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Development of a Fast and Sensitive Glucose Biosensor Using Iridium Complex-Doped Electrospun Optical Fibrous Membrane

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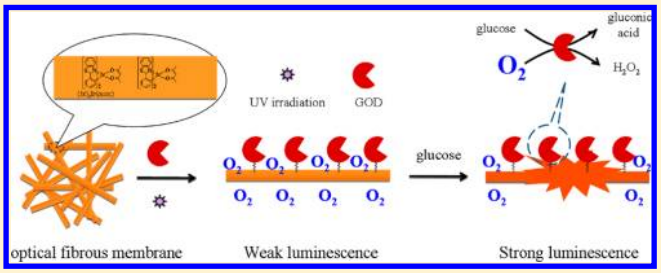
Supporting Information

ABSTRACT: Polystyrene electrospun optical fibrous membrane (EOF) was fabricated using a one-step electrospinning technique, functionalized with glucose oxidases (GOD/EOF), and used as a quick and highly sensitive optical biosensor. Because of the doped iridium complex, the fibrous membrane emitted yellow luminescence (562 nm) when excited at 405 nm. Its luminescence was significantly enhanced with the presence of extremely low concentration glucose. The detection limit was of 1.0×10^{-10} M (S/N = 3), superior to that of reported glucose biosensor with 1.2×10^{-10} M. A linear range between the relative intensity increase and the logarithm of glucose concentration was exhibited from 3.0×10^{-10} M to 1.3×10^{-4} M, which was much wider than reported results. Notably, the response time was less than 1 s. These high sensitivity and fast response were attributed to the high surface-area-to-volume of the porous fibrous membrane, the efficient GOD biocatalyst reaction on the fibers surface, as well as the fast electron or energy transfer between dissolved oxygen and the optical fibrous membrane.



Because of their high surface-area-to-volume ratio, porous structure, and efficient interaction with analytes, electrospun fibrous membranes have great potential as attractive sensor materials for sensitive and quick detection.[1−3] Generally, the surface areas of electrospun fibrous membranes are 1−2 orders of magnitude higher than those of continuous thin films.[4,5] Electrospun fibrous membrane-based optical sensors have been reported for metal ions (Fe^{3+}, Hg^{2+}),[5] organic small molecule (2,4-dinitrotoluene, methyl viologen, and phenolic compounds)[5−9] and proteins (cytochrome c, hemoglobin, and bovine serum albumin).[10] It was demonstrated that detection sensitivities of these fibrous membranes were 1−3 orders of magnitude superior to those of continuous thin films.[5−10] However, the poor detection specificity of these electrospun fibrous membrane-based optical sensors has hampered the application in real sample analysis. Nevertheless, the fast response time of the electrospun fibrous membrane-based optical sensors has not been demonstrated. So, it is still desired to develop a new and facile approach for fabricating electrospun optical fibrous membrane sensor interfaces that provide a high sensitivity, a high specificity, and a fast response time.

Glucose is a very important metabolite for living organisms. Numerous optical biosensors and electrochemical biosensors have been reported for glucose analysis. However, no optical glucose sensor based on electrospun fibrous membrane has been reported. Here glucose as a model analyte, a new porous fibrous membrane combining an electrospinning technique and a glucose oxidases (GOD) covalent immobilization technique was first fabricated. Luminescent transition-metal complexes have been widely used for fabricating optical oxygen biosensors and glucose biosensors due to their large stokes shift, strong photostability, high quantum efficiency, and high oxygen quenching efficiency.[11−15] For $(bt)_2Ir(acac)$, its quantum efficiency (0.26[16]) is significantly higher than that of $Ru(bpy)_3Cl_2$ (0.028[13]) which is widely used as a optical O_2 indicator. Its oxygen quenching efficiency (1.18×10^7 s^{-1}) was comparable to that of iridium complexes and ruthenium complexes.[14−17] So, iridium(III) bis(2-phenylbenzothiozolato-$N,C^{2'}$) acetylacetonate ($(bt)_2Ir(acac)$), was used as the luminescence probe. Using the electrospinning technique, an electrospun fibers doped with $(bt)_2Ir(acac)$) were collected and randomly oriented as a porous electrospun fibrous membrane (EOF). The EOF probably exhibits a high oxygen quenching owing to the doped $(bt)_2Ir(acac)$. To the best of our knowledge, it is the first time that $(bt)_2Ir(acac)$ was doped in electrospun fibrous membranes. Polystyrene polymer (PS) was selected as the electrospining materials because its DMF (*N*, *N*′-dimethylformamide) solution has a good disperse capability for $(bt)_2Ir(acac)$. So, $(bt)_2Ir(acac)$ molecule could be uniformly and stably doped within the PS matrix to avoid its self-quenching and leaching during the luminescent measurement. More importantly, the biofunctionalization of the PS materials

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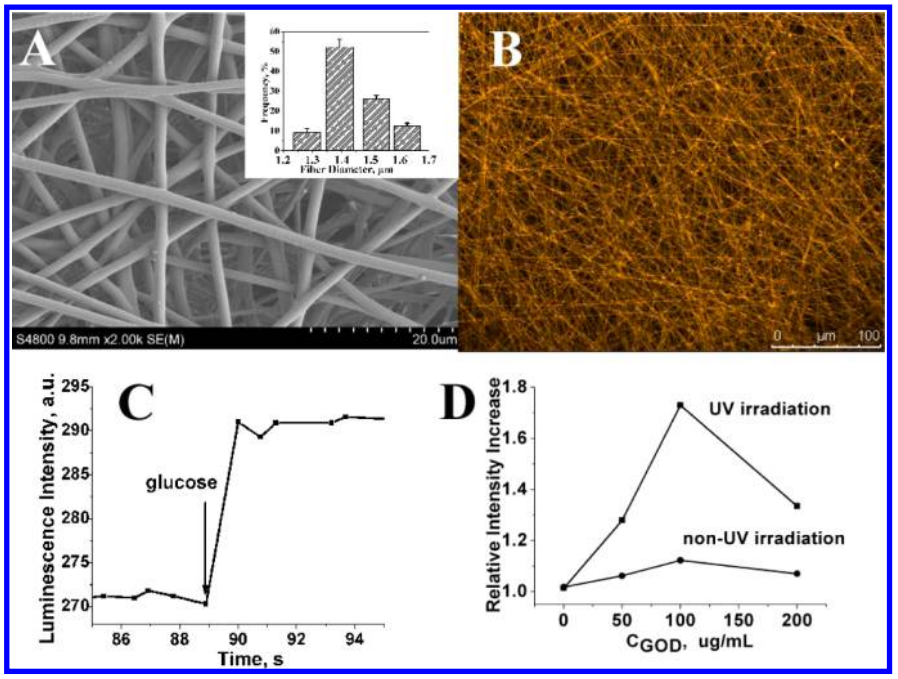

Figure 1. SEM image (A) and luminescence microscopy image (B) of the EOF. An inset shows the diameter distribution of the EOF. The fast response (C) of the EOF when the concentration of the added glucose increases from 1.7×10^{-9} M to 4.4×10^{-9} M. Effects of UV irradiation and GOD amount (D) in a 1.0 mM glucose solution. Each data was obtained from an average value of three replicate measurements. All relative standard derivation was less than 3.0% (n = 3). pH 7.0 PBS buffers.

makes it a good candidate for fabricating a biosensor surface. GOD/EOF was fabricated by covalently immobilizing GOD on the EOF surface using UV irradiation and the glutaraldehyde cross-linking, then used to detect glucose. Since the GOD/EOF have high surface area, high amount of immobilized GOD, efficient GOD biocatalyst reaction, and efficient oxygen quenching, a high sensitivity and specificity and a fast response time for detecting glucose can be achieved.

In this article, we proposed a new fabrication approach for a porous electrospun optical fibrous membrane which contains a luminescence probe within the fibrous membrane and a recognition probe on its surface. Because of the luminescence probe close to the recognition probe, the signal change reduced from the recognition events can be quick. Attributed to the immobilization of the sensitive recognition probe, an ultrasensitive, a superspecific, cost-effective, and regenerable sensing platform can be created. The proposed techniques could further be extended to develop various EOF based sensitive and fast sensors used for food safety, disease diagnosis, and clinical analysis.

EXPERIMENTAL SECTION

Chemicals. Polystyrene watch glasses were obtained from Taizhou Kejian Medical Supplies Ltd. (Jiangsu, China). Glucose oxidases (GOD, 264 Units/mg) and bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO). $(bt)_2$Ir$(acac)$ was synthesized as in refs 15 and 16 and doped to fabricate the EOF. The maximum excitation and emission wavelengths for the EOF were 330 and 562 nm, respectively. DMF (N, N'-dimethylformamide), glutaraldehyde, glucose, lactose, sucrose, fructose, tartaric acid, amber acid, and citric acid were purchased from Chengdu Chemicals (Sichuan, China). All chemicals were of analytical reagent grade and were used without further purification. Fresh human blood samples were collected in Sichuan University hospital (Chengdu, China). Redistilled water was used throughout all the experiments. The phosphate buffer solution (PBS, 0.2 M, pH = 7.0) was used in all the experiments unless specified otherwise.

Characterization. The morphology of the EOF was investigated by scanning electron microscopy (SEM) (S-4800, Hitachi, Japan) at an accelerating voltage of 5 kV. The UV irradiation of the PS fibers was done using a ZF5 portable UV analyzer (365 nm, 8-W) (Jiapeng Technology Ltd., Shanghai, China). The luminescence microscopy image in Figure 1B was performed with a Leica TCS SP5 confocal microscope (Wetzlar, Germany) at the excitation wavelength of 405 nm that is one of the usable excitation wavelengths for the confocal microscope and close to 330 nm.

All luminescence measurements were performed with the luminescence spectrophotometer F-7000 (Hitachi, Japan) with a 96-hole polyporous plate accessory at 25 ± 1 °C. The capacity of single hole of it is 300 μL that allows small-volume detections.[19] In each luminescence measurement, the final volume of the solution for testing was always 100 μL. Both excitation and emission slits were set at 2.5 nm. The excitation and emission wavelengths were always set at 330 and 562 nm unless specified otherwise.

Fabrication of EOF Membrane. The electrospinning solution was prepared by 20 wt % PS with DMF. If added, the concentration of $(bt)_2$Ir$(acac)$ was 0.2 mg/mL. The electrospinning apparatus consisted of a high-voltage power supply (series EL, Glassman High Voltage Inc.), a syringe pump, a syringe, a stainless steel needle (d = 0.55 mm), and a grounded collector (aluminum foil). The electrospinning solution was placed in the syringe. The needle was connected to the high-voltage power supply which can generate a 50 kV positive voltage. The spinning distance between the tip of the needle and the collector was about 23 cm. The positive voltage applied to polymer solutions was 17 kV. During the electrospinning

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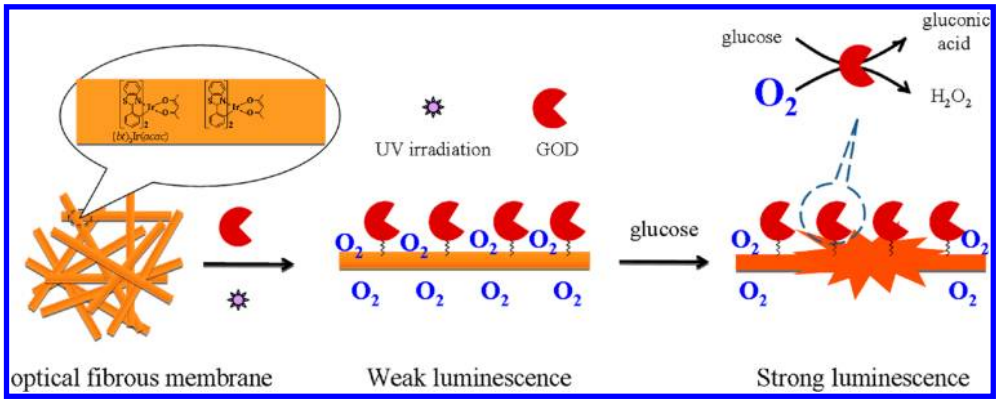
Scheme 1. Schematic Illustration of the GOD/EOF Quickly Detecting Glucose



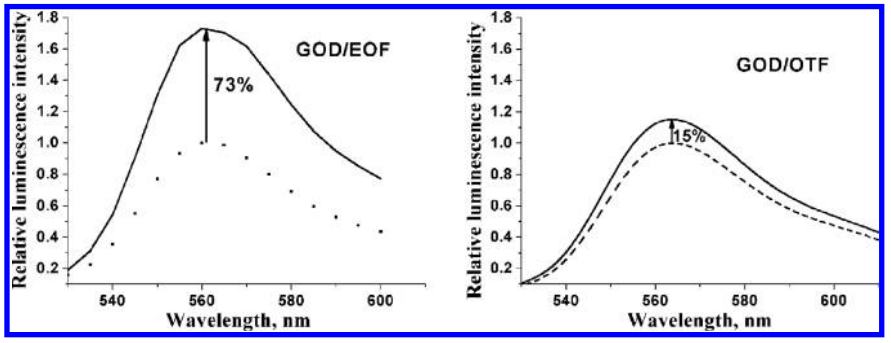


Figure 2. Different performance of the GOD/EOF and the GOD/OTF soaked in PBS buffer solution with (solid line) or without (dot line) 1.0 mM glucose.

process, one should stay at least 5 cm away from the high-voltage supply. The flow rate of the solution was set at 0.5 mL/h by the syringe pump. These fibers were evenly distributed on the substrate, and their orientations are random so that a porous membrane with an interpenetrating network structure was collected. Then the membrane was dried in an oven at 100 °C for 24 h.

Fabrication of OTF (Optical Thin Film). The OTF was fabricated using a DMF solution with the same amount of the PS and the Ir complex as these of the EOF. In brief, 10 pieces of the EOF membrane were completely dissolved with 100 μL of DMF, and 10 μL of these solution was coated on a clean regular class disk (d = 5 mm) and dried at room temperature, and the OTF was fabricated. The thicknesses of EOF and OTF were 200.2 ± 8.6 μm and 145.4 ± 5.7 μm, respectively.

Preparation of GOD/EOF and GOD/OTF. An EOF membrane was cut into a regular disk (d = 5 mm). If irradiated, the EOF membrane was placed under the ultraviolet lamp at 365 nm for 4 h. The radiation distance between the lamp and the PS membrane was 10 cm. Then the EOF membrane with or without the irradiation was soaked in a 100 μL BSA solution (1.0 wt %) for 30 min, followed by a 100 μL glutaraldehyde solution (2.0 wt %) for 30 min. In the end, it was put into a 150 μL GOD solution for 2 h. Except for GOD optimization experiments, the GOD concentration is 100 μg/mL. The EOF membrane was washed using distilled water for three times after each soaking. Then, the GOD/EOF was air-dried and stored at 4 °C. The enzyme immobilization processes for the OTF was the same as that for the EOF.

Sample Determination. Three blood serum samples were simply diluted 1000-fold by a PBS buffer (pH 7.0) to yield testing sample solutions for glucose determination. The glucose content in the blood serum was determined by the standard curve method. The recovery tests for glucose were performed by adding amounts of glucose in the sample solutions. The amounts of added glucose were then evaluated by using the proposed glucose biosensor.

RESULTS AND DISCUSSION

Sensing Strategy and Design of GOD/EOF. High sensitivity, high specificity, and fast response time are the

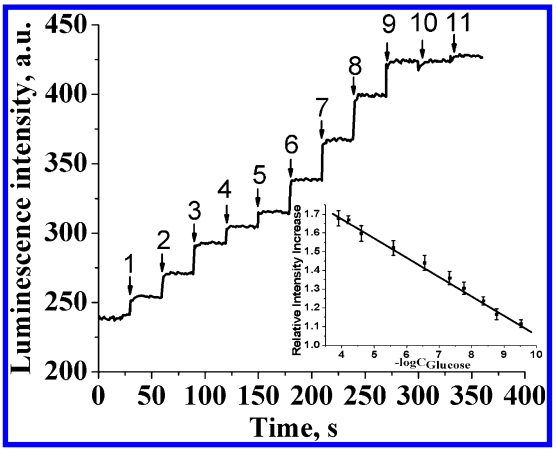


Figure 3. Typical response curves of the GOD/EOF membrane to various glucose solutions in pH 7.0 PBS buffers. The concentration of glucose: (1) 3.0×10^{-10} M; (2) 1.7×10^{-9} M; (3) 4.4×10^{-9} M; (4) 1.7×10^{-8} M; (5) 4.8×10^{-8} M; (6) 2.8×10^{-7} M; (7) 2.6×10^{-6} M; (8) 2.5×10^{-5} M; (9) 6.2×10^{-5} M; (10) 1.3×10^{-4} M; (11) 2.6×10^{-4} M. The inset shows a wide linear range from 3.0×10^{-10} M to 1.3×10^{-4} M. The slope is −0.1. r is 0.996.

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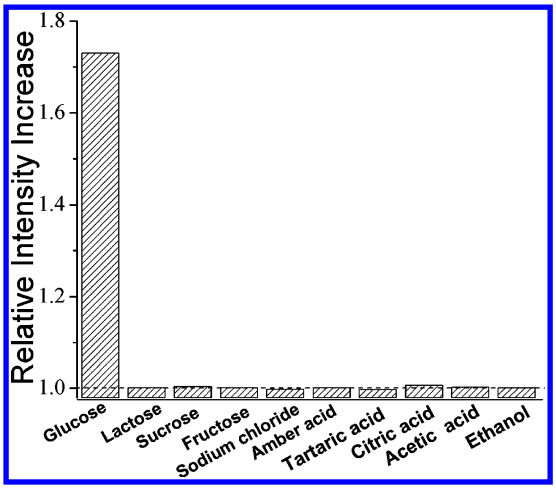

Figure 4. Interference test of the glucose biosensor at pH 7.0 PBS buffers. The concentration of glucose is 1.0 mM. Each interference concentration is 10 mM (except for 20% w/v ethanol and 14% w/v acetic acid).

most key characters for a biosensor. Here, a new fabrication approach was proposed for a porous optical biofunctional electrospun fibrous membrane that contained both a recognition probe and a sensitive luminescence probe. Glucose used as a model analyte, the GOD/EOF was fabricated using an electrospinning technique and a GOD covalent immobilization technique. A sensitive luminescence probe, $(bt)_2\mathrm{Ir}(acac)$ was used instead of a reported ruthenium complex. PS polymer solution mixing with $(bt)_2\mathrm{Ir}(acac)$ was first electrospun to an optical fibrous membrane (EOF). Because DMF solvent has a good disperse capability for $(bt)_2\mathrm{Ir}(acac)$, $(bt)_2\mathrm{Ir}(acac)$ molecule could be uniformly and stably doped within the PS matrix to avoid its self-quenching and leaching during the luminescent measurement. Owing to the high oxygen quenching efficiency (1.18×10^7 s^{-1}) of $(bt)_2\mathrm{Ir}(acac)$,[16] the luminescence from EOF might be sensitive to dissolved oxygen at low concentrations. Moreover, because of its porous and high surface-area-to-volume ratio, the EOF provides the increased immobilization amount of GOD and improved efficient GOD biocatalyst reactions. When glucose was added in the air-saturated solution, oxygen molecules in the interior of the GOD/EOF were more quickly and efficiently consumed than the other dissolved oxygen outside the membrane that was diffusing into the membrane was consumed. The dissolved oxygen concentration was regulated by the added glucose. As a result, the luminescence of the GOD/EOF was fast and significantly enhanced because the fast electron or energy transfer between the EOF and dissolved oxygen was reduced. So the GOD/EOF can be used to detect glucose quickly, sensitively and specifically. The schematic illustration of the GOD/EOF quickly detecting glucose was shown in Scheme 1.

Preparation of the GOD/EOF. The SEM image in Figure 1A shows that the obtained EOF exhibits a porous fibrous membrane and its fibers are evenly and random distributed. Its average diameter was ~1.4 μm by calculating 65 diameter values selected randomly from fibers. The EOF emits yellow luminescence when excited at 405 nm (Figure 1B). As shown in Figure 1C, after the adding of glucose, the GOD/EOF's luminescence intensity was increased greatly and reached a stable value within 1 s after the addition. Compared to reported optical glucose sensors with 3, 20, 120 s and more response time,[18−21] the GOD/EOF exhibited the fastest response time for glucose. The possible reason is that for the fibrous membrane with a high surface-to-volume ratio and a porous structure, the diffuse efficiency of both the glucose and oxygen molecule into the EOF interior could be enhanced as well as a fast electron or energy transfer between the fibers and dissolved oxygen could be realized. Both the high efficiency GOD biocatalyst reaction and the high efficiency oxygen quenching on the fibrous membrane provided the fast response time.

Here, a simple UV irradiation was used on the EOF before the GOD immobilization. It has been demonstrated that free radicals on the PS surface generated during the UV irradiation undergo oxidative reaction to generate hydrophilic groups like the −OH and −COOH groups.[22−24] A decrease of water contact angle value upon UV treatment was measured.[23] Since the distribution of biomolecules[22,23] (such as BSA) on a hydrophilic PS surface was much more uniform than that on a hydrophobic one, we supposed that GOD was uniformly immobilized on the UV irradiated EOF surface adsorbing with BSA via a glutaraldehyde cross-linking and exhibited a good sensitivity. As shown in Figure 1D, at the same glucose concentrations, the GOD/EOF by an irradiation has a more luminescence intensity increase, compared to that without irradiation. The relative standard derivation was less than 3.0% (n = 3). It was demonstrated that these irradiated PS fibers can be used as an effective biosensor support matrix for fabricating biosensors.

Moreover, both the GOD amount (Figure 1D) and pH values (not shown) were studied in detail in order to fabricate a highly sensitive biosensor. With the increase of the GOD concentration to 100 μg/mL, the intensity of the GOD/EOF increases after the addition of the same concentration glucose. However, when the GOD concentration was higher than 100 μg/mL, the intensity increase of the GOD/EOF was reduced. A possible reason could be that when the GOD concentration was so high, the chemical cross-linking of GOD to itself via glutaradehyde was dominant in comparison with that of GOD to BSA. Thus a compact cross-linked GOD membrane formed which blocked the EOF membrane's porosity. A sensitive response was observed at 100 μg/mL GOD and pH 7.0, so the optimum enzyme immobilization amount and pH condition were determined.

Table 1. Determination and Recovery of Glucose in Blood Serum Samples Using The GOD/EOF

blood serum sample	glucose concentration[a] (μM)	glucose concentration[b] (μM)	RSD[c] (%)	glucose added (μM)	glucose found[b] (μM)	recovery (%)	RSD[c] (%)
1	4.56	4.43	1.94	4.50	4.41	96.71	2.27
2	5.64	5.45	2.41	5.60	5.72	102.14	1.98
3	5.79	5.84	2.04	5.80	5.86	101.03	2.79

[a]Determined by the spectrophotometric method in the hospital. [b]Determined by this work. [c]Three replicates were performed.

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Improved Performance of the GOD/EOF for Detecting Glucose. In order to demonstrate the improved performance of the EOF for detecting glucose, we compared the luminescence intensity increase from the GOD/EOF with that from the GOD/OTF in the presence of the same concentration of glucose. As shown in Figure 2, at the glucose concentration of 1.0 mM, the luminescence intensity from the GOD/OTF was increased by 15%, while 73% from the GOD/EOF. These above improvements can be attributed to the higher surface-to-volume ratio and the more porosity structure of the EOF membrane, compared to those of the OTF.[4,5] On one hand, the immobilization amount of the GOD was enhanced. On the other hand, dissolved oxygen and glucose penetrated into the interior of the EOF membrane easily. For the GOD/EOF immobilizing the GOD on their surface and containing optical indicators within their fibers, once glucose was added, oxygen molecules in the interior of the GOD/EOF were quickly and efficiently consumed, and then the luminescence of the GOD/EOF was fast and significantly enhanced due to the fast electron or energy transfer between the fibers and dissolved oxygen.

Quantification Using the GOD/EOF. Figure 3 showed that the luminescence intensity of the GOD/EOF at 562 nm increased as the concentration of glucose increased. The inset displayed the calibration plot of $[F/F_0]$ against $-\log C$. F/F_0 stands for a relative intensity increase. F_0 is the luminescence intensity of the GOD/EOF in buffer without glucose, F is the luminescence intensity of EOF in each different concentration glucose solutions, and C is the concentration of glucose solution (M). Each data in the calibration curve was obtained from an average value of three replicate measurements. The linear equation is $[F/F_0] = -0.11 \log C + 2.1$. A linear range from 3.0×10^{-10} M to 1.3×10^{-4} M was determined with a correlation coefficient r of 0.996. The detection limit was 1.0×10^{-10} M (S/N = 3), which was better than reported results.[18−21] We previously reported a fluorescent glucose biosensor by immobilizing GOD on a porous bamboo inner shell membrane that exhibited a detection limit of 58 μM and a linear response range of 0.0−0.6 mM.[18] Mixing with GOD, a room-temperature phosphorescence TiO_2/SiO_2 nanocomposite was used to detect glucose with a detection of limit of 1.2×10^{-10} M.[19] The GOD/EOF provides a potential application in noninvasively measuring blood glucose using saliva or tear fluid. It has been reported that there is a clinical range of glucose levels between 8 and 210 μM in human saliva.[25] Owing to the GOD covalent immobilization, the GOD/EOF exhibited a good reusability. Importantly, the GOD/EOF exhibited an enhanced sensitivity and an excellent linear range, which was attributed to the efficient GOD biocatalyst reactions on an enlarged membrane surface and the fast electron transfer between the optical fibers and resolved oxygen.

Reusability, Reversibility, and the Long-Term Stability of the GOD/EOF. The reusability and the reversibility of the GOD/EOF were demonstrated by separately measuring its intensities when it was exposed to six cycles of 1.0×10^{-4} M glucose and buffer, respectively (Figure S1 in the Supporting Information). When the GOD/EOF was exposed in the 1.0×10^{-4} M glucose solution, its response time is less than 1 s and the relative standard deviation (RSD) of the measured intensities for 6 successive measurements was less than 1.0%. Whenever the GOD/EOF was moved to the buffer, its intensity went back to the origin. The signal changes were fully reversible. To test the long-term stability, the biosensor was stored at 4 °C in the refrigerator for 6 months followed by measuring its response value. When the GOD/EOF was exposed to a 1.0×10^{-4} M glucose solution, 95% of the prior luminescence intensity was detected. The bioactivity of the immobilized GOD was well maintained, which indicated that the EOF provided a biocompatible microenvironment for the enzyme molecules. The long-term stability (at least 6 months), the usability at high concentration glucose solutions (1×10^{-4} M), and a minimal detection volume (100 μL) provide the possibility of these GOD/EOF membranes being implantable in skin.

Selectivity against Interferences. Some potential interfering substances were tested one by one to evaluate the selectivity of the glucose biosensor. Their concentrations were 10 mM (except for 20% w/v ethanol and 14% w/v acetic acid). As shown in Figure 4, none of the above interferences caused any notable response from the biosensor. However, when 1.0 mM glucose solution was added, a significant enhancement of the luminescence intensity was observed. So, the GOD/EOF exhibited a good selectivity of glucose over these molecules with similar structures or molecular weights.

Blood Glucose Analysis. To illustrate the feasibility of the GOD/EOF membrane in biologically relevant matrix, it was employed to measure glucose in human blood serum. Fresh human blood samples were analyzed by both the spectrophotometric method[26] and the GOD/EOF membrane. In our work, three blood serum samples were simply diluted 1000-fold by a PBS buffer to yield testing sample solutions of pH 7.0 before determination. In addition, the recovery test was conducted. All the data are summarized in Table 1. The results obtained by the proposed glucose biosensor are satisfactory and are in good agreement with that of the sepectrophotometric method. These results demonstrate that the GOD/EOF membrane offers an excellent, accurate, and precise method for determination of glucose in a biologically relevant matrix.

■ CONCLUSION

In this paper, we proposed a simple strategy to fabricate an optical electrospun fibers sensing surface (GOD/EOF). Using an electrospinning technique, an EOF fibrous membrane doped with an iridium complex was fabricated. Owing to the UV irradiation, the enzyme GOD was covalently immobilized on the EOF surface. Luminescence spectra results demonstrated that the GOD/EOF can quickly detect glucose at the trace level. The response time is less than 1 s, and the detection limit is of 1.0×10^{-10} M (S/N = 3). An excellent linear response range between 3.0×10^{-10} and 1.3×10^{-4} M was observed. Furthermore, the reusability, the long-term stability and the selectivity of the biosensor were also studied. The enhanced detection performance of the GOD/EOF biosensor was attributed to its high surface-area-to-volume ratio, the efficient GOD biocatalyst interaction on the fibers surface, as well as the efficient electron transfer between the optical fibers and dissolved oxygen molecules.

■ 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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