Electronic Supplementary Information

Preparation of Millimeter-sized Mesoporous Carbon Spheres as an Effective Bilirubin Adsorbent and Their Blood Compatibility

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Experimental

Materials: The triblock poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) copolymer Pluronic F127 ($EO_{106}PO_{70}EO_{106}$, M_{av} = 12600) was purchased from Aldrich. Sorbitan monooleate Span-80 was purchased from Tokyo Chemical Industry. Calibration plasma kit (Composition: human plasma), PT (Prothrombin Time) high sensitivity test kit (Composition: high sensitivity rabbit brain thromboplastin, calcium ions and less than 0.1% of sodium azide) and APTT (Activated partial thromboplastin time) test kit (Composition: synthetic phospholipids, colloidal silica, calcium chloride and less than 0.1% of sodium azide) were purchased from Instrumentation Laboratory. The commercial activated carbon spheres (ACSs) for haemoperfusion were provided by Aier Instruments for Blood Purification Co.. Bilirubin was purchased from Alfa Aesar. Other chemicals, including resorcinol, furfural, hexamethylenetetramine, paraffin oil, concentrated hydrochloride acid (HCl, 36.5 wt%), n-hexane PBS solution and absolute ethanol were purchased from Sinapharm Chemical Reagent Co.. All chemicals were used as received without further purification.

Synthesis of millimeter sized Mesoporous Carbon spheres (MMCSs): 4.6 g resorcinol was dissolved in

40 g ethanol, followed by adding 6 g furfural and 0.8 g 1 wt% HCl ethanol solution. After stirring at 283 K for 30 min, 1.8 mL 2.5 M hexamethylenetetramine aqueous solution and 8 g F127 were added and the mixture was stirred for another 1.5 h. Then, the mixture was placed in a flask containing a solution of 500 mL paraffin oil and 8 g sorbitan monooleate Span-80, and the suspension was stirred at 333 K for about 2 h. Here, the stirring rate was fixed at 200 rpm. After that, the suspension was further stirred at 388 K for 4 h at a higher rate of 500 rpm. The spherical polymer particles were collected by filtration, washed by n-hexane and air-dried at 353 K. Finally, the MMCSs were obtained by pyrolyzing the polymer spheres at 1073 K for 4 h with a heating rate of 2 K min-1 under purified N_2 flow.

Hemolysis assay: The procedure is carried out as the former reference.[1] Human blood stabilized with EDTA was obtained from Shanghai Blood Center. The serum was removed from the blood by centrifugation and suction, and the red blood cells (RBC) were then washed five times with PBS solution. Following the last wash, the cells were diluted to 1/10 of their volume with PBS solution.

The diluted RBC suspension (0.3 mL) was then mixed with: a) 1.2 mL of PBS as a negative control; b) 1.2 mL deionized water as a positive control; c) 0.03 g MMCSs and 1.2 mL PBS solution; d) 0.03 g R-ACS and 1.2 mL PBS solution. For a) and b), the mixtures were vortexed and then let to rest for 2 h at room temperature. For c) and d), the mixtures were vortexed every 15 minutes and the total treatment time is also 2 h. After above treatments, the samples were centrifuged, 1 mL of the supernatants was diluted by 2 times and the absorbance of the supernatants at 541 nm was measured in UV-visible station.

Coagulation assays: For the PT and APTT measurement, 0.03 g R-ACSs or MMCSs were added into 500 μL fresh human platelet poor plasma (PPP) and the mixture was incubated for 5 min at room temperature. Then, the PT and APTT of corresponding samples were measured three times on the ACL 7000 coagulation system. And the PT and APTT of 500μL fresh human PPP were also measured for comparison. Here, Calibration plasma test kit was used for the calibration of coagulation assays, PT high sensitivity test kit was for the determination of PT, APTT test kit was for the determination of APTT.

Bilirubin adsorption experiments: For the measurements of the changes of bilirubin concentration as a function of time, 0.1 g MMCSs was added into a brown bottle containing 20 mL 250 mg/L bilirubin PBS solution under stirring at 100 rpm at room temperature. After given time, 1 mL liquid from the bottle was taken to be analyzed by UV-vis absorption spectroscopy after being diluted by 10 times. For the measurements of bilirubin equilibrium adsorption isotherm, 0.05 g MMCSs was added into a brown bottle containing 20 mL bilirbuin PBS solution with different concentrations. After a certain time period (2h), 1

mL liquid from the bottle was taken to be analyzed by UV-vis absorption spectroscopy after being diluted by 10 times.

The calibration curve of bilirubin in PBS solution was deterimined by taking the bilirubin concentration versus absorbance at 438 nm between 0 and 100 mg/mL as parameters. For this interval, the calibration curve fits the Beer-Lambert law with A = 0.0125C + 0.06644, in which C is the bilirubin concentration (mg/mL) and A is the absorbance at 438 nm.

Characterization: TEM images were obtained on a JEM-2010 electron microscope operated at 200 kV. Here, the MMCSs were ground into powder for TEM measurement. Low resolution SEM images were obtained on a JSM-6700F operated at 10 kV and high resolution SEM images were obtained on a Hitachi Model S-4800 field emission scanning electron microscope. Nitrogen sorption isotherms at 77K were measured on a Micrometitics Tristar 3000 system. Before measurement, samples were pre-treated at 373 K for 12 h under nitrogen blowing. The specific surface area and the pore size distribution were calculated from the BET and BJH data. The UV/Vis absorbance spectra were measured using Shimadzu UV-3600PC spectroscope. The PT and APTT were measured by Instrumentation Laboratory ACL 7000 Coagulation System.

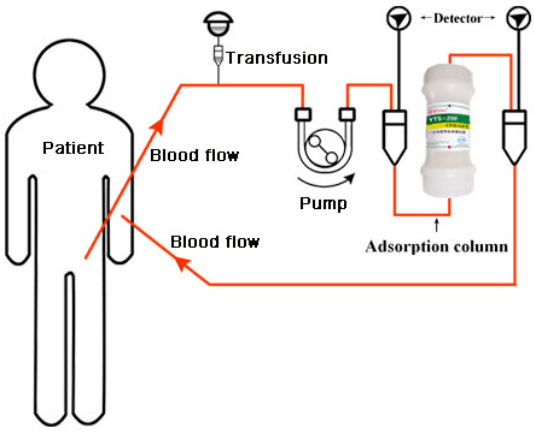

Fig. S1. Typical scheme of the practical blood perfusion and the adsorbents are added into the one-off adsorption column.[2]

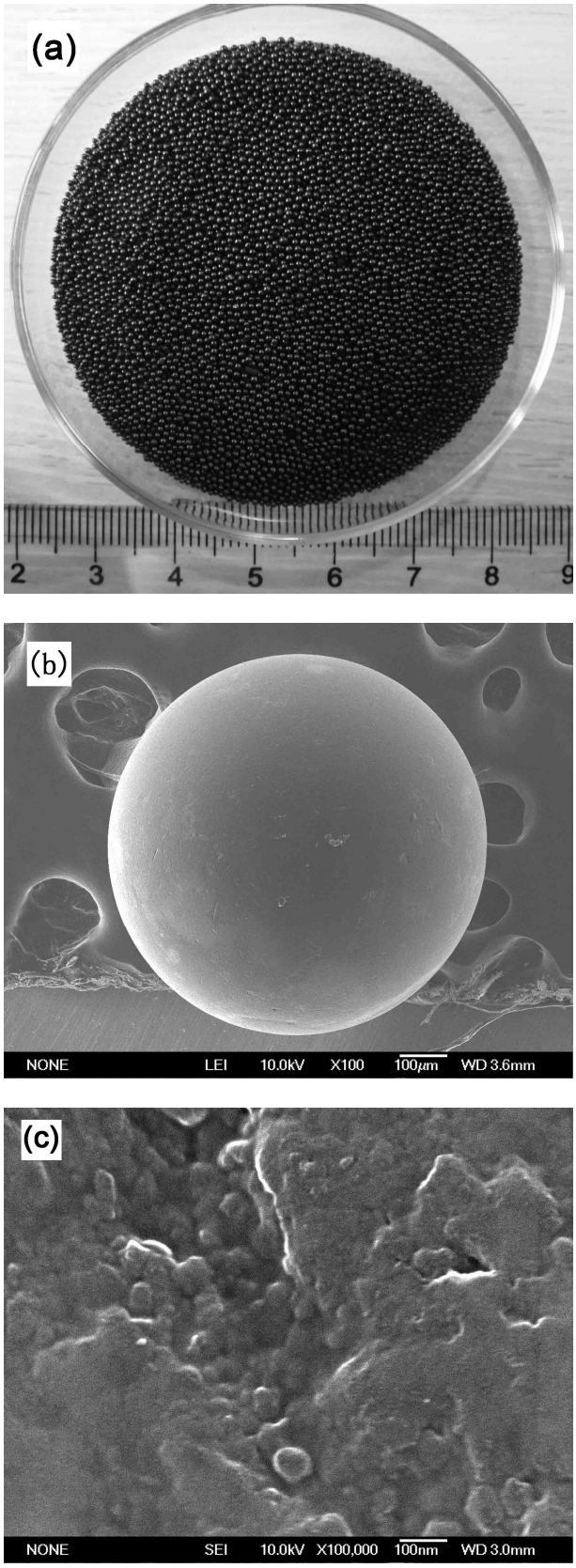

Fig. S2. (a) Macroscopic image of the activated carbon spheres (ACSs), (b) SEM image of a single activated carbon sphere (the substrate is conductive adhesive) and (c) the SEM image of microscopical structure of ACSs.

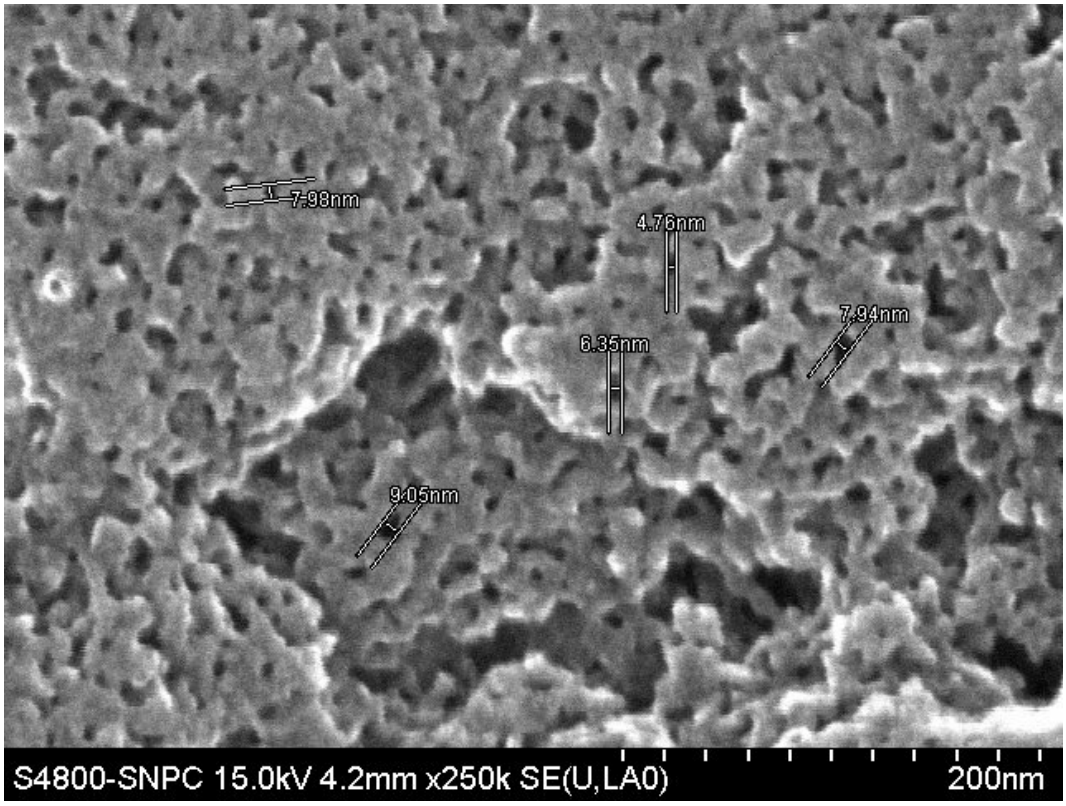

Fig. S3. The SEM image of MMCS at high magnification.

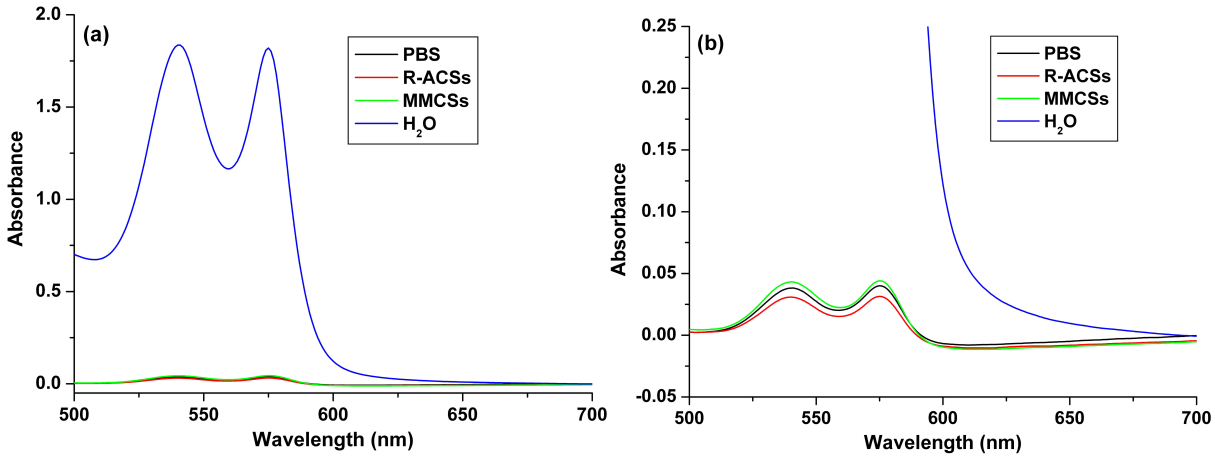

Fig.S4. The UV-visible adsorption spectra to detect the presence of hemoglobin at 541 nm. H_2O and PBS were used as a positive and a negative control. (a) is in normal scale, (b) is in greatly magnified scale.

References

1. I. Slowing, C. W. Wu, J. L. Vivero-Escoto, V. S. Y. Lin, Small 2009, 5, 57.
2. The scheme was cited from the website of Aier Instruments for Blood Purification Co: http:// www.aier-hp.com.