ACS APPLIED MATERIALS & INTERFACES

Functionalization of Monolithic and Porous Three-Dimensional Graphene by One-Step Chitosan Electrodeposition for Enzymatic Biosensor

Jiyang Liu,[†,‡] Xiaohui Wang,[†] Tianshu Wang,[‡,§] Dan Li,[‡] Fengna Xi,*[,†] Jin Wang,*[,‡,§,#] and Erkang Wang*[,‡]

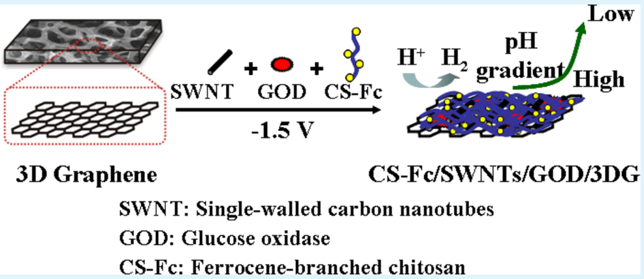
[†]Department of Chemistry, Zhejiang Sci-Tech University, Hangzhou, Zhejiang 310018, China

[‡]State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China

[§]College of Physics, Jilin University, Changchun, Jilin 130012, China

[#]Department of Chemistry, Physics and Applied Mathematics, State University of New York at Stony Brook, Stony Brook, New York 11794-3400, United States

Supporting Information



ABSTRACT: Biological modification of monolithic and porous 3D graphene is of great significance for extending its application in fabricating highly sensitive biosensors. The present work reports on the first biofunctionalization of monolithic and freestanding 3D graphene foam for one-step preparation of reagentless enzymatic biosensors by controllable chitosan (CS) electrodeposition technology. Using a homogeneous three-component electrodeposition solution containing a ferrocene (Fc) grafted CS hybrid (Fc-CS), glucose oxidase (GOD), and single-walled carbon nanotubes (SWNTs), a homogeneous biocomposite film of Fc-CS/SWNTs/GOD was immobilized on the surface of 3D graphene foam by one-step electrodeposition. The Fc groups grafted on chitosan can be stably immobilized on the 3D graphene surface and keep their original electrochemical activity. The SWNTs doped into the Fc-CS matrix act as a nanowire to facilitate electron transfer and improve the conductivity of the biocomposite film. Combined with the extraordinary properties of 3D graphene foam including large active surface area, high conductivity, and fast mass transport dynamics, the 3D graphene based enzymatic biosensor achieved a large linear range (5.0 μM to 19.8 mM), a low detection limit (1.2 μM), and rapid response (reaching the 95% steady-state response within 8 s) for reagentless detection of glucose in the phosphate buffer solution.

KEYWORDS: *3D graphene, chitosan electrodeposition, reagentless, enzyme, biosensor*

1. INTRODUCTION

Graphene, a monolayer of graphite, has attracted considerable attention because of its unique nanostructure and many extraordinary properties including exceptional conductivity, excellent thermal/chemical stability, outstanding mechanical flexibility, and environmental friendliness.[1−3] Thus, graphene has been extensively investigated in the areas of materials science and nanotechnology (e.g., nanocomposites,[4−6] nanoelectronic devices,[7] nanophotonics,[8,9] chemical and biological sensors,[10,11] and supercapacitors.[12,13]) Recently, monolithic and macroporous three-dimensional graphene (3D-G) foam with high conductivity has been prepared by chemical vapor deposition (CVD), enabling the production of high quality graphene with large quantity.[14,15] Compared to two-dimensional graphene nanosheets, 3D-G exhibits extraordinary properties including strong mechanical strengths, large surface areas, rapid charge transfer, and mass transport kinetics,[15−17] due to its seamless and porous 3D network structure. Recent reports have demonstrated that 3D-G could be served as a novel freestanding and monolithic 3D electrode and presented superior performance for supercapacitors and biofuel cells.[18,19] Compared with traditional two-dimensional planar electrodes, 3D graphene electrodes hold great promise for high sensitive electrochemical biosensors. However, the 3D-G grown by CVD is defect-free and highly hydrophobic, which imposes difficulties in its surface modification and biological applications.[20] Therefore, it is highly desired to develop facile and versatile methods for the functionalization of 3D graphene to improve its hydrophilcity and modifiability, and explore the applications in constructing novel biosensors with high performance.

Chitosan (CS) is a natural polysaccharide derived from incomplete deacetylation of chitin and displays remarkable

Received: August 18, 2014
Accepted: November 10, 2014
Published: November 10, 2014

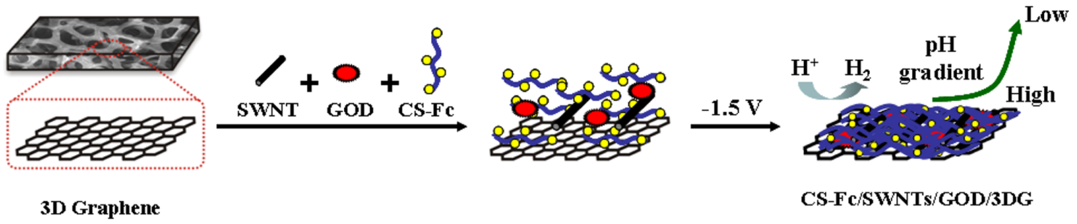


Figure 1. Schematic diagram of the fabrication of 3D graphene based reagentless enzymatic biosensors by chitosan electrodeposition.

properties such as superior film-forming ability, good adhesion, high water permeability, nontoxicity, and biocompatibility.[21−23] Those properties have promoted chitosan as one of the most promising matrices applied in enzyme immobilization.[24−27] In recent years, electrodeposition of chitosan has been demonstrated as a facile and rapid method for forming a stable chitosan sol−gel film with controllable thickness tightly attaching to the electrode surface. In this method, functional conductive materials (e.g., Au nanoparticles (NPs),[28] multiwalled carbon nanotubes (MWNTs),[11] and ionic liquid[29]), electron mediators (e.g., ferrocene,[23] and nile blue[30]), and redox enzymes (horseradish peroxidase (HRP),[29] and glucose oxidase (GOD)[23]) can be synchronously integrated into the formed chitosan biocomposite film by one-step deposition. Additionally, abundant amino groups on CS molecules can conjugate with biofunctionalized gold nanoparticles or biomolecules with specific recognition function. Therefore, electrodeposition of chitosan could provide a facile and universal way to prepare functional biocomposite films on various conductive substrates for the development of biosensors.

In this work, we demonstrated the surface biomodification of monolithic 3D graphene foam to rapidly fabricate enzymatic biosensors by using the simple and controllable CS electrodeposition method. 3D graphene foam grown by template-directed CVD was used as a free-standing electrode. GOD was chosen as a model redox enzyme, and mixed with single-walled carbon nanotubes (SWNTs)-doped ferrocene-grafted-CS (Fc-CS) homogeneous solution. Through one-step electrochemical deposition, GOD/SWNTs/Fc-CS biocomposite film was tightly attached to the surface of the 3D graphene electrode. Owing to its superior biocompatibility, the electrodeposited CS biocomposite film could provide a favorable biological microenvironment to maintain high activity of the immobilized enzymes. Fc molecules grafted on chitosan were integrated into the sensor and acted as electron mediators. Furthermore, the SWNTs acted as enhanced materials to increase the electrical conductivity of the biocomposite film and promote electron transfer of the incorporated electroactive Fc mediators, improving the sensitivity of the prepared biosensor. Collected with the unique architecture and exceptional properties of 3D graphene foam, the prepared enzymatic biosensor exhibits superior performance for glucose detection with a low detection limit, a wide detection range, high sensitivity, and good stability.

2. EXPERIMENTAL SECTION

2.1. Reagents. Chitosan (CS, 200−400 mPa·s), glucose oxidase (GOD, EC 1.1.3.4, 100 U/mg), ferrocenecarboxaldehyde (Fc-CHO, 98%), and sodium borohydride ($NaBH_4$, 98%), were obtained from Aladdin Chemistry Co. Ltd. (China). Single-walled carbon nanotubes (SWNTs) were purchased from Shenzhen Nanotech Port Co. Ltd. β-D-(+)-glucose was provided by Beijing Chemical Reagent (Beijing, China). The glucose stock solution was allowed to stand overnight at room temperature to mutarotate before use. Nafion (5 wt % in lower aliphatic alcohols and water) was purchased from Aldrich. Other chemicals were of analytical grade and were used as received. All aqueous solutions were prepared with ultrapure water (18.2 MΩ cm, Milli-Q, Millipore).

2.2. Synthesis of Ferrocene-Grafted Chitosan. Ferrocene-grafted chitosan (Fc-CS) was synthesized according to a previously reported method with slight modification.[31] In brief, CS (75.0 mg) was dispersed in a 0.1 M of acetic acid aqueous solution (15 mL). Fc-CHO (50 mg) was dissolved in 15 mL of methanol and added to the CS solution, followed by stirring for 2 h at room temperature. Then $NaBH_4$ (100 mg) was added to the above solution and the resulting mixture was stirred for another 24 h. The formed Fc-CS was precipitated with 5% NaOH. Then the yellow precipitates were separated by centrifugation and exhaustively washed with ultrapure water and methanol, respectively. The product was dried in air for a few hours and then dried at 60 °C in vacuum. Finally, the prepared Fc-CS was redissolved in an acetate buffer solution (0.2 M, pH 5.0) under ultrasonication.

2.3. Electrodeposition of Fc-CS/SWNTs/GOD Biocomposite Film onto 3D Graphene Electrode. As reported previously,[19,20] 3D graphene foam was prepared by CVD using nickel foam as the growth substrate, and ethanol was used as the precursor for graphene growth. After growth, the nickel template was completely removed by overnight incubation with HCl (3 M) solution at 80 °C to obtain a freestanding graphene foam. Subsequently, the 3D graphene foam (5 × 5 mm, 1 mm in thickness) was fixed on a glass substrate. Silver conductive paint and copper wire were used to make an electrical lead and then were insulated with silicone rubber. The 3D graphene electrode was completely prepared for further use.

Chitosan is a unique stimulus-responsive biopolymer with net charge and solubility that are pH-dependent. Therefore, CS is ideally suited for electrodeposition to prepare hydrogel film.[30] CS can disperse well in acidic conditions with a positive charge. When the pH exceeds its p*K*a (∼6.3), however, CS becomes insoluble.[28] The pH-dependent solubility of CS allows this polymer to be elecrodeposited onto various conducting substrates by increasing the local pH near the electrode surface.[23,29,30] The process for one-step preparation of reagenless enzymatic biosensor based on a 3D graphene electrode by electrodeposition is illustrated in Figure 1. The as-prepared 3D graphene electrode was immersed in a solution containing Fc-CS (2.5 mg/mL), SWNTs (0.5 mg/mL), and GOD (4 mg/mL) at −1.5 V for 120 s. At the applied potential, H^+ in the solution can be reduced to H_2 at the cathode, leading to an increased pH value near the electrode surface. When the local pH exceeds its pK_a (∼6.3), CS becomes insoluble. Therefore, the Fc-CS hydrogel film incorporated with SWNTs and GOD was electrodeposited on the 3D grahene electrode surface. After electrodeposition, the resultant Fc-CS/SWNTs/GOD/3DG electrode was rinsed with ultrapure water and then was aged in air for 2 h at room temperature. At last, 10 μL of Nafion aqueous solution (0.5%) was dropped onto the electrode surface and dried at 4 °C to form a layer of Nafion polymer film, which can serve as an effective barrier to negatively charged interferences such as UA or AA, but does not disrupt the transmission of neutral glucose molecules.[32] For comparison, the CS/GOD/3DG and Fc-CS/GOD/3DG electrodes were prepared with the same procedure. When not in use, all the modified electrodes were stored at 4 °C in a refrigerator under dry conditions.

2.4. Apparatus and Measurements. The morphology of the bare and modified 3D graphene electrodes was observed by a scanning electron microscopy (SEM) (S-4800, Hitachi, Japan). Electrochemical measurements were carried out on an electrochemical analyzer (CHI 660D, Shanghai Chenhua Equipment, China) in 0.1 M phosphate buffered solution (PBS, pH 7.0) at room temperature. A standard three-electrode system was employed for the electrochemical measurements. A platinum disk was used as auxiliary electrode. An Ag/AgCl (saturated KCl) electrode was used as the reference electrode, and a bare or modified 3D graphene electrode was used as the working electrode.

3. RESULTS AND DISCUSSION

3.1. Morphology Characterization of the 3D Graphene and Fc-CS/SWNTs/GOD Composite Film. The morphology of the 3D graphene and Fc-CS/SWNTs/GOD composite film was characterized by SEM. As shown in Figure 2a, the SEM image of CVD grown 3D graphene foam shows a well-defined macroporous network architecture with the pore diameter about 100−200 μm and exhibits a continuous graphene skeleton, suggesting that graphene sheets well retain their original structure without collapse after removing the Ni foam template. In Figure 2b, ripples and wrinkles are obviously observed on 3D graphene surface, which are mainly ascribed to the different thermal expansion coefficients of nickel and graphene.[15,33] Figure 2c is the SEM image of Fc-CS/SWNTs/GOD composite film electrodeposited on the 3D graphene surface. In contrast to the smooth graphene, the rough surface morphology of 3D graphene after electrodeposition demonstrates the formation of chitosan composite film. The magnified SEM image shows a homogeneous three-dimensional porous structure of the Fc-CS/SWNTs/GOD film (Figure 2d), which is similar to the electrodeposited chitosan film on planar glassy carbon electrodes.[23] This is because the released hydrogen bubbles could serve as dynamic template for forming hydrogel film with porous structure.[34]

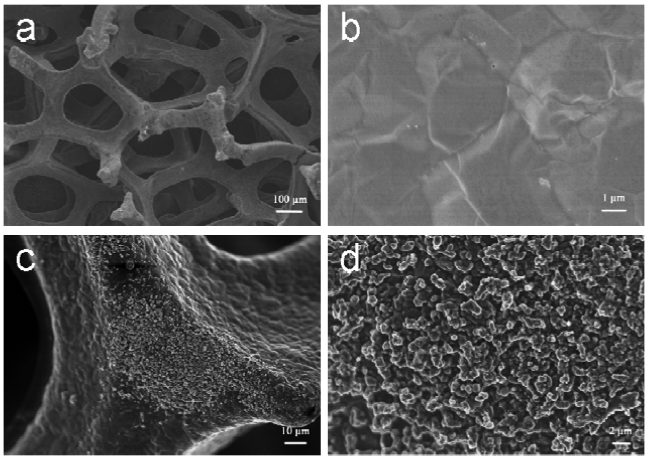


Figure 2. (a and b) SEM images of 3D graphene foam with low and high magnification. (c and d) SEM images of the Fc-CS/SWNTs/GOD composite film electrodeposited on 3D graphene with low and high magnification.

3.2. Electrochemical Characteristics of Fc-CS/SWNTs/GOD/3DG Electrode. The formation of the Fc-CS/SWNTs/GOD composite film was also characterized by cyclic voltammetry. As shown in Figure 3, the cyclic voltammograms of both 3DG (Figure 3a) and CS/GOD/3DG (Figure 3b) electrodes show relative flat curves of charging current (Figure 3b), wheareas the Fc-CS/GOD/3DG electrode displays a pair of well-defined redox peaks located at 0.207 and 0.296 V (Figure 3c), which represent the reduction and oxidation of the incorporated Fc, respectively. After being doped with SWNTs, the resultant Fc-CS/SWNTs/GOD/3DG electrode exhibits increased redox peaks and decreased peak-to-peak potential difference (Figure 3d), indicating that the SWNTs are effective to facilitate the electron transfer of the Fc grafted on CS.

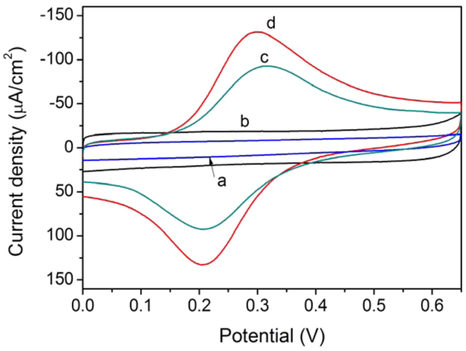


Figure 3. CV curves of the (a) 3D graphene, (b) CS/GOD/3DG, (c) Fc-CS/GOD/3DG and (d) Fc-CS/SWNTs/GOD/3DG electrodes in PBS (0.1 M, pH 7.0) at a scan rate of 100 mV/s.

Figure 4 displays the current response of the Fc-CS/SWNTs/GOD/3DG electrode at different scan rates. Both the cathodic and anodic peak currents linearly increase with increasing scan rate (inset in Figure 4), suggesting a surface-controlled electrochemical process for the electron transfer between Fc and 3DG electrode. The peak-to-peak potential difference is nearly independent of the scan rate, which also indicates that electrons can be facilely transferred between Fc and 3D graphene electrode promoted by the doped SWNTs. In addition, the Fc-CS/SWNTs/GOD/3DG electrode exhibited good stability during continuous potential scan of 50 segments (both the cathodic and anodic peak currents of the electrode decreased less than 3.6%). These results suggested that the Fc covalently grafted on CS could be stably immobilized on the 3DG surface.

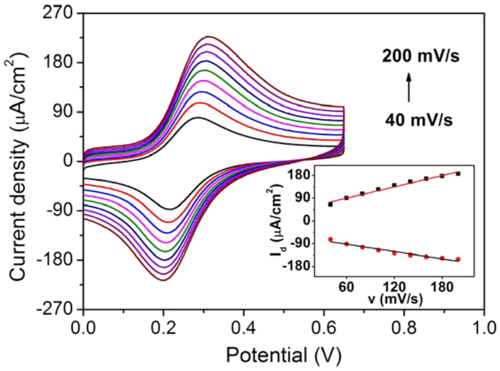


Figure 4. (a) CV curves of the Fc-CS/SWNTs/GOD/3DG electrode recorded at different scan rates (from inner to outer: 40, 60, 80, 100, 120, 140, 160, 180, and 200 mV/s) in PBS (0.1 M, pH 7.0). Inset: plots of peak currents versus scan rate.

3.3. Electrocatalytic Property of the as-Prepared Biosensor. The electrocatalytic behavior of the as-prepared biosensor on glucose oxidation was evaluated by cyclic voltammetry in 0.1 M PBS (pH 7.0). Figure 5 displays the cyclic voltammograms of the resulting Fc-CS/SWNTs/GOD/

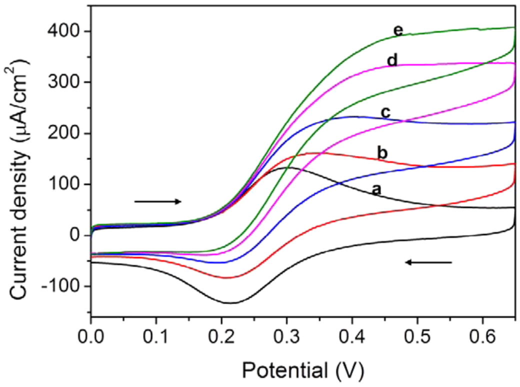


Figure 5. Bioelectrocatalysis of the Fc-CS/SWNTs/GOD/3DG electrode toward glucose in PBS (0.1 M, pH 7.0) at a scan rate of 100 mV/s. The arrows indicated the scan direction of the voltammograms. Glucose concentration was (a) 0, (b) 10, (c) 15, (d) 20, and (e) 25 mM, respectively.

3DG electrode in the presence of various concentrations of glucose. When there is no glucose in the electrolyte, the modified 3D graphene electrode shows a pair of well-defined redox peaks at 0.207 V (the cathodic peak potential) and 0.296 V (the anodic peak potential). With increasing the concentration of glucose, the cyclic voltammogram of the Fc-CS/SWNTs/GOD/3DG electrode shows an increased anodic peak current accompanied by a decreased cathodic peak current, suggesting an evident electrocatalytic oxidation of glucose at the Fc-CS/SWNTs/GOD/3DG electrode. The enzyme catalytic mechanism of glucose oxidation is proposed as follows[35,36]

$$\mathrm{GOD(FAD)} + \mathrm{glucose} \rightarrow \mathrm{GOD(FADH_2)} + \mathrm{gluconolactone} \quad (1)$$

$$\mathrm{2Fc\text{-}CS^+} + \mathrm{GOD(FADH_2)} \rightarrow \mathrm{2Fc\text{-}CS} + \mathrm{GOD(FAD)} + \mathrm{2H^+} \quad (2)$$

$$\mathrm{Fc\text{-}CS} \leftrightarrow \mathrm{Fc^+\text{-}CS} + e^- \quad (3)$$

3.4. Amperometric Detection of Glucose by the Biosensor. Figure 6 displays a typical amperometric response for the Fc-CS/SWNTs/GOD/3DG electrode to successive step additions of glucose to PBS solution under stirring. When an aliquot of glucose solution was added to the stirred PBS, an obvious increase and rapid response of the anodic current was observed. The calibration plot of the biosensor corresponding to amperometric response is shown in Figure 6b, exhibiting a large linear range of 5.0 μM−19.8 mM. Thus, the Fc-CS/SWNTs/GOD/3DG could be used for glucose detection in human blood, which has a sugar level usually in the range of 4.4−6.6 mM.[15,37] The as-prepared biosensor could reach the 95% steady-state current within 8 s, suggesting a rapid amperometric response behavior. The limit of quantity was calculated to be 4.0 μM at a signal-to-noise ratio of 10. The limit of detection was estimated to be 1.2 μM at a signal-to-noise ratio of 3, which is lower than that of those enzymatic biosensors based on GOD immobilized on 2D planar electrodes such as graphene/AuNPs/GOD/chitosan film-modified gold electrode (180 μM),[38] GOD/graphene/CS/GCE (20 μM),[10] and GOD/CdS NPs/PGE (50 μM).[39] The as-prepared 3DG based biosensor presents superior performance, which can be attributed to the extraordinary properties of 3DG foam including high electrical conductivity afforded by the seamless and multiplexed graphene network, the large active surface area and efficient mass transport supported by the 3D macroporous structure.

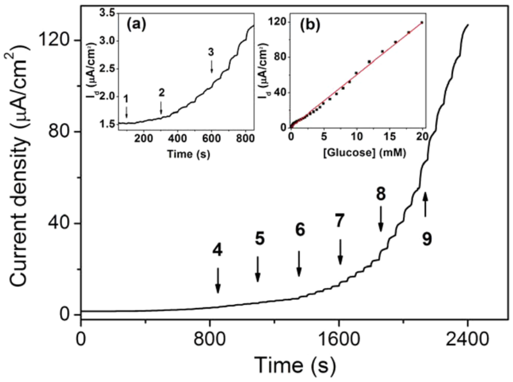


Figure 6. Amperometric response of the Fc-CS/SWNTs/GOD/3D graphene electrode upon the successive addition of 5.0 μM (1), 10.0 μM (2), 20.0 μM (3), 40.0 μM (4), 100.0 μM (5), 300.0 μM (6), 500.0 μM (7), 1.0 mM (8), 2.0 mM (9) glucose to the stirred PBS (0.1 M, pH 7.0) at 0.4 V. Inset a: the magnified curve from 50 to 850 s. Inset b: the calibration plot of the current versus the glucose concentration.

3.5. Stability, Reproducibility and Real-Sample Analysis of the Developed Sensor. The long-term stability and repeatability of this biosensor were also evaluated. When not in use, the biosensor was stored at 4 °C in a refrigerator under dry conditions. The current response (to 100 μM of glucose, $n = 7$) retained 91.6% after 7 days of storage. The biosensor showed a relative standard deviation (RSD) of 3.2% ($n = 7$) for repeatable glucose detection (100 μM), indicating that it could be used repeatedly for glucose detection. The electrode-to-electrode reproducibility was evaluated by determination of seven independently prepared electrodes under the same conditions. An RSD of 4.7% (response to 100 μM glucose) was obtained. This value is similar to those of some commercially marketed glucose sensors (RSD = 3−6%),[40−42] suggesting a good reproducibility of the developed biosensor. Human blood serum sample (1:9 diluted with PBS, pH 7.0), containing various proteins and other biomolecules, had no influence on glucose detection. When glucose (2−8 mM) was added to such serum sample, the recoveries of seven samples were between 96% and 103% (Table S1 in the Supporting Information). All the above results indicate that this biosensor is promising for glucose detection in real samples.

4. CONCLUSION

In summary, we demonstrate a facile and controllable method for biomodifcation of monolithic 3D-G foam to rapidly fabricate reagentless enzymatic biosensor by using one-step CS electrodeposition technique. Due to the perfect combination of the biocompatibility of CS and the extraordinary properties of 3D graphene foam, the as-prepared Fc-CS/SWNTs/GOD/3DG enzyme electrode presents high electrocatalytic behavior on the oxidation of glucose with a low detection limit, a wide linear range, rapid responsibility, and good stability. We also envision that the versatility of this preparation method permits fabrication of 3D-G based enzymatic biosensors by varying the used redox enzymes. This development would pave the way toward the wide application of 3D-G foam in high sensitive biosensing platforms.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Recovery assays of glucose determination in diluted human serum by the as-prepared biosensor. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*F. Xi. Tel: +86 571 86843653. E-mail: fengnaxi@zstu.edu.cn.
*J. Wang. Tel.: +86 431 85262003. E-mail: jin.wang.1@stonybrook.edu.
*E. Wang. Tel.: +86 431 85262003. E-mail: ekwang@ciac.jl.cn.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Nos. 21305127, 21190040, 11174105, and 91227114), the Science Foundation of Zhejiang Sci-Tech University (13062173-Y), and 521 talent project of ZSTU.

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