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Facile Fabrication of Highly Porous Bioelectrode Membrane by Combining Breath Figure Process and Self-Assembly Process with Amphiphilic Diblock Copolymer and Hydrophilic Biocatalyst

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Keywords: amphiphilic diblock copolymer, hydrophilic biocatalyst, self-assembly, breath figure, highly porous membrane

ABSTRACT: This study demonstrated a facile method to fabricate a bioelectrode immobilizing a hydrophilic biocatalyst via self assembling the biocatalyst with an amphiphilic diblock copolymer (di-BCP) in the breath figure process. Poly(methyl methacrylate)-*block*-poly(acrylic acid) (PMMA-*b*-PAA) as an amphiphilic di-BCP and glucose oxidase (GOD) as a hydrophilic biocatalyst were used in this model study. The electrode membrane presented a highly porous morphology, bringing a large surface area and high permeability to reactants. The sensing performances of the bioelectrode were tested to rapidly and conveniently detect glucose. The results showed that PMMA-*b*-PAA and GOD had good synergism on the electrocatalytic activity of GOD, and its current response to glucose was remarkably enhanced. The fabricated electrode presented a good reproducibility and durability. The blood glucose test provided a value well consistent with the one measured by an automatic biochemical analyzer with an enzymatic method from a hospital. This technique would be anticipated to build a platform to rapidly and conveniently construct a highly sensitive biosensor immobilizing a hydrophilic biocatalyst.

Biomembrane is one of the key components of a biosensor. Enormous endeavors have been made to construct a membrane having a high concentration of bioactive molecules[1-3], which can endow a biosensor a high sensitive to substrate molecules. Bioactive molecules could be either absorbed or chemically linked on surface of a membrane[4-8]. The biomolecules would be easily desorbed and washed away in the former. The latter needs extra steps of chemical modification. Physically embedding biomolecules into a membrane is another approach. However, the main issue is that either exposure of biomolecules or diffusion of substrate molecules could be lowered by an embedding material matrix, causing that the sensitivity is reduced and response time elongated.

A porous membrane has abundant internal channels for molecular diffusion, and a large surface area for an exposure reaction[9-10]. The method of breath figure[11-13] was a simple way to form such a membrane, and was intensively studied in the past decades, where tiny condensed moisture droplets were used as a pore template during rapid evaporation of a volatile and hydrophobic solution of a polymer in a humid chamber. In addition, self-assembly was a simple way to construct an ordered structure on electrodes[14-18], e.g., the pioneer work by James Rusling and his coworkers on layer-by-layer surface modifications. In this study, the breath figure and self-assembly were combined in one facile method to form a highly porous polymeric membrane, immobilizing a hydrophilic biocatalyst. The procedure developed in this study was described in Figure 1.

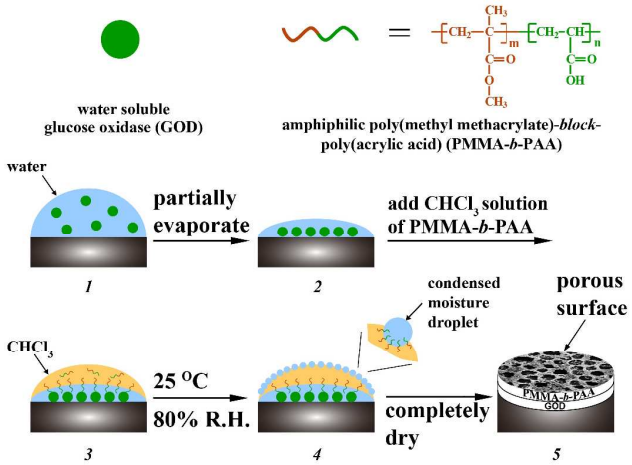

Figure 1. Scheme of formation of porous PMMA-*b*-PAA/GOD membrane on Pt electrode.

Glucose oxidase (GOD) as a model hydrophilic biocatalyst was used in this study since it was a well-understood and commercially available enzyme, and had a high catalytic ability as well as good environmental stability. Poly(methyl methacrylate)-*block*-poly(acrylic acid) (PMMA-*b*-PAA) was chosen as a model amphiphilic block copolymer (BCP) since it was a commonly used and amorphous polymer, and the PMMA block with a molecular weight of 5500 g·mol^{-1} had glass transition temperature (88 ^{o}C)[19-20] much higher than room temperature, beneficial for film formation. The PAA block was hydrophilic and able to self-assemble with hydrophilic GOD. The performances of the fabricated electrode were to be tested by attempting to rapidly and conveniently determine glucose. The reproducibility and durability of the electrode would be tested. The blood glucose concentration would be measured with the fabricated electrode and compared with a reported value from a hospital, where an enzymatic method was to be carried out to determine the concentration in parallel. This work is anticipated to build a platform capable to rapidly and conveniently construct a bioelectrode immobilizing a hydrophilic biocatalyst.

EXPERIMANTAL SECTION

Materials. PMMA-*b*-PAA (Product ID: P8349A-AAMMA with PMMA ($\overline{M}_n$ = 5500 g·mol^{-1}), PAA ($\overline{M}_n$ = 5000 g·mol^{-1}) and $\overline{M}_w / \overline{M}_n$ = 1.15) shown in Figure 2 was purchased from the Polymer Source, Inc. GOD from *Aspergillus niger* was a glycoprotein[21], containing approximately 16 wt% of neutral sugars and 2 wt% of amino sugars, and was purchased from the Sangon Biotech Company, Shanghai in a biochemical grade with a specific activity > 100 U/mg at 25 ^{o}C. The GOD was a dimmer consisting of two equal subunits and reported to have a molecular weight of 160 kDa in total as measured by gel filtration. Each subunit embedded with an electrochemical active site of one flavin adenine dinulceotide (FAD) moiety and one iron. The enzyme also contained three cysteine residues and eight potential sites for N-linked glycosylation[22]. All other chemicals used were of analytical grade and used as received. $CHCl_3$, Na_2HPO_4, and NaH_2PO_4 were purchased from the Shanghai Reagent Company. pH of a phosphate buffer (PB) solution as an electrolyte for an electrochemical measurement was adjusted to 6.5 by using 0.1 M of Na_2HPO_4 and NaH_2PO_4 in total. KBr and glucose were purchased from the Sinopharm Chemical Reagent Co., Ltd.. All aqueous solutions were prepared with deionized water. A glucose solution was prepared one night before a usage.

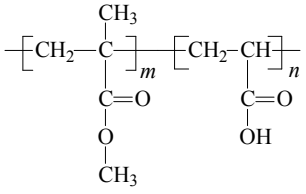

Figure 2. Chemical structure of PMMA (5.5k)-*b*-PAA(5k) with $\overline{m}$ = 55 and $\overline{n}$ = 69.

Apparatus. A three-electrode cell system was used in this study, including a saturated calomel electrode (SCE) as a reference electrode, a platinum sheet as a counter electrode (5 × 6 mm^2), and a Pt electrode (2 mm in diameter) as a working electrode on which the PMMA-*b*-PAA/GOD membrane formed. The cell enclosure was 23 mm in inner diameter, and 54 mm in height. An ultrasound machine (Model KQ-300VDE, the Kunshan Ultrasound Instruments) was used for cleaning the working electrode. A temperature and humidity controlled chamber (Model CTM-5FP01VMM, the Shanghai Shengli Company) was used for storing and drying the samples. An electrochemical workstation (Model CHI 660A by the CH Instruments) was used for amperometric measurements. Fourier transform infrared (FTIR) spectra were recorded by a Tensor 27 spectrometer. The PMMA-*b*-PAA/GOD sample for FTIR was formed on a Ni sheet (50 × 50 mm^2) as it prepared on an electrode. After being dried in the chamber, the membrane was scraped off and further dried under an infrared lamp. A small piece was grinded and tableted with KBr powder for an IR measurement. Ultraviolet-visible (UV) measurements were carried out by using a UV-2550 UV/Vis spectrophotometer. A UV sample was prepared by casting a GOD solution or a PMMA-*b*-PAA solution onto indium tin oxide (ITO) glass and drying in air, or was obtained on ITO glass as PMMA-*b*-PAA/GOD prepared on a Pt electrode and dried in the chamber. The electrolyte solution in the cell (typically 10 mL) was purged with highly purified nitrogen for at least 20 min prior to a series of experiments, and was kept under nitrogen atmosphere during the rest of the measurements. Adjustable micro-pipettes (Model Z 37146, ZX 13939, and KZ 14360 from the Shanghai Thermo Electron Corporation) were used for solution preparation and transfer. The sample film was also coated with a 40 Å layer of Au by a Bal-Tel SCD 500 sputter, and then was imaged with a XL-30E scanning electron microscope (SEM) (Philips, Netherland). A contact angle machine (OCA40, the Dataphysics Company) was used for testing surface hydrophobicity. A 3D stylus surface profilometer (Nanomap-500LS, the AEP Technology) was used to measure a membrane thickness.

Electrode Preparation. A Pt electrode with a diameter of 2 mm was polished by abrasive cloth with a layer of 0.5 and 0.05 μm of alumina grains sequentially, and then was cleaned by ultrasound in ethanol and deionized water respectively for about 2 ~ 3 mins each. The sample was rinsed with deionized water, and dried by air blowing. Amphiphilic PMMA-*b*-PAA was dissolved in $CHCl_3$, and water soluble GOD in 1.0 mM of a PB solution at pH 6.5. In order to obtain a high current response to glucose in an amperometric measurement, the mass of either GOD or PMMA-*b*-PAA was optimized by preparing a series of electrodes and running amperometric tests, and then the optimal values were chosen. In a typical electrode preparation, 8 μL of a GOD solution (1.0 mg/mL) was spread evenly onto the surface of a bald Pt electrode. The cast electrode was put in air at room temperature for about 20 min. The most of water was evaporated, and the sample

was partially dried. 6 μL of the PMMA-*b*-PAA CH_3Cl solution (0.05 wt%) was then cast on the top of the GOD layer. The sample was immediately put into a temperature and humidity controlled chamber (25 °C and 80% R.H.) for over at least half an hour until a complete drying.

Electrode Measurement. For contact angle measurements, two extra PMMA-*b*-PAA electrodes without GOD as controls were prepared by spreading 6 μL of PMMA-*b*-PAA (0.05 wt%) $CHCl_3$ solution alone onto the bald Pt electrode, and then dried either in the controlled chamber or in the open air to form either a porous or a nonporous PMMA-*b*-PAA membrane.

For amperometry measurements, three extra electrodes were prepared as controls. Their current responses to glucose were tested in 10 mL of PB solution at pH 6.5 by a successive injection of 10 μL of 1 M of glucose. The first control was a bald Pt and tested in 0.1 mg/mL of GOD solution. The second one was a porous PMMA-*b*-PAA electrode prepared by casting 6 μL of PMMA-*b*-PAA (0.05 wt%) on a Pt electrode and being dried in the controlled chamber, and was tested in 0.1 mg/mL of GOD solution. The third one was a GOD electrode prepared by casting 8 μL of GOD (1 mg/mL) and being dried in the open air, and was tested in the PB solution.

For a blood glucose test, venous blood was donated by a healthy male with fasting in a routine medical examination. The fresh blood was then tested by using three PMMA-*b*-PAA/GOD electrodes respectively. With each electrode, three drops of the blood with each in 5.0 μL were successively injected into 10 mL of 0.1 M PB solution at pH 6.5. The current responses were measured with amperometry at 600 mV, and the concentration of the glucose was read from the linear range of the calibration curve. The glucose concentration in the blood was then calculated by multiplying it with the dilution coefficient of 2000. The nine concentration values were averaged and compared with the blood glucose level in the medical report from a hospital, which was measured at the same time with an enzymatic method by an automatic biochemical analyzer (Olympus AU400).

RESULTS AND DISCUSSION

Physicochemical Properties of PMMA-*b*-PAA/GOD Membrane.

The formation of a porous morphology with a polymer alone via solvent casting and evaporation in a humid condition had been investigated by researchers[13,23-24] in the studies on the breath figure. Herein, we introduce an extra underlying layer of GOD enzyme into that approach to fabricate a PMMA-*b*-PAA/GOD composite membrane. The SEM images in Figure 3a confirmed that the PMMA-*b*-PAA/GOD membrane still had a porous morphology, which would be expected to have a large surface area and high permeability to reactants.

FTIR spectrometry was considered to be an effective way to monitor the interaction of GOD with others. In Figure S1, an IR peak at 701 cm^{-1} appeared in the spectrum of either PMMA-*b*-PAA or GOD, but disappeared in the PMMA-*b*-PAA/GOD one, which suggested some interaction between PMMA-*b*-PAA and GOD.

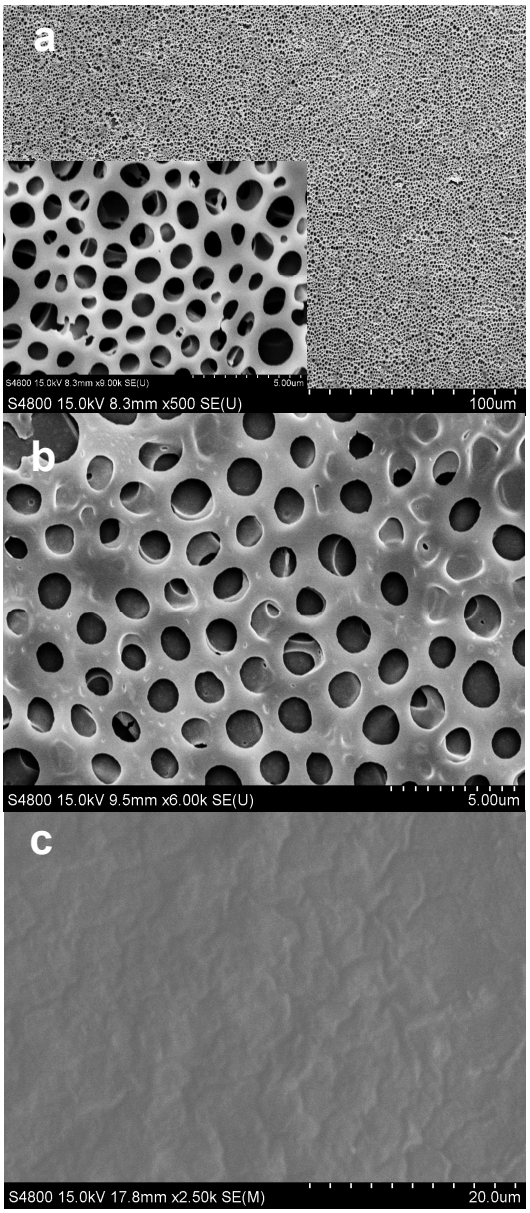

Figure 3. SEM images of porous surface of a) the PMMA-*b*-PAA/GOD membrane and b) the PMMA-*b*-PAA membrane made in a chamber at 25 °C and 80 % R.H, and c) nonporous surface of the PMMA-*b*-PAA membrane made in the open air. The inset of a) had the same magnification of b) and was of a higher magnification of a).

To compare the hydrophobicity of the PMMA-*b*-PAA membrane made in either the open air or a chamber at 25 °C and 80 % R.H. with the one of the PMMA-*b*-PAA/GOD membrane, the contact angle measurement was taken. A membrane made with either PMMA-*b*-PAA alone or PMMA-*b*-PAA/GOD was prepared. The membrane prepared in the open air had nonporous surface (Figure 3c), while the one prepared in the controlled chamber present a similar and porous surface morphology whether GOD was introduced (Figure 3a) or not (Figure 3b). The results in Figure 4 showed that the water contact angle on

PMMA-*b*-PAA/GOD (119.5°) was much higher than the one on either the porous PMMA-*b*-PAA (100.3°) or the nonporous PMMA-*b*-PAA (89.1°) membrane, suggesting that PMMA-*b*-PAA/GOD had more hydrophobic surface than PMMA-*b*-PAA and that not only the porous surface morphology (Figure 4b vs. c) but also the surface hydrophobicity (Figure 4a vs. b) attributed to the increase of the contact angle. Therefore, the introduction of GOD induced the hydrophobic PMMA blocks to enrich on the membrane surface, while the hydrophilic PAA intended to point toward to the underlying layer of the hydrophilic GOD.

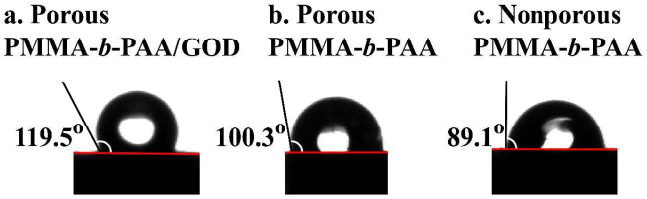

Figure 4. Water contact angle on a) porous PMMA-*b*-PAA/GOD, b) porous PMMA-*b*-PAA, and c) nonporous PMMA-*b*-PAA membranes, suggesting that not only the porosity (b vs. c) but also the surface composition (a vs. b) attributed to the increase of the contact angle and that the hydrophobic PMMA blocks enriched on the surface of the membrane a and the hydrophilic PAA blocks intended to point toward to the underlying layer of the hydrophilic GOD.

In order to better know a degree of the membrane porosity, two membranes with an equal mass were constructed respectively in a drier to form a nonporous membrane and in a controlled chamber to form a porous membrane. The thickness of the nonporous one was 3.50 ± 0.26 μm, and the porous one 7.40 ± 0.15 μm much thicker than the nonporous one, suggesting that the membrane formed in the chamber had a high porosity.

Condition Optimization. To obtain a high response to glucose, the cast mass of either GOD or PMMA-*b*-PAA was optimized by running a series of experiments, and then the optimized values were chosen. In this study 4, 6, 8, 10, 12 or 16 μL of a GOD water solution (1.0 mg/mL) was spread evenly onto the surface of the electrode 1-6, and 8 μL of the solution was spread evenly onto the surface of the electrode 7-14. Each wet electrode was dried in air at room temperature for about 20 min. The most of water was evaporated, and the electrode was partially dried. 6 μL of PMMA-*b*-PAA (0.05 wt%) was then cast on the top of the GOD layer of the electrode 1-6, and 2, 3, 4, 5, 6, 7, 8, or 9 μL of PMMA-*b*-PAA (0.05 wt%) was then cast on the top of the GOD layer for the electrode 7-14. All these samples were put into a temperature and humidity controlled chamber (25 °C and 80% R.H.) over night. After complete drying, each electrode was emerged into 10 mL of a 0.1 M PB solution (pH 6.5) to test a current response to 1.0 mM of glucose at 25 °C and 600 mV (vs. SCE). The results showed that a membrane with 6 μL of PMMA-*b*-PAA (0.05 wt%) (Figure 5b) cast on the top of 8 μg of a GOD (Figure 5a) layer provided the highest response.

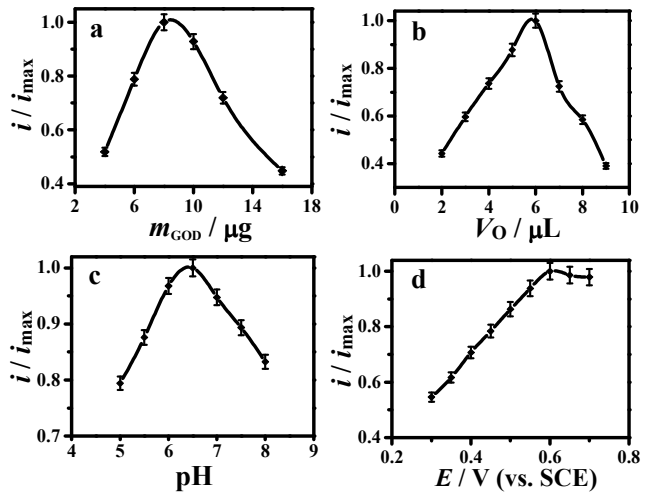

Figure 5. The effect of a) m_{GOD} cast on the electrode, b) the volume of the $CHCl_3$ solution (V_O) of PMMA-*b*-PAA (0.05 wt %) cast on the electrode, c) pH of the testing medium, and d) the potential on the current response of the PMMA-*b*-PAA/GOD electrode in 10 mL of a solution with 1.0 mM of glucose and 0.1 M of PB at 25 °C. Each set of experiments was measured under optimal conditions in other sets. 6 μL of PMMA-*b*-PAA (0.05 wt %) cast on the top of 8 μg of the GOD layer at pH 6.5 and under 600 mV provided the highest current response (i_{max} = 0.97±0.06 μA).

To find optimal testing conditions of the electrode, an electrode prepared with above optimized amount of the materials was further tested for a current response to glucose in terms of the pH, potential, and temperature. It was found that pH of 6.5 (Figure 5c), potential of 600 mV (Figure 5d), and temperature of 50 °C (Figure 6a) were the best conditions to provide the highest current responses. Since in high temperature GOD would be quickly denatured and 25 °C was the most common and convenient for a routine usage in practical applications, the room temperature was more suitable than 50 °C and therefore chosen in this study. For the pH condition, it seemed that pH 6.5 would have one more operational step to tune the blood pH from 6.5 to 7.4. However, no matter with 6.5 or 7.4, the blood specimen required a dilution by the corresponding PB solution for obtaining a sufficient volume for the measurement. There would be no operational difference between them for a dilution. However, pH 6.5 would bring out a much higher current response than pH 7.5 would, and was thus chosen.

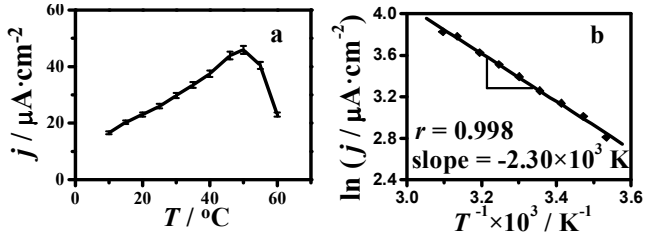

Figure 6. a) The effect of temperature on the current response of the PMMA-*b*-PAA/GOD electrode measured under 600 mV at 25 °C and pH 6.5 in a 10 mL solution of

0.1 M PB and 1.0 mM glucose; b) the linear calibration curve of ln$j$ vs. T^{-1}. E_a of 19.1 ± 0.4 kJ/mol can be extracted from the slope.

According to the Arrhenius equation $\ln k = -E_a/RT + \ln A$, where k was the reaction rate constant, E_a the reaction activation energy, R the gas constant, T temperature in Fahrenheit, and A the Arrhenius constant. Since the reaction process was proportional to the amount of electron transfer, k was proportional to a current response (i) and therefore a current density (j). ln$j$ was proportional to T^{-1}, and the corresponding slope was $-E_a/R$. From the slope of -2.30×10^3 K (r=0.998) in Figure 6b, E_a can be estimated to be 19.1 ± 0.4 kJ/mol. This value was lower than the reported ones, e.g., 20.4 kJ/mol of GOD immobilized in layered double hydroxides by Shan et al.[25], 34.5 kJ/mol of GOD in a composite film of polyaniline/poly(acrylonitrile-*co*-acrylic acid) by Shan et al.[26] suggesting that the activity of GOD immobilized in the current electrode was higher than ones in previous reports.

Electrocatalytic Activity of PMMA-*b*-PAA/GOD Electrode to Glucose. An amperometry method was considered to be an effective way to study electrochemical performances of a biosensor. The electrode was tested at a constant potential of 600 mV at pH 6.5. After the charging current approached to 0 and the baseline went flat in about 1000 s, each 5 μL and 1 M of glucose was successively injected into 10 mL of a PB solution. The current response of the PMMA-*b*-PAA/GOD electrode was 0.53 ± 0.01 μA as estimated from the height of each stage in Figure S2. For controls, the response in 10 mL of 0.1 mg/mL of GOD solution was 0.063 μA with a bald Pt electrode, and was 0.057 μA with a porous PMMA-*b*-PAA electrode prepared with 6 μL of a CH_3Cl solution of PMMA-*b*-PAA (0.05 wt%). Besides those, the response in 10 mL of a PB solution was undetectable with a GOD electrode prepared by casting and evaporating 8 μL and 1 mg/mL of GOD on a bald electrode, very likely due to dissolution of the GOD membrane into the aqueous testing medium and thus GOD was too dilute in the testing medium. The outstanding current response of the PMMA-*b*-PAA/GOD electrode suggested that the ordered arrangement of the polymer and GOD via self-assembly had a synergistic effect on enhancing the current response.

With above optimized conditions, we tested the capability of glucose catalysis of PMMA-*b*-PAA/GOD electrode. It was tested at a constant potential of 600 mV in 0.1 M of PB solution at pH 6.5 (Figure 7). From the slope equal to 1.0 μA·mM^{-1} in Figure 7d and the electrode area, the sensitivity was determined to be 31.8 ± 0.3 mA·M^{-1}·cm^{-2}. With S/N = 3, where N was noise equal to (8.0 ± 1.0) × 10^{-5} μA determined from the baseline fluctuation of i, S was signal current and can be calculated to be (2.4 ± 0.3) × 10^{-4} μA. Then the detection limit of the concentration can be calculated as 0.24 ± 0.03 μM by plug S into the linear fit curve of Figure 7d. The linear range was determined to be 0.42 ~ 6000 μM of glucose with a correlation coefficient (r) of 0.996 as shown in Figure 7d. Compared with a detection limit in other studies, e.g., an electrode based on core-shell NiO/C microspheres (the detection limit of 2 μM, and the linear range of 2 ~ 1279 μM) by Cui et al.[27], an electrode based on carbon quantum dots/octahedral Cu_2O nanocomposites (2.8 μM, and 5 ~ 5300 μM) by Li et al.[28], an electrode based on hemoglobin-capped gold nanoclusters (1.65 μM, and 100 ~ 1000 μM) by Molaabasi et al.[29] and an electrode based on porous CoO-OH nanosheet arrays (1.37 μM, and 3 ~ 659 μM) by Zhang et al.[30], the fabricated electrode in this study showed a fairly low detection limit and a moderately wide detection range for a real application. It should be noticed that a glucose concentration in blood typically varied from 0.5 to 21 mM, which was higher than our linear range. However, the volume amount of the collected blood was very tiny, and the blood specimen had to be diluted by a PB solution before the measurement, e.g., in 1000 folds. A lower limit of the range should be as low as possible. A sufficiently low value of 0.42 μM rather showed an advantage of our electrode.

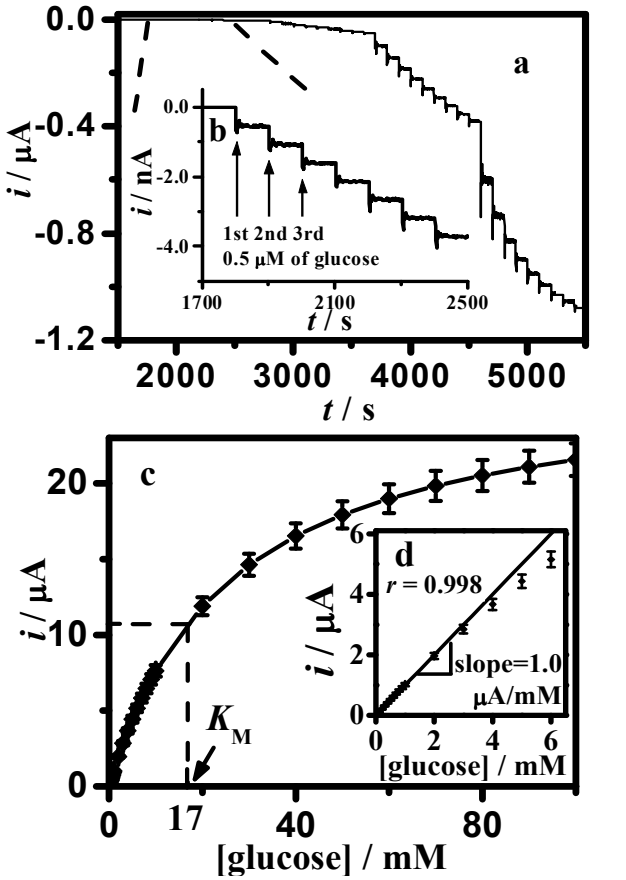

Figure 7. a) A typical curve of the steady-state current response with a successive injection of glucose into 10 mL and 0.1 M of PB solution under fast stirring (600 mV; injection interim of 100 s); b) the enlarged segment of Curve a; c) the calibration curve of PMMA-b-PAA/GOD for glucose; d) the linear range of Curve c; the sensitivity of the electrode to glucose was determined to be 31.8 ± 0.3 mA·M^{-1}·cm^{-2}. The linear range can be obtained as 0.42 ~ 6000 μM, the detection limit as 0.24 ± 0.03 μM, and K_M as 17 mM.

The Michaelis constant (K_M) has often been used to evaluate the biological activity of immobilized enzyme. Smaller K_M, higher the activity was. K_M of 17 mM was read from the concentration of glucose in Figure 7c when *i* was half of the maximum (i_{max} = 22 μA by extrapolation of the concentration to infinity). Compared with GOD immobilized electrodes in other studies, e.g., an electrode based on polypyrrole/PAMAM dendrimers/ferrocene (21.5 mM) by Crepaldi et al.[31], an electrode based on a bilayer of chitosan and Prussian blue (21 mM) by Zhu et al.[32] and an electrode based on a multilayer of poly(m-phenylene diamine)/poly(tetrafluoro ethylene)/polyurethane (24 mM) by Yang et al.[33], K_M in this study were relatively small, suggesting that the self-assembly with PMMA-*b*-PAA made the GOD relatively easier to catalyze the oxidation of glucose than the ones immobilized in above reported electrodes.

Reproducibility and Durability of PMMA-*b*-PAA/GOD Electrode. To test the reproducibility and the durability which were the keys for a bioelectrode, six porous PMMA-*b*-PAA/GOD electrodes were prepared in the same way and their current responses to 1 mM of glucose were recorded at 600 mV (vs. SCE). The relative standard deviation was calculated to be 5.5%. The electrode was then stored at 4 °C in low humidity. After each several days, the current response to glucose was recorded at room temperature. As shown in Figure S3, after 30 days of the multiple tests, the current response still can maintain 82% of the initial current (i_0). To test the operating stability of the electrode, 30 successive measurements of glucose as low as 1.0 μM provided a reproducible current response with a relative standard deviation of 10.3%. These results indicated that the electrode fabricated by this method showed a fairly good sensing reproducibility and durability.

Blood Glucose Assay. Three PMMA-*b*-PAA/GOD electrodes were prepared in the same and optimum conditions, and used to test the glucose concentration in the venous blood of fasting human. Three drops of the blood with each in 5.0 μL were successively injected into 10 mL of a PB solution at pH 6.5. The current response of each electrode was recorded at 600 mV, and the corresponding glucose concentration was read from the calibration curve in Figure 7d. The glucose concentration in the blood was calculated by multiplying it the dilution coefficient of 2000. The nine concentration values were averaged as 4.7 ± 0.8 mM. At the same time, the blood glucose was tested by a hospital with an enzymatic method in an automatic biochemical analyzer. The reported concentration in the medical report was 4.2 mM, which was well consistent with our measurements.

CONCLUSIONS

A facile method was developed in this study to fabricate a membrane immobilizing a biocatalyst, i.e. GOD. The self-assembly of hydrophilic GOD and amphiphilic PMMA-*b*-PAA brought the membrane highly porous surface, significantly enhancing the sensitivity to the substrate molecule. The performances of the fabricated model electrode were tested to rapidly and conveniently determine glucose. The results showed that PMMA-*b*-PAA and GOD had good synergism on the electrocatalytic activity of GOD, and the current response to glucose was remarkably enhanced upto 31.8 ± 0.3 $mA \cdot M^{-1} \cdot cm^{-2}$. The linear range was determined to be as wide as 0.42 ~ 6000 μM, the detection limit of 0.24 ± 0.03 μM, and K_M of 17 mM. The electrode also showed a good reproducibility and durability. Moreover, the blood glucose test with the electrode provided a value with a good consistent with the one from a medical report by an enzymatic method. This facile fabrication technique was anticipated to create a platform to rapidly and conveniently construct a highly sensitive biosensor immobilizing a hydrophilic biocatalyst.

ASSOCIATED CONTENT

Supporting Information

FTIR spectra of the electrodes, current response of the electrodes, and time stability of the electrodes (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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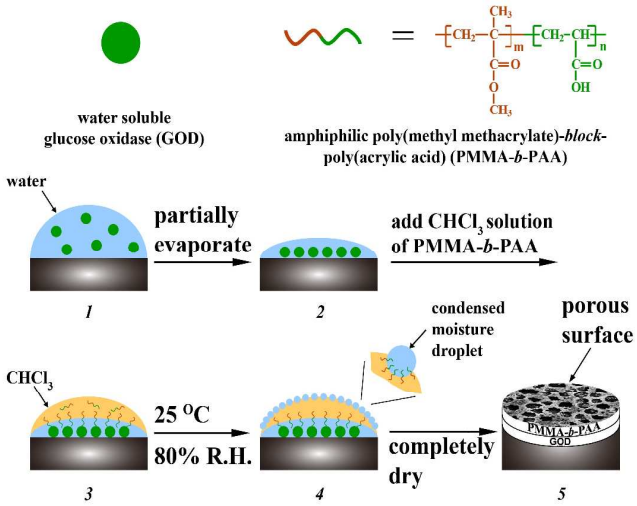
TOC Graphic


water soluble
glucose oxidase (GOD)
amphiphilic poly(methyl methacrylate)-*block*-poly(acrylic acid) (PMMA-*b*-PAA)
water
partially evaporate
add $CHCl_3$ solution of PMMA-*b*-PAA
1
2
condensed moisture droplet
porous surface
$CHCl_3$
25 °C
80% R.H.
completely dry
PMMA-*b*-PAA
GOD
3
4
5