Electronic Supplementary Information:

Fabrication and evaluation of hemoglobin-based polydopamine microcapsules as oxygen carriers

Chunmei Yu[1*], Xin Huang[1], Dongping Qian, Fengfeng Han, Linyi Xu, Yuejing Tang, Ning Bao, Haiying Gu[*]

School of Public Health, Nantong University, Nantong 226019, P. R. China

1. Experimental

1.1. Reagents and apparatus

Bovine Hb, glutaraldehyde (GA), chitosan (CS, ≥ 90 % deacetylation), hydrochloric acid (HCl), manganese sulfate ($MnSO_4$), ammonium hydrogen carbonate (NH_4HCO_3) and ethanol were obtained from Sinopharm Chemical Reagent Co., Ltd. Tris(hydroxymethyl) aminomethane (Tris), chloroauric acid ($HAuCl_4 \cdot 4H_2O$, 99.999 %, Au % > 48 %), sodium citrate and sodium hydroxide (NaOH) were purchased from Sigma (Shanghai, China) and used as received. Dopamine hydrochloride was purchased from Aladdin, China. Phosphate buffer solutions (PBS, 0.1 M) with different pH values were prepared by mixing Na_2HPO_4 and NaH_2PO_4 solutions and using 0.1 M HCl or NaOH solution to adjust the pH. All chemicals were of analytical grade and used without further purification. All the aqueous solutions were prepared with deionized water.

The morphology of PDA-Hb microcapsules was characterized by the scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) with a JSM-

[*]Corresponding author. Tel: +86 513 85012913.
E-mail address: cmyu@ntu.edu.cn; hygu@ntu.edu.cn.
[1]Both authors contributed equally.

6510 microscope. Zeta potential was measured by Zetasize Nano ZS90. UV-vis spectra were recorded using an UV-2450 spectrophotometer. Fourier transform-infrared (FT-IR) spectra were obtained with an AVATAR-370 (Nicolet) spectrometer. Confocal laser scanning microscopy (CLSM) micrographs were taken with a Leica TCS-SP2 system equipped with 100×oil-immersion objective and a numerical aperture of 1.4. Electrochemical measurements were performed with a CHI 660D electrochemical workstation (Shanghai CH Instrument Co. Ltd., China) using the modified glassy carbon electrode (GCE, 3 mm in diameter) as the working electrode (WE). A saturated calomel electrode (SCE) and a platinum wire electrode served as the reference electrode (RE) and the counter electrode (CE), respectively.

1.2. Preparation of pure Hb spheres

Particles were fabricated in a modified manner according to previous reports.[1] Briefly, 500 mg of Hb was dissolved in $MnSO_4$ solution (100 mL, 0.016 M), into which ethanol (10 mL) was rapidly poured under ultrasound treatment for 10 s. Then NH_4HCO_3 solution (100 mL, 0.16 M) was added quickly to the above mixture under ultrasound for 30 s. This system was quiescent at ambient condition for 30 min. The precipitated Hb-$MnCO_3$ particles were collected by centrifugation. Then the Hb-$MnCO_3$ particles were suspended in glutaraldehyde (0.025 %, 20 mL) for 2 h to prevent the dissociation of Hb. After centrifuged and washed with deionized water thrice, the Hb-$MnCO_3$ particles were collected. Then, the Hb-$MnCO_3$ particles were incubated in 0.1 M EDTA solution (pH 7.4) for 12 h to remove the $MnCO_3$ template. Finally, the pure Hb spheres were obtained.

1.3. Preparation of PDA-Hb microcapsules

Hb spheres were prewashed with Tris-HCl buffer (10 mM, pH 8.5) and incubated with dopamine hydrochloride solution (1 mg/mL) in Tris-HCl buffer under slight stirring at ambient condition for 12 h. Then the obtained PDA-Hb microcapsules were centrifuged (3000 rpm, 5 min) and washed with Tris-HCl buffer thrice.

1.4. Hemolysis rate test

Hemolysis rate test was conducted to investigate the blood compatibility of PDA-Hb microcapsules. Firstly, the EDTA anticoagulated whole blood of a volunteer was washed three times with physiologic saline (0.9 % w/v). Then, 1 mL normal RBCs were incubated with 50 mL physiologic saline (0.9 % w/v) to obtain RBCs diluted solution. PDA-Hb microcapsules were dispersed in physiologic saline (0.9 % w/v) and incubated at 37 °C for 30 min. Then, 2 mL RBCs diluted solution was added into 2 mL of PDA-Hb microcapsules physiologic saline solution at different concentrations or water and incubated at 37 °C for 60 min. After centrifugation at 1000 rpm for 10 min, the absorbance at 545 nm of the supernatant was read as A_t. The absorbance at 545 nm of the PDA-Hb microcapsules physiologic saline solution was read as A. Under the same conditions, an RBCs and physiologic saline (0.9 % w/v) mixture solution was used as a negative reference A_-, and an RBCs and water mixture as a positive reference A_+. The hemolysis rate was calculated via the following formula:

$$\text{Hemolytic ratio}\,(\%) = \frac{A_t - A}{A_+ - A_-} \times 100\%$$

1.5. Cytotoxicity assay

Human embryonic kidney (HEK293T) cells were seeded in 96-well plates and incubated at 37 °C with 5 % CO_2 for 24 h. Then the HEK293T cells were treated with 300 μL new culture media containing 0, 20, 40, 60 and 200 μL 0.25 mg/mL PDA-Hb microcapsules for another 24 h. After incubation, 10 μL CCK-8 solutions was added to each well and incubated with the cells for 3 h at 37 °C with 5 % CO_2 followed by measuring the absorbance at 450 nm.

The absorbance of treated wells was read as A_t. The HEK293T cells without PDA-Hb microcapsules solution were used as positive reference A_+. Under the same conditions, cell free wells were used as a negative reference A_-. The cell viability was

calculated via the following formula:

$$\text{Cell Viability}\,(\%) = \frac{A_t - A_-}{A_+ - A_-} \times 100\%$$

1.6. Fabrication of Au nanoparticles (AuNPs) and the electrode modification

For the preparation of AuNPs, 0.01 % $HAuCl_4$ solution (100 mL) was heated with vigorous stirring. Upon boiling, 1 % sodium citrate solution (0.7 mL) was dropped quickly into the $HAuCl_4$ solution. Then, the color of solution changed from light yellow to wine red color. At this stage, the solution was heated under reflux for approximately 15 min, and the solution was continuously agitated until it was cooled to room temperature. The prepared AuNPs were stored in dark at 4 °C.

Prior to modification, the glassy carbon electrode (GCE) was polished with abrasive paper and alumina slurry, followed by ultrasonically cleaned in ethanol and water and dried at room temperature. The GCE was electrodeposited at −2.0 V for 5 min in fresh prepared chitosan (CS) solution to obtain the CS film modified GCE. Then the CS/GCE was dipped in AuNPs for 20 min and washed with double-distilled water to obtain AuNPs/CS/GCE. The obtained PDA-Hb microcapsules were resuspended in pH 7.4 PBS, and 10 μL of PDA-Hb microcapsules (3 mg/mL) was dropped on the surface of the AuNPs/CS/GCE. Finally, the modified electrode was dried in the air and denoted as PDA-Hb/AuNPs/CS/GCE. For comparison, Hb/AuNPs/CS/GCE was fabricated using Hb solution (3 mg/mL) instead of PDA-Hb microcapsules according to the similar procedure.

1.7. Oxygen-carrying and releasing capacity study of PDA-Hb microcapsules

The oxygen-carrying capacity of PDA-Hb microcapsules was studied by the response currents to oxygen based on electrochemical methods. Before transfer to oxygen-free PBS (saturated N_2) for differential pulse voltammetry (DPV) detection, the PDA-Hb/AuNPs/CS/GCE was bound with oxygen by putting in saturated O_2 PBS (pH 7.4) for 30 s. Then, the modified electrode was treated in saturated N_2 PBS (pH

7.4) for 120 s to release oxygen and thereafter for detection with DPV. The above process was repeated five times. For comparison, all DPV peak currents were standardized (normalized ratio=DPV peak currents of saturation O_2 or N_2 treatment/DPV peak currents of initial saturation N_2 treatment). To investigate the oxygen-releasing ability of PDA-Hb microcapsules, PDA-Hb/AuNPs/CS/GCE was firstly put in saturated O_2 PBS (pH 7.4) for 20 min to absorb oxygen, and then the redox peaks were measured by cyclic voltammetry (CV) in oxygen-free PBS (pH 7.4).

2. Results section

2.1. CLSM images of pure Hb spheres and PDA-Hb microcapsules

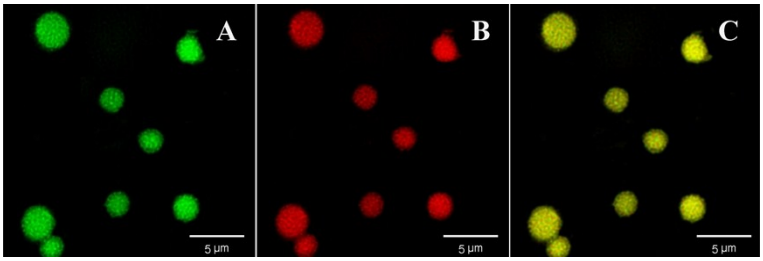

Fig. S1 CLSM images of pure Hb spheres. The samples were excited at 488 nm and three fluorescent images obtained: (A) 510–540 nm; (B) 570–600 nm and (C) overlay. Scale bars: 5 μm.

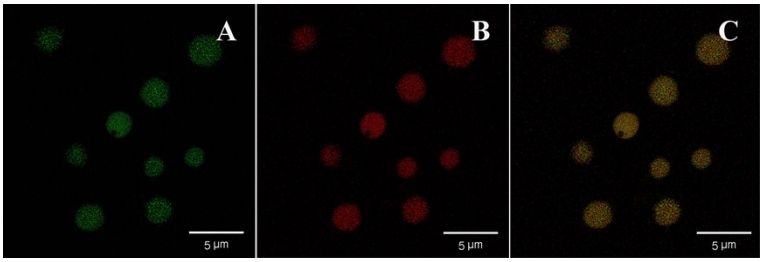

Fig. S2 CLSM images of PDA-Hb microcapsules. The experiment parameters were similar to Fig. S1.

2.2. Hb entrapment efficiency

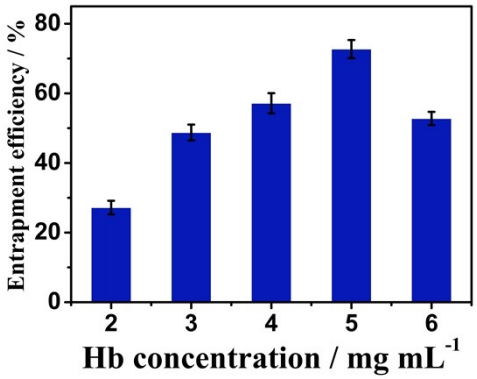

Fig. S3 The effect of initial concentration of Hb in $MnSO_4$ on Hb entrapment efficiency.

2.3. The pH stability and thermostability of the PDA-Hb microcapsules

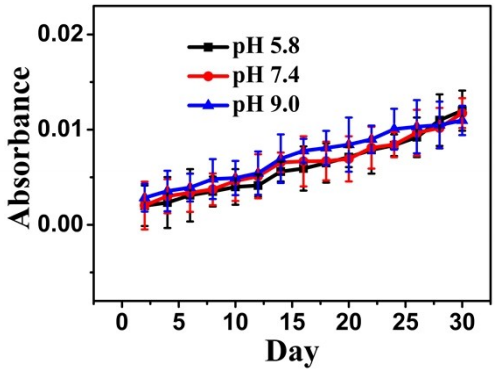

Fig. S4 The change of the absorbance of the supernatant as a function of time under different pH conditions at 25 °C.

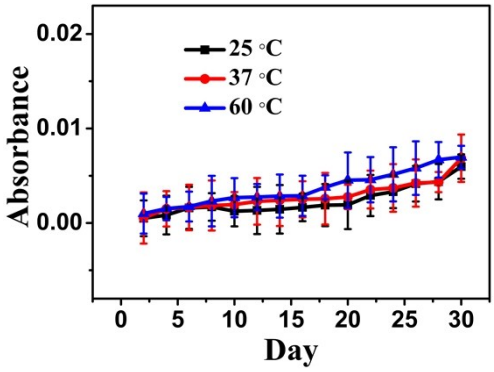

Fig. S5 The change of the absorbance of the supernatant as a function of time under different temperatures in pH 7.4 PBS.

2.4. FT-IR spectra of the PDA-Hb microcapsules

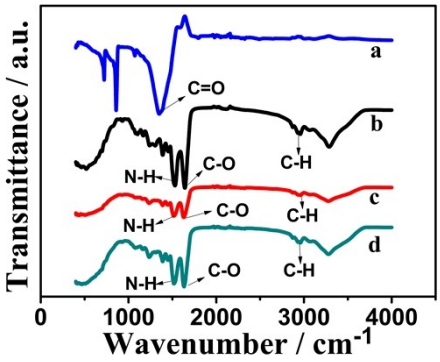

Fig. S6 FT-IR spectra of (a) $MnCO_3$, (b) Hb solution, (c) pure Hb sphere and (d) PDA-Hb microcapsules.

2.5. UV-vis analysis of the oxygen-carrying capacity of the microcapsules

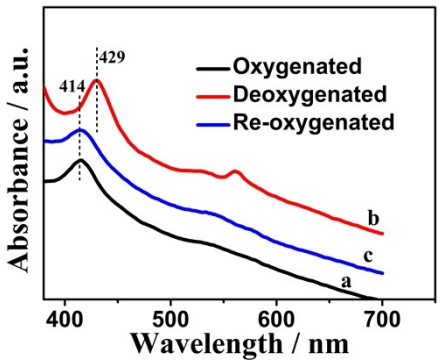

Fig. S7 UV-vis absorption spectra of (a) oxygenated, (b) deoxygenated and (c) re-oxygenated PDA-Hb microcapsules in pH 7.4 PBS (25 °C).

References

1 (a) H. G. Zhu, E. W. Stein, Z. H. Lu, Y. M. Lvov and M. J. McShane, *Chem. Mater.*, 2005, **17**, 2323–2328; (b) Y. Xiong, Z. Z. Liu, R. Georgieva, K. Smuda, A. Steffen, M. Sendeski, A. Voigt, A. Patzak and H. Bäumler, *Acs Nano*, 2013, **7**, 7454–7461.