Electronic Supplementary Information:

Layer-by-layer assembly of hemoglobin-coated microspheres for enhancing the capacity of oxygen carrying †

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1. Experimental details

1.1. Materials

Bovine hemoglobin and ethylenediamine tetraacetic acid disodium salt (Na_2EDTA) were purchased from Sinopharm Chemical Reagent Co., Ltd. Glutaraldehyde (25% water solution) was obtained from Shanghai Ling Feng Chemical Reagent Co., Ltd. Phosphate buffer solution (PBS) was prepared by mixing 0.10 M Na_2HPO_4, NaH_2PO_4 and NaCl. All chemicals were of analytical grade and used without further purification. Double-distilled water was used in all the experiments.

Electrochemical measurements were performed with a CHI 660D electrochemical workstation using the modified glassy carbon electrode (GCE, 3 mm in diameter) as the working electrode, a platinum wire as the counter, and a saturated calomel electrode (SCE) as reference electrode. The morphology and the element of Hb-coated microspheres were characterized by the transmission electron microscopy (TEM) with JEM-2100 microscope, the scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) with JSM-6510 microscope. The charges of the prepared spheres were characterized by zeta-potential measurement with Zetasizer Nano ZS-90. Electrochemical impedance spectroscopy (EIS) was performed in 0.10 M KNO_3 solution including 5.0 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ (1:1) as the supporting electrolyte with the AUTOLAB PGSTAT 302N electrochemical working station with the frequency from 1.0 mHz to 100 kHz.

1.2 Preparation of Hb-coated microspheres

0.20 g Hb was dispersed in 10 mL 0.25 M $CaCl_2$ solution and sonicated at 4 °C for 30 min.

Then the equal volume and concentration of Na_2CO_3 solution was rapidly poured into the above mixture. After 2 min, the precipitation was collected. After that, the Hb-loaded $CaCO_3$ spheres were suspended in 0.025% glutaraldehyde in pH 7.4 PBS for 2 h, followed by centrifugation and washing to obtain the Hb-$CaCO_3$ microspheres. Glutaraldehyde, a cross-linking agent, was applied to bond Hb molecules through imine linkage between aldehyde groups in glutaraldehyde and amino groups in hemoglobin. Then the particles were dispersed into Hb solutions for 12 h, followed by centrifugation and washing. The glutaraldehyde and hemoglobin were alternately adsorbed until the assembly of a desired number of hemoglobin/glutaraldehyde. Afterwards, the $CaCO_3$ template was removed by 0.20 M pH 7.0 Na_2EDTA solution. Finally, pure Hb-coated microspheres were obtained and stored at 4°C for study.

1.3 The assembly process of hemoglobin-based microspheres with glass carbon electrode

The glassy carbon electrode was first polished with abrasive paper and then with alumina slurry, followed by ultrasonically cleaned in ethanol and water and dried at room temperature. Then the glassy carbon electrode was electrodeposited at −2.0 V for 5 min in fresh prepared chitosan (CS) solution. The CS film modified GCE was washed with double-distilled water and dried in the air. Then obtained hemoglobin-coated microspheres were resuspended in pH 7.4 phosphate buffered solution, and 20 μL of this suspension was dropped on the surface of the CS modified GCE. Afterwards, the final modified electrode was then left to dry at room temperature and denoted as hemoglobin-coated spheres/CS/GCE.

1.4The study of oxygen-carrying capacity

The oxygen-carrying capacity of the Hb-coated microspheres was investigated by the response currents to oxygen based on the electrochemical methods. The modified electrodes of Hb-coated microspheres with one layer and five layers were treated in saturated oxygen device for 30 s to bind oxygen, and moved into oxygen-free device (saturated nitrogen) for differential pulse voltammetry (DPV) detection. Then, the electrodes were put in saturated nitrogen for 120 s to release oxygen and detected by DPV. This process was repeated 5 times. For better comparison, all the DPV peak currents were normalized (normalized ratio = DPV peak currents of saturation oxygen or nitrogen treatment / DPV peak currents of initial saturation nitrogen treatment).

In order to investigate the oxygen-releasing ability of Hb-coated microsphere, the Hb-coated microspheres modified electrodes were treated in saturated oxygen device for 20 min to absorb oxygen, and then measured the redox peaks by cyclic voltammetry (CV) in oxygen-free PBS (saturated N2).

2. Results section

2.1Immobilization of Hb-coated microspheres on electrodes

Electrochemical impedance spectroscopy (EIS) was utilized to monitor interfacial

information of the electrode during the assembly process[1]. As shown in Fig. S1, only a very small semicircle can be observed at bare GCE (curve a). When chitosan was electrodeposited on the surface the semicircle dramatically increased (curve b), indicated that the chitosan film acted as an insulating layer which made the interfacial charge transfer inaccessible[2]. After the Hb-coated microspheres were dropped on the chitosan modified GCE (curve c), the electron transfer resistance was obviously increased. In addition, Hb-coated microspheres showed a negative zeta potential (−12.0 ± 1.54 mV) while zeta potential of the pure $CaCO_3$ particles were positive (13.9 ± 0.83 mV). Such results suggested that Hb-coated microspheres can be successfully immobilized on the CS/GCE through the electrostatic adsorption.

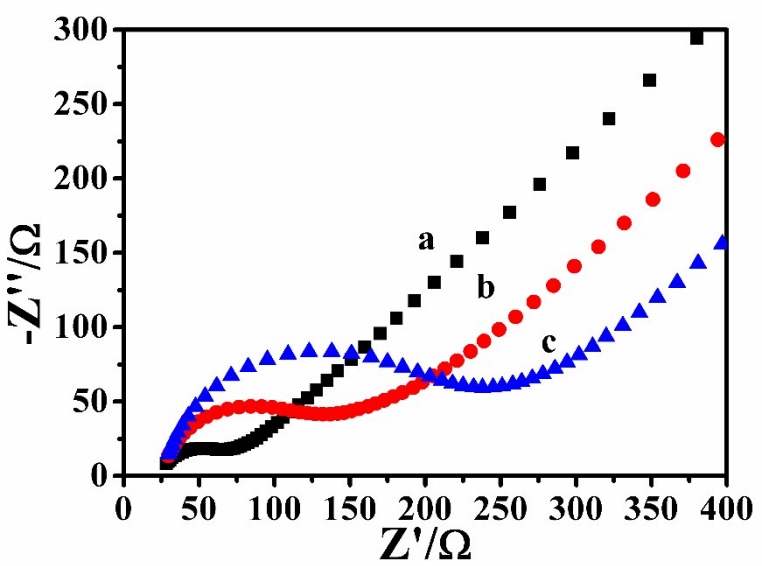

Fig. S1 EIS of bare GCE (curve a), CS/GCE (curve b), and Hb-coated spheres/CS/GCE (curve c).

References

1 S. L. Pan and L. Rothberg, *Langmuir*, 2005, **21**, 1022.

2 C. M. Yu, X. H. Zhou and H. Y. Gu, *Electrochim Acta*, 2010, **55**, 8738.