ChemPubSoc Europe

DOI: 10.1002/chem.201404747

CHEMISTRY
A European Journal
Full Paper

Biosensors

Highly Ordered Nanoporous Carbon Films with Tunable Pore Diameters and their Excellent Sensing Properties

Lichao Jia,[b] Geoffrey Lawrence,[a, b] V. V. Balasubramanian,[a] Goeun Choi,[c] Jin-Ho Choy,[c] Aboubakr M. Abdullah,[d] Ahmed Elzatahry,[e] Katsuhiko Ariga,[b] and Ajayan Vinu*[a]

Abstract: Ordered porous carbon films with tunable pore diameters, immobilized with glucose oxidase (GOD) have been fabricated and employed for the construction of a biosensor for glucose molecules. The as-prepared porous films have large specific surface areas and highly ordered porous structure with uniform pore sizes, which are critical for the immobilization of large amounts of GOD and support the promotion of heterogeneous electron transfer. The developed biosensors give enough room for the encapsulation of a high amount of GOD molecules and show excellent biosensing performance with a linear response to glucose concentration ranging from 0.5 to 9 mM and a detection limit of 1.5 μM. It is also demonstrated that the sensitivity of the biosensor can be easily tuned by modulating the pore size of carbon film as it dictates the amount of immobilization of GOD in the porous channels. The fabricated carbon-film-based biosensor has a good stability and a high reproducibility, which opens the gateway for the commercialization of this excellent technology.

Introduction

The electrochemical biosensors have generated a huge amount of research due to their inherent advantages, such as low cost, portability, high sensitivity, and good selectivity, which could make them available for various promising applications including medical diagnostics, environmental monitoring, pharmaceuticals, and food safety.[1,2] In many cases, the electron transport between the prosthetic groups of redox proteins and the electrode surface is of paramount importance for the successful preparation of highly efficient electrochemical biosensors, because it decides the efficiency of the biosensing devices. However, due to the deep-seated redox center in a cavity and instability of the biological matrix upon interaction with the electrode surface, the direct electron transfer for redox proteins is hard to achieve at conventional bare or naked electrodes.[3] Therefore, it is important to develop an electron mediator that enables not only the enzyme to immobilize onto the electrode surfaces, but also to form the efficient transport system between the enzyme and the electrode while retaining the enzymatic stability and bioactivity.[4] Furthermore, if the specific surface area or the pore diameter of support materials and the adsorption of enzymes over the support could be regulated, these factors might be used to improve the efficiency of the biosensors.

To realize these opportunities, nanomaterials, which possess the large surface to volume ratios, a high surface reactivity, a high catalytic efficiency, and a strong adsorption ability, have been widely used for the development of new devices for biological and electronic applications in recent decades.[2a,5,6] For example, carbon-based nanostructures including glassy carbon, carbon paste, carbon nanotubes, carbon fibers and functionalized carbons have been extensively utilized as supports for the biosensing applications owing to their high chemical stability, surface area, high mechanical strength, and excellent electrochemical properties.[7–9] However, these materials suffer from poor textural parameters including a broad pore-size distribution and disordered porous structure with a lot of hidden pores. In addition, in the case of carbon nanotubes, the length of the tube is too long, which makes a hurdle for the diffusion of large biomolecules to enter inside the nanochannels and access the interior part of the pores. These problems can be overcome by designing ordered porous materials with uniform pore-size distribution and excellent textural features, good electrical conductivity, and stability.

[a] G. Lawrence, Dr. V. V. Balasubramanian, Prof. A. Vinu
Australian Institute for Bioengineering and Nanotechnology
The University of Queensland, Brisbane, QLD 4072 (Australia)
E-mail: a.vinu@uq.edu.au

[b] Dr. L. Jia, G. Lawrence, Prof. K. Ariga
International Centre for Materials Nanoarchitectonics
WPI Research Centre, National Institute for Materials Science
1-1 Namiki, Tsukuba 305-0044 (Japan)

[c] G. Choi, Prof. J.-H. Choy
Center for Intelligent Nano-Bio Materials (CINBM)
Department of Chemistry and Nanoscience
EWHA Womans University, Seoul 120-750 (South Korea)

[d] Prof. A. M. Abdullah
Center for Advanced Materials
Qatar University, Doha 2713 (Qatar)

[e] Prof. A. Elzatahry
Department of Chemistry
King Saud University
2455 Riyadh 11451 (Kingdom of Saudi Arabia)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201404747.

In particular, ordered porous structures may help to avoid the diffusional limitation for the substrate to access the redox centers of immobilized proteins, thus promoting the electron transfer between the redox protein and the electrode surfaces and enhancing the performance of biocatalytic process.[7,8] Therefore, it is assumed that ordered porous material with well-defined porous structure could be a potential candidate as an appropriate host for the immobilization of biomolecules. To date, different kinds of porous materials, such as silica, metal oxides, and carbon, have been explored for immobilization of biomolecules.[9] As was expected, these materials exhibit good performance. However, these materials are in powder form and need to be cast on the substrate, which creates trouble in making a film for the fabrication of the sensing device.

In addition, modulating the device structure with a perfect control of interfaces, in which the electronic exchange occurs, or controlling the experimental process by using these powder materials to tune the biosensing performance to meet the practical requirement, is very difficult and is still a great challenge. These problems can be overcome by the direct growth of electrical mediator on the electrodes, such as silicon wafer or indium tin oxide (ITO) substrate, because it offers a good compatibility of the sensing element with the substrate and further gives the opportunity to fabricate completely integrated bioelectronic systems. Herein, we present a glucose biosensor based on porous carbon film with tunable pore diameters and well-ordered pores as an electrical mediator due to its good electrical conductivity, acceptable biocompatibility, and excellent chemical, thermal, and mechanical stability compared with those of silica and metal oxide.[10] In addition, the presence of surface functional groups on the surface of the porous carbon films can help to avoid the enzyme leaching due to the strong hydrophobic interaction and the hydrogen bonding between the glucose oxidase (GOD) and functionalized carbon surface. The prepared biosensor showed a high and reproducible sensitivity over a wide linear range and fast response, suggesting potentials for a wide range of practical applications. Importantly, the sensitivity of the biosensors can be either tuned easily by modulating the pore size of the carbon material or the amount of the GOD immobilization in the pore channels of the porous carbon films. Therefore, we believe that ordered porous carbon film with a well-controlled pore size can be a promising candidate for the construction of enzyme-based electrochemical biosensors with a high sensitivity and could make a potentially significant impact on the design and selection of electrode films for sensing various other molecules.

Results and Discussion

Figure 1 displays the representative scanning electron microscopy (SEM) images of the synthesized ordered porous carbon film using monolayer colloidal crystal as a template, which highlights the successful polymerization of carbon sources around the polystyrene sphere (PS). The porous carbon film exhibits honeycomb-like morphology, consisting of spherical pores, which are originated from the PS and uniformly arranged in a regular intervals. Each porous sphere is connected by thick carbon walls in an orderly fashion, which is attributed to the well-organized self-assembly process of PS spheres and the interaction with the carbon precursors. As can be seen in Figure 1b, the diameter of the spherical pores (470 nm) is similar with that of PS beads (476 nm) used in the synthesis, indicating that almost no detectable structural shrinkage occurred after calcination. X-ray diffraction (XRD) result (Figure S1 in the Supporting Information) reveals that the sample has a broad reflection at the 2θ value of 25.2°, which is indexed to the (002) peak and related to the turbostratic carbon structure with randomly oriented graphene layers.[11] It should be noted that ordered porous carbon film with tunable pore size can be prepared by using colloidal PSs with different diameters as templates.[12] Because the uniform porous structure could provide a significant increase of effective area for protein loading and mass transfer channel, modulating the pore size is important to control the amount of biomolecules in the porous channels and the mass transfer resistance of the biocatalytic process in glucose sensing devices for monitoring diabetes, which has been an attractive topic in bioanalysis and clinical applications. Due to the advantages of low detection limit and high sensitivity, electrochemical techniques based on the GOD is a popular method and have been widely studied.[1,2] We realize that porous carbon film with larger specific surface area and uniform pore distribution could be an attractive matrix to immobilize proteins. The GOD was loaded onto the carbon film surface and their corresponding electrochemistry properties were investigated. As shown in Figure S2 in the Supporting Information, the well-controlled hexagonal structure of the film demonstrated almost no change after the immobilization of GOD with the concentration of 10 mg mL^{-1}. The effectiveness of enzyme immobilization was examined by Fourier transform infrared (FTIR) spectroscopy (Figure 2). The porous carbon film (Figure 2a) exhibits two bands centered around 1290 and 1582 cm^{-1}, which can be assigned to C-O-C vibrations of ether groups on the surface of carbon and aromatic ring stretching coupled to highly conjugated keto groups, respectively.[13] These functional groups on the surface of the porous carbon films enhances the bonding between the GOD and the carbon, and helps to avoid leaching. Pure GOD was also analyzed by FTIR spectroscopy and the result is shown in Figure 2b. It was observed that the pure GOD possesses two bands located at 1651 cm^{-1} (amide band I) and 1537 cm^{-1} (amide band II), which is due to the C=O stretching mode, and

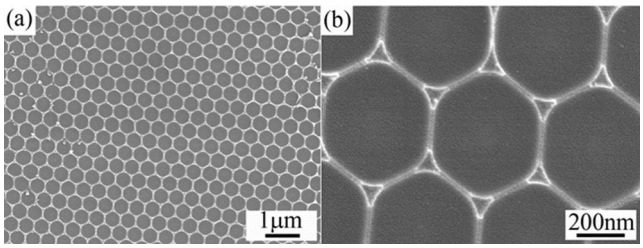


Figure 1. FESEM view of ordered porous carbon film. a) Low magnification; and b) high magnification.

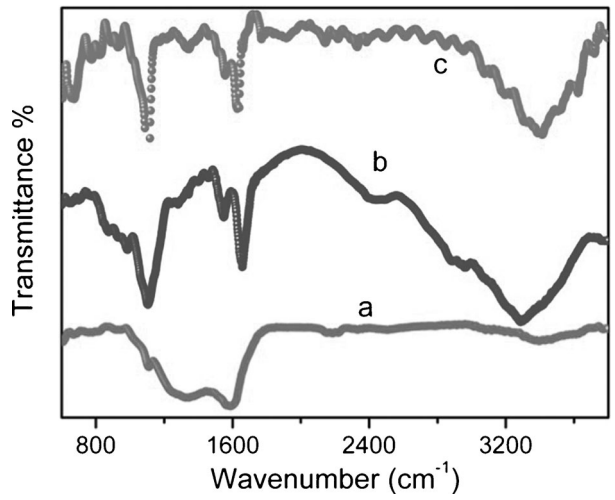


Figure 2. FTIR spectra of a) porous carbon; b) pure GOD; and c) porous carbon loaded with GOD.

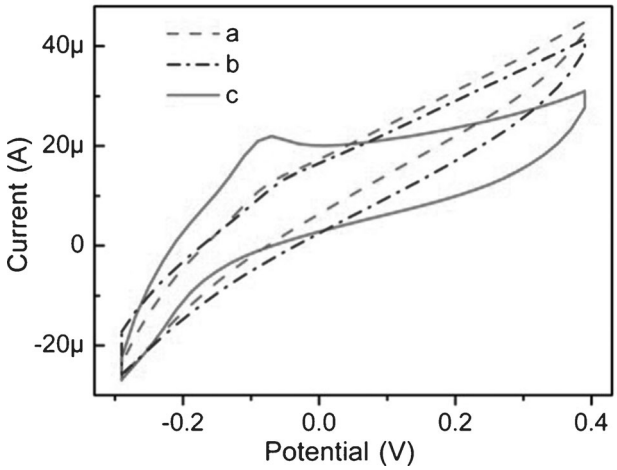


Figure 3. Cyclic voltammograms of different materials modified electrodes in 0.1 M PBS solutions at 50 mV s^{-1}: a) porous carbon/Nafion; b) GOD/Nafion; and c) porous carbon/GOD/Nafion.

the bending and the stretching mode of N–H and C–N vibrations, respectively.[2b,9c] The absorption around 1100 cm^{-1} was also observed for GOD, which might be attributed to the stretching vibration of C–O. The spectrum of GOD immobilized on the carbon film was also measured (Figure 2c). Two main amide bands can be clearly seen after subtraction of the spectra for unloaded carbon material. The ratio of intensities of the amide I and II bands before and after the immobilization of GOD indicate that the structural confirmation of GOD is retained after adsorption on carbon material. It can be assumed that the GOD molecules are perfectly immobilized in the macrochannels of the porous carbon film without denaturation. This is also supported by the fact that the peaks are shifted to lower wavenumber, which is due to the strong interaction of the amide linkages of the GOD with the keto groups on the surface of the porous carbon films.

Figure 3 presents the cyclic voltammograms (CV) of different electrodes measured in 0.1 M pH 7.0 phosphate buffered saline (PBS) solution with a scan rate of 30 mV s^{-1}. For porous carbon/Nafion modified ITO (Figure 3a), no peak could be observed, indicating both the carbon material and Nafion membrane are not electrochemically active. Meanwhile, GOD does not also exhibit any response when the enzyme alone is modified onto the ITO electrode surface, which can be ascribed to the slow electron transfer or the denaturation of GOD and ineffectiveness of Nafion in trapping GOD. In addition, a huge resistance was observed, which is clearly reflected in the distorted CV curve. However, after immobilizing GOD on the carbon material, a redox peak at −80 mV was seen, which could be attributed to the direct electrochemical behavior of GOD. This is clear evidence that porous carbon material plays an important role in improving the GOD adsorption and facilitating the electron transfer between the GOD and ITO substrate: firstly, porous carbon material has a high specific surface area and a large number of active adsorption sites on the surface, which permits a large amount of GOD molecules to immobilize on the carbon surface and makes it possible to give a high signal current; secondly, the ordered porous structure provides the favorable host matrix that isolates the GOD molecules, protecting them from self-aggregation and leaching, which is an important factor that not only facilitates the mass transport but also retains the native structure of guests; finally, the inherent good conductivity and biocompatible behavior of carbon material further favor electron transfer between the protein molecules and the electrodes.

The direct electrochemistry response of GOD, which was immobilized in the porous channels of carbon film was investigated at different scan rates with a potential range from −0.3 to 0.4 V. As shown in Figure 4, along with the increase of scan rates (10–100 mV s^{-1}), a series of perfect CV profiles were obtained. The peak current moves in a positive direction when the scan rate is increased, while the peak potentials are almost unchanged. The redox peak currents were proportional to the

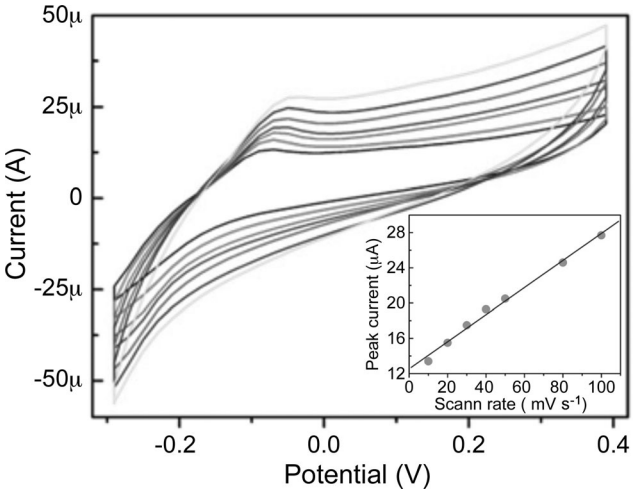


Figure 4. Cyclic voltammograms of porous carbon/GOD/Nafion modified ITO electrode in 0.1 M PBS solutions at different scan rates (10–100 mV s^{-1}). Insert: plot of peak current versus scan rate.

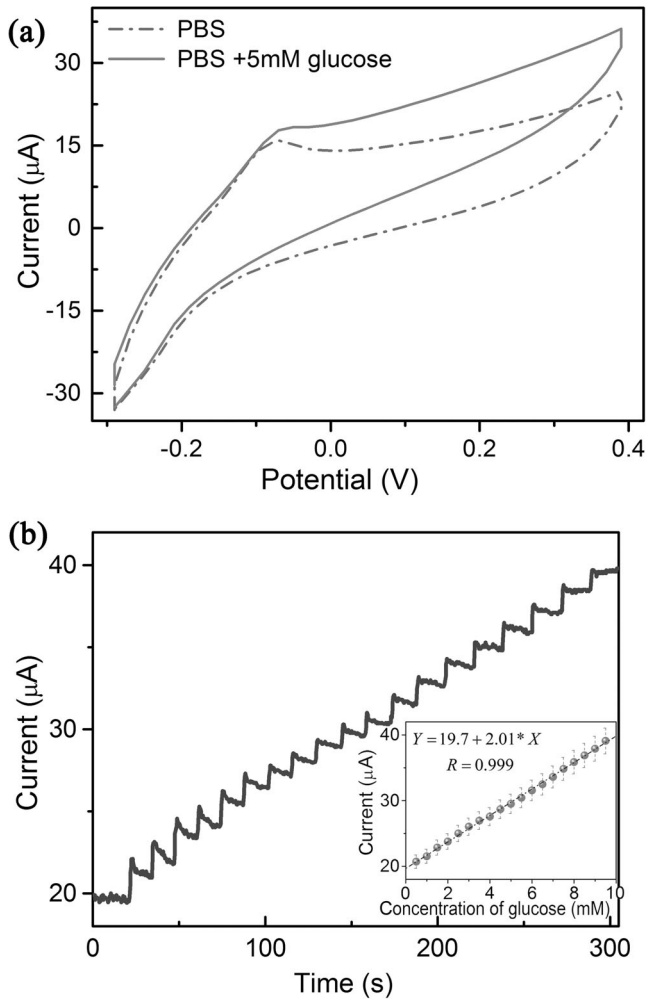


Figure 5. a) Cyclic voltammograms of porous carbon/GOD/Nafion modified ITO electrode in 0.1 M PBS solutions upon the addition of 5 mM glucose at 30 mV s^{-1}. b) Typical steady-state response of the biosensor on successive injection of 0.05 mM glucose solution into 0.1 M PBS while stirring, with an applied potential of −80 mV. Insert: the calibration curve of the sensor.

scan rates, indicating that the GOD redox reaction is a typically surface-controlled electrochemical process (Figure 4 insert).

The electrocatalytic activity of the GOD porous carbon films towards glucose oxidation was studied by increasing the concentration of the glucose in the solution and the data are shown in Figure 5a. Upon the addition of 5 mM glucose to 0.1 M PBS solution, the shape of the cyclic voltammogram for the direct electron transfer of GOD at GOD/porous carbon modified electrode changed dramatically. The reduction peak current increases with the positive shift of anodic peak, displaying a pronounced electrocatalytic between the oxidized form of GOD and glucose. It is known that D(+)-glucose is the substrate of GOD, the presence of which will result in an enzyme-catalyzed reaction and diminish the concentration of the oxidized form of GOD on the electrode surface, as shown in Equation (1):

$$\text{Glucose} + \text{GOD (FAD)} \rightarrow \text{gluconolactone} + \text{GOD (FADH2)} \quad (1)$$

GOD (FAD) is consumed while being reduced by glucose, resulting in the increasing anodic current.[14] Thus, it can be concluded that the GOD immobilized into porous carbon retains the biocatalytic activity to a certain degree and led to the increase of reduction current. Based on the increases of the electrocatalytic response, one can easily conclude that this system can be used as a glucose biosensor.

To study the bioactivity of the adsorbed protein, the constructed electrode was used as a biosensor. The typical steady-state amperometric response of the biosensor to successive additions of glucose for an air-saturated and stirred 0.1 M pH 7.0 PBS solution at the detecting potentials of −80 mV was measured. As displayed in Figure 5b, when 0.05 mM glucose was added into the 50 mL buffer solution, the current signal increased rapidly and sensitively. A clear response that is based on the oxygen reduction upon increasing the concentration of the glucose was visible. It is clear that the current is constant for a given concentration, and the response is very fast in reaching a dynamic equilibrium, generating a 95% of steady-state current signal within 5 s. This fast response can be explained by the effective transfer of the substrate and products through porous carbon matrixes containing enzymes.

In addition, the changes in current are also reversible for increasing and/or decreasing glucose concentration. Under the optimized experimental conditions, the calibration curve of the electrode presents a linear increased amperometric response to glucose ranging from 0.05 to 9 mM with a relative coefficient of 0.999 (Figure 5b insert), indicating a well-defined, typical behavior of an enzymatic kinetic reaction. The sensitivity of the GOD/porous carbon modified electrode was calculated to be 7.69 μA mM^{-1} cm^{-2}. The detection limit is estimated to be 1.5 μM at a signal-to-noise ratio of 3. Table S1 in the Supporting Information compares the electrochemical and biosensing performance among different GOD/carbon or graphene-based electrodes. It can be seen that our material exhibited a lower detection limit and an acceptable sensitivity. Furthermore, the linear range is much wider compared with other reported graphene or carbon-based electrodes, and covers the entire physiological concentration range (0.003–0.008 M) of glucose. Such an extended linear range arises from the increased immobilization of GOD due to the large surface area and porous volume of the carbon film. These results clearly reveal that the designed bioenzyme-channeling sensor is suitable for sensing of glucose in practical samples of diabetic patients.

It is known that the biosensing performance of GOD/porous carbon modified electrode is closely related with the amount of enzyme loader over the electrode support. If the quantity of immobilized GOD on the carbon surface could be changed, the corresponding amperometric response might be varied at the same time. Sequentially, the controllability of sensitivity for glucose biosensor would be realized, which could meet the application requirement of electrochemical biosensor in different fields. Thus, the influence of GOD concentration on the amperometric response has been examined carefully. As displayed in Figure 6a, with increasing of GOD concentration, the electrochemical response between GOD and electrode increases significantly and the redox peaks become clearly visible. It

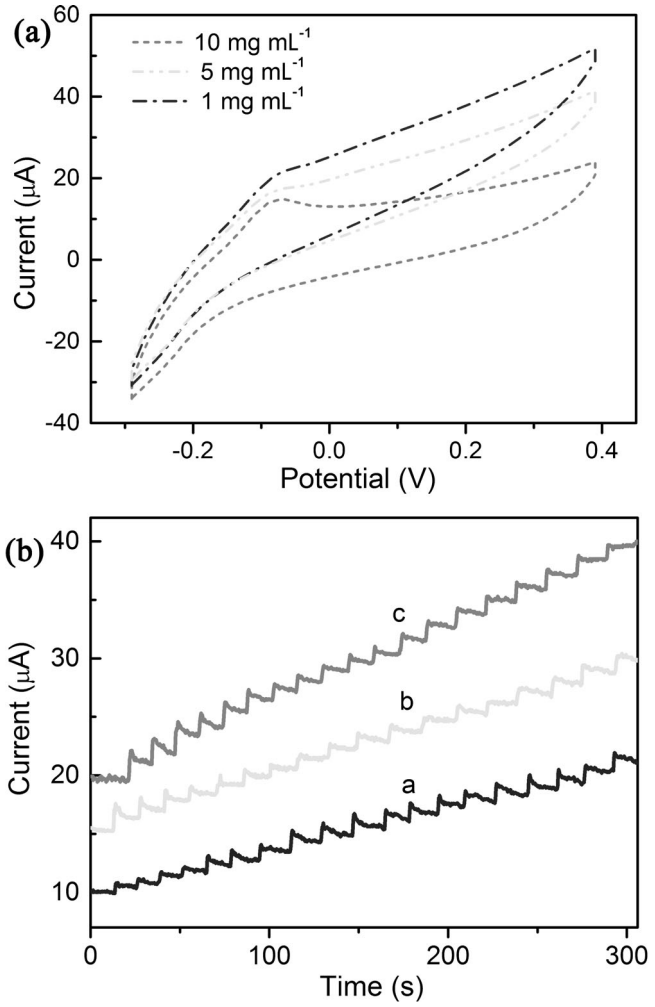


Figure 6. a) Cyclic voltammograms and b) biosensor response upon injection of 0.05 mM glucose solution of porous carbon/GOD/Nafion modified ITO electrodes with different GOD concentration in 0.1 M PBS solutions at 30 mV s^{-1}: a) 1 mg mL^{-1}; b) 5 mg mL^{-1}; and c) 10 mg mL^{-1}.

should be mentioned that the response reaches the maximum value at GOD concentration of 10 mg mL^{-1}. For all of GOD/porous carbon modified electrodes, the peak currents increase with the increasing concentration of glucose up to 9 mM (Figure 6b). Furthermore, by increasing immobilized GOD, the sensitivity of our electrode can be enhanced, evidently showing good controllability in sensing performance.

It is also evident that the immobilization of GOD is also strongly bonded with the employed electron mediator. In other words, the change of the structure for electron mediator can induce the variation of immobilization GOD, thus generating different electrochemical performance from the biosensors. For the porous carbon film displayed in this study, the synthesis method is very simple and universal. The pore size can be easily modulated by changing the diameter of PSs template, as presented in other literatures.[12] The corresponding results for GOD/porous carbon film with different pore size were shown in Figure 7. Evidently, the plots of the peak current versus concentration for all the samples also present the linear relationship over concentration from 0.05 to 9 mM. The response of the biosensor increases with the reduction of the pore size when the GOD concentration was fixed at 10 mg mL^{-1}, which is mainly due to the difference in the specific surface area that decreases significantly with increasing the pore diameter of the support.[12] The smaller pore size corresponds to the larger surface area, which significantly increases the amount of GOD adsorption on the porous channels. In addition, the surface curvature of the small pores and the density of the adsorption of the GOD molecules can also help to enhance the intermolecular interaction, which also favor the easy electron transport between the adsorbed molecules. Further, these results were confirmed by the UV/Vis adsorption of the GOD adsorbed films with different pore diameters (Figure S3 in the Supporting Information). It is clear that the adsorption quantity of GOD on the carbon film increases with decreasing the pore size. This result implies that by tuning the pore size of porous carbon film, the sensing properties of biosensor can also simply be controlled. It should also be noted that the sub-10 nm mesopores that are in the carbon walls of the samples can also enhance the electron transport.[12e] Although these pores are present in all the films irrespective of the size of the macropores, the density of these pores are larger for films with 200 nm pore size, which is also the reason for the enhancement of the sensitivity.

The stability and reproducibility of the sensor were also evaluated. When the biosensor was stored in 0.1 M pH 7.0 PBS at 4 °C in dry state and measured at intervals over several days, the response was still retained 94% value of the initial response after two weeks. This implies that the GOD/porous carbon modified ITO is very efficient for retaining the bioactivity of enzyme and can prevent the leakage from the mem-

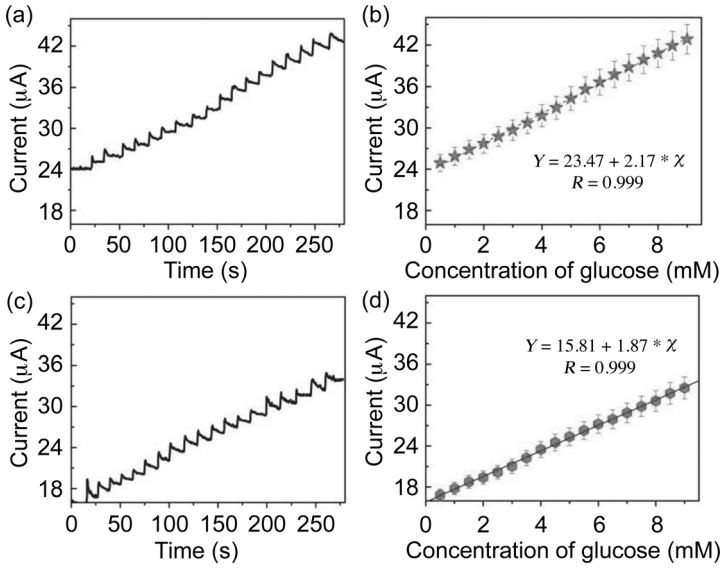


Figure 7. Typical steady-state response and calibration curve of the biosensors with different pore sizes on successive injection of 0.05 mM glucose solution into 0.1 M PBS: a),b) 200 nm; c),d) 1000 nm.

brane. In addition, the current responses of five independent sensors were also examined in PBS solution containing a glucose concentration of 5 mM, showing an acceptable reproducibility.

Conclusion

Ordered porous carbon films with well-defined geometry were fabricated and used effectively for protein immobilization and biosensing applications. The experimental results demonstrated that the new ordered porous carbon film is an attractive matrix to immobilize proteins without serious mass-transfer limitations and exhibits favorable conductivity for electron transfer and good biocompatibility. The good electron transfer can be ascribed to the unique structure with high surface area and uniform pore distribution. A glucose sensor has been developed based on the increase of the electrocatalytic response. The biosensor displayed excellent analytical performance over a wide linear range from 0.5 to 9 mM along with high sensitivity of 7.69 $\mu A\,mM^{-1}\,cm^{-2}$ and a low detection limit of 1.5 μM. Importantly, the sensing properties can be controlled easily by changing the GOD concentration or modulating the pore size of carbon film. The sensitivity of glucose biosensor decreases with the reducing of GOD concentration, while increases with the reduction of the pore size. In addition, the sensor also presents good stability and reproducibility. All these results suggest that this new support could be an excellent platform for the design of various biosensor, biocatalytic, and biofuel cell devices.

Experimental Section

Materials

Glucose oxidase (GOD; type X-S, lyophilized powder, 147000 units G^{-1} solid, from Aspergillus niger) was purchased from Sigma–Aldrich and used as received. β-D-(+)-Glucose was obtained from Nacalai tesque (Japan). Nafion solution (5 wt% in lower aliphatic alcohols) was purchased from Aldrich and was diluted to 0.5% with ethanol before use. Phosphate buffer solutions (PBS; 0.1 M, pH 7.0) were prepared by mixing stock standard solutions of K_2HPO_4 and KH_2PO_4. All other chemicals were of analytical grade and were used without further purification. All solutions were prepared with doubly distilled water.

Apparatus

The morphology of the films was observed on a Hitachi S-4800 filed emission scanning electron microscope (FESEM). The X-ray diffraction (XRD) pattern was collected on a Rigaku diffractometer by using $Cu_{K\alpha}$ ($\lambda = 0.154$ nm) radiation. FTIR spectra were recorded on a Nicolet Nexus 670 instrument. All electrochemical measurements were performed on a CHI 660B electrochemical workstation (CH Instruments, USA) by using a conventional three-electrode system, with the GOD/carbon modified ITO substrate as the working electrode, a platinum wire as the counterelectrode, and a saturated calomel electrode as the reference in a thermostated, stirred electrochemical glass cell containing 50 mL 0.1 M PBS (pH 7.0).

Preparation of ordered porous carbon film

To obtain the porous structure, monolayer colloidal crystal template comprising of polystyrene sphere with different diameter (200, 500, 1000 nm) was used as the template as reported previously in the literature.[12] Ordered porous carbon film was fabricated as follows: the 40 mL solution with 1 g of glucose and 0.1 g H_2SO_4 was taken as carbon precursor solution. First, monolayer colloidal crystal template was immersed and floated on the surface of the precursor solution. After about 10 min, the template was picked up by using a ITO substrate. Then, the templates with precursor solution were polymerized at 100 °C about 6 h and 160 °C about 6 h, respectively. Subsequently, the resulting solids were calcined at 500 °C for 3 h with the heating rate of $4\,^{\circ}C\,min^{-1}$ in nitrogen atmosphere.

GOD adsorption and preparation of GOD/carbon electrode

A series of GOD solutions (1, 5, and 10 $mg\,mL^{-1}$) were prepared by dissolving different amounts of GOD in 0.1 M pH 7.0 PBS solutions. The GOD concentrations were determined by UV absorption. UV/Vis spectra were collected on a PerkinElmer–LAMBDA 750 UV/Vis spectrophotometer. The GOD loading was calculated by subtracting the amount remaining in the supernatant liquid after adsorption from the initial amount of GOD. Protein immobilization was achieved by immersing porous carbon films on ITO substrates with 0.5 cm × 1 cm size prepared above in GOD solutions with different concentrations. After storing at 4 °C for 6 h for protein desorption, the porous carbon film on ITO substrate was picked up and dried at RT. Then, Nafion (5 μL, 0.5%) was placed on the surface of the working electrode and allowed to dry under ambient conditions for 2 h. The formed Nafion membrane was used to fix the GOD-impregnated carbon on the electrode surface.

Acknowledgements

A.V. is grateful to ARC for the award of the future fellowship and the University of Queensland for the start-up grant; A.V. also acknowledges the financial support from King Saud University to NIMS, Japan and the support from CINBM, Ewha Womans University, Korea.

Keywords: biosensors · carbon · nanostructures · porous materials · thin films

[1] a) M. Pumera, *Chem. Eur. J.* **2009**, *15*, 4970–4978; b) X. W. Mao, X. Q. Yang, G. C. Rutledge, T. A. Hatton, *ACS Appl. Mater. Interfaces* **2014**, *6*, 3394–3405; c) J. Wang, *Chem. Rev.* **2008**, *108*, 814–825; d) S. Roy, Z. Gao, *Nano Today* **2009**, *4*, 318–334; e) J. C. Claussen, A. Kumar, D. B. Jaroch, M. H. Khawaja, A. B. Hibbard, D. M. Porterfield, T. S. Fisher, *Adv. Funct. Mater.* **2012**, *22*, 3399–3405; f) S. H. Yeom, B. H. Kang, K. J. Kim, S. W. Kang, *Front. Biosci.* **2011**, *16*, 997–1023.

[2] a) B. Pérez-López, A. Merkoçi, *Adv. Funct. Mater.* **2011**, *21*, 255–260; b) S.-J. Bao, C. M. Li, J. F. Zang, X. Q. Cui, Y. Qiao, J. Guo, *Adv. Funct. Mater.* **2008**, *18*, 591–599; c) D. L. Lu, J. Cardiel, G. Cao, A. Q. Shen, *Adv. Mater.* **2010**, *22*, 2809–2813; d) C. C. Kung, P. Y. Lin, F. J. Base, Y. H. Xue, X. Yu, L. M. Dai, C. C. Liu, *Biosens. Bioelectron.* **2014**, *52*, 1–8; e) B. Xu, M. L. Ye, Y. X. Yu, W. D. Zhang, *Anal. Chim. Acta* **2010**, *674*, 20–26; f) K. Kwak, S. S. Kumar, K. Pyo, D. Lee, *ACS NANO* **2014**, *8*, 671–679.

[3] a) C. Mu, Q. Zhao, D. Xu, Q. Zhuang, Y. Shao, *J. Phys. Chem. B* **2007**, *111*, 1491–1495; b) A. Riklin, E. Katz, I. Willner, A. Stocker, A. F. Buchmann, *Nature* **1995**, *376*, 672–675.

[4] a) S. Y. Deng, G. Q. Jian, J. P. Lei, Z. Hu, H. X. Ju, *Biosens. Bioelectron.* **2009**, *25*, 373–377; b) J. F. Zang, C. M. Li, X. Q. Cui, J. X. Wang, X. W.

Sun, H. Dong, C. Q. Sun, *Electroanalysis* **2007**, *19*, 1008 – 1014; c) C. P. You, X. Li, S. Zhang, J. Kong, D. Y. Zhao, B. H. Liu, *Microchim. Acta* **2009**, *167*, 109 – 114; d) L. Tang, Y. Zhou, G. Zeng, Z. Li, Y. Liu, Y. Zhang, G. Chen, G. Yang, X. Lei, M. Wu, *Analyst* **2013**, *138*, 3552 – 3560; e) B. Liang, L. Fang, G. Yang, Y. Hu, X. Guo, X. Ye, *Biosens. Bioelectron.* **2013**, *43*, 131 – 136.

[5] a) C. Chen, Q. J. Xie, D. W. Yang, H. L. Xiao, Y. C. Fu, Y. M. Tan, S. Z. Yao, *RSC Adv.* **2013**, *3*, 4473 – 4491; b) Y. Cui, Q. Wei, H. Park, C. M. Lieber, *Science* **2001**, *293*, 1289 – 1292; c) F. Xiao, Y. Q. Li, X. L. Zan, K. Liao, R. Xu, H. W. Duan, *Adv. Funct. Mater.* **2012**, *22*, 2487 – 2494; d) A. C. Chen, S. Chatterjee, *Chem. Rev.* **2013**, *42*, 5425 – 5438; e) Y. Yu, Z. Chen, S. He, B. Zhang, X. Li, M. Yao, *Biosens. Bioelectron.* **2014**, *52*, 147 – 152.

[6] a) F. Liu, T. S. Seo, *Adv. Funct. Mater.* **2010**, *20*, 1930 – 1936; b) E. Nossol, J. G. Zarbin, *Adv. Funct. Mater.* **2009**, *19*, 3980 – 3986; c) M. Musameh, M. R. Notivoli, M. Hickey, I. L. Kyratzis, Y. Gao, C. Huynh, S. C. Hawkins, *Adv. Mater.* **2011**, *23*, 906 – 910; d) Y. Liu, T. Han, C. Chen, N. Bao, C. M. Yu, H. Y. Gu, *Electrochim. acta* **2011**, *56*, 3238 – 3247.

[7] a) C. P. You, X. Xu, B. Z. Tian, J. L. Kong, D. Y. Zhao, B. H. Liu, *Talanta* **2009**, *78*, 705 – 710; b) S. Wu, H. X. Ju, Y. Liu, *Adv. Funct. Mater.* **2007**, *17*, 585 – 592; c) L. H. Tang, Y. Wang, Y. M. Li, H. B. Feng, J. Lu, J. H. Li, *Adv. Funct. Mater.* **2009**, *19*, 2782 – 2789; d) S. Dhanekar, S. Jain, *Biosens. Bioelectron.* **2013**, *41*, 54 – 64; e) Y. Zhou, L. Tang, G. Zeng, J. Chen, Y. Cai, Y. Zhang, G. Yang, Y. Liu, C. Zhang, W. Tang, *Biosens. Bioelectron.* **2014**, *61*, 519 – 525.

[8] a) Z. H. Dai, H. X. Ju, *TrAC Trends Anal. Chem.* **2012**, *39*, 149 – 162; b) X. B. Lu, Y. Xiao, Z. B. lei, J. P. Chen, H. J. Zhang, Y. W. Ni, Q. Zhang, *J. Mater. Chem.* **2009**, *19*, 4707 – 4714; c) I. I. Slowing, B. G. Trewyn, S. Giri, V. S. Y. Lin, *Adv. Funct. Mater.* **2007**, *17*, 1225 – 1236; d) G. S. Hong, C. Li, L. M. Qi, *Adv. Funct. Mater.* **2010**, *20*, 3774 – 3783.

[9] a) X. Xu, Z. B. Tian, J. L. Kong, S. Zhang, B. H. Liu, D. Y. Zhao, *Adv. Mater.* **2003**, *15*, 1932 – 1936; b) C. K. Tsang, T. L. Kelly, M. J. Sailor, Y. Y. Li, *ACS NANO* **2012**, *6*, 10546 – 10554; c) H. Q. Wang, G. H. Li, L. C. Jia, G. Z. Wang, C. J. Tang, *J. Phys. Chem. C* **2008**, *112*, 11738 – 11743; d) H. Q. Wang, G. Z. Wang, L. C. Jia, C. J. Tang, G. H. Li, *J. Phys. D* **2007**, *40*, 6549 – 6553; e) H. Q. Wang, N. Koshizaki, L. Li, L. C. Jia, K. Kawaguchi, X. Y. Li, A. Pyatenko, Z. Swiatkowska-Warkocka, Y. Bando, D. Golberg, *Adv. Mater.* **2011**, *23*, 1865 – 1870.

[10] a) S. H. Joo, S. J. Choi, I. Oh, J. Kwak, Z. Liu, O. Terasaki, R. Ryoo, *Nature* **2001**, *412*, 169 – 172; b) C. Yu, J. Fan, B. Tian, D. Zhao, G. D. Stucky, *Adv. Mater.* **2002**, *14*, 1742 – 1745; c) B. Assfour, S. Leoni, G. Seifert, I. A. Baburin, *Adv. Mater.* **2011**, *23*, 1237 – 1241; d) X. Wang, S. Dai, *Angew. Chem. Int. Ed.* **2010**, *49*, 6664 – 6668; *Angew. Chem.* **2010**, *122*, 6814 – 6818; e) A. Vinu, T. Mori, K. Ariga, *Sci. Technol. Adv. Mater.* **2006**, *7*, 753 – 771.

[11] A. Vinu, P. Srinivasu, M. Takahashi, T. Mori, V. V. Balasubramanian, K. Ariga, *Microporous Mesoporous Mater.* **2007**, *100*, 20 – 26.

[12] a) L. Jia, W. Cai, *Adv. Funct. Mater.* **2010**, *20*, 3765 – 3773; b) L. Jia, W. Cai, H. Wang, F. Sun, Y. li, *ACS Nano* **2009**, *3*, 2697 – 2705; c) L. Jia, W. Cai, H. Wang, *J. Mater. Chem.* **2009**, *19*, 7301 – 7307; d) L. C. Jia, H. Q. Wang, D. Dhawale, C. Anand, M. A. Wahab, Q. Ji, K. Arigab, A. Vinu, *Chem. Commun.* **2014**, *50*, 5976 – 5979; e) L. C. Jia, G. P. Mane, C. Anand, D. Dhawale, Q. Ji, K. Ariga, A. Vinu, *Chem. Commun.* **2012**, *48*, 9029 – 9031.

[13] a) J. W. Shim, S.-J. Park, S. K. Ryu, *Carbon* **2001**, *39*, 1635 – 1642; b) A. Vinu, S. Hossian, P. Srinivasu, M. Miyahara, S. Anandan, N. Gokulakrishnan, T. Mori, K. Ariga, V. Balasubramanian, *J. Mater. Chem.* **2007**, *17*, 1819 – 1825.

[14] Y. Liu, M. K. Wang, F. Zhao, Z. A. Xu, S. J. Dong, *Biosens. Bioelectron.* **2005**, *21*, 984 – 988.

Received: August 6, 2014
Published online on ■■ ■■, 0000

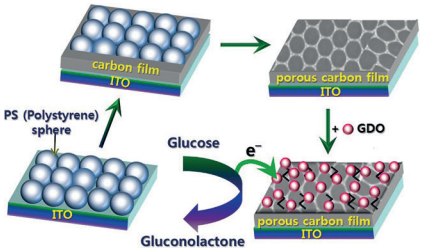
FULL PAPER

Biosensors

*L. Jia, G. Lawrence, V. V. Balasubramanian, G. Choi, J.-H. Choy, A. M. Abdullah, A. Elzatahry, K. Ariga, A. Vinu**

■■ – ■■

Highly Ordered Nanoporous Carbon Films with Tunable Pore Diameters and their Excellent Sensing Properties



Glucose sensor based on ordered porous carbon film exhibits excellent analytical performance over a wide linear range along with high sensitivity and fast response. By tuning the pore size or the biomolecule concentration, it is possible to tailor the sensing properties, suggesting that this new support could be an excellent platform for the design of various biosensors and biofuel cell devices (see scheme).