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Graphene oxide-functionalized long period fiber grating for ultrafast label-free glucose biosensor

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ABSTRACT

A label-free glucose biosensor is constructed successfully based on the long period fiber grating (LPFG) functionalized with graphene oxide (GO)-glucose oxidase (GOD) via the chemical crosslink method. GO coated on the surface of LPFG can immobilize GOD by the plentiful binding sites because of its favorable combination of exceptionally high surface-to-volume ratio. The structure and characterization of GOD-GO-modified LPFG are studied by the optical microscope, Fourier transformation infrared spectrometer (FTIR), Raman spectroscopy, scanning electron microscope (SEM) and atomic force microscopy (AFM), respectively. The reaction between GOD and glucose create gluconic acid and H_2O_2, which will lead to an evident shift of LPFG transmission spectrum due to the greater change of the surrounding refractive index (SRI). The GOD-GO-modified LPFG sensor shows a linear response with a response coefficient of 0.77 nm/(mg/mL). This biosensor has good selectivity and can be used for the detection of practical sample. The GOD-GO-modified LPFG biosensor has great prospect in the pharmaceutical research and medical diagnosis fields.

1. Introduction

Diabetes is a group of metabolic diseases characterized by high blood sugar concentration, which leads to chronic damage and dysfunction of various tissues, especially eyes, kidneys, heart, blood vessels and nerves [1]. To prevent and reduce complications associated with diabetes mellitus, close monitoring of blood glucose levels is required. Most methods are currently utilized to detect glucose concentration based on electrochemical system [2], which commonly require intricate preparation and high cost, consequently their actual applications are remarkably limited. Compared with the electrochemical method, the optical fiber-based in-situ label-free biosensors have attracted great attention because of its high sensitivity, real-time detection and effective electromagnetic resistance, providing a new method to detecting bio-parameters [3–10].

Actually, it is difficult for single-mode fibers to be directly used in biochemical sensing because of its confinement of optical field caused by the cladding. In order to introduce the guided mode into the interaction between the cladding and the surrounding environment, and to settle the question of the traditional biosensors, such as complex, time-consuming and dangerous, many microstructures and geometrically modified optical fibers have become suitable choices for the interaction between optical modes and the surrounding environment [11–16]. Compared with geometrically modified fibers requiring complete or partial removal of cladding, grating-assisted fibers retain a solid mechanical structure, especially long-period fiber grating (LPFG), which has been applied to the monitoring of biological molecules [14].

The resonance spectrum is formed by the coupling between the basement film of the line core and the cladding mode propagating in the same direction in the long period grating which is depend on the refractive index of the core cladding mode and the grating coating material. Therefore, it is necessary to select a suitable sensitive material for improving grating sensitivity. Graphene is a kind of nano-carbon material with two-dimensional grid structure which has good electrical conductivity, thermal conductivity, high strength, high transparency, large specific surface area and other excellent properties. It is widely used in micro-sensors, light-emitting diodes, catalyst carriers, composite polymer materials, super capacitors and other fields. Graphene oxide (GO) is a derivative of graphene, which has a large number of oxygen-containing functional groups such as carboxyl, hydroxyl and epoxy groups [17] at the edge and top of GO carbon layer. Oxygen-containing functional groups increase the hydrophilic properties of GO, and make it possible to modify GO chemically and further functionalize GO, as well as provide an excellent platform for immobilization [18–20]. As a result, they have been extensively used in the detection of commercial disinfectant water (hydrogen peroxide) [21], serum (urea)

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[22], agricultural hazards (methyl paraoxon) [23], food, beverage (chloramphenicol [24], caffeic acid [25]) and water (nitrite [26]). In addition, some enzymes such as glucose oxidase (GOD) and uricase have been immobilized by functionalized GO as electrochemical biosensors [17,27–29].

There are two innovation points in this work. Firstly, combining the catalysis of enzyme with biocompatibility of GO, which coated on the surface of LPFG, to realize the biosensor for low glucose concentration detection. Besides, the GO function layer will be deposited on the surface of LPFG, connecting the amino groups of the GOD via the cross-linker of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxyl succinimide (NHS) [19]. GOD immobilized on GO-LPFG (GO coated LPFG) will catalyze the glucose oxidation, generating D-glucose acid and hydrogen peroxide resulting in the change of the surrounding refractive index (SRI) and detectable spectral shift of GOD-GO-modified LPFG probe. We have investigated the effects of GOD content and the pH of solution on the properties of sensor. The arranged scheme is a valuable method for ultrafast label-free monitoring of glucose, and has broad application prospects in the field of biosensor and medical diagnosis.

2. Material and methods

2.1. Materials and instruments

Glucose oxidase (from Aspergillus niger), Na_2HPO_4, NaH_2PO_4, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxyl succinimide, nitric acid (HNO_3) and D-glucose were bought from Sinopharm Chemical Reagent Co, Ltd (China). Phosphate buffer solution (PBS) with different pH values was controlled by the volume ratio of Na_2HPO_4 and NaH_2PO_4. Graphene oxide was prepared by Hummers' method [41]and long period fiber grating was fabricated by the fused taper of carbon dioxide laser [30].

The characterization of the GO film coated on surface of LPFG was tested by Optical microscope (OM, Zeiss, Axiovert25), Raman Microscope (Renishaw, 632.8 nm light), Scanning electron microscope (SEM, JEOL Ltd., Japan, JSM-5610LV) and Atomic force microscopy (AFM, Shimadzu, SPM9700), respectively. The chemical bonds between GO and GOD were tested by Fourier transformation infrared spectrometer (FTIR, Thermo Nicolet, 4000-400 cm^{-1}).

2.2. Surface biofunctionalization of GO-LPFG with GOD

The primary functionalization process of the LPFG biosensor is depicted in Fig. 1, including the GO deposition and the GOD immobilization on the LPFG surface. Firstly, the LPFG was immersed in 5% HNO_3 solution at room temperature for 1 h to remove the pollutants, then washed three times with deionized water and ethanol, respectively. Finally, LPFG was immersed in the 1 mg/mL GO dispersions which was pretreated by ultrasound. The both ends of LPFG were fixed to keep it straight and the GO solution was kept at 50 °C, which was beneficial to the GO deposition on the LPFG surface through the dip-coating technique [11], and the operation was repeated three times. Afterwards, The LPFG coated by GO was immersed into deionized water (DI) to dislodge the dissociative GO pieces. At the same time, the two ends of the optical fiber were clamped with two segments to keep the fibers flat so as to make GO as uniform as possible. Graphene and its derivatives coated on the surface of optical fiber can also improve the performance of optical fiber devices [31,32].

The chemical bonding between GO and GOD could be realized with by mixing EDC and NHS as two cross-linking reagents. The carboxyl groups in GO layer around LPFG were activated by incubation for 1 h in an activation buffer containing 20 mM EDC and 40 mM NHS. And then LPFG was immersed in coupling buffer (1 mg/mL GOD/PBS) for 1.5 h, so that GOD was immobilized on GO coating.

Finally, the GOD-GO-modified LPFG was thoroughly washed with PBS buffered solution and DI water and dried at 4 °C. The amino groups of GODs and the carboxyl groups of GO covalently linked together, where immobilizing GOD around the surface of the LPFG. The fabricated GOD-GO-modified LPFG was used to as a sensor to detect glucose sample.

2.3. Measurement system and data analysis

The glucose LPFG sensor system consists of a light source (ASE, HY-ASE-CL-17-N-B-FP), an LPFG probe and an optical spectrum analyzer (OSA, Yokogawa AQ6370B). Its schematic diagram is shown in Fig. 2. When the probe was soaked into the glucose solution, LPFG transmission spectrum will change due to the reaction between glucose and GOD. In addition, the minimization of the bend cross-sensitivity was achieved by fixing the ends of LPFG on the three-dimensional precision platform.

3. Results and discussion

3.1. Surface morphological characterization

XRD and TEM characterizations of graphene oxide prepared by Hummers' method are shown in Figs. S1 and S2, respectively. It is important to ensure the uniformity of GO film coated on the LPFG to

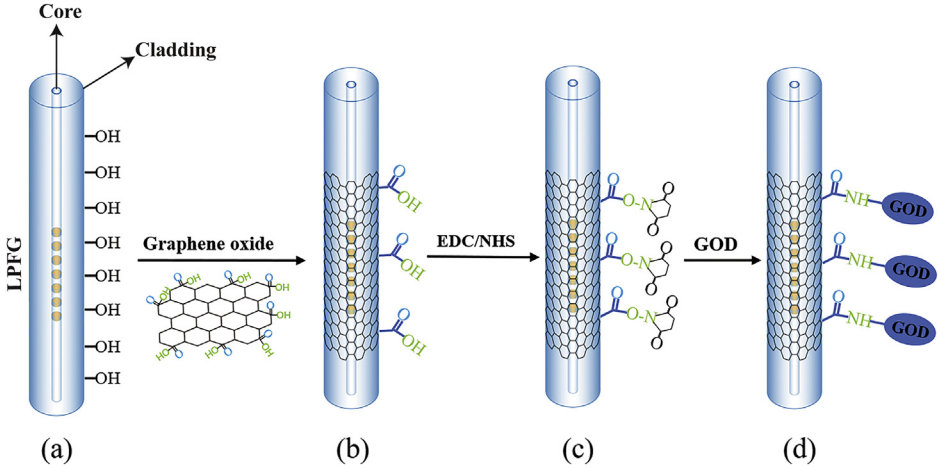

Fig. 1. Schematic illustration of fiber optic biosensor based on GO-coated LPFG: (a) FPLG surface with hydroxylation treatment, (b) GO deposition, (c) EDC/NHS-treated, (d) GOD-immobilized LPFG.

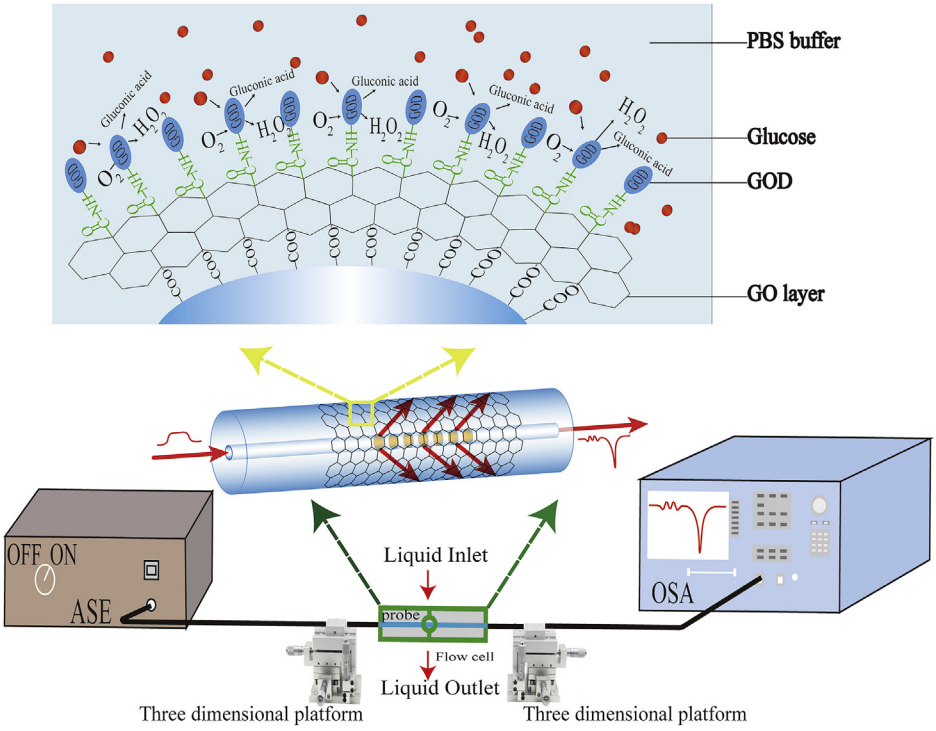

Fig. 2. Experimental setup for surrounding medium and glucose detection. The upward inset shows the schematically diagram of GOD-GO-modified LPFG.

promote the performance of the sensor. Graphene oxide coated on the surface of the LPFG was observed by optical fiber microscopy. As shown in Fig. 3 (a), GO coating was obvious on the surface of LPFG which proved the success deposition of GO on the surface of LPFG. Fig. 3 (b) shows the Raman spectra of bare LPFG and GO-coated LPFG. It is clear that the coated film exhibit D peaks (1343 cm^{-1}), G peaks (1597 cm^{-1}) and second-order 2D (2683 cm^{-1}) peaks unique to graphene oxide compared with bare fibers. The D mode is caused by local defect and disorder of GO and the first order scattering of the E_{2g} plane induces the G mode [33]. The thickness of GO layer on the optic fiber was measured at 320.62 nm by AFM as shown in Fig. 3 (c). In addition, Fig. 3 (d) shows that the surface of the optical fiber is partially uneven, which is due to the stacking of graphene sheets, but it can still be determined that the grating surface is completely covered by GO layer.

To ensure that GOD was immobilized on GO-coated LPFG sensor, the complementary information of functional groups and various qualitative bonds were recorded by FTIR as shown in Fig. 3 (e). It can be seen that the peaks of the intense bands at 1724, 1620, 1287 and 1052 cm^{-1} caused by the stretching vibrations of C=O, C=C, C-O and C-O-C groups, respectively [34,35], which indicate the existence of oxygen-containing groups from GO. After the GO-LPFG immobilization of GOD, we can see that the peaks of GO are still existed. However, the two new peaks at 1635 and 1543 cm^{-1} are observed, which were caused by the bending vibrations of the amide I and II groups [14,35,36], respectively, which indicate the existence of GOD. All in all, the FTIR spectra difference indicated that the GOD was validly covalently-immobilized on the LPFG surface coated by GO.

3.2. Sensitivity of the GOD-GO-modified LPFG sensor

In the transmission spectrum, the cladding mode was coupled to the core mode to produce the discrete resonant waves when the light transmitted along the LPFG. And the minimum transmission of the attenuation band is determined by the following equation [37]:

$$\lambda_{res} = (n_{co}^{eff} - n_{cl,m}^{eff})\Lambda \quad (1)$$

where n_{co}^{eff} is the effective refractive indices of the core, $n_{cl,m}^{eff}$ is the effective refractive indices of the cladding mode and Λ is the grating period. From Eq. (1), if n_{co}^{eff} and Λ are known, λ_{res} of the cladding mode is decided by its effective index, but its effective index depends on the SRI, which is the basic principle of SRI measurement or biological detection.

To exclude the influence of the initial background element, we define the sensitivity of the GOD-GO-modified LPFG sensor as the ratio of the change of resonant wavelengths to the changes of the measurement quantity, which is stated as Eq. (2) [38].

$$s = \frac{\Delta\lambda_c}{\Delta C} \quad (2)$$

where $\Delta\lambda_c$ (λ_c-λ_0) is the wavelength shift between the LPFG λ_{res} at C glucose concentration and the C_0 glucose concentration (the concentration of glucose is 0 mg/mL), ΔC is the change between C and C_0 of the glucose concentration.

To improve the sensitivity of the sensor for the glucose detecting, the detection conditions were optimized. Through a series of experiments, the effects of GOD concentration and solution pH were investigated and the optimum working conditions of the LPFG probe was obtained.

3.2.1. The GOD content influence for the GOD-GO-modified LPFG sensor

The concentration of GOD is a considerable element affecting the performance of LPFG sensor. Fig. S3 shows the correlation between the wavelength shifts and the GOD concentration. It is observed that the $\Delta\lambda_{1.2}$ (wavelength shift of probe at 1.2 mg/mL glucose concentration) values increases incessantly with the GOD concentration increasing from 1 to 4 mg/mL, whereas $\Delta\lambda_{1.2}$ value decreases when GOD concentration increases from 4 to 5 mg/mL. It's ascribed to the acceleration of the catalytic process of glucose with more GOD and creates more gluconic acid and H_2O_2 as well as lead to greater change in the SRI. But if the concentration is exceeded 4 mg/mL, excessive GOD concentration may lead to excessive steric hindrance, thus reducing the activity of the enzyme and affecting the performance of LPFG probe. Hence, the appropriate GOD concentration in this test is chosen as 4 mg/mL.

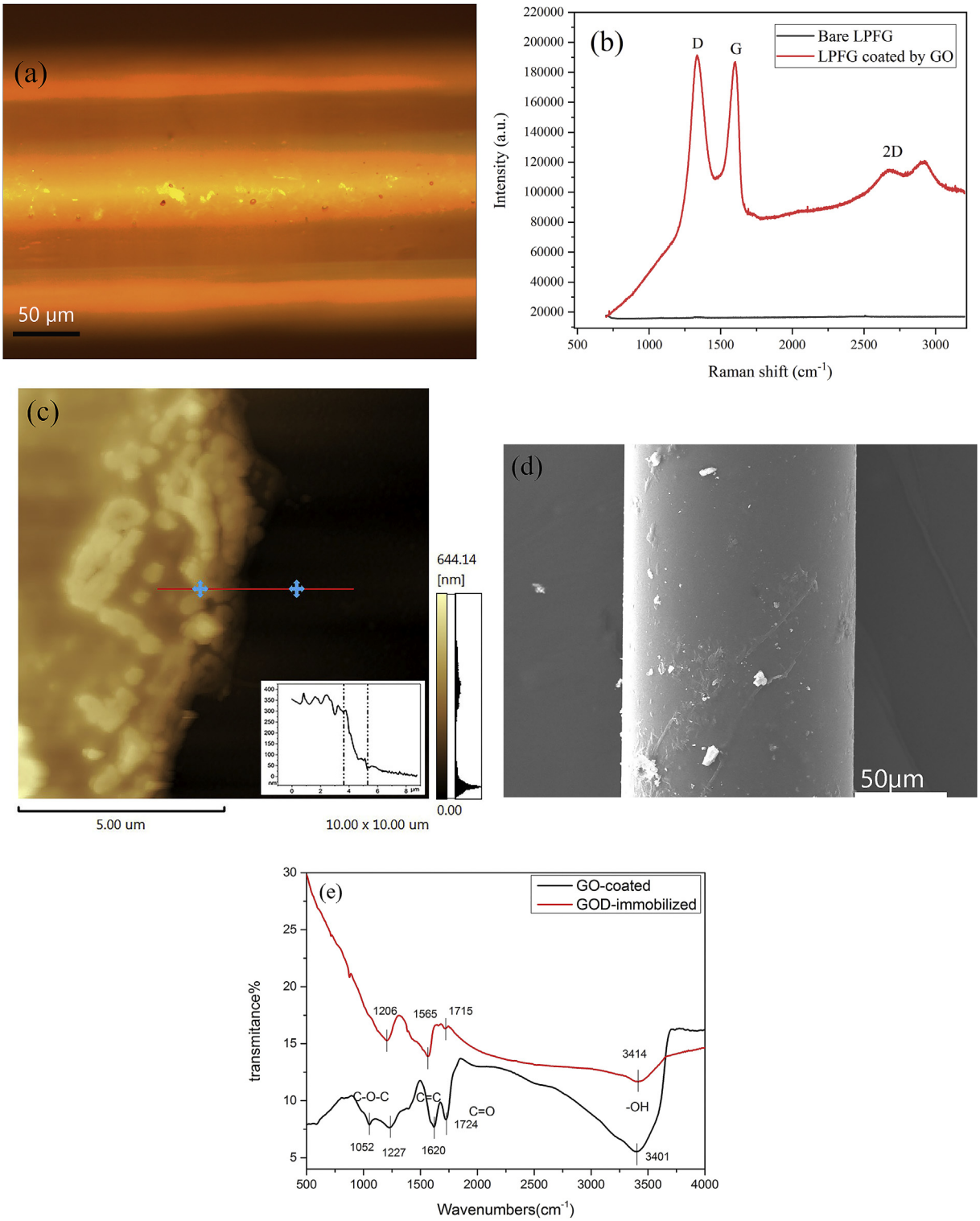

Fig. 3. (a) Optical microscopic image, (b) Raman spectra, (c) AFM (inset: height profile of GO overlay) and (d) FESEM of LPFG coated by GO, (e) FTIR spectra of GO-coated and GOD-immobilized LPFG.

3.2.2. The pH value influence for the GOD-GO-modified LPFG sensor

Enzyme activity is one of the crucial parameters affecting the performance of GOD-GO-modified LPFG sensor, and the enzyme activity is affected by the pH value. Effect of pH on the GOD-GO-modified LPFG sensor performance was tested from the range of 5.0–8.5 against a constant glucose concentration of 1.2 mg/mL. As shown in Fig. S4, the $\Delta\lambda_{1.2}$ increases gradually with increasing of pH and reaches the maximum at pH = 7.0, and then decreases as pH further increases to 8.5. This phenomenon can be explained by the point of zero charge on solid surface [39]. The enzyme activity of GOD enzyme is highest with the optimum pH value (pH = 7), which can catalyze more acid and hydrogen peroxide, and resulting in greater change in the SRI. Therefore, the appropriate value of pH for glucose detection is chosen as 7.

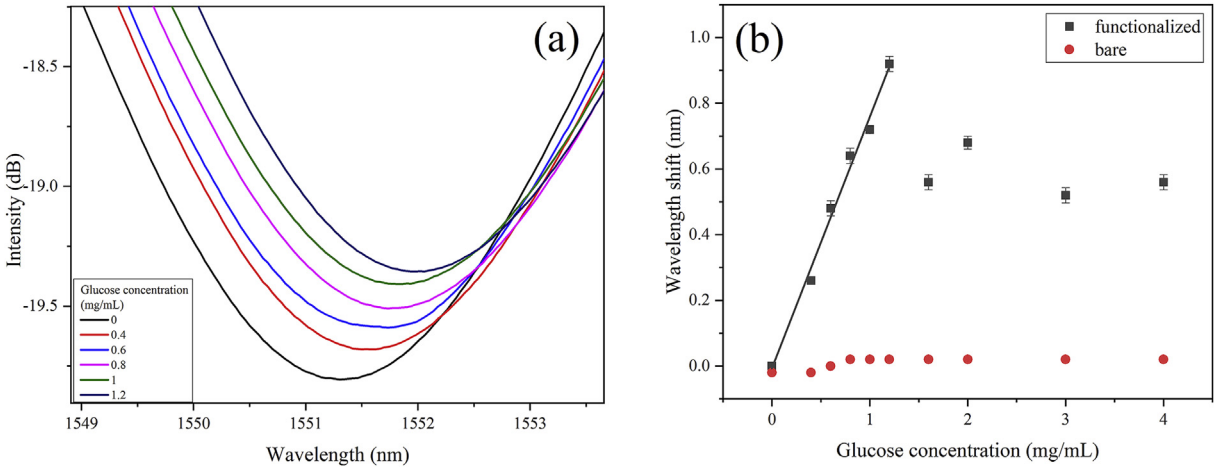

Fig. 4. Transmission spectra of optimized probe (a) and corresponding resonant wavelength shift (b) with the glucose concentration.

3.3. Performance and selectivity of optimized GOD-GO-modified LPFG sensor

The transmission spectra of the GO-LPFG sensor upon addition of different glucose concentrations were shown in Fig. 4 (a). When the grating region coated with GO and GOD is immersed in the glucose solution, the following reactions occur on the grating region between the glucose molecule and GOD:

$$\text{D-Glucose} + H_2O + O_2 \xrightarrow{GOD} \text{D-Glucose Acid} + H_2O_2 \quad (3)$$

D-Glucose molecule is oxidized and decomposed into gluconic acid and hydrogen peroxide under the action of GOD. The presence of these products will greatly affect SRI in the grating region, which further leads to significant changes in the resonance wavelength of the grating cladding mode. With the increasing of glucose concentration, the transmission spectra of LPFG shift to long wavelength because of the change of SRI caused by the by-products produced by enzymatic reaction. Fig. 4 (b) shows the correlation between the resonance wavelength shifts and the different glucose concentrations. With the concentration of glucose increases from 0 to 4 mg/mL, the resonance wavelength shifts increases to maximum with 0.92 nm. Moreover, in the linear response range (0–1.2 mg/mL), the calibration curve can be described by the equation: $\Delta\lambda_{1.2} = 0.77C - 0.0071(R^2 = 0.99)$, the sensitivity of glucose is about 0.77 nm/(mg/mL). When the concentration of glucose is low, glucose can be exposed with GOD as much as possible so that more byproducts will be produced. However, when the glucose concentration exceeded 1.2 mg/mL, GOD will become saturated and there is not much change of the resonance wavelength shift with the concentration still increasing.

The sensing performance of this work was confronted with the previous researches based on LPG [16] and 81∘-TFG in SMF-28 [40,42], as shown in Table 1. Compared with 81°-TFG sensors functionalized by APTES and GOD, the TFG functionalized by GO and GOD shows higher sensitivity, indicating that the introduction of GO and GOD can greatly improve the sensor's sensitivity to glucose. However, the industrialized production of TFG is more difficult because of the complex designs and high industrial costs. Compared with TFG, the GOD-GO-modified LPFG sensor has great advantages in industrialized production because of the industrial production of LPFG. Compare GOD-GO-modified LPFG sensor with GOD-APTES-modified LPFG sensor, replacing APTES with GO solves the potential safety hazard caused by APTES toxicity, and the sensor still maintains high sensitivity. Therefore, this sensor has great prospects in biocompatibility and environmental friendliness.

Table 1
Comparison of sensing performance between different fiber grating-based glucose sensors.

Sensing element	Length (mm)	Glucose sensitivity (nm/(mg/mL))	Measurement range (mg/mL)
TFG (with APTES)	10	0.298	0–3.0
GOD-GO-modified TFG	12	1.33	0–1.5
LPFG (with APTES)	20	0.806	0–3.0
GOD-GO-modified LPFG	20	0.77	0–1.2

Ascorbic acid, sodium ion, potassium ion and chloride ion are the staple interferences in the determination of blood glucose concentration. Consequently, we have investigated the effects of different ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, HPO_4^{2-}, ascorbic acid, fructose, urea and sucrose). Fig. 5 shows the difference between the two resonant wavelengths of the optimized sensor in the solution of all interfering substances (their concentrations are 1.2 mg/mL). Fructose and sucrose have similar structures and properties to glucose, and give the shifts of 0.28 nm and 0.3 nm at resonance wavelengths, respectively. When the optimized probe was put into the solution with other interfering substances, no obvious displacement was observed, which indicated that the optimized glucose sensor has good selectivity.

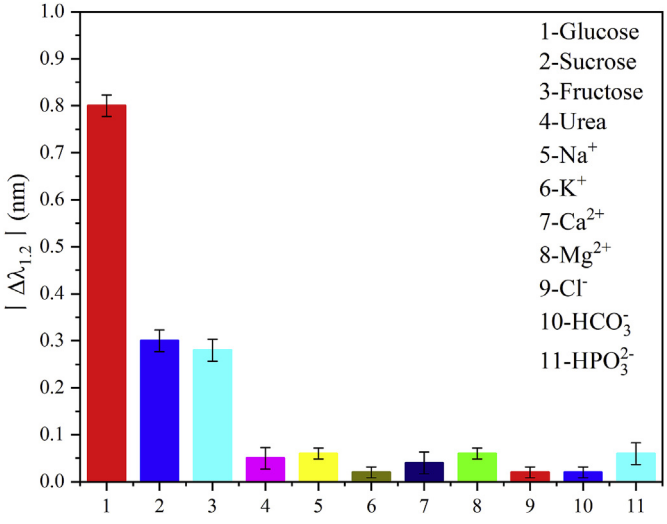

Fig. 5. Selectivity with the interfering substances concentrations of 1.2 mg/mL.

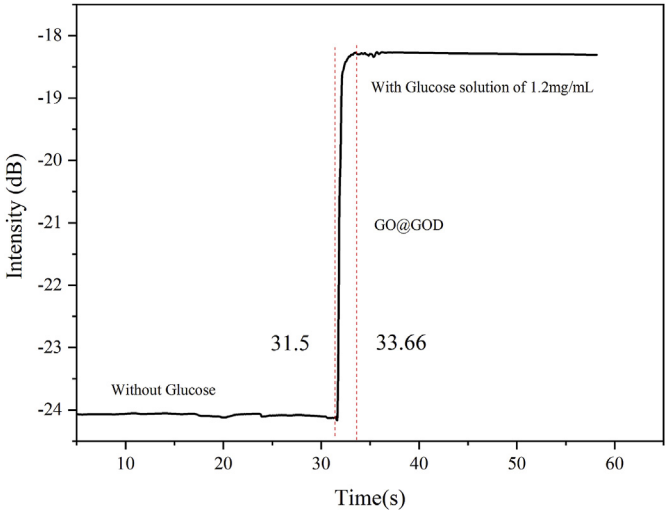

Fig. 6. Response time at1551.68 nm wavelength of LPFG sensor.

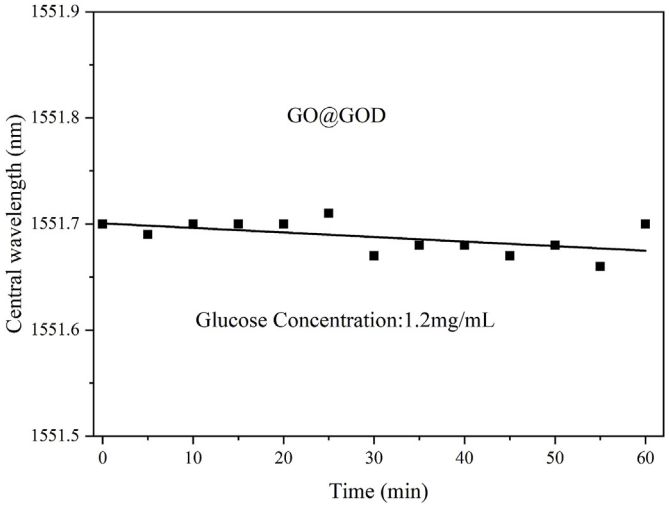

Fig. 7. Stability at 1551.68 nm wavelength of LPFG sensor.

3.4. Response time and stability of GOD-GO-modified LPFG

To study the response time of the GOD-GO-modified LPFG sensor, the optimized sensor was used to detect the glucose with 1.2 mg/mL. The response time of the fiber optic sensor is defined as the time from the fiber optic probe immersed in the glucose solution to the spectrometer receives the optical signal. As exhibited in Fig. 6, some by-products were produced by the reaction between glucose molecules with oxygen dissolved in the solution under the catalysis of GODs, causing the resonance wavelength shifts. And the response time of the optical fiber sensor is about 2.16 s.

For purpose of researching the stability of the GOD-GO-modified LPFG sensor, the optimized sensor was used to detect the glucose with 1.2 mg/mL, record every 5 min. The fluctuation of the resonant wavelength of the optimized probe within 60 min is shown as shown in Fig. 7. When the glucose concentration is 1.2 mg/mL, the resonance wavelength fluctuates around 1551.68 nm, and its average error is 0.015359.

3.5. Real sample detection

Artificial body fluids [43] (ABF) were used as actual samples to carry out recovery tests. The initial glucose concentrations in ABF were set as 0 mg/mL. The sensor was used to detect the concentration of 0.2, 0.5, 0.7 mg/mL of glucose. The detected concentration of glucose was calculated by the shift of the center wavelength of the sensor and the standard curve obtained above. All the tests were implemented in the triplicate measurements. The results are shown in Table 2. The recovery rate of each concentration is between 95% and 105%. Within the requirement of clinical test, it shows that the sensor has a small error in the detection of practical samples and the results are satisfactory.

Table 2
Determination of glucose in practical samples using the proposed sensor.

Sample	Glucose added (mg/mL)	Wavelength shift (nm)	Glucose found (mg/mL)	Recovery[a] (%)
S-1	0.20	0.14	0.191	95.50
S-2	0.50	0.38	0.503	100.60
S-3	0.70	0.54	0.711	101.57

[a] Recovery (%) is the ratio of the found glucose concentration to the added glucose concentration practical samples.

4. Conclusion

A glucose sensor based on graphene oxide was constructed by combining the advantages of chemical and optical fiber sensing, and the optimal working conditions of the sensor were explored. The optimum conditions of the sensor were determined, including the concentration of GOD (4 mg/mL) and pH (7) of solution. The sensor has excellent linear response in low concentration range (0–1.2 mg/mL) with sensitivity of ~ 0.77 nm/(mg/mL). At the same time, the sensor shows faster response speed and shorter response time (2.16 s). In addition, the biosensor has good selectivity and can be used for the detection of practical sample.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.msec.2019.110329.

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