Article

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An Innovative Glucose Biosensor Using Antibiofouling Au-F127 Nanospheres

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Quantification of the blood glucose concentration in the whole blood was not easy to achieve because the detection process was affected by many factors, such as glucose metabolism and biofouling. In this paper, we established an amperometric glucose biosensor applied in whole blood directly, which was based on the direct electron transfer of glucose oxidase (GOx) entrapped onto the Au-F127 nanospheres. Here, the Au-F127 nanospheres could provide a blood compatible surface with antifouling property for determination of glucose in whole blood. The cyclic voltammetric results indicated that GOx immobilized on the Au-F127 nanospheres exhibited direct electron transfer reaction, and the cyclic voltammogram (CV) displayed a pair of well-defined and nearly symmetric redox peaks with a formal potential of 93 mV. The biosensor had good electrocatalytic activity toward glucose with a low detection limit 3.15 pM. The glucose biosensor did not respond to ascorbic acid (AA) and uric acid (UA) at their high concentration encountered in blood. In this method, the biosensor was used for quantification of the concentration of glucose in whole blood samples. The data obtained from the biosensor showed good agreement with those from a biochemical analyzer in hospital.

KEYWORDS: *Whole Blood, Au-F127 Nanospheres, Biosensors, Glucose Oxidase.*

INTRODUCTION

Diabetes mellitus is a worldwide public health problem. Thus, millions of diabetics test their blood glucose levels daily, making glucose the most commonly tested analyte.[1] At present, primary methods of detecting blood glucose concentration are performed by biochemical analyzer and glucose meter.[2–4] For biochemical analyzer, the quantification of the concentration of glucose is mainly involved in serum samples, which are isolated from whole blood by centrifugation, but not in untreated whole blood. The test results are influenced by the different model numbers of test instruments and detection reagents, treatment processes of blood samples, factitious operations, especially additional centrifuge and too long measure time from collecting specimen of blood to examination. As for commercial glucose meter, there are some defects that can not be ignored during its operation. For example, the blood samples are obtained from fingertip peripheral but not vein, and doped easily with tissue fluid. So the accurate results of glucose concentration can not be provided by commercial glucose meter. At present, it is very difficult to design and prepare a electrochemical biosensor that can be used in whole blood directly because the biofouling of electrode surface can be developed by platelet, fibrin and blood cell adhesion in the complex environment such as whole blood media. And the biofouling of eletrode surface will bring catastrophic damage to the electron transfer between enzyme and electrode redox center. So the development of novel glucose biosensors for antifouling, rapid, highly sensitive, and selective detection is of paramount importance for blood glucose concentration monitoring in whole blood samples.

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Recent advances in nanotechnology have provided a variety of nanoparticles with highly controlled shapes, sizes, and interesting properties. These nanoparticles can bring new and unique capabilities to a variety of possible applications.[5–28] Pluronic F127 (triblock copolymer $PEO_{106}PPO_{70}PEO_{106}$) can be used as template for preparing of nanoparticles with special shapes.[29, 30] In addition, it also has good blood compatibility.[31, 32] The synthesis and blood compatibility of the rambutan-like hybrid nanospheres of Au-F127 have been reported by our group.[33] Whether we can take advantage of the cooperation effects mixing good blood compatibility and electronic conductive property of the Au-F127 nanospheres to design and prepare a novel glucose biosensor that can be used directly for whole blood samples? It is the subject of this research work.

Here, the mixture of glucose oxidase (GOx) and the Au-F127 nanospheres was immobilized on the surface of glass carbon electrode (GCE) to construct a novel enzyme biosensor. The Au-F127 nanospheres were chosen to modify the GCE for the anti-biofouling property and the fast electron transfer between GOx and GCE that due to the good blood compatibility of F127 and superior electric performance of Au nanospheres. It is worth mentioning that the antibiofouling effect of the surface of GCE was obtained that resulted from the blood compatibility of Au-F127 nanospheres because of the coherence of between blood compatibility and antifouling property.

Nowadays, novel needle sensors that try to be implanted intravascularly or subcutaneously were reported for glucose measurement. The major difficulties related to sensor stability due to blood clot and embolism are circumvented. Anti-sticking coatings were suggested and constructed on the surface of devices to prevent biofouling.[34, 35] Our researches focus on the electrode surface of glucose biosensor that modified by antibiofouling nanomaterial, which really makes a big difference from others.

The preparation of GOx/(Au-F127)/GCE biosensor was presented, and the electrochemical behavior of the biosensor immersing in whole blood was investigated in detail. In summary, we established a direct, sensitive, reliable method which has characters of inhibiting glucose metabolism and antibiofouling of electrode surface for the determination of blood glucose concentration in whole blood.

EXPERIMENTAL DETAILS

Materials

Pluronic F127 (Sigma-Aldrich Co., US) was used without further purification. Hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9%) was obtained from Alfa Aesar, a Johnson Matthey Company. GOx was purchased from Sigma-Aldrich (USA), β-D-(+)-glucose (99%) was obtained from J&K Chemical Co. Inc. (China). Phosphate buffer solution (PBS) was prepared by mixing stock standard solution of Na_2HPO_4 and NaH_2PO_4. All solutions were made up with twice-distilled water. Other reagents were of analytical grade.

Synthesis of Au-F127 Hybrid Nanospheres

The preparation method of Au-F127 hybrid nanospheres was described in the Ref. [33]. The simple synthetic process was as follows: 0.01 g $HAuCl_4$ was dissolved in 100 mL double-distilled water, then 0.75 g of Pluronic F127 powder was added to the freshly prepared $HAuCl_4$ aqueous solution. Then the mixtures were stirred for overnight at room temperature.

Characterization

Transmission electron microscopy (TEM) images were obtained by a transmission electron microscopy (JEOL-2000F, JEOL Co. Ltd., Japan). The existence of Au-F127, GOx and GOx/(Au-F127) were measured using on an UV-vis spectrophotometer (Cary 50 Conc., Australia). The spectra of Au-F127 was proved by fourier transform infrared (FTIR, Nicolet 170sx, Bruker, Germany) spectrum.

Coagulation Tests and Whole Blood Adhesion Tests of Au-F127 Hybrid Nanospheres

In order to determine the anticoagulation property of the materials, coagulation tests and whole blood adhesion studies were conducted. The coagulation tests including activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) of Au-F127 nanospheres have been investigated by our group.[31]

Whole blood adhesion tests are one of the most important steps during blood coagulation on artificial surface. The blank GCE modified with 1 nm gold nanoparticles by the help of Au-spraying device and GOx/(Au-F127) hybrid modified GCE were equilibrated with PBS (pH 7.4) for 24 h. Whole Blood that added anticoagulant was prewarmed to 37 °C and added after removing the PBS solution. After 60 min incubation at 37 °C, the GCEs were washed three times with PBS by mild shaking (fresh PBS each time) to remove non-adherent blood cells and platelets. The GCEs were then soaked in 2.5% glutaraldehyde for 30 min to fix the adhered blood cells and platelets. After washing with PBS, the blood cells and platelets adhering to the films were dehydrated in an ethanol grade series (50, 60, 70, 80, 90, 95 and 100%) for 30 min each and allowed to dry at room temperature. Then the GCEs were gold deposited in vacuum and examined by SEM (JSM Model 6300 scanning electron microscope, JEOL, Japan).

Construction of the GOx/(Au-F127) Modified Electrode

Electrochemical experiments were performed on a CHI760D electrochemical workstation (Chenhua, Shanghai, China) in a three-electrode configuration. A saturated calomel electrode (SCE) and a platinum electrode served as reference and counter electrode, respectively. All potentials given below were relative to the SCE. The working electrode was a modified GCE. Cyclic voltammogram (CV) experiments were carried out in quiescent solution at 100 $mV \cdot s^{-1}$ in 5 mL of 0.1 M PBS, and the solution was purged with high purity nitrogen prior to and blanked with nitrogen during the electrochemical experiments.

Prior to the modification, the GCE was polished to mirror-like surface with 0.3 and 0.05 μm alumina slurry followed by rinsing thoroughly with double-distilled water, and was successively sonicated in ethanol and double-distilled water for 5 min, respectively. The cleaned electrode was dried with a stream of nitrogen immediately before use. Then, 8.0 μL Au-F127 hybrid nanospheres solution was dropped onto the GCE surface and dried in the air. The pretreated GCE was cast with 8.0 μL of 6.0 $mg \cdot mL^{-1}$ GOx solution and dried in room temperature. In this way, the GOx/(Au-F127)/GCE was obtained.

RESULTS AND DISCUSSION

Characterization of Hybrid Nanoparticles of Au-F127

TEM photograph of the Au-F127 nanospheres showed the diameter in the range of 160–180 nm (Fig. S1). To confirm the formation of the Au-F127 hybrid nanospheres, the electron diffraction pattern was investigated. As shown in Figure S1(a), the diffraction pattern indicated this material was polycrystalline. A clear surface plasmon resonance (SPR) band was observed around 500 nm to 600 nm in the UV-vis absorption spectrum of Au-F127 hybrid nanospheres, which was characteristic for Au nanoparticles. FTIR was used to study the chemical structure of the hybrid nanospheres. The broad intense band of the Pluronic F127 were obtained at the 1103.4 cm^{-1}, which is distinctive of the helical structure of PEO (Fig. 1(A)).[36] A broad band was also observed at 3420.8 cm^{-1} that corresponding to the stretching vibration of the terminal hydroxyl groups in F127.[33] These results indicated that F127 had been introduced to Au-F127 nanospheres.

UV-vis absorption spectra can provide information of the micro-structural changes of enzyme. As shown in Figure 1(B), the Soret absorption bands at 380 nm and 450 nm of the native GOx solution (curve (a)) were observed.[37] Au-F127 nanocomposites had a peak from 500 nm to 600 nm which was attributed to Au, and the broad range was due to the surface plasmon coupling

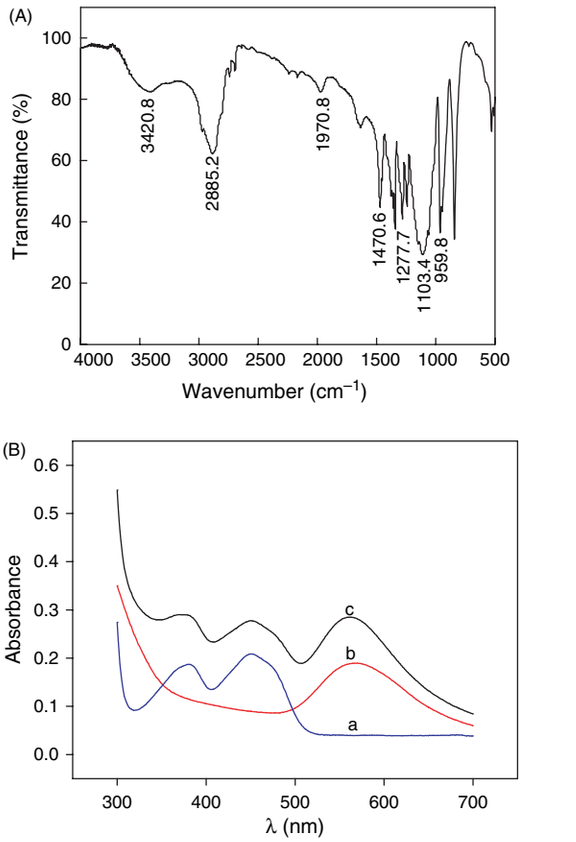

Figure 1. (A) FTIR spectrum of Au-F127. (B) UV-vis adsorption spectra of (a) GOx, (b) Au-F127, and (c) GOx/(Au-F127) film.

between closely spaced nanoparticles of Au-F127 nanospheres (curve (b)).[38] For the GOx/(Au-F127) (curve (c)), besides the same peak derived from Au, the two absorption peaks appeared at 379 nm and 449 nm, which were similar to the data of the native GOx solution. The small difference proved that the native structure of GOx was retained in the presence of Au-F127 nanospheres.[39]

Characterization of *In Vitro* Anticoagulation Properties and Whole Blood Adhesion Tests of the Au-F127 Hybrid Nanoparticles

Blood plasma APTT, PT, and TT test are commonly used to evaluate the *in vitro* anticoagulation properties of different biomaterials.[40,41] Data of APTT, PT, and TT for Au-F127 nanospheres were shown in our previous researches.[33] The results showed the Au-F127 nanospheres have anticoagulation property that attributed to long PEO chains of F127.[31–33]

The anti-biofouling property of the Au-F127 nanospheres was also evaluated by whole blood adhesion tests. And the results were observed by SEM (Fig. 2). The blank GCE modified with gold nanoparticles showed high blood cells and platelets adhesion (Fig. 2(A)). But to

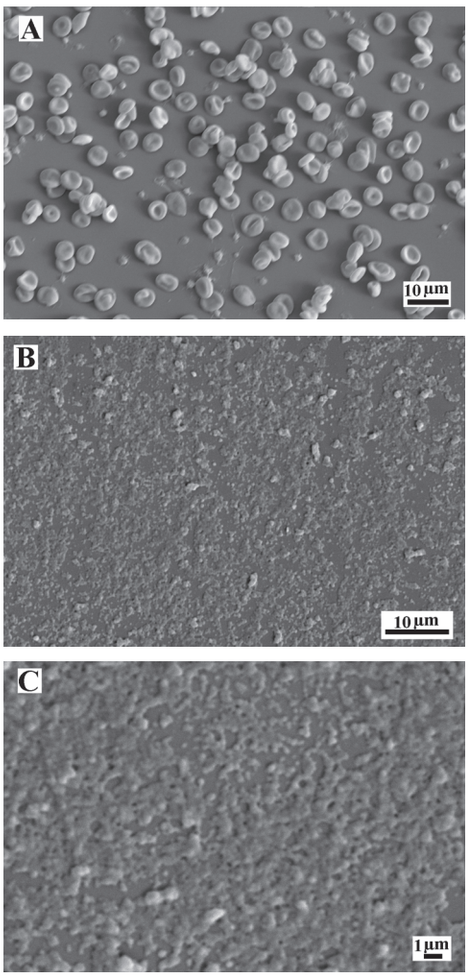

Figure 2. **SEM images of (A) blank electrode substrate modified with gold nanoparticles, (B) electrode substrate modified with GOx/(Au-F127), and (C) enlarge view from (B) exposed to human whole blood for 60 min, respectively.**

be surprised, the surface of GCE with GOx/(Au-F127) has nearly no blood cells and platelets adhered. The results of whole blood adhesion test revealed that Au-F127 nanospheres showed excellent anti-biofouling property (Figs. 2(B) and (C)). This tendency was attribute to the hydrated F127 chains attached on a surface which probably influences the microthermodynamics at the solution/surface interface and prevents adhesion of blood cells and fibrins. Further, Au-F127 nanospheres might provide a desirable microenvironment for GOx to undergo facile electron-transfer reactions.

Direct Electron Transfer Reactivity of the GOx/(Au-F127)/GCE

Cyclic voltammetry was used to evaluate the electrochemical performance of the electrodes. Figure 3(A) showed CVs of the (Au-F127)/GCE (curve (a)), GOx/F127/GCE (curve (b)) and GOx/(Au-F127)/GCE (curve (c)) and

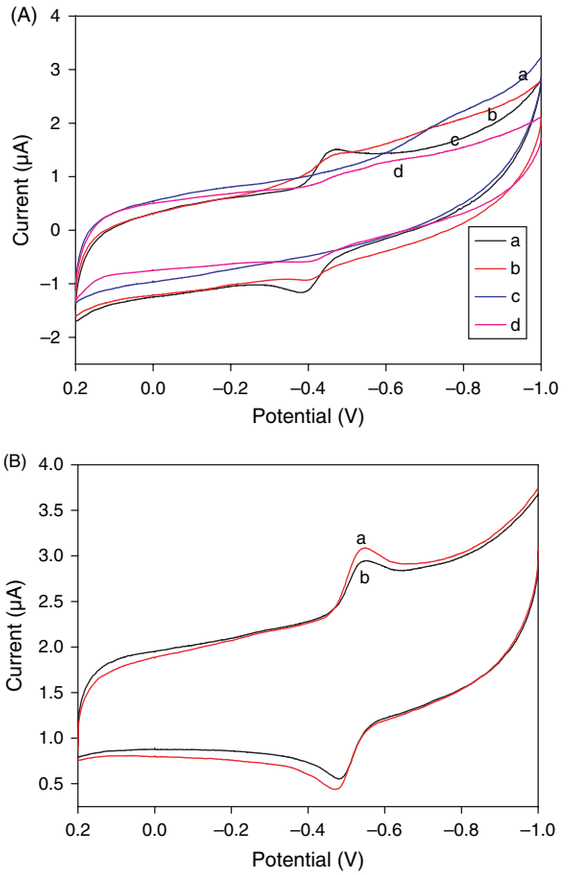

Figure 3. **(A) CVs of (a) bare (Au-F127)/GCE, (b) GOx/F127/GCE, (c) GOx/(Au-F127)/GCE, and (d) GOx/Au/GCE; (B) CVs of GOx/(Au-F127)/GCE. (a) Freshly prepared and (b) after 2 weeks stored in 0.1 M PBS (pH 7.4). Scan rate was 100 mV $\cdot$ s^{-1}.**

GOx/Au/GCE (curve (d)) in PBS (pH = 7.4) at 100 mV $\cdot$ s^{-1}. Obviously, from curve (a) and curve (c), we could get the conclusion that the response of the GOx/(Au-F127)/GCE was attributed to the redox of the electroactive center of GOx on the electrode surface. In the absence of Au or F127, the GOx/F127/GCE (curve (b)) and GOx/Au/GCE (curve (d)) could also display a couple of redox peaks of GOx. However, the response was smaller than that of the GOx/(Au-F127)/GCE (curve (c)), indicating that the presence of Au-F127 nanospheres played an important role in facilitating the electron exchange between GOx and the electrode. In addition, the anodic (E_{pa}) and cathodic (E_{pc}) peak potential were detected (scan rate of 100 mV $\cdot$ s^{-1}) at -0.383 V and -0.476 V, respectively. The ratio of anodic to cathodic peak currents was about 0.80, which indicated that GOx underwent a quasi-reversible redox process (Fe(III)/Fe(II) redox couple) on the surface of Au-F127 nanospheres modified GCE. The separation of peak potentials (ΔE_p) was 93 mV, which indicated that GOx immobilized on the surface of Au-F127 nanospheres displayed a quasi-reversible electrochemical reaction.

Figure 3(B) showed the CVs of freshly prepared GOx/(Au-F127)/GCE (curve (a)) and the GOx/(Au-F127)/GCE stored in PBS of pH 7.4 at 4 °C after 2 weeks (curve (b). Obviously, we could get the conclusion that the biosensor still remains most of its initial sensitivity after 2 weeks.

Anti-Interference of GOx/(Au-F127)/GCE Biosensor

In whole blood, there are some coexisting electroactive species such as ascorbic acid (AA), uric acid (UA) may affect the biosensors response.[42] The selectivity and anti-interference advantages of the biosensor were demonstrated (Fig. 4). The response of the biosensor was examined in the presence of different interferences with a glucose concentration of 0.1 mM. Both of the human physiological normal levels of UA and AA are about 0.2 mM.[42] Here, in the presence of AA (10 mM) and UA (10 mM), no changes in the response of the biosensor were found. Hence, a highly selective response to glucose was obtained without the use of perm-selective membrane or enzymatic preoxidation.

Effects of Sodium Fluoride in Whole Blood

While the glucose detection is finished in whole blood, but glucose metabolism by blood cells is prior to its determination. The concentrations of glucose in whole blood decrease with time due to glycolysis.[43, 44] To minimize glycolysis, blood should be collected in a tube containing heparin, immediately placed on ice and the cells should be separated from plasma within 60 min. However, glycolysis can be attenuated by inhibition of enolase with sodium fluoride.[44] The glucose concentration of whole blood is stable for 72 h at room temperature in the presence of fluoride.[45] The electrochemical measurements for glucose in whole blood were compared with the current value obtained in the absence and presence of sodium fluoride.

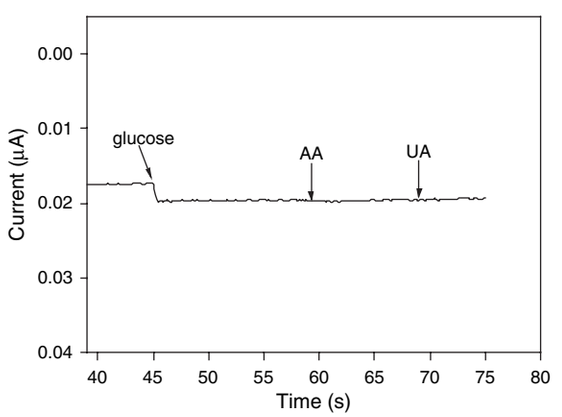

Figure 4. Amperometric responses of the biosensor upon additions of glucose (0.1 mM), AA (10 mM), and UA (10 mM), in PBS. The biosensor was biased on the potential of −0.4 V.

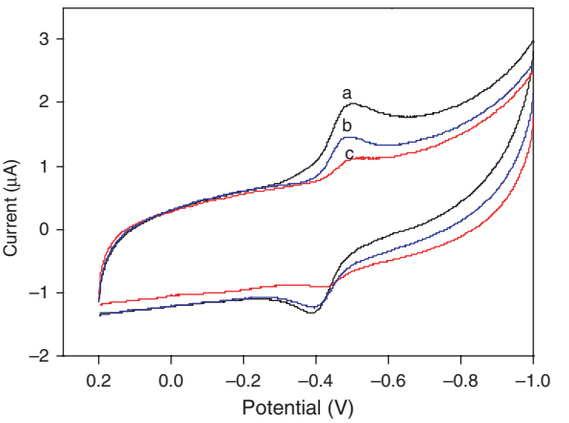

Figure 5. CVs of (a) measure in fresh whole blood sample, (b) measure after 72 h in the presence of sodium fluoride, and (c) measure after 4 h in the absence of sodium fluoride. Scan rate was 100 mV · s^{-1}.

The results of CV measurements were shown in Figure 5. Compared with the value of real-time quantitative glucose detection in the fresh whole blood sample (Fig. 5(a)), the reduce degree of the detection glucose value in the whole blood with sodium fluoride (shelved for 72 h) was significantly less than the datum obtained from the whole blood without sodium fluoride (shelved for 4 h) (Figs. 5(b) and (c)). The results indicated the importance of the presence of sodium fluoride.

Electrocatalysis of GOx/(Au-F127)/GCE to Glucose

Differential pulse voltammetry (DPV), which considered as a derivative of linear sweep voltammetry or stair case voltammetry, can be used to study the redox properties of extremely small amounts of chemicals. Different aliquots of 1×10^{-7} M glucose solution (0.3, 0.4 and 0.5 μL) were successively added to 5 mL of PBS solution under intense stirring, then CV was performed until the currents did not change any more, and DPV was immediately carried out (Fig. 6). The biosensor exhibited super highly sensitive response to glucose with a detection limit of 3.15 pM which was much lower than those of biosensors that previous reported in the literature (Table I).[46–50] As at each time, DPV was performed when the diffusion layer had grown sufficiently, the biosensor showed good reproducibility. The relative standard deviation (R.S.D.) of five successive measurements to 10 pM glucose was 5.9%. For the inter-electrode repeatability, the R.S.D. of five biosensors for detection of 10 pM glucose was 6.2%. It could be observed in Figure 6 that with the increase of glucose concentration, a shoulder peak at −0.45 V emerged gradually. The calibration curve by plotting the current response with glucose concentration was presented in the insert of Figure 6. It showed a good linear region with a concentration of glucose in the range 1–100 pM. The linear relation had a

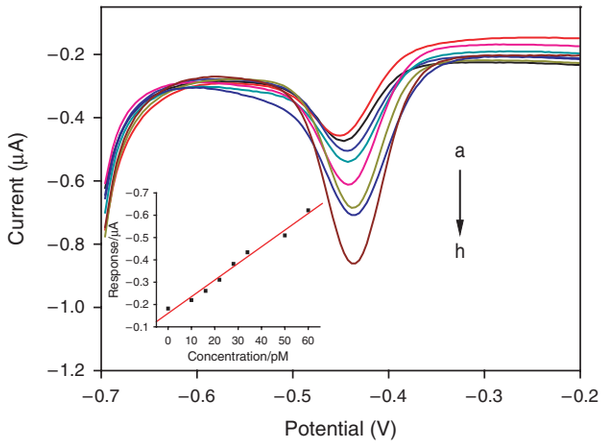

Figure 6. **DPV curves obtained at GOx/(Au-F127)/GCE in 0.1 M PBS (pH 7.4) at 25 °C with glucose of (a) 0 pM, (b) 10 pM, (c) 16 pM, (d) 22 pM, (e) 28 pM, (f) 34 pM, (g) 50 pM, (h) 60 pM; and the insert: Relationship between the peak current and the concentration of glucose.**

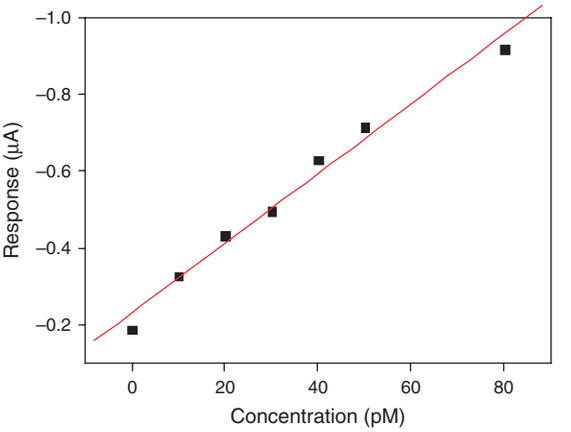

Figure 7. **Relationship between the peak current and the concentration of glucose in whole blood samples.**

regression equation of $I\ (\mu A) = -0.0747c(pM) - 0.1599$ with a correlation coefficient (R) of 0.9920.

We also investigated the linear relation of the GOx/(Au-F127)/GCE in human whole blood samples. Blood samples were supplied by volunteer, and sodium fluoride was used to prevent glucose metabolism that caused by blood cells. The current response was determined in 5 mL of 0.1 M, pH 7.4 PBS containing whole blood sample of 500 μL. The glucose concentrations in whole blood samples were determined using the standard addition method. All concentrations of glucose in detection solutions were in the linear response range (Fig. 7). The data points obtained from the GOx/(Au-F127)/GCE were the averages of the three measurements. The linear relation had a regression equation of $I\ (\mu A) = -0.00904c\ (pM) - 0.2326$ with a correlation coefficient (R) of 0.9916 (Fig. 7).

In order to verify the reliability of the biosensor, it was applied for the determination of glucose in whole blood samples. The whole blood samples were analyzed in the hospital biochemistry laboratory. Meantime, the same samples were reanalyzed with the GOx/(Au-F127)/GCE in our laboratory. The results obtained using the proposed biosensor agree well with those measured by the biochemical analyzer in hospital (Table II), which demonstrated the great potential for practical application of the biosensor and prepared for the analysis of glucose in whole blood samples.

Table I. **Comparison of detection limit of some glucose biosensors.**

Glucose biosensor	Detection limit	Refs.
GOx/ZnO nanotubes	1 μM	[46]
GOx-Nb-SWNT	10 μM	[47]
GOx-sol–gel-CNTs	50 μM	[48]
Nafion-GOx-OMC	156.52 μM	[49]
GOx-sol–gel-chitosan	10 μM	[50]
GOx/(Au-F127)	3.15 pM	This work

Table II. **Determination of glucose in whole blood samples using the GOx/(Au-F127)/GCE.**

No.	Referenced values[a] (mM)	Determined values[b] (mM)
1	3.9 ± 0.32	3.7 ± 0.35
2	4.7 ± 0.22	4.6 ± 0.34
3	5.1 ± 0.21	5.0 ± 0.25
4	5.9 ± 0.25	5.6 ± 0.29
5	13.9 ± 0.29	13.5 ± 0.38

Notes: [a]Values provided by the hospital; [b]Values determined by the GOx/(Au-F127)/GCE biosensor; they were average values of five measurements for each sample.

CONCLUSION

Recent advances in producing nanostructured materials with novel properties have stimulated research on the development of materials processing.[51–54] Moreover, many nanostructured materials can be easily modified using a wide range of biomolecules and chemical ligands.[55–58] The electrochemical glucose biosensor possesses high sensitivity and low detection limit for the real-time quantitative glucose detection in the fresh whole blood sample. These excellent performances can be attributed to Au-F127 nanospheres that can provide a good biocompatible microenvironment for maintaining enzymatic activity and keeping antibiofouling surface of GCE in whole blood. Moreover, the large specific surface of the Au-F127 nanospheres can behave as an ensemble of closely spaced but isolated nanoelectrodes in the presence of Au. The biosensor we prepared also has other advantages such as ease of construction, enhanced electrocatalysis, efficient preservation of the activity of the enzyme, and effective discrimination to the common interfering species.

Utilizing the antibiofouling property and nano-advantages of the Au-F127 nanospheres developed in this study will be the focus of our future work to detect more components in whole blood samples. This idea and technique of innovative antibiofouling nanospheres provide a promising platform for the development of novel electrochemical biosensors that can be directly used in whole blood-contact system for illness diagnosis.

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REFERENCES

1. J. Wang, Electrochemical glucose biosensors. *Chem. Rev.* 108, 814 (**2008**).
2. A. Kulkarni, M. Saxena, G. Price, M. J. O'Leary, T. Jacques, and J. A. Myburgh, Analysis of blood glucose measurements using capillary and arterial blood samples in intensive care patients. *Intens. Care Med.* 31, 142 (**2005**).
3. C. Voulgari and N. Tentolouris, The performance of a glucose-ketone meter in the diagnosis of diabetic ketoacidosis in patients with type 2 diabetes in the emergency room. *Diabetes Technol. Ther.* 12, 529 (**2010**).
4. M. H. Lin, M. C. Wu, and J. Lin, Variable classifications of glycemic index determined by glucose meters. *J. Clin. Biochem. Nutr.* 47, 45 (**2010**).
5. Z. Li, L. He, Z. Shi, H. Wang, S. Li, H. Liu, Z. Wang, and N. He, Preparation of SiO_2/polymethyl methacrylate/Fe_3O_4 nanoparticles and its application in detecting *E. coli* O157:H7 using chemiluminescent immunological method. *J. Biomed. Nanotechnol.* 5, 505 (**2009**).
6. C. Alric, J. Taleb, G. LeDuc, C. Mandon, C. Billotey, A. Le Meur-Herland, T. Brochard, F. Vocanson, M. Janier, P. Perriat, S. Roux, and O. Tillement, Gadolinium chelate coated gold nanoparticles as contrast agents for both X-ray computed tomography and magnetic resonance imaging. *J. Am. Chem. Soc.* 130, 5908 (**2008**).
7. M. C. F. Gonçalves, O. Mertins, A. R. Pohlmann, N. P. Silveira, and S. S. Guterres, Chitosan coated liposomes as an innovative nanocarrier for drugs. *J. Biomed. Nanotechnol*. 8, 240 (**2012**).
8. L. Qiu, B. Liu, Y. Peng, and F. Yan, Fabrication of ionic liquid-functionalized polypyrrole nanotubes decorated with platinum nanoparticles and their electrocatalytic oxidation of methanol. *Chem. Commun.* 47, 2934 (**2011**).
9. L. Y. Huang, T. Y. Liu, K. H. Liu, Y. Y. Liu, C. S. Chao, W. L. Tung, and M. C. Yang, Electrospinning of amphipathic chitosan nanofibers for surgical implants application. *J. Nanosci. Nanotechnol.* 12, 5066 (**2012**).
10. M. I. Z. Lionzo, G. C. Lorenzini, J. Tomedi, P. Pranke, and N. P. Silveira, Effects of the composite nanovesicles on the physical properties and cellular adhesion of chitosan films. *J. Biomed. Nanotechnol.* 8, 337 (**2012**).
11. G. H. Zhang, N. B. Yang, Y. L. Ni, J. Shen, W. B. Zhao, and X. H. Huang, A H_2O_2 electrochemical biosensor based on biocompatible PNIPAM-*g*-P (NIPAM-co-St) nanoparticles and multi-walled carbon nanotubes modified glass carbon electrode. *Sensor Actuat. B* 158, 130 (**2011**).
12. R. Rahman and D. Mazumdar, *Ab-initio* adsorption study of chitosan on functionalized graphene: Critical role of van der waals interactions. *J. Nanosci. Nanotechnol.* 12, 2360 (**2012**).
13. J. Kim, C. M. Lee, H. J. Jeong, D. W. Kim, and K. Y. Lee, Elevated anti-inflammatory effects of eicosapentaenoic acid based self-aggregated glycol chitosan nanoparticles. *J. Nanosci. Nanotechnol*. 12, 2672 (**2012**).
14. J. Fu, D. X. Wang, T. Wang, W. J. Yang, Y. Deng, H. Wang, S. G. Jin, and N. Y. He, High entrapment efficiency of chitosan/polylactic acid/tripolyphotspate nanosized microcapsules for rapamycin by an emulsion-evaporation approach. *J. Biomed. Nanotechnol.* 6, 725 (**2010**).
15. C. Mao, L. C. Jiang, W. P. Luo, H. K. Liu, J. C. Bao, X. H. Huang, and J. Shen, Novel blood-compatible polyurethane ionomer nanoparticles. *Macromolecules* 42, 9366 (**2009**).
16. W. J. Yang, J. Fu, D. X. Wang, T. Wang, H. Wang, S. G. Jin, and N. Y. He, Study on chitosan/polycaprolactone blending vascular scaffolds by electrospinning. *J. Biomed. Nanotechnol*. 6, 254 (**2010**).
17. N. Y. He, T. Wang, L. Jiang, D. X. Wang, Y. Hu, and L. Zhang, Therapy for cerebral ischemic injury with erythropoietin-containing nanoparticles. *J. Nanosci. Nanotechnol.* 10, 5320 (**2010**).
18. M. Nag, A. Patel, and N. M. Rao, Nanomaterials as *in vivo* sensors. *J. Biomed. Nanotechnol*. 7, 42 (**2011**).
19. R. Huschka, J. Zuloaga, M. W. Knight, L. V. Brown, P. Nordlander, and N. J. Halas, Light-induced release of DNA from gold nanoparticles: Nanoshells and nanorods. *J. Am. Chem. Soc.* 133, 12247 (**2011**).
20. H. SadAbadi, S. Badilescu, M. Packirisamy, and R. Wüthrich, PDMS-gold nanocomposite platforms with enhanced sensing properties. *J. Biomed. Nanotechnol.* 8, 539 (**2012**).
21. C. M. Cobley, J. Y. Chen, E. C. Cho, L. V. Wang, and Y. N. Xia, Gold nanostructures: A class of multifunctional materials for biomedical applications. *Chem. Soc. Rev.* 40, 44 (**2011**).
22. L. Xu, J. Du, Y. Deng, and N. He, Electrochemical detection of *E. coli* O157:H7 using porous pseudo-carbon paste electrode modified with carboxylic multi-walled carbon nanotubes, glutaraldehyde and 3-aminopropyltriethoxysilane. *J. Biomed. Nanotechnol.* 8, 1006 (**2012**).
23. J. Peng, C. Mao, J. H. Kim, and D. H. Kim, From nanodot to nanowire: Hybrid Au/Titania nanoarrays by block copolymer templates. *Macromol. Rapid Commun.* 30, 1857 (**2009**).
24. W. J. Yang, J. Fu, T. Wang, and N. Y. He, Chitosan/sodium tripolyphosphate nanoparticles: Preparation, characterization and application as drug carrier. *J. Biomed. Nanotechnol.* 5, 591 (**2009**).
25. T. Wang, Y. Hu, M. K. Leach, L. Zhang, W. Yang, L. Jiang, Z. Feng, and N. He, Erythropoietin-loaded oligochitosan nanoparticles for treatment of periventricular leukomalacia. *Int. J. Pharmaceut.* 422, 462 (**2012**).
26. B. Liu, Z. Li, H. Chen, Y. Deng, N. He, S. Elingarami, and J. Huang, Development of a total temperature micro-volume blended incubating and hybridizing apparatus for DNA hybridization on nanoparticles. *J. Biomed. Nanotechnol.* 8, 938 (**2012**).
27. Q. Xu, C. Mao, N. N. Liu, J. J. Zhu, and J. Shen, Direct electrochemistry of horseradish peroxidase based on biocompatible carboxymethyl chitosan-gold nanoparticle nanocomposite. *Biosens. Bioelectron.* 22, 768 (**2006**).
28. M. Singh, P. Bhatnagar, A. K. Srivastava, P. Kumar, Y. Shukla, and K. C. Gupta, Enhancement of cancer chemosensitization potential of cisplatin by tea polyphenols poly(lactide-co-glycolide) nanoparticles. *J. Biomed. Nanotechnol*. 7, 202 (**2011**).
29. M. S. Bakshi, A. Kaura, P. Bhandari, G. Kaur, K. Torigoe, and K. Esumi, Synthesis of colloidal gold nanoparticles of different morphologies in the presence of triblock polymer micelles. *J. Nanosci. Nanotechnol.* 6, 1405 (**2006**).
30. C. Mao, X. B. Chen, X. M. Hou, J. Shen, J. J. Zhu, and W. B. Zhao, Synthesis of rambutan-like hybrid nanospheres of Au-P123. *Gold Bull.* 42, 215 (**2009**).

31. C. Mao, C. X. Liang, Y. Q. Mao, L. Li, X. M. Hou, and J. Shen, Modification of polyethylene with Pluronics F127 for improvement of blood compatibility. *Colloids Surf. B* 74, 362 **(2009)**.

32. J. H. Lee, Y. M. Ju, and D. M. Kim, Platelet adhesion onto segmented polyurethane film surfaces modified by addition and crosslinking of PEO-containing block copolymers. *Biomaterials* 21, 683 **(2000)**.

33. B. Huang, Y. Chen, Q. Zhu, Z. Lu, L. Zhou, C. Mao, and J. Shen, Blood compatibility of rambutan-like hybrid nanospheres of Au-F127. *Adv. Sci. Lett.* 10, 101 **(2012)**.

34. U. Klueh, M. Kaur, D. C. Montrose, and D. L. Kreutzer, Inflammation and glucose sensors: Use of dexamethasone to extend glucose sensor function and life span *in vivo*. *J. Diabetes Sci. Technol.* 1, 496 **(2007)**.

35. M. Genshaw, Enzyme electrode for determining glucose in whole blood. *Clin. Chem.* 34, 1717 **(1988)**.

36. P. Innocenzi, L. Malfatti, M. Piccinini, and A. Marcelli, Evaporation-induced crystallization of Pluronic F127 studied *in situ* by time-resolved infrared spectroscopy. *J. Phys. Chem. A* 114, 304 **(2010)**.

37. C. M. Stoscheck, Quantitation of protein. *Method. Enzymol.* 182, 50 **(1990)**.

38. Q. H. Wei, K. H. Su, S. Durant, and X. Zhang, Plasmon resonance of finite one-dimensional Au nanoparticle chains. *Nano Lett.* 4, 1067 **(2004)**.

39. X. Ren, D. Chen, X. Meng, F. Tang, A. Du, and L. Zhang, Amperometric glucose biosensor based on a gold nanorods/cellulose acetate composite film as immobilization matrix. *Colloids Surf. B* 72, 188 **(2009)**.

40. S. E. Skrabalak, J. Chen, L. Au, X. Lu, X. Li, and Y. N. Xia, Gold nanocages for biomedical applications. *Adv. Mater.* 19, 3177 **(2007)**.

41. Z. Guo, S. Meng, W. Zhong, Q. Du, and L. L. Chou, Self-assembly of silanated poly(ethylene glycol) on silicon and glass surfaces for improved haemocompatibility. *Appl. Surf. Sci.* 255, 6771 **(2009)**.

42. H. Tang, J. H. Chen, S. Z. Yao, L. H. Nie, G. H. Deng, and Y. F. Kuang, Amperometric glucose biosensor based on adsorption of glucose oxidase at platinum nanoparticle-modified carbon nanotube electrode. *Anal. Biochem.* 331, 89 **(2004)**.

43. M. T. Sulak, O. Gokdogan, A. Gulce, and H. Gulce, Amperometric glucose biosensor based on gold-deposited polyvinylferrocene film on Pt electrode. *Biosens. Bioelectron.* 21, 1719 **(2006)**.

44. D. B. Sacks, D. E. Bruns, D. E. Goldstein, N. K. Maclaren, J. M. McDonald, and M. Parrott, Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Clin. Chem.* 48, 436 **(2002)**.

45. A. Chan, R. Swaminathan, and C. Cockram, Effectiveness of sodium fluoride as a preservative of glucose in blood. *Clin. Chem.* 35, 315 **(1989)**.

46. T. Kong, Y. Chen, Y. Ye, K. Zhang, Z. Wang, and X. Wang, An amperometric glucose biosensor based on the immobilization of glucose oxidase on the ZnO nanotubes. *Sensor Actuat. B-Chem.* 138, 344 **(2009)**.

47. P. Du, P. Wu, and C. Cai, A glucose biosensor based on electrocatalytic oxidation of NADPH at single-walled carbon nanotubes functionalized with poly(nile blue A). *J. Electroanal. Chem.* 624, 21 **(2008)**.

48. A. Salimi, R. G. Compton, and R. Hallaj, Glucose biosensor prepared by glucose oxidase encapsulated sol–gel and carbon-nanotube-modified basal plane pyrolytic graphite electrode. *Anal. Biochem.* 333, 49 **(2004)**.

49. M. Zhou, L. Shang, B. L. Li, L. J. Huang, and S. J. Dong, Highly ordered mesoporous carbons as electrode material for the construction of electrochemical dehydrogenase- and oxidase-based biosensors. *Biosens. Bioelectron.* 24, 442 **(2008)**.

50. X. Chen, J. B. Jia, and S. J. Dong, Organically modified sol–gel/chitosan composite based glucose biosensor. *Electroanalysis* 15, 608 **(2003)**.

51. F. Wang, C. Ma, X. Zeng, C. Li, Y. Deng, and N. He, Chemiluminescence molecular detection of sequence-specific HBV-DNA using magnetic nanoparticles. *J. Biomed. Nanotechnol.* 8, 786 **(2012)**.

52. L. He, Z. Li, J. Fu, F. Wang, C. Ma, Y. Deng, Z. Shi, H. Wang, and N. He, Preparation of SiO_2/(PMMA/Fe_3O_4) nanoparticles using linolenic acid as crosslink agent for nucleic acid detection using chemiluminescent method. *J. Nanosci. Nanotechnol.* 11, 2256 **(2011)**.

53. L. Tan, X. He, D. Chen, X. Wu, H. Li, X. Ren, X. Meng, and F. Tang, Highly H_2O_2-sensitive electrospun quantum dots nanocomposite films for fluorescent biosensor. *J. Biomed. Nanotechnol.* 9, 53 **(2013)**.

54. Z. Li, L. He, N. He, Y. Deng, Z. Shi, H. Wang, S. Li, H. Liu, Z. Wang, and D. Wang, Polymerase chain reaction coupling with magnetic nanoparticles-based biotin-avidin system for amplification of chemiluminescent detection signals of nucleic acid. *J. Nanosci. Nanotechnol.* 11, 1074 **(2011)**.

55. J.-I. Hahm, Polymeric surface-mediated, high-density nano-assembly of functional protein arrays. *J. Biomed. Nanotechnol.* 7, 731 **(2011)**.

56. X. Zhang, D. Li, C. Wang, X. Zhi, C. Zhang, K. Wang, and D. Cui, A CCD-based reader combined quantum dots-labeled lateral flow strips for ultrasensitive quantitative detection of anti-HBs antibody. *J. Biomed. Nanotechnol.* 8, 372 **(2012)**.

57. M. S. Islam, A. Z. Kouzani, X. J. Dai, W. P. Michalski, and H. Gholamhosseini, Design and analysis of a multilayer localized surface plasmon resonance graphene biosensor. *J. Biomed. Nanotechnol.* 8, 380 **(2012)**.

58. N. He, L. Xu, T. Wang, J. Du, Z. Li, Y. Deng, S. Li, and S. Ge, Determination of paracetamol with porous electrochemical sensor. *J. Biomed. Nanotechnol.* 5, 607 **(2009)**.