical response remained stable when the concentration of Mg^{2+} was less than 2 mM and then decreased gradually with an increase of Mg^{2+} concentration. This change was probably due to two factors: the divalent metal ions could effectively stabilize the three-way helix structure of the free aptamer. On the

other hand, an increasing metal ion concentration usually weakened the ssDNA/protein binding. Therefore, 0.1 M PBS (pH 7.0) containing 1.0 mM Mg^{2+} was chosen as incubation buffer in the experiment.

The amount of $\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}@\text{Au}$ composite employed for the detection was also optimized. This study was carried out in the range of 20-300 μL of the composite (0.017 g/mL) as shown in Fig. S4. Therefore, 200 μL of 0.017 g/mL of $\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}@\text{Au}$ was chosen as the optimal amount to construct electrochemical platform. The amount of aptamer- SiO_2 -MB might have an influence on the determination of the target protein, and the experiments with different concentration of aptamer- SiO_2 -MB were carried out by adding increasing amount of aptamer- SiO_2 -MB under a fixed amount of PDGF. As shown in Fig. S5, 150 μL of 3.09×10^{-7} M of the probe aptamer- SiO_2 -MB was employed in the subsequent work.

3.4 Electrochemical responses with different concentration

This biosensor relies on the electrochemical transduction of the specific hybridization between the aptamer probe and its targets. Electrochemical responses of the target protein PDGF with different concentration could be quantitatively analyzed. In the experiments, boronic acid functionalized $\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}@\text{Au}$ composite, aptamer- SiO_2 -MB composite was incubated with the target protein of different concentrations, then the peak currents from the MB redox reaction were measured. As shown in Fig. 4 (curve a to f), it was clear that the relative electrochemical intensity increased with increasing concentration of PDGF, implying their concentration-dependent sensitivity. There was a good linear relationship between the electrochemical change and the PDGF concentration over the range of 0.79-19.9 fM of protein concentration for sensitive PDGF quantitation (Fig. 4). The resulting linear regression is $Y = 0.5987 + 0.4676X$ (X stands for the value of the 10^{-15} M, and Y is the value of the DPV current, unit: μA), with a correlation coefficient of 0.998.

In addition, an extremely low detection limit of 0.22 fM was obtained based on the signal-to-noise ratio of 3, which was attributed to the high efficiency of the electron transfer mediator for $\text{SiO}_2@\text{Fe}_3\text{O}_4@\text{PDA}@\text{Au}$ composite. To the best of our knowledge, this detection limit is comparable or better than some other analytical methods for PDGF detection. The detection limit is lower than the reported optical methods, such as 1.1 nM with a colorimetric

strategy^[37], 167 pM with a fluorescence resonance energy transfer modes for PDGF^[40]. The detection limit achieved in the present work is also superior or close to reported electrochemical studies (0.02 pM of an amplified amperometric aptasensor for selective detection of protein using catalase-functional DNA–PtNPs dendrimer as a synergetic signal amplification label^[45], 20 fM of an electrochemical aptasensor for PDGF detection based on layered MoSe₂–Gr composites and exonucleaseIII-aided signal amplification^[21]). A straightforward comparison between this work and the previous reports is shown in Table 1^[19-21, 37-45]. Obviously, the proposed electrochemical aptasensor had a good analytical performance for the detection of PDGF-BB, which must respond for the excellent electrical conductivity and biocompatibility of SiO₂@Fe₃O₄@PDA@Au composites. Most importantly, the proposed sensing system is much more simple, inexpensive and controllable since only one oligonucleotide sequence was used to fabricate the sensor and no extra amplification means was needed.

To evaluate the reproducibility of the aptamer sensor, six assays were performed following identical processing steps. Their responses toward 1.61×10^{-14} M target protein gave an average DPV response of 8.89 μ A with a relative standard deviation of 5.42%, indicated that the sensor could be used for analysis with excellent reproducibility. The stability of the biosensors was also investigated by measuring the current response of 1.61×10^{-14} M target protein every 7 days. The response current decreased to 96.67% after 7 days, with a relative standard deviation of 4.69%, indicating excellent long-term stability. The high stability and reproducibility of the biosensors reflected the high effectiveness of the binding between the functional groups and the PDGF-binding aptamer, which also endowed the biosensor potential for use as a long-term reusable biosensor in clinical applications.

Fig. 4 DPV responses of the sensor after the addition of target PDGF in different concentrations (curves a–f: a: 7.90×10^{-16} M, b: 2.54×10^{-15} M, c: 8.86×10^{-15} M, d: 1.41×10^{-14} M, e: 1.57×10^{-14} M, f: 1.99×10^{-14} M). Inset is the plot of the reduction peak current of MB versus the target PDGF concentration. The incubation was performed in 0.1 M PBS (pH 7.0) containing 1.0 mM Mg^{2+} by adding 200 μL of $\text{SiO}_2@ \text{Fe}_3\text{O}_4@ \text{PDA} @ \text{Au}$ suspension, 200 μL aliquots of PDGF solution, 150 μL of the aptamer- SiO_2 -MB, at 37 $^\circ\text{C}$. The electrochemical detection was performed in 0.1 M PBS solution. Error bars show the standard deviations of measurements taken from independent experiments with at least five distinct sensors. pH = 7.0 PBS. This followed the linear equation: $Y = 0.5978 + 0.4676 \cdot X$ (PDGF) (10^{-15} M), with a correlation of $R = 0.998$.

Table 1. Comparison of different aptasensors for PDGF-BB determination

Methods	LOD	Linearity range	Ref
colorimetric	1.1 nM	2.0 – 80 nM	[37]
colorimetric	200 pM	0.2–8.2 nM	[38]
fluorescence	370 pM	1–50 nM	[39]
fluorescence	167 pM	0.167–1.167 nM	[40]
chemiluminescent	0.68 pM	1×10^{-3} –10 nM	[41]

SERS	0.5 pM	1×10^{-3} – 5×10^{-2} nM	[42]
Electrochemical	0.6 pM	0.002–40 nM	[43]
Electrochemical	8.3 pM	0.017–1660 nM	[44]
Electrochemical	0.02 pM .	0.00005–35 nM	[45]
Electrochemical	5 fM	5×10^0 – 5×10^4 fM	[19]
Electrochemical	1.78 fM	1.78 fM–178 pM	[20]
Electrochemical	20 fM	0.0001–1nM	[21]
Electrochemical	0.22 fM	0.79–19.9 fM	This work

3.5 Selectivity of the sensor

PDGF is an extracellular protein that binds to its membrane growth factor receptor for biological function. The selectivity of the aptamer sensor was also examined using some other proteins in serum. A fixed amount of the aptamer-SiO₂-MB was incubated with either PDGF or some other proteins such as IgG, lysozyme, thrombin, BSA, hemoglobin and etc. About 10¹¹ fold concentration of IgG, thrombin, IgM, lysozyme, BSA and Hb were incubated and detected with the sensor. The peak currents were shown in Fig. 5. It could be seen that no significant DPV responses were obtained for these interfering proteins. Also, no significant cross-reactivity was detected for these proteins. So the comparisons clearly showed that the control proteins had nearly no effect on the detection by using proposed aptasensor, which ascribed to the highly selective recognition and binding between aptamer and target protein. It indicates that a high sensitivity of PDGF detection could be achieved in complex biological samples.

Fig. 5 Selectivity analysis of the electrochemical sensor

The incubation was performed in 0.1 M PBS containing 1.0 mM Mg^{2+} by adding 200 μ L of $SiO_2@Fe_3O_4@PDA@Au$ suspension, 200 μ L aliquots of a certain concentration of protein solution, 150 μ L of the aptamer- SiO_2 -MB, for 30 min. a:c(PDGF)= 1.61×10^{-14} M b:c(IgG)= 8.21×10^{-3} M c:c(thrombin)= 4.81×10^{-3} M d:c(lysozyme)= 7.01×10^{-3} M e:c(BSA)= 5.23×10^{-3} M f:c(Hb)= 6.49×10^{-3} M. The DPV was performed in 0.1 M PBS solution with the scan from 0.40 to -0.80 V. Error bars show the standard deviations of measurements from five independent experiments.

3.6 Application of the sensor for PDGF determination in human serum

PDGF-BB is a cancer-related protein which has been directly implicated in the cell transformation process and in tumor growth and progression^[46]. Studies reveal that over expression of PDGF BB within tumors results in increased pericyte coverage, suggesting that PDGF BB signaling is also essential for tumor growth and the cancerous pericyte recruitment process^[47]. Therefore, the detection of PDGF BB in human serum is significant for early diagnosis and treatment of cancer. In clinical samples, the concentration of PDGF in the blood serum and plasma has been found to be in the range: (3.92-20.29 ng/mL in human serum and 0.10-0.69 ng/mL in human plasma^[6]). Based on the performance of our developed biosensor, the sensitivity of our present method was determined to be 0.22 fM. This sensitivity would be feasible for PDGF detection in blood serum samples. To test the applicability of this assay, three human serum samples were collected and assayed by the aptamer biosensor. Human serum samples were diluted 5 times with PBS buffer. After dilution, the incubation and electrochemical detection were carried

out as the procedure mentioned above. As displayed in Table 2, the recoveries were ranged from 92.50% to 106.7%, indicating that our assay possessed a great selectivity and anti-interference capability, even in complex media, and might therefore find practical utility in clinical settings.

Table 2. PDGF-BB concentration in human serum samples detected by the proposed method

Sample No	Found (10^{-8}g.mL^{-1})	Added (10^{-8}g.mL^{-1})	Found (10^{-8}g.mL^{-1})	Recovery	RSD%(n=3)
1	1.55	1.50	2.99	96.00	8.64
		1.20	2.68	94.17	7.65
2	1.03	1.50	2.59	104.0	4.68
		1.20	2.14	92.50	5.57
3	1.23	1.50	2.83	106.7	3.96
		1.20	2.36	94.17	8.95

The incubation and electrochemical detection were carried out as the subsection 2.10. The R.S.D. were derived from three independent experiments.

4. Conclusions

In this work, we developed a novel highly sensitive assay to quantify PDGF-BB by integrating boronic acid functionalized magnetic composites and $\text{SiO}_2\text{-MB}$ signal probe. This strategy had several excellent features. First, the large surface active sites and good conductivity of the yolk-egg-like magnetic composite ($\text{SiO}_2\text{@Fe}_3\text{O}_4\text{@PDA@Au}$) provided a promising sensing platform. Second, the application of the efficient magnetic isolation and enrichment was attractive for the electrochemical detection of protein which was commonly affected by the presence of nonhybridized aptamer adsorbates. Third, boronic acid functionalized magnetic composite exhibited good biocompatibility, specificity for protein enrichment. Particularly, the $\text{SiO}_2\text{@Fe}_3\text{O}_4\text{@PDA@Au}$ nanocomposite could be firmly immobilized onto the electrode benefitting from the magnetic property, which eliminated the complicated electrode modification process in other methods. The related fabricated electrochemical biosensors exhibited considerably low detection limit of 0.22 fM. To sum up, the experimental results demonstrated that such coupling magnetic $\text{SiO}_2\text{@Fe}_3\text{O}_4\text{@PDA@Au}$ nanocomposite with the $\text{SiO}_2\text{-MB}$ tags, had the potential to be used for ultrasensitive quantitative analysis of PDGF-BB or other glycoproteins in complex samples.

Acknowledgments

The authors are grateful to the financial support by the National Science Foundation of China (No 21305086). The Natural Science Foundation of Shanghai City (13ZR141830), Research Innovation Program of Shanghai Municipal Education Commission (14YZ138), the Special Scientific Foundation for Outstanding Young Teachers in Shanghai Higher Education Institutions (ZZGJD13016, ZZGJD13018).

References

- [1] H. Narimatsu, H. Sawaki, A. Kuno, H. Kaji, H. Ito, Y. Ikehara, A strategy for discovery of cancer glyco - biomarkers in serum using newly developed technologies for glycoproteomics, *Febs Journal*. 277 (2010) 95-105.
- [2] L. P. Qiu, Z.-S. Wu, G. L. Shen, R. Q. Yu, Highly sensitive and selective bifunctional oligonucleotide probe for homogeneous parallel fluorescence detection of protein and nucleotide sequence, *Anal. Chem.* 83 (2011) 3050-3057.
- [3] A. Csordas, A. E. Gerdon, J. D. Adams, J. Qian, S. S. Oh, Y. Xiao, H. T. Soh, Detection of proteins in serum by micromagnetic aptamer PCR (MAP) technology, *Angew. Chem. Int. Ed.* 49 (2010) 355-358.
- [4] D. Bronzert, P. Pantazis, H. Antoniadis, A. Kasid, N. Davidson, R. Dickson, M. E. Lippman, Synthesis and secretion of platelet-derived growth factor by human breast cancer cell lines, *Proc. Natl. Acad. Sci.* 84 (1987) 5763-5767.
- [5] D. F. Bowen-Pope, T. W. Malpass, D. M. Foster, R. Ross, Platelet-derived growth factor in vivo: levels, activity, and rate of clearance, *Blood*. 64 (1984) 458-469.
- [6] K. Leitzel, W. Bryce, J. Tomita, G. Manderino, I. Tribby, A. Thomason, M. Billingsley, E. Podczaski, H. Harvey, M. Bartholomew, Elevated plasma platelet-derived growth factor B-chain levels in cancer patients, *Cancer Res.* 51 (1991) 4149-4154.
- [7] F. Qu, H. Lu, M. Yang, C. Deng, Electrochemical immunosensor based on electron transfer mediated by graphene oxide initiated silver enhancement, *Biosens. Bioelectron.* 26(2011), 4810-4814.
- [8] M. Mascini, I. Palchetti, S. Tombelli, Nucleic acid and peptide aptamers: fundamentals and bioanalytical aspects, *Angew. Chem. Int. Ed.* 51 (2012) 1316-1332.
- [9] X. Ouyang, R. Yu, J. Jin, J. Li, R. Yang, W. Tan, J. Yuan, New strategy for label-free and time-resolved luminescent assay of protein: conjugate Eu^{3+} complex and aptamer-wrapped carbon nanotubes, *Anal. Chem.* 83 (2011) 782-789.
- [10] F. Chen, Y. Liu, C.Y. Chen, H. Gong, C.Q. Cai, X.M. Chen, Respective and simultaneous detection tumor markers CA125 and STIP1 using aptamer-based fluorescent and RLS sensors, *Sensors Actuat. B:Chem.* 245 (2017) 470-476.
- [11] Q. Anjum, G. Yasar, H.N. Javed, Label-free capacitance based aptasensor platform for the detection of $\text{HER}_2/\text{ErbB}_2$ cancer biomarker in serum, *Sensors Actuat. B:Chem.*

220(2015)1145-1151.

- [12] Z. Zhu, C. Wu, H. Liu, Y. Zou, X. Zhang, H. Kang, C. J. Yang, W. Tan, An Aptamer Cross - Linked Hydrogel as a Colorimetric Platform for Visual Detection, *Angew. Chem. Int. Ed.* 122(2010) 1070-1074.
- [13] L. Y. Yang, C. C. Fu, H. L. Wang, S. P. Xu, W. Q. Xu, Aptamer-based surface-enhanced Raman scattering (SERS) sensor for thrombin based on supramolecular recognition, oriented assembly, and local field coupling, *Anal. Bioanal. Chem.* 409(2017) 235-242.
- [14] L. X. Fang, K. J. Huang, Y. Liu, Novel electrochemical dual aptamer-based sandwich biosensor using molybdenum disulfide/carbonaerogel composites and Au nanoparticles for signal amplification, *Biosens. Bioelectron.* 71(2015) 171–178.
- [15] L. Rivas, C. C. Mayorga-Martinez, D. Quesada-González, A. Zamora-Gálvez, A. dela Escosura-Muñiz, A. Merkoçi, Label-free impedimetric aptasensor for ochratoxin-A detection using iridium oxide nanoparticles, *Anal. Chem.* 87(2015) 5167-5172.
- [16] B. Wu, R. Jiang, Q. Wang, J. Huang, X. Yang, K. Wang, W. Li, N. Chen, Q. Li, Detection of C-reactive protein using nanoparticle-enhanced surface plasmon resonance using an aptamer-antibody sandwich assay, *Chem. Commun.* 52(2016) 3568-3571.
- [17] W. Dong, Y. Li, D. Niu, Z. Ma, J. Gu, Y. Chen, W. Zhao, X. Liu, C. Liu, J. Shi, Facile synthesis of monodisperse superparamagnetic Fe₃O₄ core@hybrid@Au shell nanocomposite for bimodal imaging and photothermal therapy, *Adv. Mater.* 23 (2011) 5392-5397.
- [18] Z. H. Zhang, C. P. Guo, S. Zhang, L. H. He, M. H. Wang, D. L. Peng, J. F. Tian, S. M. Fang, Carbon-based nanocomposites with aptamer-templated silver nanoclusters for the highly sensitive and selective detection of platelet-derived growth factor, *Biosens. Bioelectron.* 89 (2017) 735–742.
- [19] J. Jun, J. S. Lee, D. H. Shin, J. Jang, Aptamer-functionalized hybrid carbon nanofiber FET-Type electrode for a highly sensitive and selective Platelet-Derived Growth Factor biosensor, *ACS Appl. Mater. Interfaces.* 6(2014) 13859-13865.
- [20] J. S. Lee, W. Kim, S. Cho, J. Jun, K. H. Cho, J. Jang, Multidimensional hybrid conductive nanoplate based aptasensor for platelet-derived growth factor detection, *J. Mater. Chem. B.* 4(2016) 4447-4454.
- [21] K. J. Huang, H.L.Shuai, J.Z. Zhang, Ultra sensitive sensing platform for platelet-derived growth factor BB detection based on layered molybdenum selenide–graphene composites and Exonuclease III assisted signal amplification, *Biosens. Bioelectron.* 77(2016)69–75.
- [22] E. Baldrich, F. X. Munoz, Carbon nanotube wiring: A tool for straightforward electrochemical biosensing at magnetic particles, *Anal. Chem.* 83(2011), 9244–9250.
- [23] P. Miao, Y. G. Tang, L. Wang, DNA Modified Fe₃O₄@Au magnetic nanoparticles as selective probes for simultaneous detection of heavy metal ions, *ACS Appl. Mater. Interfaces.* 9(2017), 3940–3947.
- [24] J. E. Lee, N. Lee, H. Kim, J. Kim, S. H. Choi, J. H. Kim, T. Kim, I. C. Song, S. P. Park, W. K. Moon, Uniform mesoporous dye-doped silica nanoparticles decorated with multiple magnetite nanocrystals for simultaneous enhanced magnetic resonance imaging, fluorescence imaging, and drug delivery, *J. Am. Chem. Soc.* 132 (2009) 552-557.
- [25] Y. Xiong, C. Deng, X. Zhang, Development of aptamer-conjugated magnetic graphene/gold nanoparticle hybrid nanocomposites for specific enrichment and rapid analysis of thrombin by MALDI-TOF MS, *Talanta.* 129 (2014) 282-289.

- [26] H. Lee, S.M. Dellatore, W. M. Miller, P. B. Messersmith, Mussel-Inspired Surface Chemistry for Multifunctional Coatings, *Science*, 318 (2007) 426-430.
- [27] D. Fapyane, E. E. Ferapontova. Enhanced electron transfer between gold nanoparticles and horseradish peroxidase reconstituted onto alkanethiol-modified hemin. *Electrochem. Commun.* 70 (2016) 39–42.
- [28] L. Z. Zhao, C. C. Li, H. L. Qi, Q. Gao, C. X. Zhang, Electrochemical lectin-based biosensor array for detection and discrimination of carcinoembryonic antigen using dual amplification of gold nanoparticles and horseradish peroxidase, *Sensors Actuat. B:Chem.* 235 (2016) 575–582.
- [29] G. Presnova, D. Presnov, V. Krupenin, V. Grigorenko, A. Trifonov, I. Andreeva, O. Ignatenko, A. Egorov, M. Rubtsov, Biosensor based on a silicon nanowire field-effect transistor functionalized by gold nanoparticles for the highly sensitive determination of prostate specific antigen, *Biosens. Bioelectron.* 88(2017) 283-289.
- [30] J. Wang, Y. Wang, M. Gao, X. Zhang, P. Yang, Multilayer hydrophilic poly(phenol-formaldehyde resin)-coated magnetic graphene for boronic acid immobilization as a novel matrix for glycoproteome analysis, *ACS Appl. Mater. Interfaces.* 7 (2015) 16011-16017.
- [31] H. Li, H. Wang, Y. Liu, Z. Liu, A benzoboroxole-functionalized monolithic column for the selective enrichment and separation of cis-diol containing biomolecules, *Chem. Commun.* 48 (2012) 4115-4117.
- [32] S. Zhang, X. Hu, X. Yang, Q. Sun, X. Xu, X. Liu, G. Shen, J. Lu, R. Yu, Background eliminated signal-on electrochemical aptasensing platform for highly sensitive detection of protein, *Biosens. Bioelectron.* 66(2015) 363-369.
- [33] S. R. Torati, V. Reddy, S.S. Yoon, C.G. Kim, Electrochemical biosensor for *mycobacterium tuberculosis* DNA detection based on gold nanotubes array electrode platform, *Biosens. Bioelectron.* 78(2016) 483-488.
- [34] A. McQuistan, A. J. Zaitouna, E. Echeverria, R.Y. Lai, Use of thiolated oligonucleotides as anti-fouling diluents in electrochemical peptide-based sensors, *Chem. Commun.* 50 (2014) 4690-4692.
- [35] M. Zhang, X. He, L. Chen, Y. Zhang, Preparation of IDA-Cu functionalized core-satellite Fe₃O₄/polydopamine/Au magnetic nanocomposites and their application for depletion of abundant protein in bovine blood, *J. Mater. Chem.* 20(2010)10696-10704.
- [36] M. Zhang, X. Zhang, X. He, L. Chen, Y. Zhang, A self-assembled polydopamine film on the surface of magnetic nanoparticles for specific capture of protein, *Nanoscale*, 4 (2012) 3141-3147.
- [37] H. Zhang, F. Y. Li, H. L. Chen, Y. H. Ma, S. D. Qi, X. G. Chen, L. Zhou, Au NPs colorimetric sensor for detecting platelet-derived growth factor-BB based on isothermal target-triggering strand displacement amplification, *Sensors Actuat. B:Chem.* 207 (2015) 748–755.
- [38] H. Li, Y. Zhu, S. Dong, W. Qiang, L. Sun, D. Xu, Fast functionalization of silver decahedral nanoparticles with aptamers for colorimetric detection of human platelet-derived growth factor-BB, *Anal. Chim. Acta.* 829 (2014) 48-53.
- [39] J. J. Liu, X.-R. Song, Y. W. Wang, A.-X. Zheng, G. N. Chen, H. H. Yang, Label-free and fluorescence turn-on aptasensor for protein detection via target-induced silver nanoclusters formation, *Analytica Chimica Acta*, 749 (2012) 70-74.
- [40] J. Liang, R. Wei, S. He, Y. Liu, L. Guo, L. Li, A highly sensitive and selective aptasensor based on graphene oxide fluorescence resonance energy transfer for the rapid determination of oncoprotein PDGF-BB, *Analyst*, 138 (2013) 1726-1732.

- [41] S. Bi, B. Luo, J. Ye, Z. Wang, Label-free chemiluminescent aptasensor for platelet-derived growth factor detection based on exonuclease-assisted cascade autocatalytic recycling amplification, *Biosens. Bioelectron.* 62 (2014) 208-213.
- [42] C. W. Wang, H. T. Chang, Sensitive Detection of Platelet-Derived Growth Factor through surface-enhanced raman scattering, *Anal. Chem.* 86 (2014) 7606-7611
- [43] J. Han, Y. Zhuo, Y.Q. Chai, R.Yuan, Y. Xiang, Q. Zhu, N. Liao, Multi-labeled functionalized C₆₀ nanohybrid as tracing tag for ultrasensitive electrochemical aptasensing, *Biosens. Bioelectron.* 46(2013) 74-79.
- [44] W. Song, H. Li, H. Liang, W. B. Qiang, D. K. Xu, Disposable electrochemical aptasensor array by using in situ DNA hybridization inducing silver nanoparticles aggregate for signal amplification, *Anal. Chem.* 86(2014), 2775–2783.
- [45] J. Zhang, Y. L. Yuan, S. B. Xie, Y. Q. Chai, R. Yuan, Amplified amperometric aptasensor for selective detection of protein using catalase-functional DNA–PtNPs dendrimer as a synergetic signal amplification label, *Biosens. Bioelectron.* 60(2014)224–230.
- [46] J. Floege, T. Ostendorf, U. Janssen, M. Burg, H. H. Radeke, C. Vargeese, S.C. Gill, L.S. Green, N. Janjic, Novel Approach to Specific Growth Factor Inhibition in Vivo: Antagonism of Platelet-Derived Growth Factor in Glomerulonephritis by Aptamers, *Am J Pathol.* 154 (1999) 169-179.
- [47] N. Song, Y. Huang, H. Shi, S. Yuan, Y. Ding, X. Song, Y. Fu, Y. Luo, Overexpression of platelet-derived growth factor-BB increases tumor pericyte content via stromal-derived factor-1 α /CXCR4 axis, *Cancer res.* 69 (2009) 6057-6064.

Graphical abstract

The boronic acid functionalized with hybrid magnetic composites were synthesized and used as the matrix for the enrichment and detection PDGF.