**Real-time amperometric monitoring of cellular hydrogen peroxide
based on electrodeposited reduced graphene oxide incorporating
adsorption of electroactive methylene blue hybrid composites**

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ABSTRACT

This paper demonstrates a novel strategy for real-time monitoring of cellular small biomolecules based on graphene hybrid composites film, which was fabricated by one step electrodeposited reduced graphene oxide (ERGO) incorporating adsorption of electroactive methylene blue (MB) onto glassy carbon electrode (GCE). UV-visible spectroscopy, FT-IR spectroscopy, electrochemistry and scanning electron microscopy were employed for systematical characterization of GO-MB adsorptive nanostructure. The ERGO-MB/GCE shows more favorable electron transfer kinetics for potassium ferricyanide and potassium ferrocyanide probe molecules, which are important electroactive compounds, compared with bare GCE, MB/GCE and ERGO/GCE. The electrochemical behaviors of dissolved oxygen and hydrogen peroxide (H_2O_2) at ERGO-MB/GCE were investigated by cyclic voltammetry, suggesting that the modified electrode exhibits enhanced electrocatalytic activity towards H_2O_2 . Under physiological condition, the ERGO-MB/GCE showed a linear amperometric response from 0.5 μM to 11.68 mM for H_2O_2 , with the detection limit of 60 nM ($\text{S/N}=3$), and also showed high sensitivity ($10.2 \mu\text{A mM}^{-1} \text{cm}^{-2}$) and short response time within 2 s for H_2O_2 detection. Besides, the reproducibility, stability and anti-interference ability of the proposed nonenzymatic H_2O_2 biosensor were also proved. The developed method has been successfully applied to determination of H_2O_2 released from living cells, which could be clearly distinguished cancer cells including Hep3B, HepG2 and HeLa cells from normal cells HEK 293. This assay shows great potential for nonenzymatic determination of H_2O_2 in physiological and pathological investigations.

Keywords: Cellular hydrogen peroxide; Graphene; Methylene blue; Electrochemistry; Cancer cell; Biosensor

1. Introduction

The level of hydrogen peroxide (H_2O_2) has been suggested as a potential biomarker of whole body oxidative stress. H_2O_2 is a major reactive oxygen species in living organisms, which is produced by almost all oxidases in mitochondria and can diffuse out freely through membranes to reach various cellular compartments. Maintaining H_2O_2 at an appropriate level is essential for intracellular signaling transduction and normal cell functions due to oxidative damage resulting from cellular imbalance of H_2O_2 is connected to aging and severe human diseases such as cancer and cardiovascular disorders [1]. Therefore, to monitor the H_2O_2 level in biological environment, especially in cellular environment is an important and significant interest in biology and biomedicine. Comparison of the conventionally colorimetric methods for the H_2O_2 detection [2, 3], electrochemical based H_2O_2 sensors have attracted considerable attention in recent years due to their merits of providing sensitive, accurate and simple, inexpensive, compact and more convenient for on-site assay [4]. Among those reported electrochemical H_2O_2 sensors are the most commonly based on catalytic reduction of H_2O_2 by a natural enzyme, i.e. horseradish peroxidase (HRP), which may efficiently catalyze H_2O_2 dependent one electron oxidation [5, 6]. However, as a natural enzyme, though the low detection limits and high specificity obtained, HRP is not readily available, poorly electrical conductivity and stability. The outstanding chemical/physical properties of nanomaterials, i.e. gold [7], MoS_2 [8] and $\text{PtPd-Fe}_3\text{O}_4$ [9] nanoparticles, Pt nanowire [10], carbon nanotubes [11] and inorganic/organic doped carbon aerogels [12] etc. have been explored as alternative electrode materials or the electrode modification materials for the construction of electrochemical H_2O_2 sensors [13, 14].

Graphene, a two-dimensional honeycomb lattice of sp^2 carbon atoms, is ideally

suited for implementation in electrochemical biosensors applications since it was discovered by Geim and co-workers in 2004 [15] due to its intrinsic properties, including the electronic, optical and mechanical properties associated with its planar structure [16, 17]. Especially, graphene-based hybrids composites exhibit superior performance compared with the individual components because of the synergistic effects between each component [18, 19]. As a representative example, graphene is regarded as an ideal platform has been hybridized with gold [20], platinum [21], silica [22] and CuS [23] nanoparticles, silver nanocrystals [24], prussian blue [25], ferrocene [26] and hemin protein [27] for the determination of H_2O_2 . Graphene oxide (GO) is electrically insulating materials due to its disrupted sp^2 bonding networks, always served as a good precursor to be reduced graphene oxide (RGO) for recovered the electrical conductivity by restoring the π -network [28]. Compared with chemical reduction [29] and thermal reduction [30], electrochemical reduction [31, 32] prepared method is green, fast, free reagent and will not result in contamination of the reduced material. To the best of our knowledge, there being the π - π stacking and hydrophobic interactions between graphene and methylene blue (MB) which a polynuclear aromatic compounds and possessing good electrochemical properties and excellent electrocatalytic activities [33, 34]. MB is expected to enhance H_2O_2 reduction catalysis from the following aspects: MB is used to decorate the surface of graphene nanosheets and graphene itself have excellent electron transfer properties, the combination of this organic-inorganic nanocomposite is proving to be a very powerful strategy for the detection of specific targets; Moreover, MB which has good electrochemical properties and excellent electrocatalytic activities, incorporated in the graphene matrix could effectively shuttle electrons between the base GCE and the specific targets. Therefore, the MB recommended here, which further contributes to

the enhancement of electronic, electrochemical and/or electrocatalytic signal measurement in terms of selectivity and sensitivity [35].

This work describes a novel strategy for real-time monitoring of cellular H_2O_2 based on graphene hybrid composites film, which was fabricated by one step electrodeposited reduced graphene oxide (ERGO) incorporating adsorption of electroactive MB onto glassy carbon electrode (GCE). The interaction of GO with MB was studied with UV-vis spectroscopy and FT-IR spectroscopy. The electrochemically reduced and electrodeposition process of GO-MB was investigated by cyclic voltammetry. Additionally, the surface morphology of the modified electrode was characterized by scanning electron microscopy. And the electrochemical properties of the modified electrodes and the electrocatalytic activities toward the reduction of dissolved oxygen and H_2O_2 were investigated. The reproducibility, stability and anti-interference ability of the modified electrode were also examined.

2. Experimental

2.1. Reagents and apparatus

Graphene oxide (GO) was prepared from natural graphite powders by a modified Hummers method [36, 37]. The as-synthesized GO was dispersed in 10 mM sodium phosphate buffer saline (PBS, pH 7.40) containing 100 mM KCl to yield a yellow-brown suspension (1.0 mg mL^{-1}). Then $100 \text{ }\mu\text{M}$ Methylene blue (MB) solution was mixed with 1.0 mg mL^{-1} GO solution and ultrasonicated for 15 min to get a MB functionalized GO (GO-MB) dispersion solution. MB, hydrogen peroxide (H_2O_2), ascorbic acid (AA), uric acid (UA), dopamine (DA), lysine (Lys), glycine (Gly), and phobol 12-myristate-13-acetate (PMA) were purchased from Sigma. All reagents

were of analytical grade, and Millipore Milli-Q water (18 M Ω cm) was used throughout.

UV-vis spectra were recorded with a Lambda 950 UV-vis spectrophotometer (Perkin Elmer, USA). Fourier transform infrared (FT-IR) measurements were performed with a System 2000 infrared spectrometer (Perkin Elmer, USA). Transmission electron microscopic (TEM) image was obtained using H-600 electron microscope (Hitachi, Japan). Scanning electron microscopic (SEM) images were obtained using TM-1000 tabletop microscope (Hitachi, Japan). All electrochemical measurements were performed using CHI 760D electrochemical workstation (Co. CHI, TX) with a conventional three-electrode system comprised of platinum wire as auxiliary electrode, a saturated Ag/AgCl electrode as reference, and bare or modified glassy carbon electrode (GCE, diameter 3.0 mm) as working electrode. 10 mM PBS (pH 7.40) containing 100 mM KCl was used as electrolyte. Electrolyte solutions were deoxygenated with high purity nitrogen for 10 min prior to each experiment. All measurements were carried out at room temperature under the nitrogen atmosphere.

2.2 Culture and collection of cells

HEK 293, HeLa, Hep3B, and HepG2 cells were obtained from Shanghai Institute of Cell Biology in the Chinese Academy of Sciences. Cells were cultured in IMDM (GIBCO, Grand Island) supplemented with 10% fetal bovine serum (Sigma), penicillin (100 $\mu\text{g mL}^{-1}$), and streptomycin (100 $\mu\text{g mL}^{-1}$) in an incubator (5% CO₂, 37 °C). All cells were collected, separated from the medium by centrifugation at 1000 rpm for 5 min, and then washed twice with sterilized 10 mM sodium phosphate buffer saline (PBS, pH 7.40). The sediments were resuspended in 10 mM PBS (pH 7.40) to obtain cell suspension with the final concentration of cells (1.0×10^7 cells mL⁻¹).

2.3. Preparation of modified electrodes

The GCE was successively polished to a mirror finish using 0.3 and 0.05 μm alumina slurry followed by rinsing thoroughly with water. After successive sonication in 1:1 nitric acid, acetone, and doubly distilled water, the electrode was rinsed with doubly distilled water and allowed to dry at room temperature. And then, the clean GCE was performed electrodeposited reduction by cyclic voltammetric in the GO-MB adsorptive dispersion with a magnetic stirring and N_2 bubbling. The scan was performed between 0.60 V and -1.5 V at a rate of 50 mV s^{-1} , and the loading amount of reduced graphene oxide incorporating adsorption of electroactive methylene blue hybrid composites was controlled by 10 potential cycles (denoted as ERGO-MB/GCE). For comparison, MB/GCE and ERGO/GCE were prepared using GCE in the MB ($100 \mu\text{M}$ MB) and GO (1.0 mg mL^{-1} GO) solution with the same condition as that for the ERGO-MB/GCE, respectively. All modified electrodes obtained were rinsed with water thoroughly.

3. Results and discussion

3.1. UV-vis spectroscopic characterization of GO with MB

The interaction of GO with MB was studied with UV-vis spectroscopy. As shown in Fig. 1, the UV-vis spectrum of the GO dispersed in 10 mM PBS (pH 7.40) shows an absorption peak at 226nm (curve a). The spectrum of MB in 10 mM PBS (pH 7.40) displays a strong absorbance at 664 nm and 292nm (curve b), which are characteristic absorbance peaks of the MB monomer in aqueous solution. The shoulders at 618 nm and 246 nm are ascribed to the absorbance of the MB dimer in aqueous solution [38, 39]. The spectrum of the GO-MB (curve c) displays a absorption peaks at 595 nm, 288 nm and 248 nm compared with that of MB, which is maybe attributed to that the π - π stacking interaction between GO and MB, as well as the electrostatic attraction. The results are in accord with that reported for adsorption of MB onto graphene [33],

while are different from that of MB onto graphite [38] and carbon nanotubes [39]. They are maybe attributed to the fact that the interaction between MB and graphene or GO is stronger than that between MB and graphite as well as between MB and carbon nanotubes. The results suggested that MB molecules were adsorbed onto GO nanosheets, and the GO-MB adsorptive nanostructure in aqueous solution was obtained.

Fig. 1

3.2. FT-IR spectroscopic characterization of GO with MB

Fig. 2 shows the FT-IR spectra of GO, MB and GO-MB. The FT-IR spectrum of GO (curve a) shows that the bands around 1638 cm^{-1} , 1618 cm^{-1} , 1458 cm^{-1} and 1130 cm^{-1} . These confirm that the successful oxidation of graphite and there are a lot of hydrophilic groups, i.e., -OH, -COOH and epoxides in the surface of GO obtained. The FT-IR spectrum of MB (curve b) exhibits its ring stretch at 1593 cm^{-1} , the symmetric stretch of C-N at 1325 cm^{-1} , and stretching vibration of C-C at 1167 cm^{-1} . Similar to the conclusion drawn from the UV-vis spectra (Fig. 1), the adsorption of MB onto the GO was also substantial, based on the FT-IR spectra, since the characteristic spectrum of GO-MB (curve c) was changed clearly comparison of the spectrum of GO.

Fig. 2

3.3. Preparation and electrochemical characterization of ERGO-MB/GCE

Fig. 3A shows the typical cyclic voltammograms of GO-MB hybrid composite was electrochemically reduced and electrodeposition on GCE, where two oxidation peaks at -0.28 V (I) and at $+0.10\text{ V}$ (II), and two reduction peaks at -0.30 V and -1.2 V (III and IV) are observed during the consecutive redox process. The oxidation peak

I and reduction peak III are attributed to the electrochemically reversible redox of adsorptive MB. And the oxidation peak II is probably attributed to the oxygen-containing groups on the graphene plane that are too stable to be reduced by the cyclic voltammetry [35], either contribution of poly(methylene blue) polymer formed on the surface of ERGO. The reduction peak IV is attributed to the irreversible electrochemical reduction of GO [35]. The persistent increase of peak currents with consecutive potential scans indicates that the deposition of conducting reduced GO incorporating adsorption of electroactive MB hybrid composite film on the GCE has successfully been obtained. This is supported by the cyclic voltammograms (Fig. 3B) in which the characteristic redox wave of MB (I and III) is still clearly observed when the ERGO-MB/GCE is re-cycled in the pure phosphate buffer in the absence of MB. The result is similar to that the continuous adsorption and growth of MB onto the graphene [33] and single-walled carbon nanotubes [39] on GCE. Additionally, the small redox peak potential separation (ΔE_p) and a near unity of the ratio of i_{pa}/i_{pc} , characteristic of the fast electron transfer of adsorbed MB on the surface of reduced graphene oxide modified electrode. Fig. 3C depicts the cyclic voltammograms of the ERGO-MB/GCE in phosphate buffer in the range of the scan rates from 10 to 600 mV s⁻¹. The data in Fig. 3D were abstracted from Fig. 3C. From lines a and b in Fig. 3D, it can be seen that the oxidation peak currents linearly increase with the scan rates while the reduction peak currents linearly decrease with the scan rates. And the peak potentials are nearly unchanged with increasing the scan rates (lines c and d in Fig. 3D). This further demonstrates that the electrochemical process at the ERGO-MB/GCE is a surface-confined process with a fast electron transfer [40].

Fig. 3

3.4. Morphologic characterization of ERGO-MB/GCE

The morphologies of GO sheets and GO-MB adsorptive composites were characterized by TEM, and the surface of bare GCE and ERGO-MB/GCE prepared were characterized by SEM, respectively. Fig. 4A reveals that the graphite oxide was entirely exfoliated as individual GO sheets in phosphate buffer solution (pH 7.40). And the morphology of GO-MB adsorptive composites (Fig. 4B) does not change appreciably after the adsorbed MB on the surface of GO. Compared with the SEM image of a clean GCE surface (Fig. 4C), it can be seen that the ERGO-MB hybrid composite film exhibits a curly morphology consisting of a thin wrinkling paperlike structure, and distribute homogeneously on the GCE surface (Fig. 4D), which again indicates that the EGRO-MB hybrid composite is constructed on the surface of GCE.

Fig. 4

3.5. Electrochemical reactivity of ERGO-MB/GCE for $Fe(CN)_6^{3-/4-}$ probes

The electron transfer kinetics of potassium ferricyanide and potassium ferrocyanide, which are important electroactive probes, at ERGO-MB/GCE was investigated. Fig. 5 depicts cyclic voltammograms (A) and electrochemical impedance spectra (B) obtained at the bare GCE, MB/GCE, ERGO/GCE and ERGO-MB/GCE in 10 mM PBS containing 2.0 mM $K_3Fe(CN)_6$ /2.0 mM $K_4Fe(CN)_6$ /0.1 M KCl. From Fig. 5A, it can be seen that a pair of redox peaks with the oxidation-to-reduction peak potential separation (ΔE_p) of 70 mV and the ratio of oxidation peak current to reduction peak current (i_{pa}/i_{pc}) is close to 1.0 appears at the bare GCE (curve a). While at the MB/GCE (curve b), the redox peak currents slightly increase (the rate of increase for i_{pa} and i_{pc} were 6.0% and 6.8%, respectively.) and the value of ΔE_p negligibly change to 72 mV. While reduced graphene oxide was

electrodeposited on the surface of GCE (at the ERGO/GCE, curve c), the redox peak currents clearly increase (the rate of increase for i_{pa} and i_{pc} were 24.6% and 21.5%, respectively.) and the value of ΔE_p slightly change to 61 mV. While at the ERGO-MB/GCE (curve d), the redox peak currents distinctly increase (the rate of increase for i_{pa} and i_{pc} were 32.5% and 30.1%, respectively.) and the value of ΔE_p also slightly change to 61 mV. This probably be attributed to the fact that ERGO-MB/GCE has a large surface area due to its wrinkle-like structures (Fig. 4D) and good electronic conductivity [41, 42], as well as the synergistic effects between ERGO and MB, compared with other electrodes tested.

The capability of electron transfer in four electrodes including GCE, MB/GCE, ERGO/GCE and ERGO-MB/GCE were investigated by electrochemical impedance spectroscopy (Fig. 5B). By fitting the data using the Randle equivalent circuit (insert of Fig. 5B), the electron transfer resistances (R_{et}) obtained were $305 \pm 10 \Omega$ for the bare GCE (curve a), $56 \pm 5 \Omega$ for the MB/GCE (curve b), $18 \pm 2 \Omega$ for the ERGO/GCE (curve c) and $8 \pm 1 \Omega$ for the ERGO-MB/GCE (curve d). This indicates that the $\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_6^{4-}$ probes have fast electron transfer kinetics at the ERGO-MB/GCE. This can probably be attributed to the rapid charge transfer of graphene being enhanced by the intimate interaction between MB and graphene and their highly conductive nature, in which MB acts synergistically with graphene to facilitate charge transfer by decorating the graphene thin-film [34, 35]. As was expected, the electrochemical performances and applications of reduced graphene oxide incorporating MB hybrid composite are worthy of further exploration.

Fig. 5

3.6. Electrochemical catalysis of ERGO-MB/GCE for dissolved oxygen and H_2O_2

The electrocatalytic behavior of ERGO-MB hybrid composite toward reduction of dissolved oxygen and H_2O_2 were investigated. The cyclic voltammetry measurements were performed in 10 mM PBS (pH 7.40) under an air-saturated atmosphere and purging with nitrogen gas using the ERGO-MB/GCE, and the results of purged time for 0 min (curve a), 2 min (curve b) and 10 min (curve c) are shown in Fig. 6A. The current of the cathodic peak ($E_{\text{pc}} = -0.30$ V) decreased and that of the anodic peak ($E_{\text{pa}} = -0.28$ V) increased with positive shifts of peak potentials when the purged time of nitrogen gas was extended. This indicates that employed ERGO-MB hybrid composite on the electrode surface can catalyze the reduction of dissolved oxygen [43]. Furthermore, the cyclic voltammetry measurements were performed in 10 mM PBS (pH 7.40) under an N_2 -saturated atmosphere using the ERGO-MB/GCE and the results of increasing with H_2O_2 concentrations are shown in Fig. 6B. It can be seen that the current of the cathodic peak increased and that of the anodic peak decreased with negative shifts of peak potentials when the H_2O_2 content was enhanced. The efficient electrocatalytic activity of ERGO-MB/GCE towards H_2O_2 is most likely attributed to the excellent conductivity and large electroactive surface area due to its wrinkle-like structures [44].

Fig. 6

3.7. Amperometric determination of H_2O_2

The amperometric response of the ERGO-MB/GCE to the successive addition of H_2O_2 at -0.45 V in 10.0 mL of 10 mM PBS (pH 7.40) under a N_2 -saturated atmosphere besides the plotted calibration curve are shown in Fig. 7. As is shown in the low concentration range (the inset of Fig. 7A), a quick amperometric response is observed within 2 s at 95% steady state current. Furthermore, under physiological

condition, the ERGO-MB/GCE showed a wide linear amperometric response from 0.5 μM to 11.68 mM for H_2O_2 , and the linear equation was calibrated as $I (\mu\text{A}) = -0.72 C (\text{mM}) - 0.50$ with the correlation coefficient of 0.9999 (Fig. 7B). The detection limit (defined as 3σ , where σ is the standard deviation of the blank) and the sensitivity were 60 nM and $10.2 \mu\text{A mM}^{-1} \text{cm}^{-2}$ [45], respectively. These performances are improved or comparable with those of electrochemically nonenzymatic H_2O_2 biosensors in the reported literatures (Table 1).

Fig. 7

Additionally, the reproducibility, stability and anti-interference ability of the H_2O_2 biosensor were also examined. The repetitive measurements of one biosensor for 0.5 mM H_2O_2 showed good reproducibility with a relative standard deviation (RSD) of 1.0% ($n=9$), and the paralleled preparation of five biosensors for 0.5 mM H_2O_2 with RSD of 3.0%. After 30 days, the current-generating ability of the sensor yet maintained at around 87% of the initial value under the same concentration of H_2O_2 . The anti-interference ability of the sensor for various typical interferences including ascorbic acid (AA), lysine (Lys), uric acid (UA), glycine (Gly) and dopamine (DA) was tested under physiological condition in which no apparent interferences are observed (Fig. 8).

Fig. 8

3.8. Real-time amperometric monitoring of cellular H_2O_2

The developed sensor was applied to the real-time monitoring of H_2O_2 released from living cells including human embryonic kidney cells (HEK 293, a normal cell line), human hepatoma cancer cells (Hep3B and HepG2) and human cervical cancer

cells (HeLa). Phobol 12-myristate-13-acetate (PMA) was employed as a stimulating agent, which can induce the H_2O_2 released from cancer cells within a short period of time [46]. As is shown in Figure 9B, under identically physiological conditions, no obvious change in the current response at the ERGO-MB/GCE was observed for normal cells HEK 293 (curve b) upon the addition of PMA, while distinct changes in the current signal were observed for cancer cells including Hep3B (curve a), HepG2 (curve c) and HeLa (curve d) cells upon the addition of PMA. And the comparison is shown in Figure 9C, the results indicate that the released H_2O_2 from cancer cells could be well detected, which was clearly distinguished from normal cell lines. The amount of H_2O_2 released from cancer cells was determined by measuring the current change and then calculated from the standard curve displayed in the Figure 7B. The current changes of Hep3B, HepG2 and HeLa cells obtained were about 29.3 nA, 20.3 nA and 14.5 nA, corresponding to 40.7 μM , 28.2 μM and 20.1 μM H_2O_2 in cancer cell solution, respectively. These results were similar to those of previous reports [43, 47]. Thus, it was firmly demonstrated that the prepared ERGO-MB/GCE shows excellent analytical performance for real-time sensing H_2O_2 , i.e., high sensitivity and selectivity, quick response and broad linear range, and potential for physiological and pathological investigations.

Fig. 9

4. Conclusion

In summary, a novel 2-D hybrid material has been developed by one step electrodeposited reduced graphene oxide (ERGO) incorporating adsorption of electroactive methylene blue (MB) onto glassy carbon electrode (GCE). The ERGO-MB/GCE fabricated was used as a nonenzymatic H_2O_2 biosensor for the

electrochemical detection of H₂O₂ in aqueous medium under physiological conditions. The sensor exhibits attractive performance for sensitive and selective detection of H₂O₂ with broad linear range, low detection limit, and quick response. The developed sensor has been employed to selectively detect cancer cells (Hep3B, HepG2 and HeLa) with the distinguishability from normal cells (HEK 293). This assay will potentially be applied to nonenzymatic determination of H₂O₂ in physiological and pathological investigations and real-time monitoring of other cellular small biomolecules.

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Figure Captions

Fig. 1. UV-vis spectra of GO (black curve a), MB (blue curve b) and GO-MB adsorptive nanostructure (red curve c) in 10 mM PBS (pH 7.40) aqueous solution.

Fig. 2. FT-IR spectra of GO (black curve a), MB (blue curve b) and GO-MB adsorptive nanostructure (red curve c).

Fig. 3. (A) Cyclic voltammograms obtained on GCE in 100 mM KCl-10 mM PBS (pH 7.40) containing 1.0 mg mL^{-1} GO and $100 \text{ }\mu\text{M}$ MB at 50 mV s^{-1} . (B) Cyclic voltammograms obtained on GCE (curve a), MB/GCE (curve b), ERGO/GCE (curve c) and ERGO-MB/GCE (curve d), respectively, in 100 mM KCl-10 mM PBS (pH 7.40) at 100 mV s^{-1} . (C) Cyclic voltammograms and (D) the plots of peak currents and potentials of anodic and cathodic versus scan rate obtained on ERGO-MB/GCE in 100 mM KCl-10 mM PBS (pH 7.40) at scan rate (from inner to outer), 10, 20, 40, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550 and 600 mV s^{-1} , lines a and b are the plots of peak currents, and lines c and d are the plots of peak potentials versus scan rate.

Fig. 4. TEM images of GO (A) and GO-MB (B), SEM images of GCE (C) and ERGO-MB/GCE (D).

Fig. 5. Cyclic voltammograms (A) and electrochemical impedance spectra at the GCE (curve a), MB/GCE (curve b), ERGO/GCE (curve c) and ERGO-MB/GCE (curve d) in 100 mM KCl-10 mM PBS (pH 7.40) containing $2.0 \text{ mM K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$ (1:1). Scan rate: 100 mV s^{-1} . Frequency range: 10^5 to 1 Hz; potential: 0.24 V; perturbation signal: 5 mV. The inset shows the Randle equivalent circuit applied to fit the impedance spectroscopy and fitted data are solid lines.

Fig. 6. (A) Cyclic voltammograms obtained on ERGO-MB/GCE in 100 mM KCl-10

mM PBS (pH 7.40) under an air-saturated atmosphere (curve a), 2 min N₂ inlet (curve b) and 10 min N₂ inlet (curve c). (B) Cyclic voltammograms obtained on ERGO-MB/GCE in 100 mM KCl-10 mM PBS (pH 7.40) containing a) 0, b) 5, c) 10, d) 15, e) 20, f) 25, g) 30 and h) 35 mM H₂O₂ under a N₂-saturated atmosphere. The scan rate is 100 mV s⁻¹.

Fig. 7. (A) Amperometric current-time response curve of ERGO-MB/GCE at -0.45 V upon successive addition of H₂O₂ into 100 mM KCl-10 mM PBS (pH 7.40) under a N₂-saturated atmosphere. The inset shows the amplified response curve at low concentrations. (B) Calibration curve of steady-state currents vs H₂O₂ concentration.

Fig. 8. (A) Amperometric current-time response curve of ERGO-MB/GCE at -0.45 V upon sequential addition of 0.5 mM H₂O₂, 0.5 mM ascorbic acid (AA), 0.5 mM lysine (Lys), 0.5 mM uric acid (UA), 0.5 mM glycine (Gly) and 0.5 mM dopamine (DA) into 100 mM KCl-10 mM PBS (pH 7.40) under a N₂-saturated atmosphere.

Fig. 9. (A) Schematic diagram for real-time amperometric monitoring of cellular H₂O₂. (B) Amperometric response of the ERGO-MB/GCE in the presence of Hep3B (curve a), HEK 293 (curve b), HepG2 (curve c) and HeLa (curve d) cells upon addition of 10 µL of 1.0 µg/mL PMA to 10 mM PBS (pH 7.40) at -0.45 V, respectively. (c) Comparison in cellular assay of H₂O₂ detection for Hep3B, HEK 293, HepG2 and HeLa cells.

Table 1. Comparison of the performance of modified glassy carbon electrodes for nonenzymatic determination of H₂O₂.

Electrode modified materials	Linear range (μM)	LOD (μM)	Sensitivity (μA mM ⁻¹ cm ⁻²)	Applied potential (vs. Ag/AgCl)	Reference
AuNPs/silica sol-gel	2.5 - 45	0.00315	/	-0.55 V	[7]
MoS ₂ nanoparticles	0.005 - 0.100 0.100 - 100	/	2580	-0.30 V	[8]
PtPd-Fe ₃ O ₄ NPs	0.02 - 0.1 2 - 14000	0.005	/	-0.25 V	[9]
Pt nanowire	5 - 3000	9.6	1360000	0 V	[10]
Prussian blue/ polyaniline/CNTs	0.008 - 5	0.005	526430	0 V	[11]
Reduced graphene oxide-PtNPs	0.5 - 3475	0.2	459	-0.08 V	[21]
Graphene oxide -SiNPs/chitosan	5.22 - 10430	2.6	18.78	0.68 V	[22]
CuS/RGO	5 - 1500	0.27	/	-0.27 V	[23]
Silver nanocrystals/ ERGO	20 - 10000	3	183.5	-0.50 V	[24]
Prussian blue/ graphene oxide	5.0 - 1200	0.122	408.7	0.15 V	[25]
Ferrocene-graphene	50 - 4750 5750 - 14750	4.15	/	0.5 V	[26]
Hemin-graphene/ AuNPs	0.3 - 1800	0.11	2774.8	-0.25 V	[27]
ERGO-MB	0.5 - 11680	0.060	10.2	-0.45 V	This work

Figure(1)

Figure(2)

Figure(3)

Figure(4)

Figure(5)

Figure(6)

Figure(7)

Figure(8)

Figure(9)