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Boronic acid functionalized magnetic composites with sandwich-like nanostructures as a novel matrix for PDGF detection

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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(SiO_2@Fe_3O_4@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 SiO_2@Fe_3O_4@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 (SiO_2-MB) were prepared to immobilize aptamer. In the presence of PDGF, the aptamer labeled SiO_2-MB (SiO_2-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.

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-

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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 multi-dimensional 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 $MoSe_2$–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]. Fe_3O_4@Au magnetic nanocomposites have been used as the selective electrochemical probes for the simultaneous detection of Ag^+ and Hg^{2+} detection [23]. Combined with the above mentioned, a simple method for fabricating yolk-egg-like magnetic composites (SiO_2@Fe_3O_4@PDA@Au) supported electrochemical sensor for PDGF is proposed. Compared with previous reports, the current strategy presents several advantages. (1) the SiO_2 cores in the composite not only improve their good aqueous dispersion and colloid stability [24], but also can be used as the support for Fe_3O_4 NPs. (2) the Fe_3O_4 NPs have high specific surface area, stability and magnetic properties, which give rise to enrichment of magnetic targets [25]. Moreover, the immobilized Fe_3O_4 NPs on the surface of SiO_2 cores facilitate the coating of PDA in view of its hydrophilic property. (3) The outer PDA shell protects Fe_3O_4 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 (SiO_2@Fe_3O_4@PDA@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 SiO_2-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 SiO_2-MB NPs exhibited high biocompatibility and high electron-transfer efficiency as a mediator. Subsequently, the aptamer labeled SiO_2-MB(denoted as aptamer-SiO_2-MB) could be used to specifically bind with PDGF. The fabricating procedure of PDGF aptasensor by a sandwich format of SiO_2@Fe_3O_4@PDA@Au, PDGF and aptamer-SiO_2-MB has been shown in Fig. 1. Firstly, the SiO_2@Fe_3O_4 composite was synthesized by sol-gel method and high temperature decomposition process. Secondly, PDA was deposited onto the surface of SiO_2@Fe_3O_4 composite in tris-buffer solution(pH 8.5). The PDA shell on the SiO_2@Fe_3O_4@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 SiO_2@Fe_3O_4@PDA@Au composite, which could be applied to selectively enrich glycoproteins(PDGF). Meanwhile, in the presence of the target protein, aptamer-SiO_2-MB was easily conjugated onto the surface of the SiO_2@Fe_3O_4@PDA@Au composite via the specific binding between aptamer and PDGF. After the PDGF was captured by SiO_2@Fe_3O_4@PDA@Au composite, the SiO_2@Fe_3O_4@PDA@Au/PDGF/aptamer-SiO_2-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.

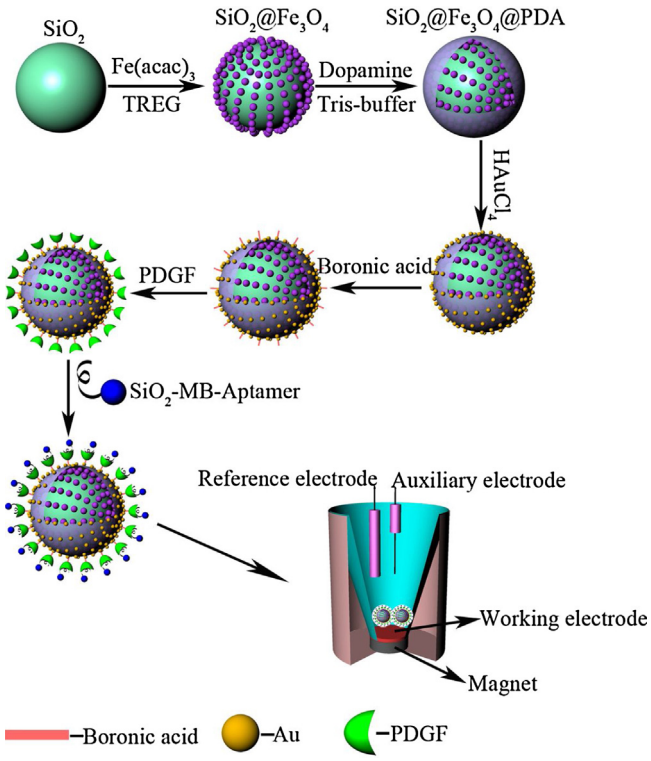

Fig. 1. Schematic representation for the configuration of the biosensor for PDGF.

2. Experimental

2.1. Chemicals

Analytical reagents such as iron(III) acetylacetonate ($Fe(acac)_3$), ethanol, tetraethoxysilane(TEOS), 4-mercaptophenylboronic acid, diethylenetriamine(DETA), dopamine hydrochloride, glutaric