Direct Electrochemistry of Glucose Oxidase on Nail-like Carbon and Its Biosensing for Glucose

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Abstract
Nail-like carbon (NLC) was synthesized by a simple hydrothermal method. It was the first time that a novel electrochemical biosensing of glucose was explored based on the glucose oxidase (GOx)-NLC-chitosan (CHIT) glassy carbon electrode. Morphology and structure of NLC were characterized by scanning electron microscope; meanwhile the chemical composition was determined by X-ray diffraction and energy dispersive X-ray spectroscopy. The cyclic voltammetry of immobilized GOx showed a pair of quasireversible redox peaks with the formal potential ($E^{\circ\prime}$) of -0.458 V and the peak-to-peak potential separation was 47 mV at a scan rate of 100 mV s^{-1}. The present biosensor has a linear range of glucose from 0.02 to 1.84 mM (correlation coefficient of 0.9991) and detection limit of 0.01 mM ($S/N = 3$). Compared with the previous reports based on the carbon material biosensor, it has a high sensitivity of 165.5 μA mM^{-1} cm^{-2} and low apparent Michaelis–Menten constant of 0.506 mM. Thus, the NLC may have potential applications in the field of bioelectrochemistry, bioelectronics and biofuels.

Keywords: Biosensor, Direct electrochemistry, Glucose oxidase, Nail-like carbon, Glucose

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1. Introduction

In recent years, increasing attention has been focused on the direct electron transfer (DET) between enzyme and electrode surface. Because it not only can provide information about electron transfer mechanism of proteins but also is the foundation for fabricating the third–generation biosensors and bioelectronics [1].

Glucose oxidase (GOx) contains a flavin adenine dinucleotide (FAD) redox center and can catalyze oxidation of glucose to gluconlactone. Therefore, it has been extensively used to monitor the glucose level and fabricate glucose biosensors. However, DET is extremely difficult between proteins and electrode [2], because the active site of GOx, FAD is deeply embedded within a protective protein shell.

In order to improve the electron transfer of GOx and achieve its DET, many materials such as nanoparticles [3, 4], room temperature ionic liquid [5, 6] and organic polymers [7] have been used for enhancing the electron transfer. Among the various materials, carbon nanomaterials (CNMs), such as carbon nanotubes (CNTs) [2, 8–10], is one of the most favorable materials that was used to promote the electron transfer of redox proteins. Since CNTs were discovered by Iijima in 1991 [11], CNMs have attracted considerable attention all over the world. Different structures of CNMs have been successfully realized, such as carbon nanofibers (CNFs) [12, 13], highly ordered mesoporous carbon (OMC) [14, 15], flower-like carbon [16], tree-like carbon [17], carbon hollow spheres [18, 19] etc. Due to their remarkable surface area, high thermal resistance and high chemical stability, they have a wide range of applications including serving as electrode materials for lithiumion batteries [20], catalyst [21, 22], hydrogen storage [23] and biosensor [24, 25]. Especially, with the subtle electronic properties, CNTs, CNFs and OMC as the modified materials for electrode, have been studied and used in biosensor [9, 10, 26–28]. Accordingly, it is necessary to develop novel types of CNMs and apply them for the construction of the biosensor.

In this work, the NLC was successfully synthesized using a simple hydrothermal method and the morphology and chemical composition were characterized by scanning electron microscope, X-ray diffraction and energy dispersive X-ray spectroscopy. An electrochemical biosensor of glucose was fabricated based on the GOx-NLC-CHIT film modified GCE. Direct electron transfer and bioelectrocatyalytic activity of immobilized enzyme were investigated by cyclic voltammetry.

2. Experimental

2.1. Reagents

GOx (E.C. 1.1.3.4, 182 U mg^{-1}, Type X–S from *Aspergillus niger*), CHIT (MW $5-6\times10^5$, >90% deacetylation) was

purchased from Shanghai Yuanju Biotechnology Co., Ltd (China). Ferrocene (Fc) (97%, Aldrich), glutaraldehyde (50% water solution), β-D(+)-glucose and carbon tetrachloride were analytical grade and used without further purification. 0.1 mol L^{-1} phosphate buffer solution (PBS) was used as the supporting electrolyte. Other reagents were analytical reagent grade and doubly distilled water was used in all the experiments.

2.2. Apparatus

X-ray diffraction (XRD) experiment was performed with a Shimadzu XD-3A X-ray diffractometer (Japan) using Cu–Ka radiation ($k = 0.15418$ nm). The scan rate was 4° min^{-1}. Scanning electron microscopic (SEM) measurements were carried out on a scanning electron microscope (JEOL JSM-6380) at 20 kV. Samples were placed in aluminum stubs and then coated with a gold layer by sputtering in order to enhance their conductivity. The chemical composition in samples was investigated by an energy dispersive X-ray spectroscopy (EDS) attached to JSM-6390A SEM. A Model CHI660A Electrochemistry Workstation (Chenhua Instruments in Shanghai, China) was employed for all the electrochemical techniques. A three-electrode system, where a standard saturated calomel electrode (SCE) served as reference electrode, a platinum wire electrode as the auxiliary electrode, and the modified electrodes (GCE) as the working electrode. All the electrochemical experiments were conducted at room temperature (about 25 °C).

2.3. Electrode Preparation

A 1.0 wt. % chitosan (CHIT) solution was prepared by dissolving certain amount of CHIT into a 0.5 wt.% acetic acid (HAc) and diluted with distilled water.

The nail-like carbon (NLC) was synthesized by a simple hydrothermal method. In a typical synthesis, 3 mg of the Fc powder was mixed with 6 mL CCl_4 solution followed by hydrothermal treatment of the mixture at 280 °C in a 10 mL Teflon-lined autoclave for 24 h. After reaction, the black precipitate was separated by filtration, washed with absolute ethanol and distilled water until the filtrate reached clarified. Then the black sample was dried in a vacuum oven at 50 °C for 24 h.

Prior to use, a GCE was polished on a polishing cloth with alumina of successively smaller particles (1.0 μm and 0.05 μm diameter, respectively). Then the electrode was cleaned by ultrasonication in ethanol and distilled water, respectively. Typically, 1.0 mg NLC was dispersed in 1.0 mL of CHIT solution, thus 1.0 mg mL^{-1}, with the help of ultrasonic agitation. Then, 5 mg of GOx and 50 μL of glutaraldehyde solution (0.5%) were added into the above 1 mL solution. The mixture was hand-mixed completely and allowed to be left for overnight at 4 °C in a refrigerator. The glucose biosensor was constructed by coating a drop of the resulting solution 10 μL the resulting solution onto the GCE to obtain GOx-NLC-CHIT | GCE.

3. Results and Discussion

3.1. Characterization of NLC

Figure 1a shows the X-ray diffraction (XRD) of the NLC sample. The peaks located at 26.3° can be assigned to the (002) reflection of the NLC. Besides, the peak of (100) is inconspicuous, which indicates that the crystallization of the NLC is poor and no characteristic peaks of impurities appeared. The EDS of the NLC (see Fig. 1b) shows C, Cl and O, no peaks of Fe element could be found in the spectra, indicating that iron have been completely eliminated. The peak of chlorine may be attributed to the reason that chlorine ion may be participate in the formation of the NLC [29, 30]. The atomic ratio of C:Cl was 92.75 : 4.31, promising the high purity of the NLC.

Figure 1c and d show the SEM images of the NLC. It can be seen that the samples are nail-like structures composed of carbon. The structures are unique and ordered. Each nail has one end, the tip, outside and the other end, the cap, bound to the other nails. Large particles are caused by agglomeration of nail-like carbon. The particle size of the cap of the nails ranges from 0.7 to 1 μm and the length range of the body of the nails is from 2 to 4 μm. Because of the good conductivity and unique of spatial trends, the NLC could improve the rate of the electron transfer and the immobilization of enzyme.

3.2. Cyclic Voltammetry of GOx-NLC-CHIT | GCE

Figure 2 shows cyclic voltammograms (CVs) of GOx-NLC-CHIT | GCE (a), GOx-CHIT | GCE (b), NLC-CHIT | GCE (c) and bare GCE (d). No peak is observed at bare GCE and NLC-CHIT | GCE. GOx-NLC-CHIT | GCE exhibits an anodic peak at -0.434 V and a cathodic peak at -0.481 V with the peak-to-peak separation (ΔE_p) of 47 mV at scan rate of 100 mV s^{-1}, indicating a fast and direct electron transfer reaction occurred. The formal potential $E^{\circ\prime}$ ($E^{\circ\prime} = (E_{pa} + E_{pc})/2$ of the GOx modified electrode is about -0.458 V (vs. SCE) that is close to the values reported previously [31, 32]. By contrast, no peaks are observed at both NLC-CHIT | GCE (c) and bare GCE (d). Meanwhile, only very weak peaks are detected for GOx/CHIT/GCE. This indicates that electrochemical reaction is almost reversible and NLC could provide an excellent microenvironment for GOx and facilitate the electron transfer of GOx.

3.3. Effect of Scan Rate

Figure 3 showed the CVs for GOx-NLC-CHIT | GCE with different scan rates in the range of 100 – 1000 mV s^{-1}. Both the anodic and cathodic peaks currents are linearly propor-

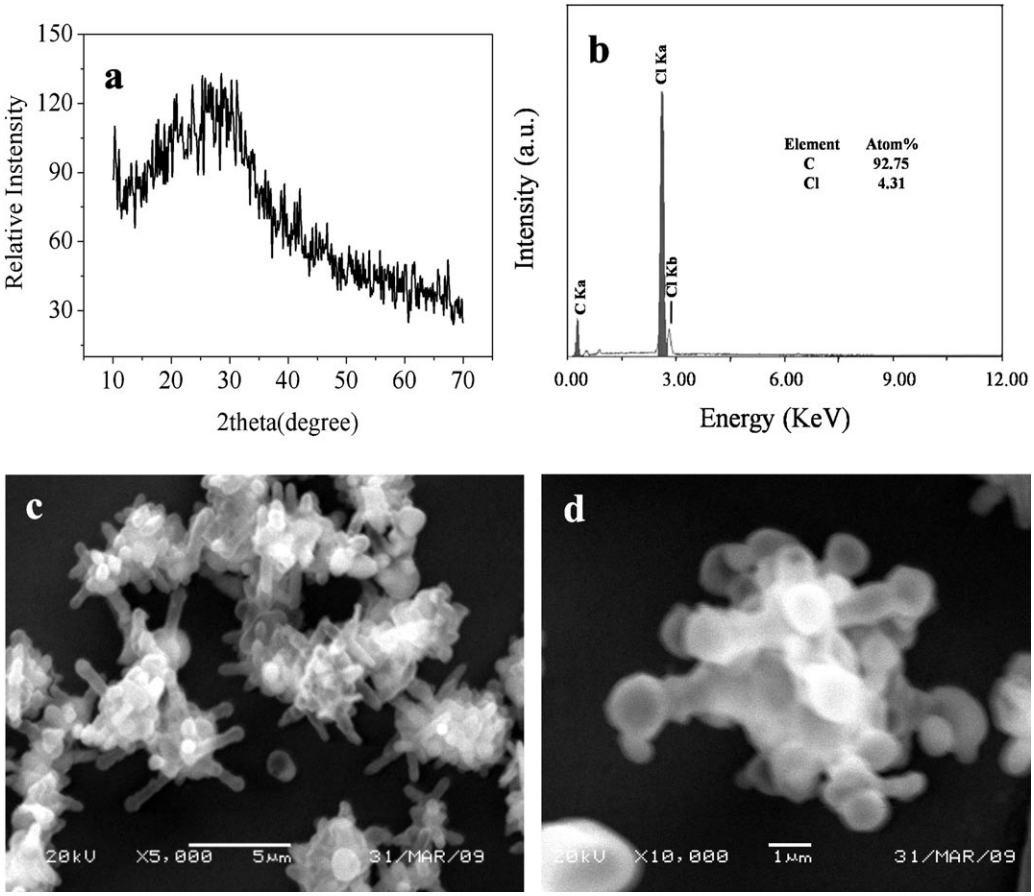

Fig. 1. The XRD spectra of NLC (a); the EDS of NLC (b); SEM images of prepared NLC with low (c) and high c (d).

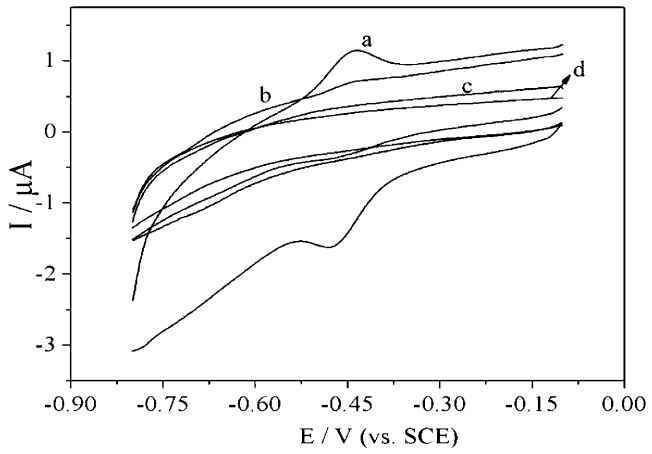

Fig. 2. CVs of different modified electrodes: GOx-NLC-CHIT | GCE (a), GOx-CHIT | GCE (b), NLC-CHIT | GCE (c) and bare GCE (d) in 0.1 M PBS (pH 6.98) at a scan rate of 100 mV s^{-1} under nitrogen atmosphere.

tional to the scan rate in the range of 100 – 1000 mV s^{-1} (Fig. 3, inset), the linear regression equation is I_{pa} (μA) = $-0.6314 - 0.00428\,\nu$ (mV s^{-1}), $R = 0.9982$, $I_{pc} = -0.03077 + 0.00328\,\nu$ (mV s^{-1}), $R = 0.9986$. This indicates that the electron transfer process for GOx-NLC-CHIT | GCE is a surface confined electrode process.

According to the equation of $I_p = n^2F^2\nu A\Gamma^*/4RT = nFQ\nu/4RT$ [33], n is calculated as 1.75, meaning that 2e transfer is involved. The surface concentration of electroactive proteins in the film (Γ^*) was calculated to be 2.10×10^{-10} mol cm^{-2} from the slope of the $I_p - \nu$ curve. This value is much larger than the theoretical value (2.86×10^{-12} mol cm^{-2}) for the monolayer of GOx on the bare electrode surface [34], suggesting that the NLC provides a large surface area for enzyme immobilization. According to the Laviron model [33], the slopes of the peak potential (E_p) vs. lg ν can be calculated for both the cathodic and the anodic peaks and the obtained value of α is 0.53 for GOx, so the electron transfer rate constant (k_s) was estimated to be 4.37 s^{-1}. This value is larger than the value, 1.7 s^{-1}, obtained from GOx immobilized on singlewalled carbon nanotubes [31], 1.53 s^{-1} for CNTs-Nafion [35] and boron-doped CNTs 1.56 s^{-1} [36]. These can further suggest that the NLC can promote the electron exchange between active site of GOx and the electrode.

3.4. Influence of pH

CVs of modified electrode were recorded at different pH solution (pH 4.0 – 8.0) (Fig. 4). As is shown in Figure 4, both anodic and cathodic peak potentials of GOx shift to negative

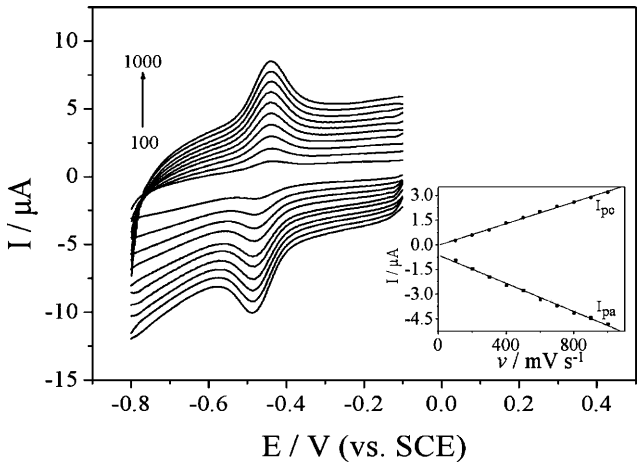

Fig. 3. CVs of GOx in 0.1 M PBS (pH 6.98) at different scan rates on GCE. Scan rate: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 mV s^{-1}. Inset is linear relation of I_p and ν at the GOx-NLC-CHIT | GCE.

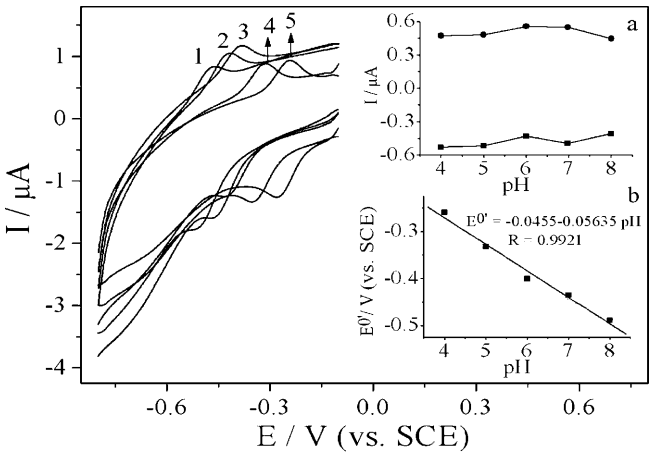

Fig. 4. CVs of GOx-NLC-CHIT | GCE in N_2-saturated PBS of various pH: 1: 8.0, 2: 6.98, 3: 6.0, 4: 5.0, 5: 4.0 at a scan rate of 100 mV s^{-1}. Inset graph: (a): plot of I vs. pH values, (b): plot of $E^{\circ\prime}$ vs. pH.

direction, indicating that proton is present in the electron transfer process of GOx. That was due to the redox process of FAD. It undergoes a redox reaction as follows:

$$\text{GOx-FAD} + 2e^- + 2H^+ \rightleftharpoons \text{GOx-FADH}_2$$

Since it is a two-electron coupled with two-proton reaction, peak potentials should be pH-dependent. The formal potential ($E^{\circ\prime}$) versus pH gives a straight line with the slope of -56.35 mV pH^{-1} (Fig. 4b Inset), the slope is close to the theoretical value (-58.6 mV pH^{-1}). This indicates that the numbers of electron and proton transferred in the electrochemical reaction are equal. As shown in Figure 4a, the pH gave the best response being between 4.0 and 6.98, the maximum response current can be observed at pH 6.98, and similar results were also obtained in other modified electrode [37, 38]. The optimal pH was related to the dissociation of functional groups such as imidazolium and sulphydryl, of histidine and cysteine amino acid residues in the GOx [37], whereas a decrease appeared at about pH 5.0 to 6.0, which may be affected by the modified materials of the NLC. In addition, there was another high current response at pH 4.0 to 5.0, while the free GOx shown a maximum activity around pH 5.1 [39], this result indicated that the NLC-CHIT film has not altered the activity of GOx.

3.5. Electrocatalytical Behavior of the GOx-NLC-CHIT | GCE

Figure 5 shows the CVs of the GOx-NLC-CHIT | GCE in air-saturated 0.1 M PBS without (curve 0) and with (curves 1 – 15) the addition of various concentrations of glucose at a scan rate of 100 mV s^{-1}. When glucose is added to this O_2-saturated buffer solution, the cyclic voltammetry shows a decrease of cathodic peak current (Fig. 5). This is because the consumption of dissolved O_2 led to the decrease of O_2 concentration [32, 40]. Based on the decrease of the reduction current, the concentration of glucose in the range of 0.02 – 1.84 mM had a linear relation. The linear regression equation is obtained as ΔI_{pc} (μA) = 11.75 $C_{glucose}$ (mM) + 1.048 ($n = 11$, $R = 0.9991$) with a detection limit of 0.01 mM ($S/N = 3$). The sensitivity of 165.5 μA mM^{-1} cm^{-2} (the electrode area is 0.071 cm^2). The apparent Michaelis – Menten constant (K_M) that gives an indication of the enzyme-substrate kinetics can be obtained from the Lineweaver – Burk equation [41]. The K_M is estimated to be 0.506 mM, implying that the GOx-NLC-CHIT | GCE exhibits a higher affinity for glucose. For comparison, the analytical performance of the proposed sensor and some other glucose biosensors were listed in Table 1. The high sensitivity and low K_M of the biosensor implies that NLC can provide superior conductivity for electron transfer; the CHIT-NLC film provides biocompatible microenvironment to maintain enzymatic activity.

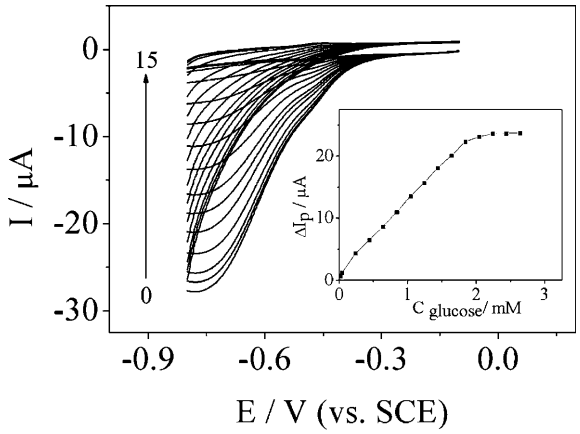

Fig. 5. CVs of GOx-NLC-CHIT | GCE in 0.1 M O_2-saturated PBS(0), the N_2-saturated PBS(15), curves (1) – (14) are in the presence of different concentration of glucose: 0.02, 0.04, 0.24, 0.44, 0.64, 0.84, 1.04, 1.24, 1.44, 1.64, 1.84, 2.04, 2.24, 2.44 mM, at a scan rate 100 mV s^{-1}. Inset: plot of ΔI_p vs. $C_{glucose}$.

Table 1. Comparison of analytical performance of some glucose biosensors.

Biosensor	Line range (mM)	Detection limit (μM)	K_M (mM)	Sensitivity (μA mM^{-1} cm^{-2})	Ref.
GOx-C-ZnO [a]	0.01 – 1.6	1	1.54	35.3	[3]
GOx-BCNT [a]	0.05 – 0.3	10	0.2	111.57	[36]
GOx-MWCNT-CHIT [a]	0 – 7.8	–	8.2	–	[42]
$\{GOx\text{-}CHIT\text{-}MWNT\}_6$-PB [b]	0 – 7	50	–	8.017	[43]
Nafion-GOx-SWCNT [a]	0 – 6	60	8.5	–	[44]
Nafion-GOx-MWCNT [a]	0.025 – 2	4	–	–	[45]
GOx-MWNT-Fc-CHIT [a]	0.012 – 3.8	3	3.12	25	[46]
$[GOx\text{-}PDDA]_3$-$[SDS\text{-}MWNT\text{-}PDDA]_3$-MPS [d]	2 – 2.2	10	5.15	5.6	[47]
Nafion-GOD-MC [a]	0.1 – 1	90	–	138.31	[48]
$(GOx\text{-}Au\ NPs\text{-}MWCNT)_9$ [c]	0.1 – 10	6.7	14.9	–	[49]
GOx-NLC-CHIT [a]	0.02 – 1.84	10	0.506	165.5	this work

[a] GCE, [b] ITO, [c] Pt electrode, [d] Au-Ti-PET electrode.
(BCNT: boron-doped carbon nanotubes, SWCNT: singlewall carbon nanotubes, MWCNT: Multiwalled carbon nanotubes, C–ZnO: carbon-decorated ZnO nanowire, PB: Prussian blue, Fc: ferrocenecarboxaldehyde, PET: poly(ethylene terephthalate), MPS: 3-mercapto-1-propanesulfonic acid, sodium salt, MC: mesoporous carbon)

3.6. Stability and Repeatability of the GOx-NLC-CHIT | GCE

The stability of the GOx-NLC-CHIT | GCE is evaluated by examining the cyclic voltammetric responses of GOx-NLC-CHIT | GCE in N_2-saturated PBS. No significant changes after multiple scans (> 50 cycles) is observed, indicating the good stability of this modified electrode.

The sensor can keep a constant current when successively swept for 50 cycles in the presence of 1.0 mM glucose. The reproducibility of the glucose biosensor was also investigated by detecting 1.0 mM glucose successively for 15 days, and its response to glucose remains about 92% compared to that of 15 days before. Thus, the NLC modified electrode was found to exhibit excellent stability, reproducibility and might be used in glucose biosensor fabrications.

4. Conclusions

In sum, the unique NLC has been successfully synthesized using a hydrothermal method through controlling the mass ratio of Fc and CCl_4. Furthermore, based on the GOx-NLC-CHIT film modified GC electrode, an electrochemical biosensor of glucose was fabricated. GOx in the film of the GOx-NLC-CHIT modified electrode exhibited a fast direct electron transferand can catalyze the reduction of dissolved oxygen without damaging its bioactivity and native structure. The biosensor displayed a higher sensitivity of 165.5 μA mM^{-1} cm^{-2} and a lower K_M of 0.506 mM than that of previous reports for glucose response, ascribing to the good conductivity, novel morphology and high biocompatibility of the NLC. Accordingly, the NLC can be applied as a useful material for bioelectrochemistry, bioelectronics and biofuels.

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6. References

[1] F. A. Armstrong, H. A.O. Hill, N. J. Walton, *Acc. Chem. Res.* **1988**, *21*, 407.
[2] G. C. Zhao, L. Zhang, X. W. Wei, *Anal. Biochem.* **2004**, *329*, 160.
[3] J. P. Liu, C. X. Guo, C. M. Lia, Y. Y. Li, Q. B. Chi, X. T. Huang, L. Liao, T. Yu, *Electrochem. Commun.* **2009**, *11*, 202.
[4] A. Salimi, E. Sharifi, A. Noorbakhsh, S. Soltanian, *Biosens. Bioelectron.* **2007**, *22*, 3146.
[5] C. S. Shan, H. F. Yang, J. F. Song, D. X. Han, A. Ivaska, L. Niu, *Anal. Chem.* **2009**, *81*, 2378.
[6] W. Sun, D. D. Wang, G. C. Li, Z. Q. Zhai, R. J. Zhao, K. Jiao, *Electrochim. Acta* **2008**, *53*, 8217.
[7] T. Kuwahara, K. Oshima, M. Shimomura, S. Miyauchi, *Synth. Met.* **2005**, *152*, 29.
[8] A. Salimi, B. Kavosi, R. Hallaj, A. Babaei, *Electroanalysis* **2009**, *21*, 909.
[9] D. Ragupathy, A. L. Gopalan, K. P. Lee, *Electrochem. Commun.* **2009**, *11*, 397.
[10] M. Holzinger, L. Bouffier, R. Villalonga, S. Cosnier, *Biosens. Bioelectron.* **2009**, *24*, 1128.
[11] S. Iijima, *Nature* **1991**, *354*, 56.
[12] I. J. Teng, P. K. Chuang, C. T. Kuo, *Diamond Relat. Mater.* **2006**, *15*, 1849.
[13] M. C. Diaz, J. M. Blackman, C. E. Snape, *Appl. Catal. A* **2008**, *339*, 196.
[14] R. Ryoo, H. J. Sang, S. Jun, *J. Phys. Chem. B* **1999**, *103*, 7743.
[15] J. J. Feng, J. J. Xu, H. Y. Chen, *Biosens. Bioelectron.* **2007**, *22*, 1618.

[16] J. M. Shen, Y. T. Feng, *J. Phys. Chem. C* **2008**, *112*, 13114.
[17] P. M. Ajayan, J. M. Nugent, R. W. Siegel, B. Wei, Ph. Kohler–Redlich, *Nature* **2000**, *404*, 243.
[18] Y. Wang, F. B. Su, J. Y. Lee, X. S. Zhao, *Chem. Mater.* **2006**, *18*, 47.
[19] H. S. Qian, F. M. Han, B. Zhang, Y. C. Guo, J. Yue, B. X. Peng, *Carbon* **2004**, *42*, 761.
[20] W. M. Zhang, J. S. Hu, Y. G. Guo, S. F. Zheng, L. S. Zhong, W. G. Song, L. J. Wan, *Adv. Mater.* **2008**, *20*, 1160.
[21] H. B. Zhao, L. Li, J. Yang, Y. M. Zhang, H. Li, *Electrochem. Commun.* **2008**, *10*, 876.
[22] W. L. Xu, X. C. Zhou, C. P. Liu, W. Xing, T. H. Lu, *Electrochem. Commun.* **2007**, *9*, 1002.
[23] B. Z. Fang, M. S. Kim, J. H. Kim, J. S. Yu, *Langmuir* **2008**, *24*, 12068.
[24] Q. L. Sheng, J. B. Zheng, *Biosens. Bioelectron.* **2009**, *24*, 1621.
[25] K. B. Male, S. Hrapovic, J. M. Santini, J. H.T. Luong, *Anal. Chem.* **2007**, *79*, 7831.
[26] X. B. Lu, J. H. Zhou, W. Lu, Q. Liu, J. H. Li, *Biosens. Bioelectron.* **2008**, *23*, 1236.
[27] L. N. Wu, X. J. Zhang, H. X. Ju, *Anal. Chem.* **2007**, *79*, 453.
[28] C. P. You, X. W. Yan, Y. Wang, S. Zhang, J. L. Kong, D. Y. Zhao, B. H. Liu, *Electrochem. Commun.* **2009**, *11*, 227.
[29] N. A. Katcho, E. Urones-Garrote, D. Avila-Brande, A. Gomez-Herrero, S. Urbonaite, S. Csillag, E. Lomba, F. Agullo-Rueda, A. R. Landa-Canovas, L. Carlos Otero-Diaz, *Chem. Mater.* **2007**, *19*, 2304.
[30] Z. Yao, X. Zhu, X. X. Li, Y. Xie, *Carbon* **2007**, *45*, 1566.
[31] A. Guiseppi-Elie, C. H. Lei, R. H. Baughman, *Nanotechnology* **2002**, *13*, 559.
[32] J. D. Zhang, M. L. Feng, H. Tachikawa, *Biosens. Bioelectron.* **2007**, *22*, 3036.
[33] E. Laviron, *J. Electroanal. Chem.* 1979, *100*, 263.
[34] J. Z. Xu, J. J. Zhu, Q. Wu, Z. Hu, H. Y. Chen, *Chin. J. Chem.* **2003**, *21*, 1088.
[35] C. X. Cai, J. Chen, *Anal. Biochem.* **2004**, *332*, 75.
[36] C. Y. Deng, J. H. Chen, X. L. Chen, C. H. Xiao, L. H. Nie, S. Z. Yao, *Biosens. Bioelectron.* **2008**, *23*, 1272.
[37] S. Brahim, D. Narinesingh, A. Guiseppi-Elie, J. *Mol. Catal. B–Enzym.* **2002**, *18*, 69.
[38] M. C. Tsai, Y. C. Tsai, *Sens. Actuators B: Chem.* **2009**, *141*, 592.
[39] H. Bright, M. Appleby, *J. Biol. Chem.* **1969**, *244*, 3625.
[40] Y. Y. Sun, H. Y. Wang, C. Q. Sun, *Biosens. Bioelectron.* **2008**, *24*, 22.
[41] F. R. Shu, G. S. Wilson, *Anal. Chem.* **1976**, *48*, 1679.
[42] Y. Liu, M. K. Wang, F. Zhao, Z. A. Xu, S. J. Dong, *Biosens. Bioelectron.* **2005**, *21*, 984.
[43] Y. J. Zou, C. L. Xiang, L. X. Sun, F. Xua, *Electrochim. Acta* **2008**, *53*, 4089.
[44] X. Q. Liu, L. H. Shi, W. X. Niu, H. J. Li, G. B. Xua, *Biosens. Bioelectron.* **2008**, *23*, 1887.
[45] Y. C. Tsai, S.-C. Li, J. M. Chen, *Langmuir* **2005**, *21*, 3653.
[46] J. D. Qiu, W. M. Zhou, J. Guo, R. Wang, R. P. Liang, *Anal. Biochem.* **2009**, *385*, 264.
[47] X. B. Yan, X. J. Chen, B. K. Tay, K. A. Khor, *Electrochem. Commun.* **2007**, *9*, 1269.
[48] K. Q. Wang, H. Yang, L. Zhu, Z. S. Ma, S. Y. Xing, Q. Lv, J. H. Liao, C. P. Liu, W. Xing, *Electrochim. Acta* **2009**, *54*, 4626.
[49] B. Y. Wu, S. H. Hou, F. Yin, Z. X. Zhao, Y. Y. Wang, X. S. Wang, Q. Chen, *Biosens. Bioelectron.* **2007**, *22*, 2854.

