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# Construction of a novel electrochemical biosensor based on a mesoporous silica/oriented graphene oxide planar electrode for detecting hydrogen peroxide†

Kun-Chao Lu,<sup>a</sup> Ji-Kui Wang,<sup>\*a</sup> Dong-Hai Lin,<sup>ID \*b</sup> Xue Chen,<sup>a</sup> Shi-Yu Yin<sup>a</sup> and Guo-Song Chen<sup>a</sup>

A constant magnetic field (CMF) was used to arrange the orientation of graphene oxide (GO) which was modified on a self-made screen-printed electrode. We evaluated the efficiency of this method for potential analytical application towards the sensing of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). Mesoporous silica (MS)-encapsulated horseradish peroxidase (HRP) was immobilized on the electrode with vertically arranged GO to construct an  $\text{H}_2\text{O}_2$  sensor (denoted as CMF/GO/HRP@MS). The linear range of the response of the CMF/GO/HRP@MS sensor to  $\text{H}_2\text{O}_2$  was 0.1–235  $\mu\text{M}$ , and the detection limit was as low as 0.01  $\mu\text{M}$ . The results demonstrated that the vertical arrangement of GO resulting from the CMF on the electrode surface could increase the electron transfer rate. The excellent selectivity and anti-interference ability of this sensor to  $\text{H}_2\text{O}_2$  in physiological samples may be attributed to the synergistic effect of mesoporous silica, GO and constant magnetic field.

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## Introduction

Maintaining hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) concentration at a normal level is essential to achieve the physiological activities of cells.<sup>1–3</sup> Low  $\text{H}_2\text{O}_2$  concentration in physiological samples requires higher sensitivity of the detection method. Compared with other analytical methods,<sup>4–6</sup> electrochemical detection methods have the characteristics of high sensitivity, low detection limit, and convenience, so they are widely used.<sup>7–10</sup> In this work, we chose mesoporous silica (MS) as a carrier to encapsulate horseradish peroxidase (HRP) on the electrode surface to increase the loading of the enzyme and maintain good biological activity.<sup>11–13</sup> However, HRP has a huge spatial structure, and its redox center is surrounded by polypeptide chains.<sup>14</sup> Therefore, it is difficult to achieve direct electron transfer between the enzyme and the electrode surface.

To achieve more efficient electron transfer between the enzyme and the electrode surface, Komori *et al.* accelerated the electron transfer between HRP and the electrode by modifying the electrode surface with carbon nanotubes;<sup>15</sup> Yang *et al.* improved the direct electron transfer of the enzyme by growing ZnO/Cu nanocomposites on the electrode surface.<sup>16</sup> Graphene

oxide (GO) is a common 2D material used for electrode modification.<sup>11,17</sup> The in-plane resistance of graphene oxide is much lower than the interlayer resistance, and the electron transport rate of GO is affected by the spatial orientation.<sup>18</sup> GO may be orientated and aligned by a magnetic<sup>19</sup> or electric field.<sup>20</sup>

Although GO is a non-magnetic nanomaterial, due to the magnetic response of orbiting electrons, diamagnetism is a universal property of materials. Diamagnetism has been used to align non-magnetic nanomaterials, but usually requires a huge field.<sup>21</sup> Smaller magnetic field has been used to arrange graphite and graphene sheets, but only with the help of magnetic or paramagnetic nanoparticles to enhance electrical conductivity.<sup>22</sup> Without the assistance of magnetic nanoparticles, Lin *et al.* investigated for the first time the magnetic response and alignment of graphene flakes with a weak magnetic field.<sup>23</sup> Smarzewska *et al.* examined the effect of constant magnetic field strength on activation of sensors modified with GO.<sup>19</sup> They observed that applying a constant magnetic field (0.4 T) could significantly increase the electroactive area of sensors modified with a graphene oxide monolayer.

Here, an electrochemical biosensor based on mesoporous silica/oriented graphene oxide was constructed. Mesoporous silica (MS) was chosen as a carrier to encapsulate horseradish peroxidase (HRP) on the electrode surface to increase the loading of the enzyme and maintain good biological activity of HRP. Then HRP@MS was immobilized on the electrode with vertically arranged GO by applying a weak constant magnetic

<sup>a</sup>School of Chemistry and Molecular Engineering, Nanjing Tech University, Nanjing 210009, China. E-mail: wjk@njtech.edu.cn

<sup>b</sup>School of Environmental and Materials Engineering, College of Engineering, Shanghai Polytechnic University, Shanghai 201209, China. E-mail: dhlin@sspu.edu.cn

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field (CMF) (0.4 T) from small commercial magnets to construct an  $\text{H}_2\text{O}_2$  sensor (denoted as CMF/GO/HRP@MS) on the self-made screen-printed electrode. The CMF/GO/HRP@MS composite based sensor exhibited high sensitivity, excellent selectivity, lower detection limit, good reproducibility and anti-interference ability. Therefore, the CMF/GO/HRP@MS sensor was applied to analyse  $\text{H}_2\text{O}_2$  in real human serum samples and was found to have good application prospects in the detection of  $\text{H}_2\text{O}_2$  in physiological samples.

## Experimental

### Reagents and apparatus

$\text{K}_4[\text{Fe}(\text{CN})_6]$  and  $\text{K}_3[\text{Fe}(\text{CN})_6]$  were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. KCl was obtained from Shanghai Shenbo Chemical Co., Ltd. Uric acid (UA), ascorbic acid (AA) and dopamine (DA) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Anhydrous ethanol was acquired from Wuxi Yasheng Chemical Co., Ltd. Nafion (5 wt%) was obtained from Shanghai Format New Energy Technology Co., Ltd. Graphene oxide (GO) and mesoporous silica (MS) were obtained from Jiangsu Xianfeng Nanomaterials Technology Co., Ltd. Horseradish Peroxidase (HRP) (500KU/2.26 g lyo.) was purchased from Roche Diagnostics, USA. All the reagents used were of analytical grade. All the solutions were prepared with ultrapure water (Milli-Q, 18 M $\Omega$  cm). Neodymium magnets were purchased from Jianku Co., Ltd.

The morphologies of the samples were examined using a scanning electron microscope (SEM) (JEM-2100, JEOL). The enzyme HRP on MS was characterized by FTIR (Nicolet iS5, Thermo Fisher, USA) and UV-Vis spectroscopy (UV-3600, Shimadzu, Japan). The Raman spectra were recorded on a micro-Raman spectrometer (785 nm, ThermoFisher DXR™, USA) in the wavenumber range of 500–3000  $\text{cm}^{-1}$ . Electrochemical measurements were carried out on an electrochemical workstation (CHI 660E, Chenhua Instruments, Shanghai, China).

### Magnetic field orientation and electrode modification

**HRP@MS nanocomposites.** 20 mg MS and 10 mg HRP were dispersed in 1 mL PBS buffer solution (pH 7.0) and the mixture was oscillated overnight at room temperature. The unadsorbed HRP was removed using centrifugation (8000 rpm, 10 min) and then, HRP@MS was washed 5 times with PBS buffer solution to obtain HRP@MS nanocomposites.

**CMF/GO/HRP@MS electrode.** GO was dispersed in Milli-Q water with the assistance of ultrasonication to prepare a GO solution (1 mg  $\text{mL}^{-1}$ ). A mixture of 5.0  $\mu\text{L}$  GO solution and HRP@MS (volume ratio 1 : 1) was dropped on the screen-printed electrode's (homemade)<sup>24</sup> surface that was positioned horizontally, and then it was placed midway between two parallel neodymium magnet pieces (see Fig. S1†) until the modified solution had completely dried. Then 5.0  $\mu\text{L}$  Nafion (0.15 wt%) solution was coated on the electrode surface. Thus the CMF/GO/HRP@MS electrode with constant magnetic field oriented GO was obtained. The distribution of the magnetic field in space near the electrode was simulated by using

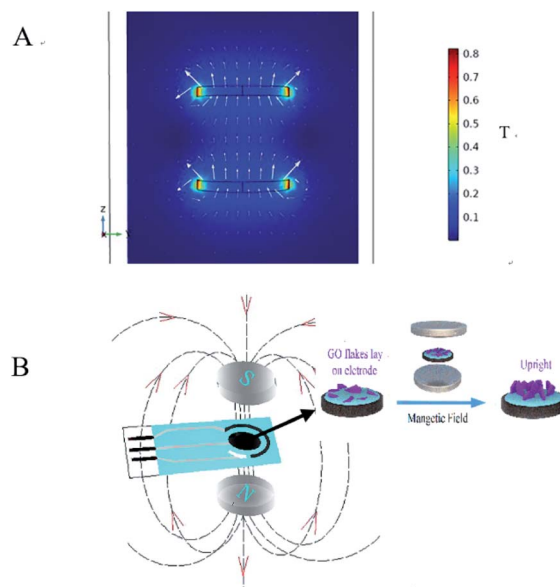


Fig. 1 (A) Simulation of the distribution of the constant magnetic field (CMF) using COMSOL software and (B) electrode modification process under constant magnetic field (CMF).

COMSOL software. From the simulation results (Fig. 1A), it could be seen that the magnetic induction lines were perpendicular to the surface of the plane electrode and were evenly distributed. The HRP@MS electrode and GO/HRP@MS electrode were used in the control experiment.

### Characterization of HRP

Prior to the evaluation of immobilized enzyme HRP on MS using FTIR and UV-Vis spectroscopy, free HRP was removed by centrifugation. FTIR samples of HRP, MS and HRP@MS were prepared using the KBr tableting method.

### Characterization of electrochemical properties

The HRP@MS electrode, GO/HRP@MS electrode, and CMF/GO/HRP@MS electrode were used to test their electrochemical performance by cyclic voltammetry (CV) in an oxygen-free PBS buffer system (pH 7.0), and the sweep voltage varied from  $-0.1$  V to  $0.4$  V in steps of  $0.01$  V  $\text{s}^{-1}$ . The electrochemical properties of the electrode were characterized in an  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  system by CV with different scan rates (0.01, 0.02, 0.03, 0.05, 0.10, 0.15 and  $0.20$  V  $\text{s}^{-1}$ ). Electrochemical impedance spectroscopy (EIS) of all electrodes was performed in a mixed solution containing 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  + 0.1 M KCl; the frequency range was 0.01 to  $10^5$  Hz, and the amplitude was 10 mV. The  $\text{H}_2\text{O}_2$  response current of the electrodes was recorded under a constant potential of 0.08 V (vs. Ag/AgCl).

## Results and discussion

### Characterization of immobilized HRP by UV-Vis and FTIR

Since the immobilization of enzymes on solid surfaces might lead to the loss and denaturation of their specific natural

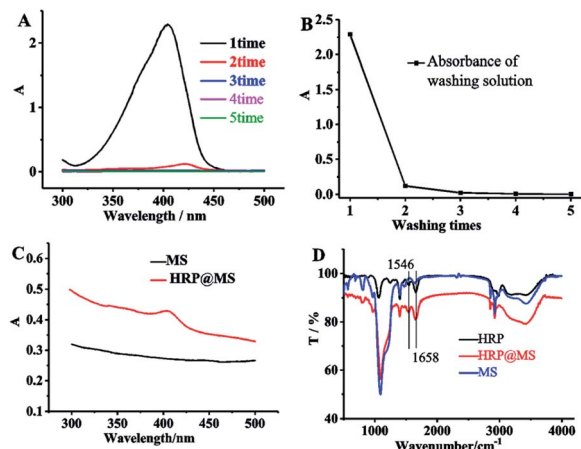


Fig. 2 (A) UV-Vis absorption spectra of HRP@MS in PBS (pH 7.0) with different washing times; (B) the intensity of UV-Vis spectra of washing solution with different washing times; (C) UV-Vis spectra of MS and HRP@MS; and (D) FTIR spectra of HRP (black solid line), HRP@MS (red solid line) and MS (blue solid line).

conformations, UV-Vis spectroscopy was adopted to monitor structural changes in immobilized HRP. It can be seen from Fig. 2A and B that the absorbance gradually decreased with the increase of washing times. When washed more than three times, the absorbance dropped to almost zero, indicating that the free HRP adsorbed on the surface of MS had been completely removed. Fig. 2C shows the UV-Vis spectra of MS (black line) and HRP@MS washed five times (red line) in PBS (pH 7.0). The strong absorption peak at 407 nm was a typical Soret band of HRP.<sup>25</sup> By comparing the UV absorption spectra of the two, it was found that HRP had been fixed to MS. Fig. 2D shows a comparison of the FTIR absorption spectra of HRP (black line), HRP@MS (red line) and MS (blue line). The amide I and II bands of HRP@MS complexes are located at 1658  $\text{cm}^{-1}$  and 1546  $\text{cm}^{-1}$ , respectively, which were also basically the same as those of free HRP.<sup>26</sup> MS did not have this characteristic peak at this position. Both UV-Vis and FTIR absorption spectrum results confirmed that HRP had been successfully incorporated into MS.

### SEM characterization of CMF oriented GO

Without a CMF, most GO was in a natural lying state on the electrode during the modification process (Fig. 3A). Fig. 3B shows the distribution of HRP@MS on the surface of the bare carbon electrode. When the nanocomposite was directly incorporated on the surface of the bare carbon electrode, the surface of the electrode was uneven due to the uneven distribution of HRP@MS, and the consistency of the surface of the electrode was poor. While the surface of the electrode was covered by GO, the HRP@MS loading capacity of the electrode was enhanced, the dispersion was more uniform and the surface state of the electrodes remained consistent (Fig. 3C). After curing, most GO was in an approximately upright state, as shown in Fig. 3D. The external CMF (0.4 T) allows GO to spread parallel to the magnetic induction lines. Due to the conductive characteristics of GO, the in-plane resistance of GO is much smaller than the

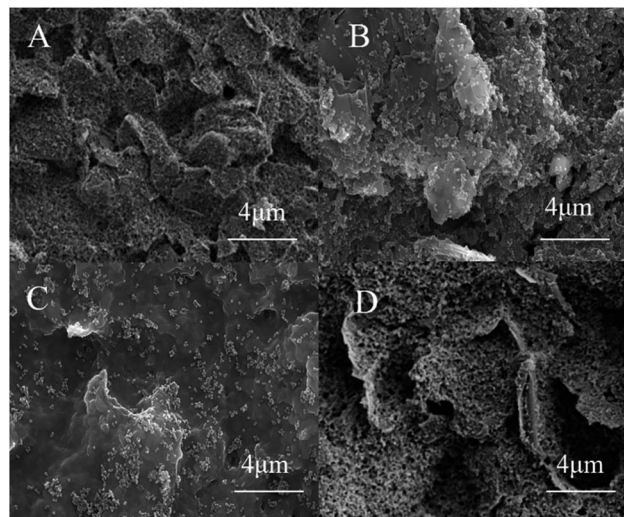


Fig. 3 SEM images of (A) GO; (B) HRP@MS; (C) GO/HRP@MS; and (D) CMF/GO/HRP@MS modified on a screen-printed electrode.

interlayer resistance. Therefore, the charge transfer ability of a vertically oriented GO electrode is better than that of a natural lying GO electrode.

Raman studies were performed before and after applying the CMF in order to evaluate the formation of more edges on GO. The Raman spectra were recorded on a ThermoFisher DXR<sup>TM</sup> (785 nm) micro-Raman spectrometer in the wavenumber range of 500–3000  $\text{cm}^{-1}$ . The peaks at around 1350  $\text{cm}^{-1}$  and 1590  $\text{cm}^{-1}$  could be attributed to the lattice defect peak (D peak) of carbon and the ordered  $\text{sp}^2$  carbon plane peak (G peak), respectively (Fig. 4). The ratio of the relative intensity of the D peak and G peak ( $I_D/I_G$ ) can be used to evaluate the crystal structure and graphitization degree of carbon-based materials.<sup>27,28</sup> When the CMF was applied,  $I_D/I_G$  increased from 1.02 to 1.13, indicating that the atomic arrangement was less orderly and the defect degree was increased. There are more edges on GO after applying the CMF.

### Electrochemical characterization

In order to interstage the CMF effect of electrochemical performance of modified electrodes, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed in PBS buffer (pH 7.0) and an  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  system, respectively. CV curves were recorded for HRP@MS (red line), GO/HRP@MS (blue line), and CMF/GO/HRP@MS (pink line) electrodes at a speed of 0.01  $\text{V s}^{-1}$  in PBS buffer (pH 7.0) containing a 0.1 M supporting electrolyte, respectively (Fig. 5A). A pair of redox peaks were obtained on the HRP@MS electrode, and the oxidation and reduction peak potentials were about 0.291 V and 0.087 V, respectively. The carbon electrode did not show the corresponding redox peaks at the same potential. Compared with the HRP@MS electrode, the GO/HRP@MS electrode increased the current intensity by 75%, which indicated that GO improved the direct electron transfer between the electrode and HRP. Because the in-plane conductivity of GO is better than its interlayer conductivity,<sup>18</sup> GO oriented with the

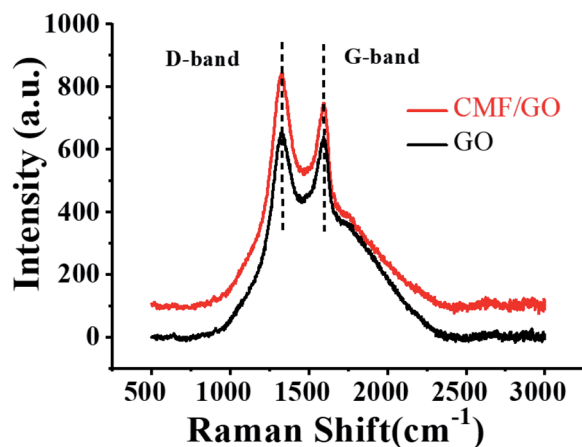


Fig. 4 Raman spectra of GO (black line) and CMF/GO (red line).

CMF could increase the electrical conductivity of the electrode.<sup>19</sup> Therefore, the peak current intensity of the CMF/GO/HRP@MS electrode was the largest, which was about 30% higher than that of GO/HRP@MS.

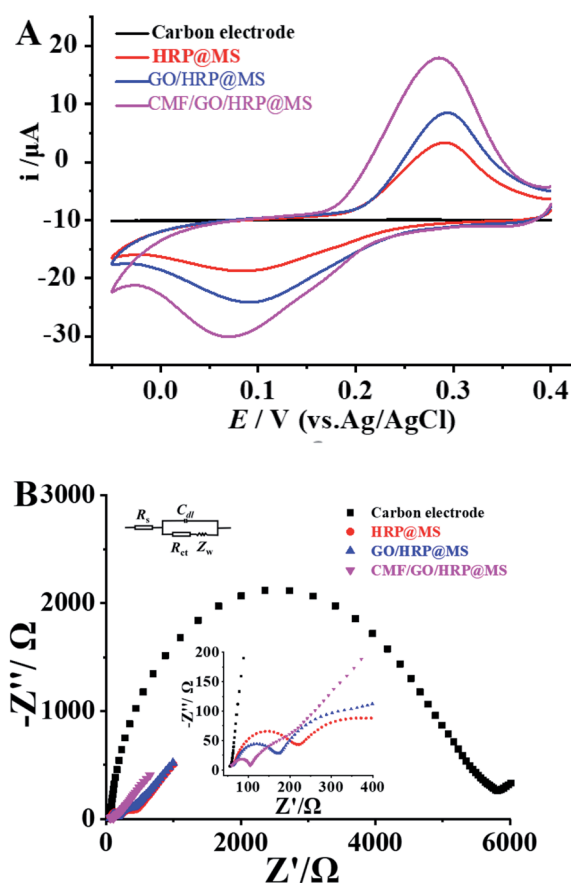


Fig. 5 (A) CV and (B) EIS curves of HRP@MS, GO/HRP@MS, CMF/GO/HRP@MS and carbon electrodes in PBS buffer (pH 7.0) and an  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  system, respectively. The insets show the enlarged Nyquist plots and the Randles equivalent circuit employed for all fits.  $R_s$  is the solution resistance,  $C_{dl}$  is the electrode double layer capacitance,  $Z_w$  is the Warburg impedance and  $R_{ct}$  is the charge transfer resistance at the electrode interface.

From the current of modified electrode, we can obtain the apparent coverage of HRP on the electrode surface (eqn (1)).<sup>29,30</sup>

$$T = \frac{Q}{nFA} \quad (1)$$

where  $Q$  represents the electric quantity of the oxidation and reduction process ( $C$ ),  $F$  is the Faraday constant ( $96\,487\text{ C mol}^{-1}$ ),  $n$  is the number of electrons transferred during the redox reaction ( $n = 1$ ), and  $A$  is the effective area of the electrode ( $\text{cm}^2$ ). It was calculated that the apparent coverage of HRP on the HRP@MS and GO/HRP@MS electrodes was  $7.18 \times 10^{-11}\text{ mol cm}^{-2}$  and  $8.59 \times 10^{-11}\text{ mol cm}^{-2}$ , respectively. The CMF made GO distribute vertically on the electrode surface, which increased the surface area of the electrode. Therefore, the apparent coverage of HRP on the CMF/GO/HRP@MS electrode was the largest, reaching  $1.02 \times 10^{-10}\text{ mol cm}^{-2}$ .

The experimental results of the three electrodes at different scanning rates are provided in the ESI (Fig. S2†). Based on the Randles-Sevcik formula (eqn (2)).<sup>31,32</sup>

$$i_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} \nu^{1/2} c \quad (2)$$

where  $A$  is the effective area of the electrode ( $\text{cm}^2$ ),  $D$  is the diffusion coefficient of the reactant ( $\text{cm}^2\text{ s}^{-1}$ ),  $n$  is the electron transfer number of the electrode reaction,  $\nu$  is the sweep rate ( $\text{V s}^{-1}$ ), and  $c$  is the concentration of the reactant ( $\text{M}$ ). The effective surface area of the bare carbon electrode and GO/HRP@MS electrode was  $0.27\text{ cm}^2$  and  $0.35\text{ cm}^2$ , respectively. With the help of the CMF, vertically distributed GO on the electrode surface improved the utilization of the electrode and increased the effective area of the CMF/GO/HRP@MS electrode ( $0.40\text{ cm}^2$ ). The experimental results of the increase of effective area further proved the success of the magnetic field orientation experiment.

Compared with CV, EIS can better describe the solution/electrode interface and the kinetics of electron transfer. It can be seen from Fig. 4B that  $R_{ct}$  of the bare carbon, HRP@MS, GO/HRP@MS and CMF/GO/HRP@MS electrodes decreases sequentially. In order to determine the apparent hetero-electron transport rate constant ( $K_{app}$ ) for each electrode, the following formula (eqn (3)) was used:<sup>33</sup>

$$K_{app} = \frac{RT}{n^2 F^2 A R_{ct} c} \quad (3)$$

where  $R$  is the ideal gas constant ( $8.314\text{ J (mol K)}^{-1}$ ),  $T$  is the temperature ( $298\text{ K}$ ),  $F$  is the Faraday constant ( $96\,487\text{ C mol}^{-1}$ ),  $n$  is the number of electrons transferred during the redox reaction ( $n = 1$ ),  $A$  is the effective area of the electrode ( $\text{cm}^2$ ),  $R_{ct}$  is the charge transfer resistance ( $\Omega$ ), and  $c$  is the concentration of the redox substance ( $\text{M}$ ).

The experimental results show that the  $R_{ct}$  of the GO/HRP@MS electrode is much lower than that of the HRP@MS electrode. The  $K_{app}$  of HRP@MS and GO/HRP@MS electrodes was  $2.98 \times 10^{-3}\text{ cm s}^{-1}$  and  $3.38 \times 10^{-3}\text{ cm s}^{-1}$ , respectively. The results indicate that GO accelerated the electron transfer rate.  $R_{ct}$  of the CMF/GO/HRP@MS electrode is the lowest ( $K_{app} = 7.01 \times 10^{-3}\text{ cm s}^{-1}$ ); since the in-plane electron transfer rate of

GO is better than the interlayer electron transfer rate, its electron transfer rate is doubled.

### Evaluation of $\text{H}_2\text{O}_2$ detection performance

The detection performance of HRP@MS (blue line), GO/HRP@MS (red line) and CMF/GO/HRP@MS (black line) sensors for  $\text{H}_2\text{O}_2$  is shown in Fig. 6A. (The inset shows an enlargement of the marked region at low  $\text{H}_2\text{O}_2$  concentration.) When  $5\ \mu\text{M}$   $\text{H}_2\text{O}_2$  was added to PBS buffer, the three electrodes displayed a rapid current response and reached a steady state in a short time. By adding  $5\ \mu\text{M}$   $\text{H}_2\text{O}_2$  continuously, the linear response range and calibration curves of different electrodes can be obtained (Table 1). Fig. 6B shows the calibration curve for the CMF/GO/HRP@MS sensor (the line in the inset of Fig. 6B is a low concentration fitting curve). The linear range is wider than that of the other two electrodes, and its detection limit is as low as  $0.01\ \mu\text{M}$ . Therefore, the CMF/GO/HRP@MS electrode has the widest linear range, the lowest detection limit and the highest sensitivity compared with the other two electrodes in the ESI (Fig. S3†).

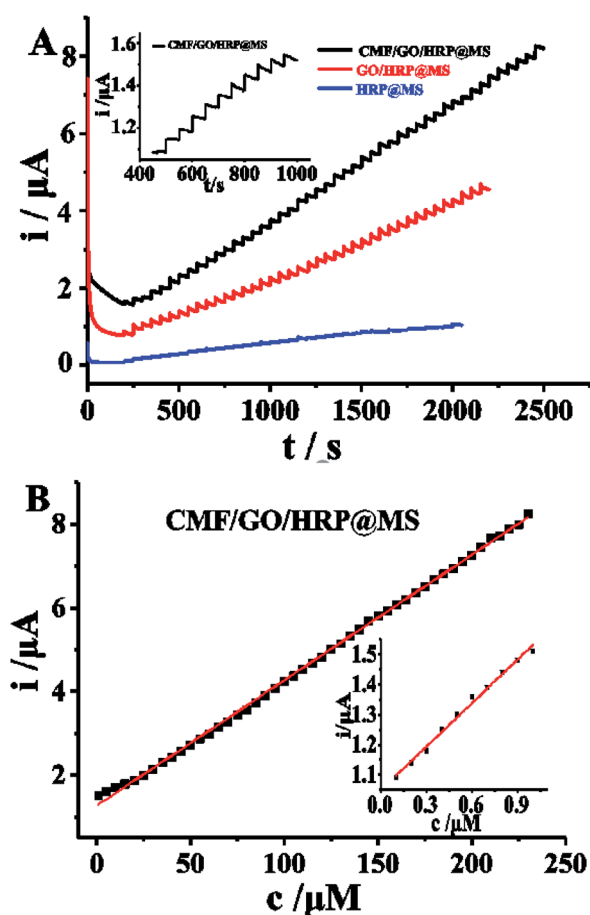


Fig. 6 (A) The  $i$ - $t$  curves of HRP@MS, GO/HRP@MS and CMF/GO/HRP@MS electrodes in PBS buffer (pH 7.0); the inset shows the enlarged view of the marked region at a low concentration of  $\text{H}_2\text{O}_2$ . (B) Calibration curve between the steady-state current on the CMF/GO/HRP@MS electrode and  $\text{H}_2\text{O}_2$  concentration; the line in the inset of Fig. 5B is a low concentration fitting curve.

Table 1 Comparison of  $\text{H}_2\text{O}_2$  sensors in the literature

| Electrode         | Linear range/ $\mu\text{M}$ | Detection limit/ $\mu\text{M}$ | Ref.      |
|-------------------|-----------------------------|--------------------------------|-----------|
| Inkjet printed Ag | 100–6800                    | 5                              | 35        |
| BiNDs/GaN         | 10–1000                     | 5                              | 36        |
| BPQDs@ZnO/GCE     | 0.5–10 000                  | 2.5                            | 37        |
| HRP/AgNPs/Au      | 3.3–9400                    | 0.78                           | 38        |
| HRP@MS            | 5–160                       | 1.802                          | This work |
| GO/HRP@MS         | 2–195                       | 0.318                          | This work |
| CMF/GO/HRP@MS     | 0.1–235                     | 0.01                           | This work |

The low limit of detection in the CMF/GO/HRP@MS sensor may be attributed to a combination of two factors, (A) an increase in surface area, and (B) the presence of more edges in CMF/GO/HRP@MS. Mesoporous silica was chosen as a carrier to increase the loading of the enzyme and maintain good biological activity. Graphene oxide was used to assist direct electron transfer between the enzyme and the electrode surface. A constant magnetic field (CMF) was applied to orientate and align graphene oxide to enhance electrical conductivity. GO, with strong paramagnetic properties, may intensively interact with a magnetic field. When paramagnetic material GO was placed in the magnetic field, the surface and structure of graphene oxide was under the influence of tensile forces and structural stress.<sup>19</sup> In addition, Roslan *et al.*<sup>34</sup> illustrated that a CMF causes an increase of mechanical stress and changes the structure of carbon nanomaterials. Graphene oxide is a highly reactive hexagonal lattice made of  $\text{sp}^2$  hybridized carbon atoms. In such structures, one can distinguish two factors that affect their chemical reactivity: the distortion angle of the  $\text{sp}^2$  hybridization towards  $\text{sp}^3$  hybridization and incomplete overlapping of p orbitals of adjacent carbon atoms. This is reflected in the values of  $\text{sp}^2$  hybridization distortion angles. Therefore, the reactivity of carbon nanostructures is caused by structural stress. The latter increase is responsible for the increase in reactivity and may be caused by the magnetic field. Under the influence of the magnetic field, we observed increased electroactive surface area and more edges on GO after applying the CMF, which have been confirmed by CV and Raman results, respectively. Therefore, the limit of detection of the CMF/GO/HRP@MS sensor may be attributed to a combination of two factors, (A) an increase in surface area, and (B) the presence of more edges in CMF/GO/HRP@MS.

### Anti-interference ability and reproducibility

The anti-interference ability of the CMF/GO/HRP@MS sensor was investigated. From Fig. 7A, the current increased after addition of  $20\ \mu\text{M}$   $\text{H}_2\text{O}_2$ , but the addition of the other three substances (UA, AA, and DA) did not cause an obvious current response. After the addition of  $20\ \mu\text{M}$   $\text{H}_2\text{O}_2$ , the current increased again, indicating that the sensor had good anti-interference ability and better selectivity to  $\text{H}_2\text{O}_2$ .

The reproducibility of the sensor was further examined, and the current response was separately recorded 4 times by adding  $20\ \mu\text{M}$   $\text{H}_2\text{O}_2$  to the anaerobic PBS buffer (pH 7.0). The

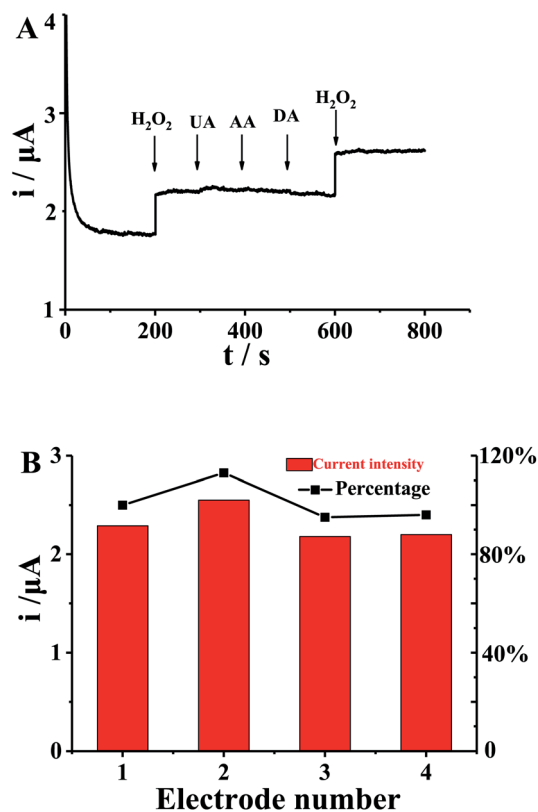


Fig. 7 (A) Amperometric response of the CMF/GO/HRP@MS electrode with the addition of 20 μM H<sub>2</sub>O<sub>2</sub>, UA, AA, and DA; (B) the response of four CMF/GO/HRP@MS electrodes in anaerobic PBS containing 20 μM H<sub>2</sub>O<sub>2</sub>.

results showed that the relative standard deviation (RSD) of the four independent CMF/GO/HRP@MS electrodes for the detection of H<sub>2</sub>O<sub>2</sub> was 7.28% (Fig. 6B), indicating that the individual reproducibility of the sensors was good. The experimental results of HRP@MS and GO/HRP@MS sensors can be found in the ESI (Fig. S4†). From the experimental results, it can be seen that both HRP@MS and GO/HRP@MS sensors had good anti-interference ability, but their stability was not as good as that of the CMF/GO/HRP@MS sensor. The RSD results of H<sub>2</sub>O<sub>2</sub> detection obtained from the four independent HRP@MS and GO/HRP@MS sensors were 12.59% and 11.96%. The long-term stability of the sensor is important for practical application. When the CMF/GO/HRP@MS modified electrode was stored at 4 °C in a refrigerator and subjected to day-by-day calibrations at room temperature, the electrode could retain over 90% of the initial value in response to 0.3 μM H<sub>2</sub>O<sub>2</sub> after 14 days, indicating that this vertically aligned orientation of the CMF/GO/HRP@MS sensor has good long-term stability for practical application.

#### Real sample detection

To test the feasibility of detecting H<sub>2</sub>O<sub>2</sub> in biological samples, the standard addition method was used to determine H<sub>2</sub>O<sub>2</sub> in human serum. The sample was diluted 60 times<sup>39</sup> with a PBS solution (pH 7.0), and 5 μM, 20 μM, and 100 μM H<sub>2</sub>O<sub>2</sub> were

Table 2 Detection of H<sub>2</sub>O<sub>2</sub> in real samples

| Sample  | Added/μM | Detected/μM | RSD/% | Detection rate/% |
|---------|----------|-------------|-------|------------------|
| Serum 1 | 5        | 4.48        | 5.63  | 89.6             |
| Serum 2 | 20       | 19.1        | 1.86  | 95.5             |
| Serum 3 | 100      | 91.4        | 2.79  | 91.4             |

added to the sample, respectively. Three groups of parallel experiments were performed for each concentration. The CMF/GO/HRP@MS sensor has a satisfactory response to H<sub>2</sub>O<sub>2</sub> in human serum and has a high detection rate (Table 2). In summary, the H<sub>2</sub>O<sub>2</sub> sensor based on the CMF/GO/HRP@MS electrode has good application prospects in the detection of H<sub>2</sub>O<sub>2</sub> in physiological samples. The experimental results of the HRP@MS and GO/HRP@MS electrodes can be found in the ESI (Tables S1 and S2†). The detection rate of the GO/HRP@MS electrodes surpassed 80%, while the sensitivity of the HRP@MS electrode in the low concentration range was relatively narrow and the recovery was poor.

## Conclusion

In this paper, a constant magnetic field (CMF) was developed to orientate graphene oxides (GO) on the self-made screen-printed electrode surface to improve the electrode conductivity and detection sensitivity for H<sub>2</sub>O<sub>2</sub>. The experimental results demonstrated that the 'vertical' arrangement of GO on the electrode surface improved the direct electron transfer of HRP on the electrode. Additionally, the HRP loading of the GO-modified electrode increased. The enhanced sensitivity of the CMF/GO/HRP@MS based sensor may be attributed as followed: firstly, mesoporous silica was chosen as a carrier to increase the loading of the enzyme and maintain good biological activity. Secondly, graphene oxide was used to assist direct electron transfer between the enzyme and the electrode surface. Thirdly, a weak constant magnetic field (CMF) (0.4 T) from small commercial magnets was applied to orientate and align graphene oxide to enhance electrical conductivity. This CMF/GO/HRP@MS composite based sensor has high sensitivity, excellent selectivity, lower detection limit, good anti-interference ability and reproducibility towards H<sub>2</sub>O<sub>2</sub>. The standard addition method was used to analyse H<sub>2</sub>O<sub>2</sub> in real human serum samples and the results indicated that the sensor prepared by this method has good application prospects in the detection of H<sub>2</sub>O<sub>2</sub> in physiological samples.

## Conflicts of interest

There are no conflicts of interest to declare.

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