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Antibody recognition by a novel microgel photonic crystal

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ABSTRACT

In this study, a easy-to-prepare biosensor for the sensitive detection of the antibody (Ab) protein was developed using a novel microgel photonic crystal (MPC). The MPC was fabricated by the spin-coated self-assembly method with the monodisperse Ab-sensitive poly (methyl methacrylate-acrylamide-glutaraldehyde-hapten) (P(MMA-AM-GA-HP)) microgels. Morphology characterization showed that the P(MMA-AM-GA-HP) microgels possessed round shapes and the large specific surface area, and the formed MPC had a highly ordered three dimensional (3D) periodically-ordered structure with the desired structural color. The Ab-response event of the P(MMA-AM-GA-HP) microgels can be directly transferred into a readable optical signal through a change in Bragg reflection of the periodic structure of the MPC. With the sensory system, the sensitive and selective detection of Ab was achieved without labeling techniques and expensive instruments. Therefore, this easy and sensitive detection system has great potential for next generation of the bioassay platform for clinical diagnosis and other applications.

1. Introduction

Photonic crystals (PCs) are dielectric optical materials consisted of spatially arranged periodic nanostructures and exhibits brilliant structural colors based on Bragg reflection and lattice spacing [1]. These materials also can modulate light with a certain wavelength or frequency. Therefore, PCs have received extensive attention in the field of biosensors and the formed PC biosensors held many advantages over other existing competing biosensing technologies, including cost-effective fabrication and short assay time [2,3].

There is a growing demand for the development of the biosensors to the sensitive and selective detection of biomarcomolecules to facilitate clinical diagnosis and other applications. For this purpose, there have been many studies of PC biosensors to detect various proteins, such as bovine serum albumin (BSA) [4–6], protein A [7], protein G [8], immunoglobulin Gamma (IgG) [9,10] and so on [11–13]. Among them, the functionalization of the hard PCs (usually silica or titanium-based PCs) around constitutes one of the key steps in biosensor development. Choi and co-workers [9] developed a biosensor for the detect of IgG using silica PCs embedded within a protein A treated hydrogel, and the limt of detection (LOD) was at the concentration of 0.5 mg mL^{-1}. Chen et al. [14] fabricated a type of silica PC modified with molecular imprinted gel as a biosensor to detect hemoglobin bovine at 1 mg mL^{-1}. By combination of the molecular imprinted technique and silica PC, Li et al. [15] developed a self-reporting sensor to measure BSA. Cunningham et al. [16] developed a biosensor using nanorod-coated TiO_2 PCs for the detection of IgG protein. Asher's research group [12] utilized carbohydrate containing responsive hydrogel to treat polystyrene PCs and developed a biosensor for lectin proteins. Although many methodologies for the hard PC functionalization have been described, such PC biosensors have many disadvantages: firstly, additional functionalization of the hard PCs structure around is time-consuming and complex process and easily to destroy the periodic PC structure, thus causing weakly and messy optical signals; secondly, the response mechanism of these PC biosensors is not derived from the PC itself because the hard PCs are non-responsive, but rather the modification matrix around the PC, which making them suffer from the low sensitivity [17].

However, a novel photonic sensor platform using microgel photonic crystal (MPC) would be an interesting alternative for easy and sensitive detection of proteins. The MPC is a class of soft PC materials with the highly ordered microgels arrays [18,19]. In contrast to the hard PCs, MPC are stimuli-responsive. In addition, MPCs are intrinsically defect-tolerant, thanks to the soft nature of the particles, which is a favorable to fabricate the large PC patterns. Thus, some MPC sensors have been developed for sensitive monitoring temperature [20,21], pH [22], osmotic [23,24] and pressure [25], using poly (methyl methacrylate) (PMMA) microgels, poly (acrylamide-acrylic acid) (PAM-AA) microgels, poly (N-isopropyl-acrylamide) (PNI-AM) microgels. Our research group [26–28] has constructed several molecular imprinted MPC

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chemical sensors using PMMA microgels. Liu et al. [17] and Hong et al. [29] developed the glucose-sensitive biosensors utilizing poly (N-isopropylacrylamide-3-acrylamidophenylboronic acid) (P(NIPAM-PBA)) and poly (Methyl methacrylate-N-isopropylacrylamide-3-acrylamidophenylboronic acid) (P(MMA-NIPA-AAPBA)) respectively. Recently, Jia's research group [30] utilized PNI-AM microgel colloidal arrays doped with P(NIPAM-PBA) microgels to develop a highly sensitive glucose biosensor. Therefore, all these techniques make them promising for high sensitivity and accurate biomacromolecules detection.

In this work, we have constructed a new type of a MPC biosensor containing Ab-responsive poly (methyl methacrylate-acrylamide-glutaraldehyde-hapten) (P(MMA-AM-GA-HP)) microgels for the sensitive detection of antibody (Ab) protein. This MPC was fabricated by the spin-coated self-assembly method to form 3D, highly-ordered periodic arrays of P(MMA-AM-GA-HP) microgels without extra-functionalization around the PC structure, and can directly create a readable optical signal based on Ab-response of the MPC itself, which endowed the MPC biosensor for proteins with higher sensitivity compared with the current PC biosensors reported.

2. Materials and methods

2.1. Materials

All of chemicals and solvents used were of analytical reagent grade. The hapten syntheses and verification were described in the ESI (Fig. S1). The monoclonal Ab protein against the hapten was obtained from Shanghai Biotechnology Institute (Shanghai, China). GA, AM, MMA, potassium peroxydisulfate (KPS), methanol, active carbon and the IgG were purchased from Tianjin Regent Corp. (Tianjin, China).

2.2. Fabrication of the MPC

As shown in Fig. 1, the monodisperse P(MMA-AM) microgels were synthesized firstly by the suspension polymerization method. Concretely, the AM (140 mg) and KPS (0.06 g) were dissolved in the MMA (3 mL), and then degassed with nitrogen for 20 min to remove the dissolved oxygen. The obtained solution was moved into a four-neck round-bottom flask and allowed to polymerization at 80 °C under the nitrogen gas for 45 min. Then, 10 mL GA aqueous sulution (1%) was added for the further polymerazation at 37 °C for 2 h. By centrifugation at 5500 rpm for 6 min, the obtained microgels with 203 (± 10) nm were separated from the resulting emulsion. After washing microgels 3 times with PBST buffer, the microgels were fully dispersed in the hapten solution (10 mL, 40 nmol mL^{-1}) and shaken for 2 h at 37 °C. Aminoacetic acid (AA) (80 mmol mL^{-1}) was then added and shaken for 2 h at 37 °C to block the remaining binding sites of the microgels surface. After remove excess water, the obtained P(MMA-AM-GA-HP) microgels (0.4 mL) was poured into a hanging-coating instrument (Shanghai San-Yan Technology, China) and spin-coated on the slide surface at a rate of 3500 rpm/min until the solution dried to get the high ordered MPC with a structural color. At the same time, a control one was fabricated using the same procedure without the addition of the hapten molecules.

2.3. Characterization of the MPC

Using a Thermo S-4800 high-resolution field emission scanning electron microscopy (SEM), the morphological characteristics of the MPC were examined. Optical properties of the MPC were recorded by a digital camera, and the optical reflection peak intensity value (PIV) change was determined with a HR2000 high resolution fiber spectrometer with a tungsten halogen light source (Ocean Optics, Dunedin, FL, USA).

2.4. Development of the MPC biosensor for Ab protein

A portable biosensor was constructed and shown in Fig. 2. In order to avoid the interference of stray light, this platform was placed in a dark box. In this system, a halogen lamp with 25.4 mm^2 filter slides was selected as the light sources for its monochrome, stability, and compactness. The incident light then was entered the multimode fiber with a diameter of 200 μm and injected into the MPC that was placed in the test trap. Meanwhile, the reflection PIV changes were captured by a reflection probe and send to a high-sensitivity UV/visible spectrometer. The sensing performance of the biosensing platform was monitored by reflection PIV, and then incubated into Ab solution (0–14 μg mL^{-1}) respectively.

3. Results and discussion

3.1. Preparation of the MPC

Fig. 1 displayed the preparation protocol of the Ab-sensitive P(MMA-AM-GA-HP) microgels. The P(MMA-AM-GA-HP) microgels were fabricated by modifying P(MMA-AM) microgels with GA and hapten as previously reported [31,32]. In this preparation, some reaction conditions can adjust the microgel size including the amount of hapten, GA, AM and MMA, reaction temperatures and rotation speed. Among them, small sized microgels were polymerized by using a smaller amount of hapten, GA, AM and MMA, higher temperatures or faster rotation speed. In contrast, larger size microgels were obtained by using more amounts of hapten, GA, AM and MMA, a slower rotation speed or lower temperature. The prepared P(MMA-AM-GA-HP) microgels were then spin-coated into form the Ab protein sensitive MPC with the highly ordered photonic pattern. For the most PC biosensors for proteins [9–15], generally there have been extra functionalization steps after the PC structure formation, which usually consuming takes hours, days, or even months. For our previous molecular imprinted MPC materials [25–27], the removal of the imprinted target chemical molecules was required spending tens of minutes. Therefore, the preparation of this MPC containing Ab-sensitive P(MMA-AM-GA-HP) microgels was an easy and time-saving process.

As shown in Fig. 2, the MPC was characterized by a 3D soft multilayers structure composed of periodic Ab-responsive P(MMA-AM-GA-HP) microgels arrays. Accordingly, the inherent high affinity of P(MMA-AM-GA-HP) microgels allowed the MPC to specifically recognize and bind the target Ab and be transferred into changes of optical signals

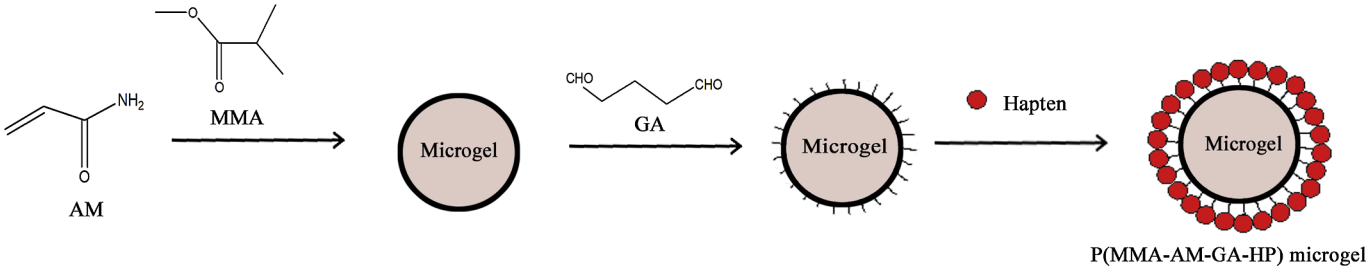

Fig. 1. Schematic illustration of the procedure used for P(MMA-AM-GA-HP) microgels preparation.

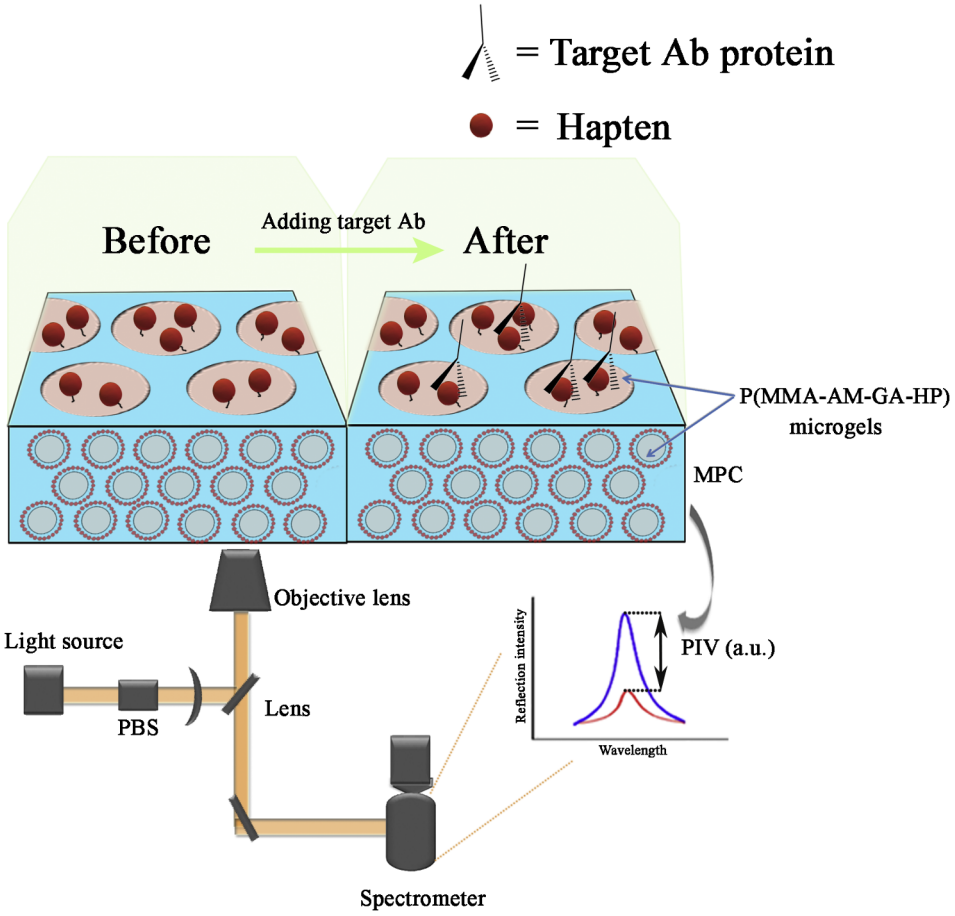

Fig. 2. The sensing mechanism of the MPC biosensor.

due to changes in the periodic structure of the MPC itself. The sensing behavior of the MPC was then monitored by the all-fiber biosensing platform. As a result, this MPC biosensor can produce reflection PIV changes according to target Ab protein concentration due to their special structures consisted of P(MMA-AM-GA-HP) microgels.

3.2. Morphology of the MPC

Fig. 3A and B displayed the SEM images of the P(MMA-AM-GA-HP) microgels and the MPC materials. The SEM images revealed that the P (MMA-AM-GA-HP) microgels were still uniform in size and shape, and showed the large specific surface area and high density, which were favorable for the transmission and adsorption of molecules in the material (Fig. 3A). As shown in Fig. 3B, there was the monodispersity of the MPC indirectly and a regular close-packed micorgels arrangement. This better monodispersity created a larger integrated area with fewer cracks and the maximum size of the crystalline domains reached about 23–58 μm in this study, which would be caused by intrinsically defect-tolerant abilitys caused by the soft nature of the microgels. As a result, this large and highly ordered periodic structure of P(MMA-AM-GA-HP) microgels array would enable the MPC not only to immediately generate optical signals, but also to sensitively respond to target analytes.

The reflection wavelength λ max for the MPC is determined by the Bragg equation:

$$\lambda_{max} = 1.633d\sqrt{n^2 - sin\theta^2}$$

where d is the average diameter of the microgel, n is the average refractive index of the material, and θ is the angel of incident light [23,25]. When θ is constant, reflection wavelength λ max is proportional to microgel diameter d. Therefore, different microgel diameter would cause different reflection wavelength and structural color of the MPC. In this work, the prepared MPC showed a blue-green color, which caused by the Bragg reflection of visible light from these well-ordered and periodic 3D opal structures formed in the MPC, in Fig. 3B.

3.3. The MPC biosensor for Ab protein

To optimize the detection performances of the biosensor, the sensing conditions were optimized, such as ambient light interference and pH value of the reaction medium. The reaction medium at pH 7.2 make the biosensing platform have a better response to Ab (6 μg mL^{-1}), which was then selected for the following experiment (Fig. S2). In addition, even under the faint external light interference, we found that the specific reflection peak was disappeared, leading to an ill-defined optical signal (not shown). Thus we putted the biosensing platform in a closed darkroom to monitor the antigen-antibody reaction.

Fig. 4A showed the sensing response of the biosensor to a series concentration of Ab. Upon exposure to 0.25 μg mL^{-1} Ab in PBS (pH 7.2), the reflection PIV decreased by 3.96 a.u. It was indicated that the MPC biosensor was sensitive to the Ab protein. In view of LOD, the sensitivity was improved compared with other PC biosensors for proteins [9–13]. This higher sensitivity would be resulted from the following reasons. The most of reported PC biosensors for protein were composed of the hard PC of non-responsive spheres embedded in stimulus-responsive bulk hydrogel matrix. Unfortunately, the slow swelling rate of the bulk hydrogel film would cause the slow response for target protein and further affect the sensitivity of the biosensors. Furthermore, the functional hydrogel immobilization on the PC structure

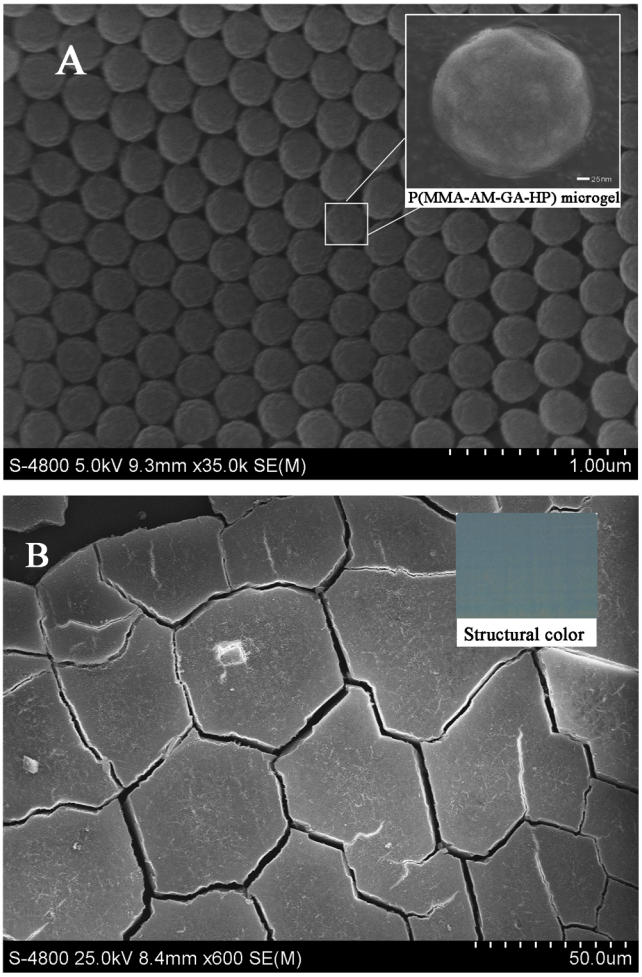

Fig. 3. The SEM image and photographic image of the MPC and the P(MMA-AM-GA-HP) microgels: (A) The SEM image of the MPC (35,000×) and a P (MMA-AM-GA-HP) microgel. (B) The SEM image (600,000×) and the photographic image of the MPC.

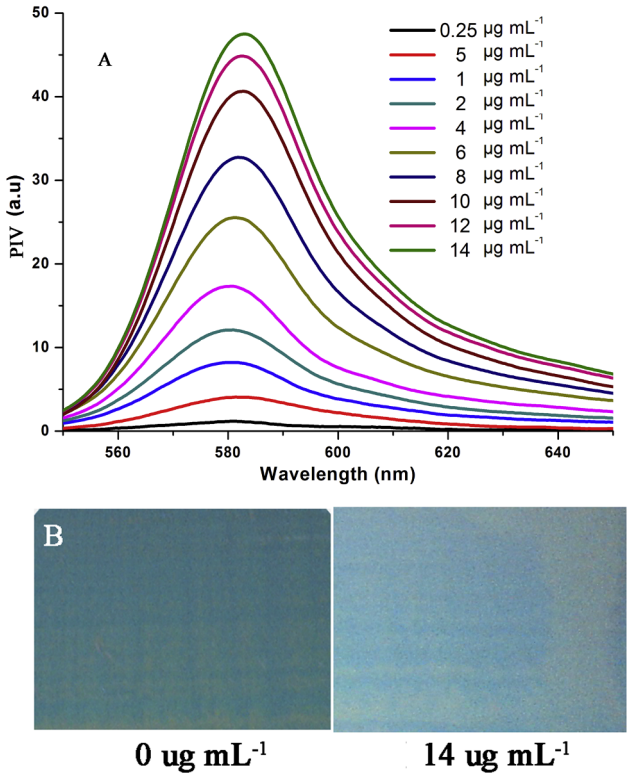

Fig. 4. (A) The response behavior of the biosensor for Ab with different concentration. (B) Photographic images displaying the color changes of the biosensor to 14 μg mL^{-1} of Ab.

around can be easily to destroy the periodic structure, thus creating weakly and messy optical signals. However, our MPC materials was consisted of the stimuli-responsive P(MMA-AM-GA-HP) microgels. This format not only avoided extra-functional process around the PC, but also the microgels had a much faster response rate than bulk gels.

With the Ab concentration ranged from 0.25 μg mL^{-1} to 14 μg mL^{-1}, the reflection PIV changes of the MPC gradually decreased, which was accompanied by color changes (from blue-green to amaranth), as shown in Fig. 4B. The phenomenon would be caused by the following reasons. At first, when Ab molecules entered the MPC, a multitude of simultaneous secondary bonds can be formed between the P(MMA-AM-GA-HP) microgels and the Ab molecules due to immune recognition reaction. These secondary bonds formation made the P (MMA-AM-GA-HP) microgels of the MPC swell, probably because of the duildup of Donnan potential between microgels phase and the bulk solution phase during the binding and recognition process of Ab to P (MMA-AM-GA-HP) microgels [2]. With more and more Ab molecules binding on the microgel, the size of microgels increased and caused in an increase in the interplanar spacing followed by the reflection PIV reducing. In addition, the increase of the effective refractive index would be one of the factors leading to reflection PIV decrease [3].

To further clarify the molecular recognition properties of the biosensor, control experiments were performed by a control one fabricated under the same preparation conditions as those for the MPC but in the absence of hapten molecules. Different from the response behavior of the MPC, the reflection PIV of the control one caused a slight fluctuation, probably because of some nonspecific adsorption (Fig. 5A). So it was learned that the immune recognition response of the MPC was responsible for the observed results. The selectivity of the MPC was investigated with BSA and the IgG as the reference proteins in Fig. 5B. Apart from obvious reflection PIV changes of the MPC to Ab molecules, there was a low response to BSA and the IgG. It was suggested that the MPC possessed good selectivity for Ab proteins.

4. Conclusions

We have developed a new strategy for the highly sensitive detection of Ab protein based on the MPC biosensor containing P(MMA-AM-GA-HP) microgels. The constructed sensor system emphasized that through special structural changes of the MPC material, trace target Ab proteins can be specifically recognized and sensitively responded to create a readable optical signal without using label techniques and expensive detection instruments. In addition, this biosensor biosensor not only released from the dependence of bulk hydrogel response and complex fabrication processes, but also exhibited the higher sensitivity than other PC biosensors reported before. To our knowledge, this was one of the most sensitive PC biosensors for proteins yet reported. Therefore, in the future, this new approach can be applied to many sensor systems for sensitive recognition in clinical diagnosis, drug testing and life science. We will conduct additional studies on the improvement of the color sensitivity of our material for the purpose of detecting target molecules in actual samples by the naked eyes without the help of spectrometers.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

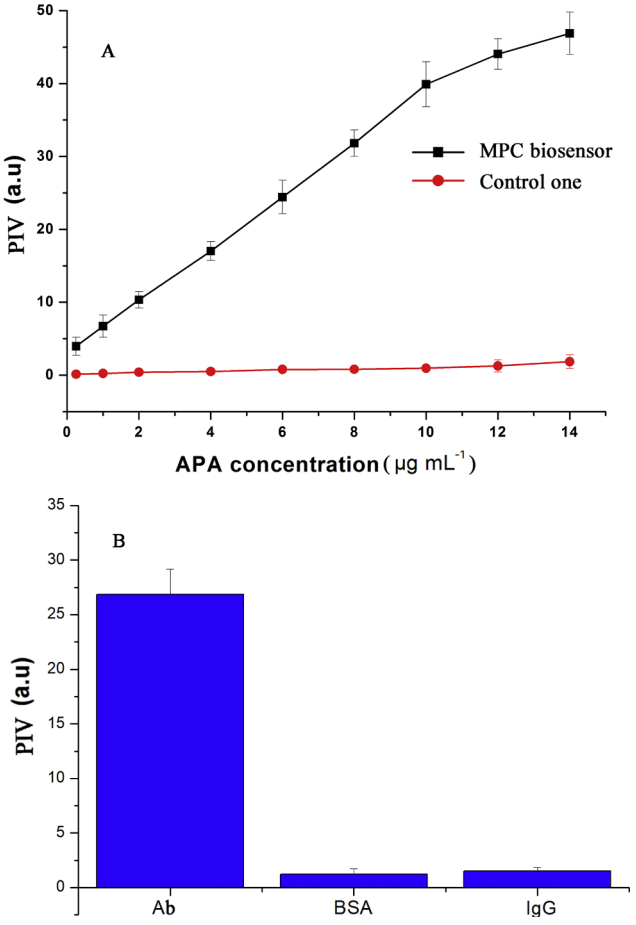

Fig. 5. (A) The response behavior of the control one for Ab with different concentration. (B) The response behavior of the biosensor for Ab, BSA and IgG.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgement

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.12.001.

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