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Letter

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Electrochemical Biosensing Platform Using Hydrogel Prepared from Ferrocene Modified Amino Acid as Highly Efficient Immobilization Matrix

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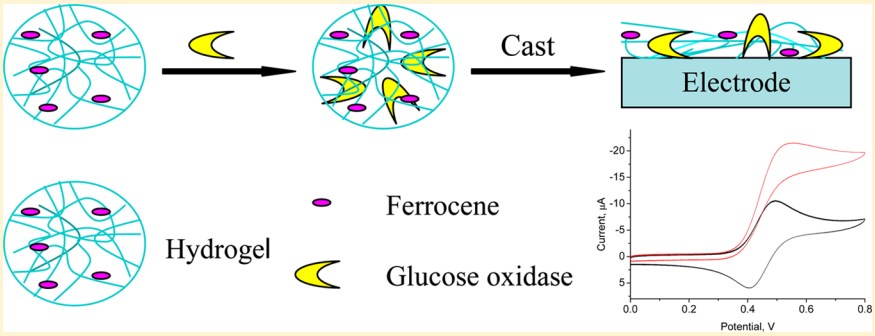
Supporting Information



ABSTRACT: To increase the loading of glucose oxidase (GOx) and simplify glucose biosensor fabrication, hydrogel prepared from ferrocene (Fc) modified amino acid phenylalanine (Phe, F) was utilized for the incorporation of GOx. The synthesized hydrogel displays good biocompatibility and contains a significant number of Fc moieties, which can be considered as an ideal matrix to immobilize enzymes for the preparation of mediator-based biosensors. The hydrogel was studied by scanning electron microscopy, which indicated that it was composed of nanofibers with a diameter of around 50−100 nm and length extended to 1 mm. With the addition of GOx into the hydrogel and by directly dropping the resulting biocomposite onto the electrode surface, a glucose biosensor, that displays good performance due to improved enzyme loading and efficient electron transfer, can be simply constructed. The favorable network structure and good biocompatibility of the hydrogel could effectively avoid enzyme leakage and maintain the bioactivity of the enzymes, which resulted in good stability of the biosensor. The biosensor was utilized for the detection of glucose in blood samples with results comparable to those obtained from the hospital. The hydrogel as a functional component of an amperometric biosensor has implications for future development of biosensors and for clinical applications.

Enzyme-based electrochemical biosensors that combine the specific recognition of enzymes toward targets with electrochemical transduction methods have the advantages of high sensitivity, low cost, and simple instrumentation.[1−4] Such advantages endow these biosensors with applications in clinical diagnosis, environmental monitoring, food quality control, and so on.[5−7] The immobilization of enzymes is one of the key steps in developing high-performance biosensors, since it will affect the loading as well as the bioactivity of the enzymes.[8−10] Different methods have been studied to achieve efficient enzyme immobilization, such as covalently binding enzymes onto substrate surface or incorporating enzymes into different matrixes.[11−13] Compared to the immobilization of enzymes onto substrate surface, incorporation of enzymes into matrix has the potential to increase the enzyme loading as well as to protect the enzyme from the surrounding environment. Although significant work has been done in this area, efforts are still needed for further improvement.

Besides the enzyme immobilization, another issue that needs to be considered is the electron transfer between the enzymes and electrode, as the redox centers of the enzymes are usually deeply embedded in the thick protein shell. To solve this problem, artificial electron transfer mediators, such as ferrocene (Fc) and its derivatives, are introduced to facilitate the electron transfer.[14−16] For the successful preparation of biosensors with good performance, enzyme and mediator are both required to be stably immobilized onto the electrode surface for the

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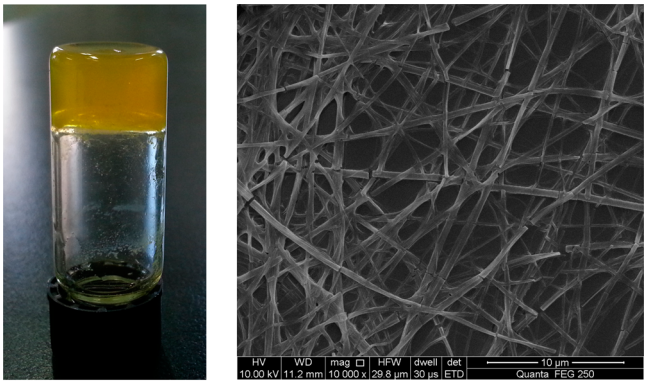
efficient transfer of the biorecognition events into current signal.

Recently, we discovered that ferrocene (Fc) modified phenylalanine (Phe, F) monomers (Fc-F) aggregated in water via a rapid self-assembly mechanism to form stable hydrogels.[17] The hydrogels prepared from such self-assembly methods have gained great interest because of its facile preparation process and functionalization flexibility.[18−20] Compared to other reported hydrogels prepared from peptide and amino acid, Fc-F is characterized by its exceptionally simple molecular structure. In this work, considering the above-mentioned requirements for developing good performance biosensors, the hydrogel as new and efficient immobilization matrix for enzymes in aqueous suspension was explored to develop glucose oxidase (GOx)-based glucose biosensors. The hydrogel was prepared by first coupling Fc onto F (Fc-F) and subsequently dissolving the Fc-F complex into organic solvent followed by dilution with a phosphate buffer. The as formed redox hydrogel displays a yellow color (Figure 1A). The critical



(A) (B)

Figure 1. (A) Image of the synthesized hydrogel. (B) SEM characterization of the hydrogel.

gelation concentration (w/w) of Fc-F is ∼0.3% (3 mg/mL) at room temperature, which means it holds a great number of water molecules. The large number of water molecules contained in the hydrogels provides a favorable microenvironment for GOx. The detailed procedure for the synthesis of the hydrogel and relevant characterization are provided in the Supporting Information. After the addition of GOx into the hydrogel, the resulting biocomposite contains both GOx and mediator Fc. The good biocompatibility of the hydrogel could well maintain the bioactivity of the GOx, and the large number of Fc moieties contained in the hydrogel acted as a mediator for GOx. Hence, the synthesized hydrogel can be considered as an ideal matrix to immobilize GOx for the preparation of glucose biosensors.

Such a glucose biosensor that utilizes an artificial mediator has the advantage of high sensitivity when compared to glucose biosensors that are based on oxygen as natural mediator or are based on the direct electron transfer of GOx.[21,22] Typically, artificial mediator and GOx are separately immobilized onto an electrode surface.[23,24] However, with our designed hydrogel, the electrochemical biosensor for glucose can be simply prepared by directly dropping the GOx incorporated hydrogel onto the substrate surface.

The synthesized hydrogel was characterized by scanning electron microscopy (SEM). As shown in Figure 1B, the hydrogel was composed of mainly nanofibers with diameters ranging from 50 to 100 nm and a length that can extend to 1 mm. A possible mechanism for the formation of the nanofibers is that Fc-F monomers first quickly assembled to dimers, and then, the dimers continue to assemble into fibers.[17] The nanofibers act as entangled matrices for holding large amounts of water and thus lead to the formation of the hydrogels. The synthesized hydrogels are very stable and can retain the original gel state for up to nine months when stored at pH 7.0.

To simplify electrode fabrication, GOx can be incorporated into the hydrogel by simply mixing the hydrogel and the GOx solution. After dropping the GOx incorporated hydrogel onto the electrode surface and drying naturally, another chitosan layer was added onto the electrode to stabilize the hydrogel. Figure 2 displays the cyclic voltammetry (CV) response of the

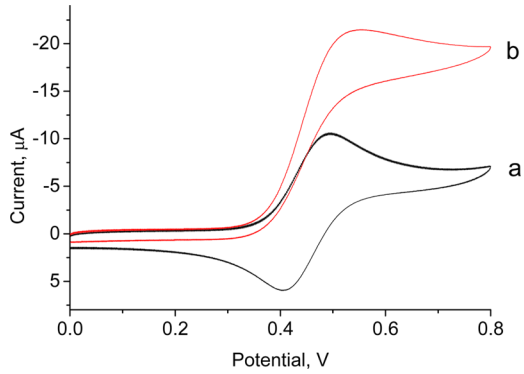


Figure 2. Cyclic voltammetry (CV) response of the glucose biosensor in buffer in the absence (a) and presence (b) of 10 mM glucose. Scan rate, 0.1 V/s.

electrode in buffer. It can be seen that the electrode displays a pair of reversible redox peaks at 0.41 and 0.47 V, respectively (curve a), demonstrating the facile electron transfer of Fc at the electrode surface. After the addition of 10 mM glucose (10 μL of sample) into the buffer, a significant increase of the oxidation current and a dramatic decrease of the reduction current were observed, a typical response of Fc mediated enzyme-catalyzed reaction (curve b).[24,25] The obvious catalytic behavior can be ascribed to the inherent properties of the hydrogel, since (1) the hydrogel can obviously provide a large space to in situ incorporate more enzyme molecules by the one-pot protocol and prevent the enzyme bioactivity loss and (2) the electron transfer among enzyme, mediator, and the electrode is greatly facilitated due to the direct contact of the enzyme with Fc moieties in the hydrogel, which shortened the electron transfer pathway. The control experiment for the electrode modified by the hydrogel but without GOx does not generate an obvious response toward glucose addition.

The effect of GOx concentration on the performance of the biosensor was studied. With increasing the concentration of GOx to 10 mg/mL, the sensitivity of the biosensor to glucose was increased (Supporting Information, Figure S1). Further increase of the GOx concentration resulted in the leakage of the GOx from the hydrogel, thus affecting the stability of the biosensor response. Thus, an optimal GOx concentration of 10 mg/mL was selected for further experiment.

Figure 3A shows a typical current−time response of the biosensor to successive addition of glucose. At the potential of 0.5 V, well-defined amperometric step responses are observed for consecutive glucose injections. The response was fast with

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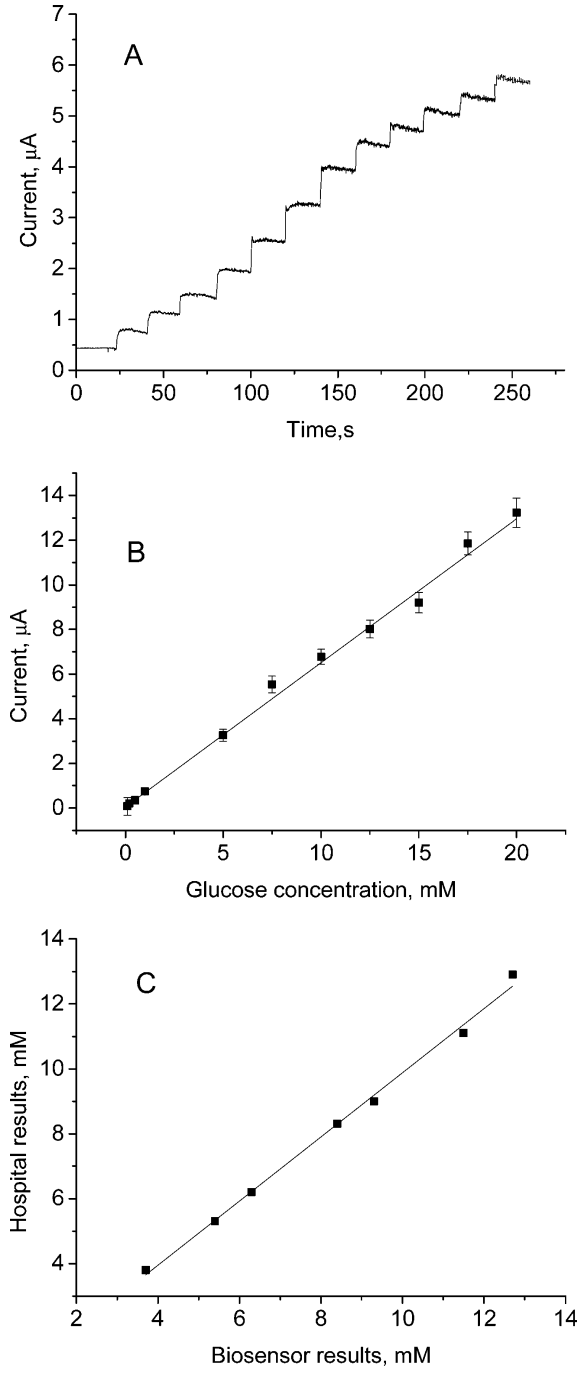

Figure 3. (A) Typical current–time response of the biosensor to successive addition of 1 mM glucose. Potential, 0.5 V. (B) Calibration curve of the biosensor to different concentrations of glucose. Error bar = standard deviation (n = 5). (C) Comparison of the glucose level in blood samples determined with the biosensor and the hospital colorimetric method.

steady-state current reached within 5 s. The calibration curve shows that the linear range of the biosensor to glucose was from 0.1 to 20 mM (Figure 3B) with a detection limit of 50 μM (S/N = 3). The satisfactory performance of the prepared biosensors again proved the attractive merits of the hydrogel.

Different kinds of gels have been studied for the incorporation of enzymes. However, compared to the silica-based sol–gel that has been widely used as matrix for enzyme immobilization, the synthesis of our hydrogel does not require harsh chemical conditions (e.g., strong acid).[26,27] Recent studies also reported metal nanoparticle doped gels for encapsulation of GOx,[8,10] but all these gels do not have redox functionality. While other redox hydrogels have also been studied for the preparation of mediator-based biosensors, such as by attaching redox functions (e.g., $Os^{2+/3+}$) to poly(4-vinylpyridine) (PVP) or poly(*N*-vinylimidazole) (PVI), the synthesis of these hydrogels are rather complex.[28,29]

The apparent Michaelis–Menten constant (K_m^{app}), which gives an indication of the enzyme–substrate kinetics, can be calculated from the Linweaver-Burk equation:[30]

$$1/I_{ss} = 1/I_{max} + K_m^{app}/I_{max} \times C$$

In the equation, I_{ss} is the steady-state current after the addition of substrate, I_{max} is the maximum current under saturated substrate conditions, and C is the concentration of substrate. A value of 8 mM is obtained for the hydrogel modified electrode, which is lower than that of the native enzyme (~33 mM).[31,32] The K_m^{app} value in this work is also lower than 23 mM for GOx entrapped in silica hybrid material[33] and 17 mM for GOx immobilized through the layer-by-layer method,[34,35] indicating GOx entrapped in the hydrogel exhibits higher enzymatic activity and higher affinity for glucose.

To further study the performance of the proposed biosensor, the selectivity, repeatability, reproducibility, and stability of the biosensor were investigated. The selectivity of the biosensor was first investigated. The response of the biosensor to 0.1 mM ascorbic acid, uric acid, and acetaminophen were recorded, which were negligible compared to the response of the biosensor to 1 mM glucose (Supporting Information, Figure S2). The repeatability was examined by the successive detection of 5 mM glucose using a single electrode. A relative standard deviation (RSD) of 5.3% was obtained for 10 successive detections. The reproducibility was studied by six biosensors prepared independently using the same batch of hydrogel, and a RSD of 6.7% was obtained using the prepared biosensors for the detection of 5 mM glucose. The immobilized GOx in the hydrogel film was also relatively stable. When the modified electrode was scanned continuously in the glucose solution, the voltammetric response decreased slowly and remained at ~90% of the initial response after 100 cycles. The long-term stability of the biosensor was also tested. When the biosensor was not in use, it was stored in buffer at 4 °C. The response of the biosensor toward 5 mM glucose was tested intermittently, and no significant signal variation was observed after two weeks, indicating good stability of the immunosensor. After one month, the response of the biosensor decreased to about 80% of its initial values (Supporting Information, Figure S3).

On the basis of the good performance of the biosensor described above, the glucose biosensor was applied for determining glucose concentrations in blood samples donated by healthy and diabetic persons. Results were compared with those determined by the hospital using the colorimetric method (with a commercially available glucose assay kit, in which GOx oxidizes glucose to produce H_2O_2 that reacts with a dye to generate color, Figure 3C). As can be seen, glucose contents determined by the two methods agree well and a plot of the glucose concentration obtained by the two methods gave a straight line with a correlation coefficient of 0.991, indicating the reliability of the biosensor results.

Recent technologies for immobilization of GOx include immobilization onto modified paper[36] and different nanomaterials, such as TiO_2 nanorods,[37] palladium-helical carbon

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nanofiber (Pd-HCNF) hybrid nanostructures,[38] and magnetic nanoparticles.[39] However, encapsulation of the enzyme into hydrogel, as demonstrated here, offers several advantages such as improved stability of the enzymes. Because hydrogel is a three-dimensional matrix, it permits increased enzyme loading and simplified electrode fabrication compared to other technologies for immobilization of GOx.

In summary, we demonstrated GOx can be successfully incorporated into the prepared hydrogel. By casting the GOx incorporated hydrogel onto the electrode surface in one step, both the enzyme and mediator are immobilized onto electrode. The prepared glucose biosensor exhibited good performance for the electrochemical detection of glucose, such as high sensitivity, wide linear range, short response time, and good stability. The favorable results were attributed to the biocompatible microenvironment provided by the hydrogel and the significant amount of Fc moieties contained in the hydrogel. The glucose biosensor prepared here can serve as a model electrochemical biosensing platform for the development of other enzyme-based biosensors. The main advantage of this biosensor preparation method is its simplicity, which could be applied for the mass production of glucose biosensors and found wide potential commercial applications.

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. 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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