Electronic Supplementary Material (ESI) for RSC Advances

A cathodic electrogenerated chemiluminescence biosensor based on luminol and hemin-graphene nanosheets for cholesterol detection

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Materials: Graphene oxide was purchased from Nanjing xianfeng nano Co. (Nanjing, China). Hemin, cholesterol oxidase (ChOx, EC 1.1.3.6, ≥50 units/mg, from Brevibacterium sp.), cholesterol ($C_{27}H_{46}O$, M_r: 386.67, ≥99% purity, from lanolin), and Triton X-100 ($C_{34}H_{62}O_{11}$, MW: 646.85) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Phosphate buffer solutions (PBS, containing 0.9% NaCl) with pH 7.4 were prepared with 0.05 M KH_2PO_4 and 0.05 M Na_2HPO_4. The stock solution was prepared by dissolving cholesterol in the mixture of 2-propanol and Triton X-100, and then diluted it only with Triton X-100 solution for preparing standard solutions. Other chemicals used were of analytical grade and were used as received. Double distilled water was used throughout this study.

Apparatus and measurements: Cyclic voltammetry (CV) was performed with a CHI 600D electrochemical work station (Shanghai Chenhua Instruments Co., China). The ECL emission was monitored with a model MPI-A electrochemiluminescence analyzer (Xi'an Remax Electronic Science & Technology Co. Ltd., China) with the

voltage of the photomultiplier tube (PMT) set at 600 V in the process of detection. All experiments were performed with a conventional three-electrode system. The modified glassy carbon electrode (GCE) as working electrode, a platinum wire as counter electrode and a saturated calomel electrode (SCE) or Ag/AgCl (sat. KCl) as reference electrode. The UV-Vis absorption spectra were recorded in the range of 200-800 nm, using a UV-Vis spectrometer (UV-Vis 8500). All the electrochemical experiments were carried out at room temperature.

Preparation of hemin-graphene nanosheets: According to Guo's work[1], hemin-graphene nanosheets (H-GNs) were synthesized with a simple wet-chemical strategy through the π-π interactions. First, 20.0 mL of the homogeneous graphene oxide dispersion (0.5 mg/mL) was mixed with 20.0 mL of 0.5 mg/mL hemin aqueous solution and 200.0 μL of ammonia solution, followed by the addition of 30 μL of hydrazine solution. After being vigorously stirred for a few minutes, the vial was put in a water bath (60 ℃) for 3.5 h. Finally, the product was obtained by filtration and washed several times. The obtained H-GNs can be redispersed readily in water by ultrasonication. Additionally, the preparation of pure graphene was similar to H-GNs except no addition of hemin.

Construction of the cholesterol biosensor: Glassy carbon electrode (GCE, Φ = 4 mm) was polished with 0.3 and 0.05 μm alumina slurry, and then ultrasonically cleaned in ethanol and water thoroughly. After it was allowed to dry at room temperature, 10 μL H-GNs dispersed solution was dropped on the GCE. Subsequently, 5 μL ChOx (1 mg/mL in 0.1 M PBS, pH 7.0) solutions were dropped on the surface of

the electrode to construct a cholesterol biosensor (noted as ChOx/H-GNs/GCE). For comparison, ChOx/GNs/GCE was prepared similarly. The modified electrodes were stored at 4 °C for future use.

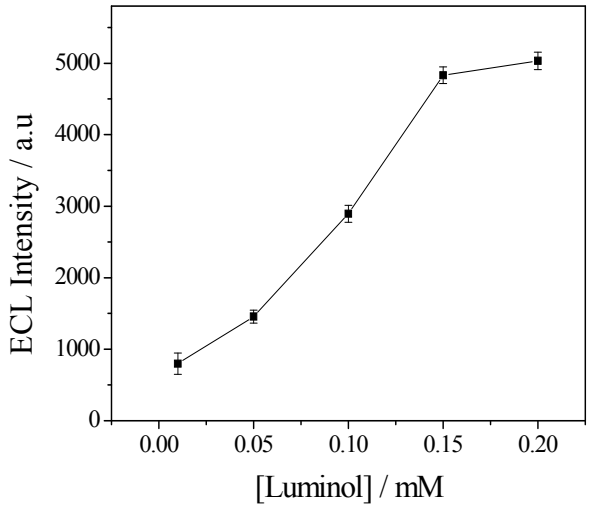

Fig. S1 Effect of luminol concentration on the ECL responses to cholesterol at ChOx/H-GNs/GCE in 0.05 M PBS (pH 7.4). Scan rate: 100 mV/s.

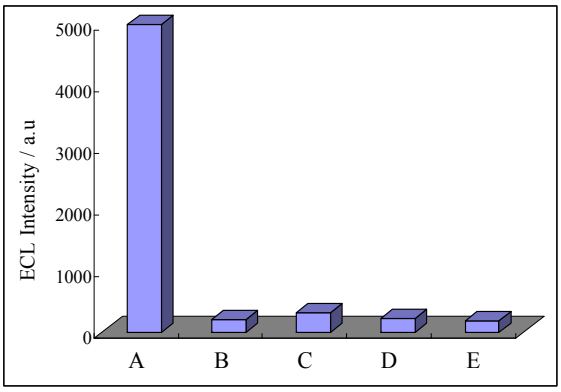

Fig. S2 The ECL responses of luminol (0.15 mM) at ChOx/H-GNs/GCE to (A) 0.38 mM cholesterol, (B) 2 mM ascorbic acid, (C) 2 mM uric acid, (D) 2 mM dopamine, and (E) 2 mM glycine in 0.05 M PBS (pH 7.4). Scan rate: 100 mV/s.

Table S1 Comparison of performance of some cholesterol sensors

Electrode materials	Determine method	Linear range (μM)	Detection limit (μM)	Refs.
ChOx/Chi-IL/MWNT(SH)-Au	Chronoampertry	500-5000	—	2
ChOx-PPy/Pt	Chronoampertry	25-300	5.7	3
Gold electrode polymerized with 2-MBI	Molecularly imprinted polymers	5-30	0.42	4
ChOx/p(pyrrole)/p(HEMA)	Chronoampertry	500-1500	120	5
ChOx/H-GNs/GCE	ECL	0.17-1120	0.06	This work

ChOx, cholesterol oxidase. Chi, chitosan. IL, ionic liquid. MWNTs, multiwall carbon nanotubes. PPy, polypyrrole. MBI, maslach burnout inventory. p(HEMA), poly(2-hydroxyethyl methacrylate).

Table S2. Application of the biosensor for determination the recovery of cholesterol.

Sample	Detected[a] (μM)	Added (μM)	Found[a] (μM)	Recovery (%)
1	10.0	10.0	20.5±0.8	102.5
2	10.0	15.0	25.7±1.5	102.8
3	15.0	25.0	44.8±1.8	112.0
4	75.0	75.0	150.8±2.0	100.5
5	150.0	200.0	345.1±0.9	98.6
6	300.0	400.0	699.4±0.6	99.9

All samples were analyzed using standard addition method (n = 3).
[a] Mean value ± standard deviation (n = 3).

References

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