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Ultrasonic Multi-analyte Electrochemical Immunoassay Based on Metal-ions Functionalized Titanium Phosphate Nanospheres

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Abstract:
A novel multi-analyte electrochemical immunoassay was developed for ultrasensitive detection of human cardiopathy biomarkers cardiac troponin I (cTnI) and human heart-type fatty-acid-binding protein (FABP) by using metal-ions functionalized titanium phosphate nanospheres (TiP-metal ions) as labels. The metal-ions could be detected directly through square wave voltammetry (SWV) without metal preconcentration and the distinct voltammetric peaks had a close relationship with each sandwich-type immunoreaction. The position and size of the peaks reflected the identity and level of the corresponding antigen. The large amount of metal ions loading on the TiP nanospheres greatly amplified the detection signals and the good biocompatibility of graphene nanoribbons (GONRs) retained good stability for the sandwich-type immunoassay. The proposed immunoassay exhibited high sensitivity and selectivity for the detection of cTnI and FABP. The linear relationships between electrochemical signals and the concentrations of cTnI and FABP were obtained in the range of 0.05 pg/mL-50 ng/mL and 0.05 pg/mL-50 ng/mL, respectively. The detection limits of cTnI and H1gG were 1 fg/mL and 3 fg/mL (S/N=3), respectively. Moreover, the immunoassay accurately detected the concentrations of cTnI and FABP in human serum samples, which were demonstrated to have excellent correlations with standard ELISA method. The results suggested that the electrochemical immunoassay would be promising in the point-of-care diagnostics application of clinical screening of acute myocardial infarction (AMI) biomarkers.

Keywords: ultrasonic; multi-analyte; titanium phosphate; metal-ions; electrochemical immunoassay

1. Introduction

The detection of biomarkers plays a crucial role in basic medical researches as well as in clinical diagnostics.1,2 Immunoassay based protocols employing antibody-antigen interaction is one of the most important analytical techniques in the quantitative detection of biomarkers due to the highly specific molecular recognition of immunoreaction.3 Because of the increasing need of in-time control of various diseases, the development of sensitive, rapid and low cost immunoassay methods has become a great challenge. In comparison with other immunological methods such as fluorescence,4 chemiluminescence,5 surface-plasmon resonance,6 or quartz crystal microbalance,7,8 electrochemical immunoassay9 has attracted considerable interest for its intrinsic advantages such as good portability, low cost, and high sensitivity. Therefore, different
electrochemical immunosensors, particularly amperometric immunosensors, have been developed and extensively applied to the determination of biomarkers.

The advantages of nanotechnologies have offered alternative approaches for sensitive and low-cost detection of protein biomarkers. Various nanomaterials including colloidal gold nanoparticles (AuNPs), carbon nanotubes (CNTs), bionanospheres, and magnetic beads have been used as excellent carriers for the preparation of labels by loading numerous signal tags such as enzymes, quantum dots, oligonucleotide, and dyes. The prepared labels can greatly amplify the transduction signal of a recognition event in bioassays and simplify the detection steps. However, traditional metal nanoparticles or QDs based labels required complicated and time-consuming acid dissolution step and metal preconcentration before electrochemical detection. As a result, there is still a great challenge in developing novel probes to simplify detection steps and reduce detection time. Recently, our group has successfully synthesized the porous titanium phosphate nanoparticles by using surfactant sodium dodecyl sulfate (SDS) as the structure-directing agent, which were demonstrated to have excellent ion-exchange property with Cd, and the nanocomposite can be loaded large amount of Cd. The Cd functionalized TiP nanoparticles were demonstrated to be used as signal tags for electrochemical detection of HigG.

Herein, we prepare the TiP nanospheres by using docusate sodium salt (AOT) as the structure-directing agent, which is different from that of our previous work (SDS). Thus, the TiP nanospheres exchanged with different metal ions such as Cd and Zn fabrication of versatile labels for simultaneous electrochemical detection of cardiopathy biomakers. Graphene nanoribbons (GONRs) have attracted a great deal of attention as a two-dimensional carbon material since they have been reported. Apart from the layered structure with a large theoretical specific surface area, GONRs bear abundant oxygen-containing surface groups, such as hydroxyl, epoxide, carbonyl and carboxyl groups. The presence of such groups not only allows the GONRs to be well dispersed in water to yield a colloidal stable suspension, but also offers potential application as nanoscale substrates for the fabrication of flexible GONRs-based immunosensors. In this work, GONRs was used for antibodies immobilization, which is also a novelty of our present work.

Compared to the traditional single-analyte immunoassay, the multiplexed immunoassay is more efficient in clinical application since it can quantitatively detect a panel of biomarkers in a single run with improved diagnostic specificity. Moreover, the multiplexed immunoassay can shorten analytical time, enhance detection throughput, and decrease sampling volume and detection cost. In clinical analysis, simultaneous determination of acute myocardial infarction (AMI) biomarkers possesses important application to the screening and diagnosis of AMI. In recent years, electrocardiographic investigation has been the main method for establishing AMI diagnosis; however, only 57% of AMI patients reveal electrocardiogram changes. As a result, there has been considerable interest in developing new protocols for AMI diagnosis. Human cardiac troponin I (cTnI), a cardiac muscle protein, has been recognized as a principle diagnostic marker for myocardial damage. Compared with other biochemical markers of myocardial injury, such as creatine kinase-MB isoenzyme and myoglobin, cTnI has an excellent cardiospecificity. Human heart-type fatty-acid-binding protein (FABP), a cytosolic protein mainly expressed by myocytes, might have potential as an early cardiac marker. It appears in plasma 1-3 h after cardiac damage, and may be the earliest available plasma marker of acute myocardial injury. It may have better diagnostic accuracy than other cardiac markers in the early stages after symptom
Multiplexing immunoassay for AMI biomarkers improves the detection reliability and accuracy of cardiopathy. Human IgG (HIgG) is a glycoprotein mainly responsible to combat chronic infectious diseases and for surveillance against recurrence of infections, and is a standard model for many immunoassay methods. Here, HIgG can be used as a model protein to evaluate applicability our proposed immunoassay.

Scheme 1 (A) Preparation procedures of Ab$_2$ functionalized TiPR-Cd$^{2+}$ and TiPR-Zn$^{2+}$ as probes and (B) schematic illustration of the stepwise immunosensor fabrication process and interaction of antibody-antigen. Ab$_1$ and Ab$_2$ represent the primary antibody (capture antibody) and tracer secondary antibody (signal antibody), respectively.

In this work, we designed a novel tracer, metal-ions functionalized titanium phosphate nanospheres (TiP-metal ions), to label the signal antibody (Ab$_2$) and developed an ultrasensitive immunoassay method by combining graphene oxide nanoribbons (GONRs) immobilized with capture antibodies (Ab$_1$) to facilitate the signal amplification (Scheme 1). The TiP nanospheres were synthesized with docusate sodium salt (AOT) as the structure-directing agent. Due to the outstanding ion-exchange capability, a large amount of metal ions such as Cd$^{2+}$ and Zn$^{2+}$ were incorporated into the TiP nanospheres to form TiP-metal ions hybrids. cTnI antibodies and HIgG antibodies were conjugated with TiP-Cd$^{2+}$ and TiP-Zn$^{2+}$ to fabricate anti-cTnI-TiP-Cd$^{2+}$ and anti-FABP-TiP-Zn$^{2+}$ bioconjugates. The metal ions in the bioconjugates could be detected directly without acid dissolution and preconcentration, which would greatly simplify the detection steps and reduce the detection time. Based on these bioconjugates as probes, a novel and facile strategy for simultaneous electrochemical detection of AMI biomarkers was developed. To demonstrate the multiplex immunoassay can be used in other multiplex protein detection, HIgG was used as a model protein to replace FABP in this work. The results indicated that the multiplex immunoassay also possess excellent applicability in other proteins detection.

2. Experimental Section

2.1 Materials and Reagents

Human HIgG, rabbit anti-human IgG, goat anti-human IgG and the HIgG ELISA kit were purchased from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Anti-cTnI antibodies, cTnI antigens and the clinical serum samples were obtained from the First Affiliated Hospital of Nanjing Medical University. Bovine serum albumin (BSA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide
(NHS), poly-(allylamine hydrochloride) (PAH) and Tween-20 were from Sigma-Aldrich. Multi-walled carbon nanotubes (MWCNTs, CVD method, purity > 95%, diameter 40-60 nm, length 5-15 µm) were used as received from Shenzhen Nanotech Port Co., Ltd. Glutaraldehyde (GLU) was obtained from Shanghai Reagent Company (Shanghai, China). Tetrabutyl titanate, docusate sodium salt (AOT) and H₃PO₄ were from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Phosphate buffer saline (PBS) with different pH values were prepared by mixing the stock solution of NaH₂PO₄ and Na₂HPO₄, and then adjusting the pH with 0.1 M NaOH and H₃PO₄. HAc/NaAc solutions with different pH values were prepared by mixing the stock solutions of HAc and NaAc. All other reagents were of analytical reagent grade and used without further purification. Ultrapure fresh water obtained from a Millipore water purification system (MilliQ, specific resistivity is larger than 18 MΩ, S.A., Molsheim, France) was used in all run.

2.2 Apparatus

All electrochemical immunoassay measurements were performed on a CHI 660D workstation (Chenhua, Shanghai, China) with a conventional three-electrode system comprised of a platinum wire as the auxiliary, a saturated calomel electrode as the reference, and the modified GCE as the working electrode. Scanning electron micrographs (SEM) were obtained with a Hitachi S-4800 scanning electron microscope. The high resolution transmission electron micrographs (HRTEM) were measured on a JEOLJEM 200CX transmission electron microscope using an accelerating voltage of 200 kV, which equipped with an energy dispersive X-ray spectrometer (EDS). Atomic absorption spectrophotometer (AAS) was monitored by a Hitachi 180-80 spectrometer.

2.3 Preparation of Signal Antibodies (Ab₂)-TiP-metal Ions Probes

First, TiP nanospheres were synthesized according to the literature. Briefly, 5 g of AOT was dissolved into 25 g ethanol, and H₃PO₄ (10 g) was added to get a turbid solution. Then a mixture of TBOT with ethanol (1.75 g/25 g) was fast dropped into the AOT/ethanol solution to get a stable mixture solution with the aid of ultrasonic. The mixture was stirred at 80 °C for 6 h. The solid product was washed with ethanol and DI water for several times to remove the residual phosphoric acid and surfactant.

For ion-exchange, 1 mL of TiP nanospheres (40 mg/mL) were dispersed in 17 mL of 10 mM Cd(NO₃)₂ and Zn(NO₃)₂ aqueous solution respectively and stirred at 50 °C for 24 h. The resulted hybrid nanospheres were obtained by centrifugation and rinsed with water for several times. The TiP-metal ions products were dispersed in 2 mL DI water to get a dispersion of TiP-Cd²⁺ and TiP-Zn²⁺ with the concentration of 20 mg/mL. Next, 1 mL of the TiP-metal ions hybrids were dispersed in 1 mL of PAH (2 mg/mL) aqueous solution and sonicated for 20 min. Then, the hybrids were washed with DI water and dispersed in 1 mL GLU (wt. 0.25%) and sonicated for 5 min. After washing with DI and PBS for three times, 100 µL of anti-cTnl (1 mg/mL) solution were added into the TiP-Cd²⁺ hybrids and 100µL of anti-FABP (1 mg/mL) solution were added into the TiP-Zn²⁺ hybrids respectively and shaken for 6 h. After centrifuged, the obtained bioconjugates were further washed with PBS for at least three times and resuspended in 4 mL PBS as the assay solution.

2.4 Fabrication of Immunosensor

Graphene oxide nanoribbons (GONRs) were prepared from MWCNTs according to the
Method reported by James M. Tour. The antibody-labeled GONR conjugate was prepared through the following approach: 1 mL GONR (0.2 mg/mL) colloid solution was centrifuged at 18000 rpm for 20 min, and the supernate was removed. Then, 0.4 mL of aqueous solution of 10 mg/mL EDC and 5 mg/mL NHS was added to activate the carboxylic groups onto the surface of the GONRs for 1 h. The mixture was then centrifuged at 15000 rpm for 5 min, and the supernate was removed. Then, 100 µL of antibody solution (1 mg/mL) was added into the GONRs solution. The final volume was adjusted to 200 µL by adding PBS and was allowed to stand overnight at 4 °C, followed by the addition of 800 µL of 1% BSA solution. The precipitate was then collected after a second centrifugation at 12 000 rpm for 10 min. Finally, the resulting conjugate was resuspended in 200 µL of PBS with 1% BSA to stabilize the functionalized GONRs (GONR-Ab) and to minimize nonspecific adsorption during the immunoassay.

The fabrication of the sandwich immunosensor was illustrated in Scheme 1B. The GCE with a diameter of 3 mm was polished using 0.3 and 0.05 µm alumina slurry followed by rinsing thoroughly with water. After successive sonication in 1:1 nitric acid, acetone and water, the GCE was allowed to dry at room temperature. 5 µL of GONR-Ab composite solution was dropped on the fresh pretreated GCE and allowed to dry at 4 °C overnight.

2.5 Measurement Procedure
To carry out the immunoreaction and electrochemical measurement, the modified electrode was first incubated with a 20 µL drop of the target antigens (cTnI and FABP) or serum samples with different concentrations for 60 min at 37 °C as the immunoreaction could obtain higher efficiency at this temperature, followed by washing with PBST for three times. Next, it was further incubated with 20 µL of Ab2-TiP-metal ions bioconjugates solution for 60 min at 37 °C and then washed thoroughly with PBST to remove non-specifically bounded conjugates. Finally, the electrochemical measurement was carried out in 3 mL of pH 4.6 HAc/NaAc. SWV scan from -1.4 V to -0.2 V with a pulse amplitude of 25 mV, a pulse frequency of 15 Hz and a quiet time of 2 s was performed to record the electrochemical responses at -0.8 V and -1.1 V for simultaneously quantitative measurement of cTnI and FABP.

3. Results and discussion
3.1 Fabrication of Ab2-TiP-metal Ions Probes
TiP nanospheres were obtained with AOT as the structure-directing agents. The TiP nanospheres showed uniform morphology and size distribution, good dispersion, and the average diameter was about 50 nm (Figure S1A, Supporting Information). Recent research has indicated that TiP nanoparticles have ion-exchange property. After ion-exchanging with Cd2+ and Zn2+, the morphologies of TiP-metal ions hybrids did not change (Figure S1B and S1C). This result indicated that the ion-exchange process would not destroy the structure of TiP nanospheres. Elemental compositions of TiP, TiP-Cd2+ and TiP-Zn2+ nanomaterials were analyzed with EDS, as shown in Figure S2. Signature peaks of Na, Ti, O and P were observed for TiP nanospheres. Meanwhile, the presence of cadmium and zinc in the TiP-metal ions hybrids were confirmed, which were shown in Figure S2 (b) and (c). The weight percentages of Cd and Zn in the hybrids were also measured by using AAS. The results revealed that 58.6 mg of cadmium and 21.9 mg of zinc were loaded onto 1.0 g of TiP, respectively. The large amount of metal-ions loading on TiP nanospheres could be facilitated by the structure and large amount of hydroxyl groups.
Due to the incorporation of large amount of metal ions (Cd$^{2+}$ and Zn$^{2+}$), the TiP-metal ions hybrids can be further used as labels in bioassay. As shown in Figure 1, the antibodies were obviously trapped on the surface of TiP-Cd$^{2+}$ and TiP-Zn$^{2+}$ hybrids, indicating the successful fabrication of Ab$_2$-TiP-Cd$^{2+}$ and Ab$_2$-TiP-Zn$^{2+}$ nanoprobes.

3.2 Fabrication of the Electrochemical Immunosensor.

In this work, GONRs were used as biosensor platforms for immobilization of proteins, which showed good biocompatibility and remarkably enhance the sensitivity of the proposed immunosensor. Because of the carboxyl groups on GONRs (Figure S3, Supporting Information), antibodies can be bound to GONRs by crosslinking between carboxyl groups and amino groups. Figure S4 showed the HRTEM images of as-prepared GONR. The GONR had a diameter with 400-600 nm, which was much wider than the diameter of MWCNT (40-60 nm). This result indicated that GONRs were successfully obtained by exfoliation of MWCNTs. The length of GONR was 3–4 µm, which was in accordance with the length of MWCNT. As shown in Figure S5 (Supporting Information), the average thickness of GONRs is about 8 nm.

3.3 Immunoassay Using Ab$_2$-TiP-metal Ions Probes

Metal elements are commonly used as trace labels in electrochemical immunoassay, which provide an elegant way to detect multiple proteins in connection with highly sensitive stripping voltammetric detection of the metal components. The metal ions in the nanoprobes were directly tested by highly sensitive SWV, as can be seen in Figure 2A. The stripping peaks at –1.1 V and –0.8 V are ascribed to the oxidation of zinc and cadmium, respectively. The position of two peaks could reflect the identity of corresponding antigens.

3.4 Analytical Performance

The electrochemical performance of the immunosensor would be influenced by many factors. Herein, we investigated the dependence of the SWV peak current under different experimental variables, including the incubation time and the pH value of detection solution (Figure S6, Supporting Information). As a result, the incubation time with 60 min and 0.2 M HAc/NaAc (pH 4.6) was selected as the electrolyte for the electrochemical immunoassay.

Under optimized assay conditions, the peak currents were enhanced with the increasing concentration of analytes, as shown in Figure 2A. The calibration plots showed good linear relationships between the peak currents and the logarithm of the analyte concentrations. For cTnI (Figure 1B), the linear regression equations were $i(\mu A) = 31.50 + 6.86 \log C_{cTnI}$ (ng/mL) in the range from 0.05 pg/mL to 50 ng/mL with a correlation coefficient of 0.996. For FABP (Figure 1C),

![Figure 1](image_url)
the linear regression equations were $i(\mu A) = 10.16 + 2.07 \log C_{\text{FABP}} \text{ (ng/mL)}$ in the range from 0.05 pg/mL to 50 ng/mL with a correlation coefficient of 0.998. The detection limits for cTnl and FABP were 1.0 fg/mL and 3.0 fg/mL (S/N=3), respectively, which were much lower than previous studies for cTnl$^{40,41,43}$ The low detection limits might be attributed to the enormous loading of metal ions, which greatly amplified the peak signals. The wide linear ranges for two analytes were also very significant for practical application. To study if the proposed multiplex immunoassay can be used in other proteins multiplex detection, HlgG was used as a model protein to investigate the analytical performance. The details for simultaneous detection of cTnl and HlgG were described in Supporting Information. The results (Figure S7, Supporting Information) indicated our proposed multiplex immunoassay can be widely used in other proteins detection.

Figure 2 (A) Typical SWV of the immunosensors for different cTnl and FABP concentration (from a to e: 0, 0.00005, 0.005, 0.5, 5 ng/mL cTnl and FABP, respectively). (B) and (C) The resulting calibration curve of proteins plotted on a semi-log scale.

3.5 Specificity, Reproducibility and Stability of the Immunoassay

The specificity played an important role in analyzing biological samples.$^{44}$ Some proteins such as carcinoembryonic antigen (CEA), bovine serum albumin (BSA) and neuron specific enolase (NSE) were used as the possible interferences to evaluate the specificity of the proposed immunosensor. The electrochemical signals of 5 pg/mL FABP and 5 pg/mL cTnl were compared with that containing interferential substance of 50 pg/mL. The peak currents showed minimal difference when the incubation solution contained interferences (Figure 3). When using HlgG to perform the immunoassay, some similar antibody molecules, such as human immunoglobulin E (HlgE), human immunoglobulin M (HlgM) were also used as the possible interferences to evaluate the specificity of the proposed immunoassay (see Figure S8 and S9, Supporting Information). The results indicated that HlgE and HlgM could not interfere the detection of HlgG and cTnl. The proposed immunosensor showed high specificity, which could be further used for detection of FABP and cTnl in complicated samples.
Figure 3 The specificity of the proposed immunosensor at the concentration of 0.5 ng/mL cTnI and 0.5 ng/mL H1gG with the interfere concentrations of (a)(d) no interferes, (b)(e) 2 ng/mL H1gE, (c)(f) 2 ng/mL H1gM.

The reproducibility of the proposed immunoassay was assessed by the relative standard derivations (RSD) of intra- and inter-assays, which were listed in Table S1 and Table S2. The intra-assay precision of the developed immunoassay was valued by detecting four samples containing 0.0005, 0.005, 0.05 and 0.5 ng/mL of cTnI and FABP, respectively. Each sample was measured five times using five parallelly prepared immunosensors. For different concentrations of cTnI and FABP, the RSD values of intra-assay were less than 5.6% and 4.0%, respectively. In addition, the inter-assay precision was estimated by measuring one sample with five immunosensors made at the same GCE independently. From Table S1, the RSD values were less than 4.0% and 4.4% for different concentration of cTnI and FABP, respectively. These results indicated that the proposed multi-analyte immunoassay possessed acceptable precision and reproducibility.

Furthermore, when the immunosensor was stored at 4 °C for 20 days, it remained 89% of initial responses for both cTnI and FABP. This result indicated the good stability of the immunosensor which might be attributed to the good biocompatibility of GONRs since they could retain the bioactivity of proteins. The slow decrease of responses might be due to the gradual deactivation of the immobilized biomolecules.

3.6 Evaluation of Cross-Reactivity

An excellent immunosensor must exclude cross-reactivity between analytes and non-specific antibodies. The GONRs were employed to immobilize the two different capture antibodies (Ab1) for cTnI and FABP. The cross-reactivity was evaluated by comparing the amperometric responses of two analytes to those containing only one analyte. As expected, the amperometric responses showed minimal difference when the incubation solution contained one or two kinds of analytes (Figure 4). In the absence of cTnI and FABP, no obvious SWV signal was observed at -0.8 V or -1.1 V (curve a), which confirmed that non-specific reaction was negligible in the sandwich immunoassay. While an obvious peak appeared at -1.1 V when the incubation solution only containing 0.05 ng/mL FABP (curve b). When both 0.05 ng/mL FABP and cTnI added, two signal peaks were obtained in curve d. The SWV peaks at -1.1 V (curve b and curve d) had the same current value, indicating that the presence of cTnI had no influence on FABP. Similarly, a sharp peak was obtained at -0.8 V when the incubation solution only containing 0.5 ng/mL cTnI (curve c), which matched the peak at -0.8 V in curve d well. The result also confirmed that FABP had no influence on cTnI. Thus, the detection of FABP and cTnI would not cause interference with each other and the cross-reactivity between them could be negligible. In addition, the cross reactivity was also investigated by simultaneous detection of 0.5 ng/mL H1gG and cTnI (Figure S10, Supporting Information). The results indicated H1gG and cTnI could not cause crosslinking with each other.
3.7 Application in Analysis of Clinical Serum Samples

Table 1: Assay Results of Clinical Serum Samples Using the Proposed Method and ELISA Method

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Detection of cTnI (ng/mL)</th>
<th>Detection of FABP (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proposed method[a]</td>
<td>ELISA method</td>
</tr>
<tr>
<td></td>
<td>19.42 62.16 30.80 37.7 5.90</td>
<td>19.62 62.34 30.38 39.3 5.59</td>
</tr>
<tr>
<td></td>
<td>-1.0 0.3 1.7 -4.1 5.5</td>
<td>RSD within 5.5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Relative standard deviation (%)</td>
</tr>
<tr>
<td></td>
<td>Sample no.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proposed method[a]</td>
<td>ELISA method</td>
</tr>
<tr>
<td></td>
<td>1.661 21.255 9.959 86.7 4.823</td>
<td>1.749 21.032 9.896 89.6 4.863</td>
</tr>
<tr>
<td></td>
<td>-5.0 1.1 0.6 -3.2 -0.8</td>
<td>RSD within 5.0%</td>
</tr>
</tbody>
</table>

[a] Average value of three successive determinations.

To evaluate the analytical reliability and application potential of the designed immunoassay method, the assay results of cTnI and FABP in human serum samples using the proposed method were compared with the reference values obtained by the commercial ELISA method. The serum samples came from normal persons and AMI patients. As a result, the concentrations of cTnI could reflect the real situation of clinical diagnosis. As shown in Table 1, the assay results have the RSD within 5.5% for cTnI and -5.0% for FABP detection, indicating feasibility of the proposed method for serum sample. For the simultaneous detection of cTnI and H1gG (Table S3, Supporting Information), the results also proved the wide application of our proposed immunoassay methods.

4. Conclusions

In summary, a novel ultrasensitive multiplexed immunoassay based on metal-ions functionalized titanium phosphate nanospheres as labels was successfully developed. The Ab2-TiP-metal ions probes not only provide a high concentrate of metal ions for signal
amplification, but also simplify the detection steps by excluding the striping process. The proposed electrochemical immunoassay showed acceptable stability, reproducibility, and accuracy, and excellent performance for simultaneous detection of cTnI and FABP with no obvious cross-reactivity. The method described here opens a new approach for simple, sensitive, and simultaneous determination of cardiopathy biomarkers, and it can also be extended for the detection of other relative biomarkers, which shows great potential in point-of-care application for accurate clinical disease diagnostic.

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References

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