Int. J. Electrochem. Sci., 12 **(2017)** 7262 – 7271, doi: 10.20964/2017.08.67

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Fabrication of Electrochemical Immunosensor for Interferon-γ Determination and Its Application of Tuberculosis Diagnosis

Xianhua Wang[1], *Xiuxia Han*[1], *Aiguo Ma*[4,*], *Lei Chen*[1], *Hui Liang*[1], *Aishan Litifu*[2], *Feng Xue*[3]

[1] The Institute of Human Nutrition, Medical College of Qingdao University, No. 38 Dengzhou Road, Qingdao 266021, P.R China.
[2] Department of Respiratory Medicing, Xinjiang Uygur Autonomous Region Chest Hospital, The Xinjiang Uygur Autonomous Region, P.R China.
[3] Tuberculosis Department of The Xinjiang Uygur Autonomous Region Center for Disease Control and Prevention, the Xinjiang Uygur Autonomous Region, P.R China.
[4] The Institute of Human Nutrition, Medical College of Qingdao University, Qingdao, P.R China.
[*]E-mail: aiguoma1956@163.com

Received: 5 May 2017 / *Accepted:* 16 June 2017 / *Published:* 12 July 2017

Tuberculosis (TB), as one kind of infectious diseases caused by Mycobacterium tuberculosis, has killed countless numbers of people especially in underdeveloped countries. In our study, a label-free electrochemical immunosensor for the detection of interferon gamma (IFN-γ) was prepared for the first time. ZnO NPs prepared by hydrothermal method were used for the immobilization of IFN-γ monoclonal antibody. The increased impedance values displayed linear relationship with the logarithmic value of IFN-γ concentrations within the range of 0.0001 to 0.1 ng/mL. In addition, the detection limit was 0.12 pg/mL (S/N=3). The as-proposed immunosensor demonstrated promising potential usage in the diagnosis of tuberculosis in time owing to its simple operation, excellent sensitivity and stability.

Keywords: Tuberculosis; Interferon gamma; ZnO NPs; Hydrothermal; Electrochemical impedance spectroscopy

1. INTRODUCTION

Tuberculosis (TB), as one kind of infectious diseases that occurred three million years ago, has been world's second-deadliest infectious disease and killed countless numbers of people. TB is caused by the bacterium *Mycobacterium tuberculosi* and generally affects the lungs. World Health Organization (WHO) declare TB as “global emergency” in April 1993. After years of hard work, TB

can be completely cured, and the survival rate of patients with pulmonary tuberculosis increased significantly as well. However, the global eradication of TB is still difficult owing to the emergence of acquired immune deficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV) infection. Therefore, more attention should be paid to TB. The emergence of certain new forms of TB such as drug-resistant TB (DR-TB) [1-4], extensively drug-resistant TB (XDR-TB) and multidrug-resistant TB (MDR-TB) [5, 6] hampered the effective control of TB. Most recently, due to the insufficient monitoring of drug resistance and improper use of antibiotics, some rare but potentially dangerous forms of TB including super XDR-TB, totally drug-resistant TB (TDR-TB) and extremely drug-resistant TB (XXDR-TB) have emerged especially in India and China [7-9].

At present, 85% of patients with TB can be cured with good treatment. However, a global epidemic may occur in such a situation that MDR- and XDR-TB is undiagnosed and treated with normal treatment, particularly for those people co-infected with HIV [10, 11]. Therefore, developing a rapid and accurate technique for the detection of *M. tuberculosis* is crucial to the control of TB. Nevertheless, current point-of-care (POC) detection method has some problems such as slow speed, low rate of accuracy and limited to active TB infection, leading to the inefficient reduction of TB-related deaths. As to the detection of TB, the technique should be fast, accurate, cheap and field-applicable. In addition, the discrimination between active TB and DR-TBs should be achieved in order to initiate effective treatment at the most common stage in diagnose. Since 2001, IFN-γ release assays in two formats composed of ELISA (*e.g.*, QuantiFERON®-TB, GFT) and enzyme-linked immunospot assay (ELISPOT, *e.g.*, T-SPOT® TB test) have been developed and implemented [12]. Specifically, the concentration of IFN-γ can be determined by QuantiFERON®-TB (including QFT-Gold and QFT-Gold In-Tube), and the number of IFN-γ that can produce antigen-specific T lymphocytes is determined by T-SPOT® TB test. The IFN-γ release assays was achieved by measuring the release of T cell by IFN-γ (host cellular immune response) under the stimulation of whole blood with MTB-specific antigens including culture filtrate protein 10 (CFP10), early-secreted antigenic target 6 (ESAT6) and TB7.7 (an additional antigen used in modified IGRA kits). The IFN-γ release assays method has some disadvantages. The distinguishment between people with active disease and LTBI are not effective, in despite that the affects by prior BCG vaccination can be avoided [13]. Moreover, the IFN-γ release assays are time-consuming (24 h) and expensive owing to the requirement of significant instrumentation and well-trained person. Electrochemical immunosensors have attracted numerous attention in the last decades owing to its simple operation, excellent sensitivity and high selectivity [14-17]. Because of the electrochemically inert of most antigen and antibody molecules, electrochemical impedance spectroscopy (EIS) has been employed for the direct determination of immunospecies [18, 19]. In comparision with assay methods, EIS method is cheaper because none additional materials (e.g., labeled antibodies) are required. Moreover, the non-destructive testing EIS can be employed for the detection of electrical properties of the biological interface [20]. Therefore, EIS have been widely used in a large number of biological studies [17, 21-30].

In this study, a novel electrochemical impedance immunosensor for the sensitive detection of IFN-γ was developed for the first time. The sensor was fabricated with ZnO modified electrode on which IFN-γ monoclonal antibody was immobilized. The concentration of IFN-γ was determined by detecting the change of impedance values after the specific reaction between IFN-γ antigen and IFN-γ

antibody. The sensitivity of as-prepared immunosensor was greatly improved by the usage of ZnO owing to its significant role as favourable platform for the load of proteins with high capacity. The as-prepared immunosensor demonstrated various advantages such as simple operation, low cost, label-free and high sensitivity, which is superior to the conventional assay. The immunosensor is demonstrated to be a potential method for the early diagnosis of TB infections.

2. EXPERIMENTS

2.1. Chemicals

Purified recombinant IFN-γ antigen and monoclonal IFN-γ antibody were supplied by Shandong agricultural research institute. Bovine serum albumin (BSA) and Nafion were purchased from Sigma Aldrich. Zinc nitrate hexahydrate, absolute ethanol, chloroform and hydrazine were supplied by Sinopharm Chemical Reagent Co. Ltd. Phosphate buffer saline (PBS buffer) with the concentration of 0.1 M and pH of 7.0 was prepared by mixing NaH_2PO_4 with Na_2HPO_4. All reagents are analytic reagents, and distilled water is used throughout the entire experiments.

2.2. Preparation of ZnO nanoparticles

To prepare the ZnO nanoparticles, 1 mL of hydrazine solution (2 wt%) was poured into 10 mL of zinc nitrate solution (50 mM), and then the slurry with gray color was stirred for 1 h. Subsequently, the mixed solution was transferred into a Teflon-lined stainless steel autoclave (30 mL) and heated at 140 °C for 4 h. The solution was treated with centrifugation and the collected ZnO nanoparticles were dried at 70 °C in an oven.

2.3. Preparation of IFN-γ immunosensor

Prior to use, the GCE electrode was pre-treated with the following procedures: polished with 0.03 and 0.05 μm alumina slurry, washed with distilled water thoroughly, sonicated in nitric acid-water mixture (v/v=1:1), ethanol and distilled water in sequence, and then dried in air. ZnO NPs suspension was prepared by adding 2.0 mg of ZnO NPs into 1.0 mL of distilled water, and the solution was treated with ultrasonic and stirring in order to achieve complete dissolution. Subsequently, 0.5 mL of monoclonal IFN-γ antibody solution (400 μg/mL) was added into 0.5 mL of as-prepared ZnO NPs suspension. The pre-treated GCE electrode was modified by dropping 5.0 μL of resulting mixture onto the surface of electrode, and then kept at 4 °C for 12 h. 5.0 μL of Nafion solution with the concentration of 1.0% was added to the surface of IFN-γ antibody/ZnO NPs/GCE electrode in order to hinder the leakage of IFN-γ antibody. BSA solution (10 mg/mL) was used for blocking the non-specific sites. The reaction occurred at 37.5 °C for 30 min. Finally, the IFN-γ immunosensor was washed three times with PBS solution. The fabricated immunosensor was stored at 4 °C before use.

For immunosensor fabrication, each electrode was denoted as bare GCE, ZnO/GCE, anti-IFN-γ/ZnO/GCE, BSA/anti-IFN-γ/ZnO/GCE and IFN-γ/BSA/anti-IFN-γ/ZnO/GCE.

2.4. Immunoassay measurement

A CHI 660D electrochemical workstation (CH Instruments, Shanghai, China) with conventional three-electrode system (bare or modified glassy carbon electrode (GCE) with diameter of 3 mm, calomel electrode (SCE) and platinum wire as working, reference and auxiliary electrodes, respectively) was employed for all electrochemical experiments. Cyclic voltammetry and EIS experiments were performed with PBS solution containing 0.1 M KCl and 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ as electrolyte and 100 mV/s as scan rate. As to EIS experiments, the amplitude of applied potential was 10 mV and the frequency ranges from 10^{-1} to 10^5 Hz.

3. RESULTS AND DISCUSSION

Cyclic voltammetric experiments were perfomed to study the electrochemical behaviour of electrode after each assembly step. CV experiments of $Fe(CN)_6^{3-/4-}$ were carried out at bare GCE, ZnO/GCE, anti-IFN-γ/ZnO/GCE, BSA/anti-IFN-γ/ZnO/GCE and IFN-γ/BSA/anti-IFN-γ/ZnO/GCE, respectively, and the results were shown in Fig. 1. Specifically, PBS solution (0.1 M, pH 7.0) containing $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (5.0 mM) and KCl (0.1 M) was used as electrolyte, and 50 mV/s as scan rate. It was found that the redox currents obtained on GCE electrode after stepwise modifications showed a gradual decrease, indicating that the electron transfer from $Fe(CN)_6^{3-/4-}$ probe to the surface of electrode was greatly hindered. These changes are attributed to an increased interfacial concentration of the anionic probe ($[Fe(CN)_6]^{3-/4-}$) due to its strong affinity toward the polycationic (NH^{3+}) layer of the amino groups of the BSA [31]. Particularly, the current of cathonic and anodic peaks showed an obvious decrease at electrodes modified with IFN-γ antibody or BSA, indicating the significant inhibition of electron transfer.

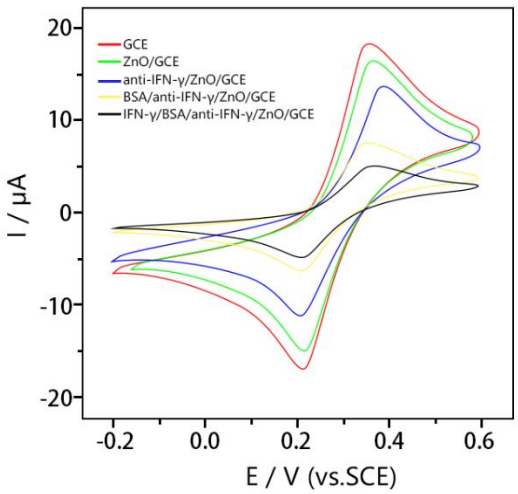

Figure 1. Cyclic voltammograms of $Fe(CN)_6^{3-/4-}$ at bare GCE, ZnO/GCE, anti-IFN-γ/ZnO/GCE, BSA/anti-IFN-γ/ZnO/GCE and IFN-γ/BSA/anti-IFN-γ/ZnO/GCE.

Fig. 2 showed the detailed process for detecting IFN-γ. Firstly, the prepared IFN-γ immunosensor was incubated in IFN-γ antigen solution with different concentrations at 37.5 °C for 80 min, and then rinsed with PBS throughly. The prepared ZnO and immunosensor were characterized with different means. The results indicated that ZnO provided a hydrophilic, favorable and high capacity platform for loading of proteins, which thus improves the sensitivity of the resultant immunosensor [32]. Then EIS experiments were performed in 0.1 M KCl and 0.1 M PBS (pH 7.0) solution containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$, and the change of the impedance of immunosensor was recorded.

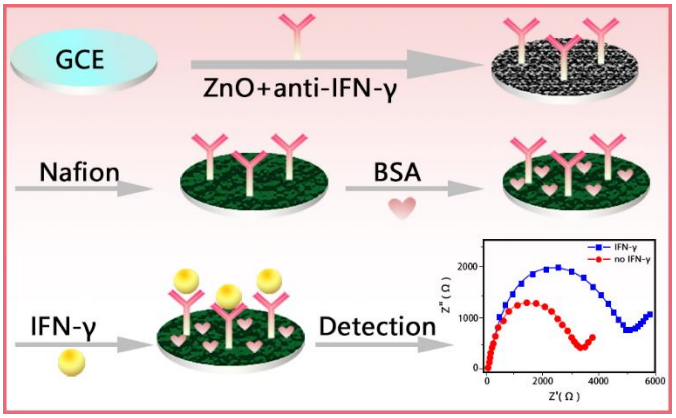

Figure 2. Schematic illustration of the immunoassay process for detecting IFN-γ by fabricated immunosensor.

In general, the change of impedance during the modification process was measured by EIS experiments. The Faradaic impedance spectra achieved in each modification process were shown in Fig. 3 A. The diameter of semicircle calculated from the Nyquist plot corresponds to the electron transfer resistance (R_{ct}). As can be deduced from the impedance spectrum obtained at bare GCE, the transfer resistance was low owing to the very small semicircle. The R_{ct} value showed a slight increase when GCE was modified with ZnO NPs, indicating the successful formation of ZnO NPs on the surface of GCE. Obviously, R_{ct} showed a great increase for anti-IFN-γ/ZnO/GCE, suggesting that IFN-γ antibody was successfully loaded on the surface of ZnO NPs modified GCE. R_{ct} further increased after the block treatment to anti-IFN-γ/ZnO/GCE with BSA. In contrast, the linear portion coinciding with the diffusion limited electron shift occurred at comparatively lower frequencies. It can be proposed that the spectra were similar to those of Randle's equivalent circuit in theory [33, 34]. When the fabricated immunosensor was used for the detection of IFN-γ antigen, R_{ct} was found to increase furtherly, which could be ascribed to the significant hinder effect of formed protein layer for the diffusion of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ probe toward the surface of electrode.

FRA software of Autolab was used to process impedance data on the base of Randles equivalent circuit. Fig. 3B showed a modified Randles equivalent circuit and the corresponding fitted graph based on one measured spectrum, indicating that the circuit model was consistent with the measured system over the whole measured frequency range. The circuit consists of electron transfer

resistance (R_{ct}), solution resistance (R_s), Warburg impedance (Z_W), and interfacial double layer capacitance (C_{dl}) between electrode and solution. Usually, R_s represents the bulk properties of electrolyte solution, and Z_W represents the diffusion feature of the redox probe in solution. Both R_s and Z_W are constant regardless of the electrode surface. The impedance data was fitted to Randles equivalent circuit with a constant phase element (CPE) instead of classical capacitance due to the complicated interface between electrode and electrolyte in this study. The result also indicated that R_{ct} is a suitable signal for sensing the interfacial properties of the prepared immunosensor during all these modification steps.

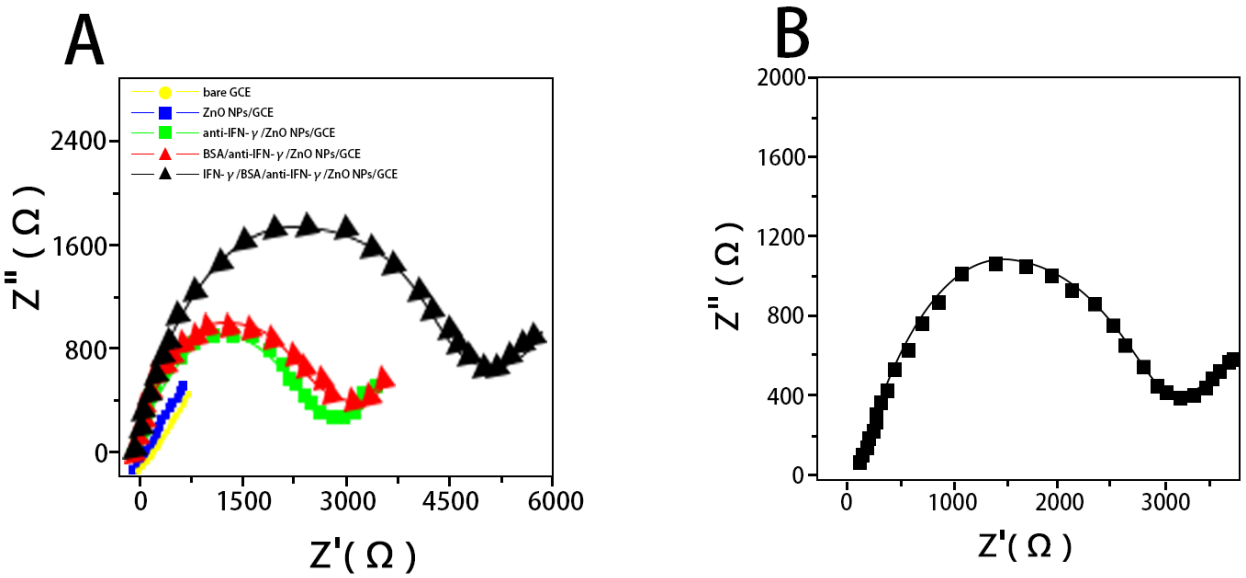

Figure 3. (A) Nyquist plots obtained on bare GCE, ZnO NPs/GCE, anti-IFN-γ/ZnO NPs/GCE, BSA/anti-IFN-γ/ZnO NPs/GCE and IFN-γ/BSA/anti-IFN-γ/ZnO NPs/GCE. (B) Experimental and fitted Nyquist plots of impedance spectra.

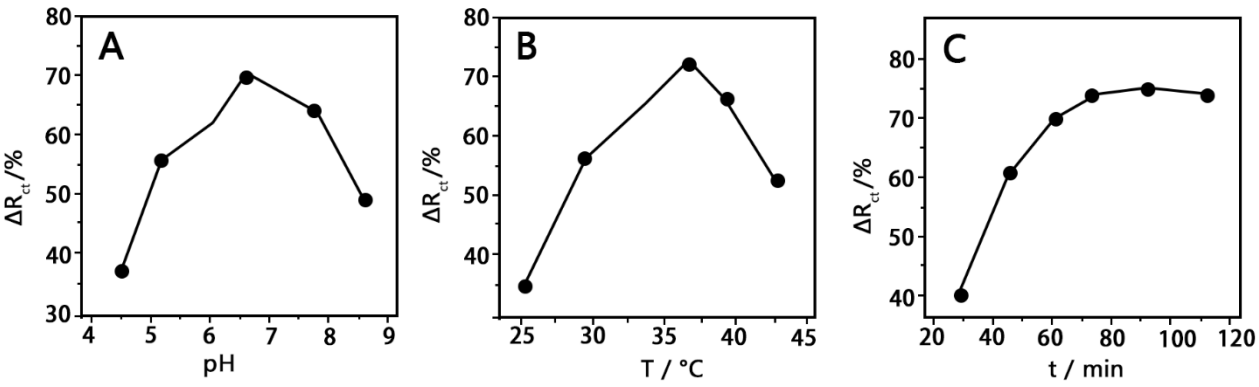

Figure 4. Effect of (A) solution pH, (B) incubation temperature and (C) time on the change of R_{ct} values with the concentration of IFN-γ being 0.01 ng/mL.

R_{ct} will change as the interface properties of electrodes change due to the load of target proteins on the surface of ZnO NPs modified GCE. Non-Faradaic impedance biosensors perform impedance measurement in the absence of any redox probe. Bacteria detection is based on the impedance change upon the attachment of bacterial cells on an interdigitated microelectrode in the absence of any redox probe in the sample solution. Therefore, various factors including incubation time, temperature and pH of the solution that affect antibody-antigen interaction should be optimized. Firstly, the influence of pH on R_{ct} values was investigated with the concentration of IFN-γ being 0.01 ng/Ml and the results were shown in Fig. 4A. It was found that ΔR_{ct} value first increased then decreased within pH range of 4.0-9.0, and the maximum ΔR_{ct} value was obtained at pH of 7.0. Thus, pH of 7 was chosen for the immunoreaction. The effect of incubation temperature within range of 25-45 °C on R_{ct} values was examined with the concentration of IFN-γ being 0.01 ng/mL as well. As can be seen from Fig. 4B, 37.5 °C was the optimum incubation temperature with maximal change of R_{ct}. In addition, Fig. 4C showed the effect of incubation time on ΔR_{ct} values with the concentration of IFN-γ being 0.01 ng/mL. The electrochemical response increased until reached the plateau at 80 min with increasing incubation time. In conclusion, the obtained optimum parameters for immunoassay of IFN-γ were as follows: pH value 7.0, incubation temperature 37.5 °C and time 80 min.

The performance of fabricated immunosensor for the determination of IFN-γ at different concentrations was observed and the results were shown in Fig. 5. As shown from the Nyquist plots of impedance spectra (Fig. 5A), the diameter of semicircle increased with increasing concentrations of the IFN-γ. As can be seen from the calibration curve (Fig. 5B) of IFN-γ, the R_{ct} change displayed a linear relationship with logarithm of IFN-γ ranging from 0.0001 to 0.1 ng/mL. The detection limit was calculated to be 0.12 pg/mL (S/N=3). The sensing performance of the proposed sensor was compared with recently reported sensors, as shown in Table 1.

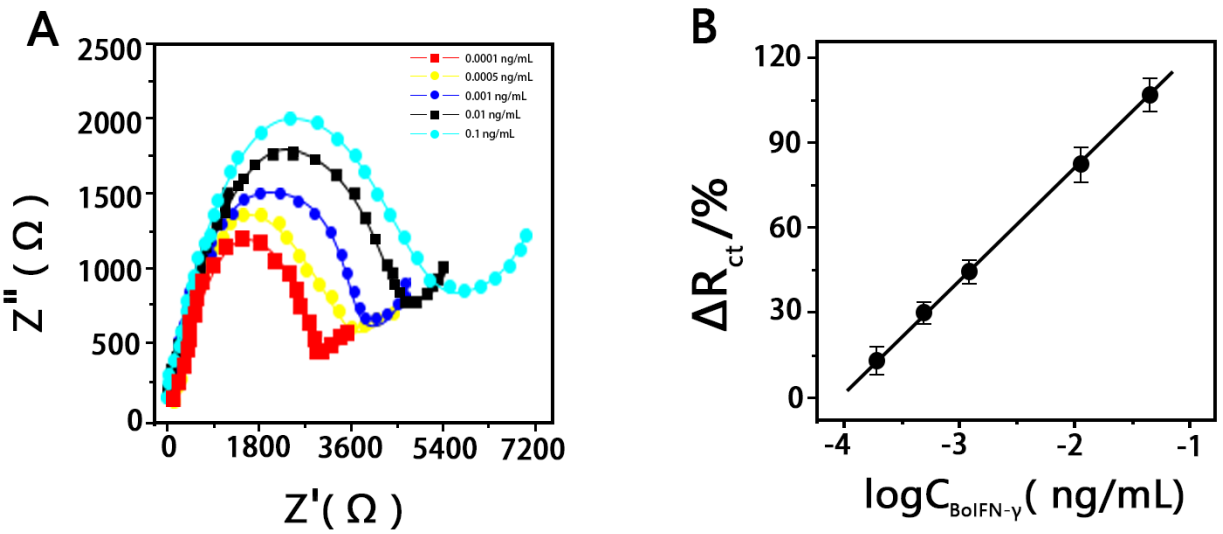

Figure 5. (A) Impedance spectra of the as-prepared immunosensor upon incubated with different concentrations of IFN-γ (0.0001, 0.0005, 0.001, 0.01 and 0.1 ng/mL). (B) Calibration curve for IFN-γ.

For the sake of evaluating the reproducibility of as-proposed IFN-γ immunosensor, intra- and inter-assay coefficients of variation (CV) was measured at the concentration of IFN-γ being 0.01 ng/mL. The intra-assay CV for six replicative measurements was 4.1%, indicating the excellent reproducibility of IFN-γ immunosensor. When six immunosensors fabricated in six batches were employed, the inter-assay CV was 3.9%, suggesting the good fabrication reproducibility of IFN-γ immunosensor. In addition, no obvious change of R_{ct} values was found after its storage at 4 °C in refrigerator for 60 days, indicating the excellent microenvironment supplied by ZnO NPs for retaining the bio-activity of immobilized antibody.

Table 1. Performance comparison of the IFN-γ/BSA/anti-IFN-γ/ZnO/GCE and other IFN-γ determination methods.

Method	Linear range	Detection limit	Reference
Micropatterned aptamer-modified electrodes	0.05-0.2 ng/mL	4 pg/mL	[35]
Enzyme-linked immunospot assay	—	—	[36]
Electron paramagnetic resonance	0.05-20 ng/mL	—	[37]
IFN-γ/BSA/anti-IFN-γ/ZnO/GCE	0.0001-0.1 ng/mL.	0.12 pg/mL	This work

The recovery performance of developed IFN-γ immunosensor was investigated by spiking IFN-γ at different concentrations (0.0002, 0.0005, 0.001, 0.005 and 0.01 ng/mL) in bovine serum samples. A BD™ Cytometric Bead Array (CBA) Human IFN-γFlex Set (Bead B8) was used for comparison. As can be seen from the obtained recoveries (Table 2), the fabricated immunosensor demonstrated outstanding accuracy for label-free detection of IFN-γ.

Table 2. Recoveries of as-proposed immunosensor for the detection of IFN-γ.

Sample	Added (ng/mL)	Found (ng/mL)	Recovery (%)	RSD (%)	IFN-γ ELISA kit
1	0.0005	0.00051	102	3.2	0.00052
2	0.001	0.00107	107	1.9	0.00105
3	0.005	0.00496	99.2	2.2	0.00494
4	0.01	0.01041	104.1	4.0	0.01043

4. CONCLUSIONS

In conclusion, an electrochemical impedance immunosensor for label-free detection of IFN-γ was developed for the first time. The as-proposed immunosensor was fabricated using ZnO NPs modified GCE on which IFN-γ monoclonal antibody was immobilized. The resultant IFN-γ immunosensor demonstrated various advantages such as simple manipulation, ultrahigh sensitivity, excellent specificity, wide linear range, good reproducibility and stability as well. As indicated by the recovery experiments, the proposed immunosensor with high accuracy demonstrated potential usage in the detection of IFN-γ in practical samples.

ACKNOWLEDGEMENT
The work was supported by National Natural Science Foundation of China (81673160) and The Xinjiang Uygur Autonomous Region Natural Science Foundation (2016D01B015).

References

1. M. Espinal, S. Kim, P. Suarez, K. Kam, A. Khomenko, G. Migliori, J. Baéz, A. Kochi, C. Dye and M. Raviglione, *Jama*, 283 (2000) 2537.
2. A. Pablos-Méndez, M. Raviglione, A. Laszlo, N. Binkin, H. Rieder, F. Bustreo, D. Cohn, C. Lambregts-van Weezenbeek, S. Kim and P. Chaulet, *N. Engl. J. Med.*, 338 (1998) 1641.
3. S. Dooley, W. Jarvis, W. Marione and D. Snider, *Annals of Internal Medicine*, 117 (1992) 257.
4. S. Sharma and A. Mohan, *Chest Journal*, 130 (2006) 261.
5. C. Chiang, R. Centis and G. Migliori, *Respirology*, 15 (2010) 413.
6. P. Farnia, M. Masjedi, M. Merza, P. Tabarsi, G. Zhavnerko, T. Ibrahim, H. Kuan, J. Ghanavei and P. Farnia, *Int J Clin Exp Med*, 3 (2010) 308.
7. G. Migliori, G. De Iaco, G. Besozzi, R. Centis and D. Cirillo, *Euro surveill*, 12 (2007) E070517.
8. S. Parida, R. Axelsson-Robertson, M. Rao, N. Singh, I. Master, A. Lutckii, S. Keshavjee, J. Andersson, A. Zumla and M. Maeurer, *Journal of Internal Medicine*, 277 (2015) 388.
9. A. Velayati, M. Masjedi, P. Farnia, P. Tabarsi, J. Ghanavi, A. ZiaZarifi and S. Hoffner, *Chest Journal*, 136 (2009) 420.
10. T. Frieden, L. Sherman, K. Maw, P. Fujiwara, J. Crawford, B. Nivin, V. Sharp, D. Hewlett, K. Brudney and D. Alland, *Jama*, 276 (1996) 1229.
11. N. Gandhi, N. Shah, J. Andrews, V. Vella, A. Moll, M. Scott, D. Weissman, C. Marra, U. Lalloo and G. Friedland, *American Journal of Respiratory and Critical Care Medicine*, 181 (2010) 80.
12. G. Mazurek, J. Jereb, P. LoBue, M. Iademarco, B. Metchock and A. Vernon, *MMWr Recomm rep*, 54 (2005) 49.
13. K. Dheda, R. van Zyl Smit, M. Badri and M. Pai, *Current Opinion in Pulmonary Medicine*, 15 (2009) 188.
14. M. Yang, A. Javadi and S. Gong, *Sensors and Actuators B: Chemical*, 155 (2011) 357.
15. G. Lai, F. Yan and H. Ju, *Anal. Chem.*, 81 (2009) 9730.
16. X. Li, X. Yang and S. Zhang, *TrAC Trends in Analytical Chemistry*, 27 (2008) 543.
17. L. Wang, J. Lei, R. Ma and H. Ju, *Anal. Chem.*, 85 (2013) 6505.
18. X. Chen, Y. Wang, J. Zhou, W. Yan, X. Li and J. Zhu, *Anal. Chem.*, 80 (2008) 2133.
19. X. Li, L. Dai, Y. Liu, X. Chen, W. Yan, L. Jiang and J. Zhu, *Adv Funct Mater*, 19 (2009) 3120.
20. H. Chen, J. Jiang, Y. Huang, T. Deng, J. Li, G. Shen and R. Yu, *Sensors and Actuators B: Chemical*, 117 (2006) 211.
21. B. Derkus, E. Emregul, C. Yucesan and K. Emregul, *Biosensors and Bioelectronics*, 46 (2013) 53.
22. R. Elshafey, C. Tlili, A. Abulrob, A. Tavares and M. Zourob, *Biosensors and Bioelectronics*, 39 (2013) 220.
23. A. Montrose, S. Cargou, F. Nepveu, R. Manczak, A. Gué and K. Reybier, *Biosensors and Bioelectronics*, 49 (2013) 305.
24. Y. Wang, J. Ping, Z. Ye, J. Wu and Y. Ying, *Biosensors and Bioelectronics*, 49 (2013) 492.
25. J. Ma, Y. Luo, R. Packard, T. Ma, Y. Ding, P. Abiri, Y. Tai, Q. Zhou, K. Shung and R. Li, *Sensors and Actuators B: Chemical*, 235 (2016) 154.
26. R. Packard, X. Zhang, Y. Luo, T. Ma, N. Jen, J. Ma, L. Demer, Q. Zhou, J. Sayre and R. Li, *Annals of Biomedical Engineering*, 44 (2016) 2695.
27. W. Weng, C. Jhou, H. Xie and T. Pan, *Journal of The Electrochemical Society*, 163 (2016) B710.
28. X. Liu, M. Marrakchi, D. Xu, H. Dong and S. Andreescu, *Biosensors and Bioelectronics*, 80 (2016) 9.

29. P. Norouzi, H. Haji-Hashemi, B. Larijani, M. Aghazadeh, E. Pourbasheer and M. R Ganjali, *Current Analytical Chemistry*, 13 (2017) 70.
30. A. Ahammad, A. Al Mamun, T. Akter, M. Mamun, S. Faraezi and F. Monira, *Journal of Solid State Electrochemistry*, 20 (2016) 1933.
31. J. Zhao, C. Bradbury, S. Huclova, I. Potapova, M. Carrara and D. Fermín, *J Phys Chem B*, 109 (2005) 22985.
32. T. Ramalingam, R. Gieseck, T. Acciani, K. Hart, A. Cheever, M. Mentink-Kane, K. Vannella and T. Wynn, *The Journal of Pathology*, 239 (2016) 344.
33. R. Chand, D. Han, S. Neethirajan and Y. Kim, *Sensors and Actuators B: Chemical*, 248 (2017) 973.
34. M. Huang, H. Li, H. He, X. Zhang and S. Wang, *Analytical Methods*, 8 (2016) 7413.
35. Y. Liu, T. Kwa and A. Revzin, *Biomaterials*, 33 (2012) 7347.
36. T. Taguchi, J. McGhee, R. Coffman, K. Beagley, J. Eldridge, K. Takatsu and H. Kiyono, *Journal of Immunological Methods*, 128 (1990) 65.
37. C. Pellat, Y. Henry and J. Drapier, *Biochemical and Biophysical Research Communications*, 166 (1990) 119.