Supplementary Information for Manuscript:

Hollow mesoporous carbon spheres – An excellent bilirubin adsorbent

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Experimental Section

Materials: The active carbon for haemoperfusion was from Aier Instruments for Blood Purification Co.. Octadecyltrimethoxysilane (C18TMS, GC, ≥90%) was from Fluka. Bilirubin (97%) was from Alfa Aesar. Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer Pluronic P123 ($EO_{20}PO_{70}EO_{20}$) was from BASF Chemical Company. Albumin from human serum (96-99%) was from Sigma. Aluminium sulfate ($Al_2(SO_4)_3 \cdot 18H_2O$), aqueous ammounia, tetraethyl orthosilicate (TEOS, 98%), sodium hydroxide, oxalic acid, furfuryl alcohol and phosphate buffer saline (PBS, pH 7.4) were from Sinapharm Chemical Reagent Co.. The active carbon was dewatered by heating for 24 h at 120 ℃ before use and other chemicals were used without further purification.

Preparation of CMK-3: First, mesoporous silica SBA-15 template was prepared according to the literature.[1] In a typical preparation, the starting composition was 4.0 g of Pluronic P123, 8.5 g of TEOS, 120 g of HCl (2M) and 30 g of H_2O. After the reaction at 40 ℃ for 24 h, the mixture was then transferred into autoclaves and hydrothermaly treated at 100 ℃ for 24 h. The solid products was filtered, washed with distilled water, dried at 100 ℃, and calcined in air at 550 ℃

for 5 h.

The CMK-3 was prepared according to the literature.[2] Typically, 1.5 mL of furfuryl alcohol and a small quantity of oxalic acid were dissolved in 10 mL ethanol. This solution was incorporated into 1 g of SBA-15 by a wetness impregnation technique. After evaporating the ethanol and polymerizing furfuryl alcohol at 80 ℃, the residual unpolymerized furfuryl alcohol was evaporated at 150 ℃. The composite was thermal-treated in nitrogen at 850 ℃ to carbonize the polyfurfuryl alcohol. The silica template in the composite was removed by twice washing with heated 2M NaOH solution. The template-free mesoporous carbon (CMK-3) was collected by filtering, washed with water and ethanol, and dried at 80 ℃.

Preparation of HMCSs: Synthesis of silica core/mesoporous shell (SCMS) templates: 3.14 mL of aqueous ammonia (32 wt.%) was added into a solution containing 74 mL of ethanol and 10 mL of deionized water. 6 mL of TEOS was added into above-prepared mixture at 30 ℃ under vigorous stirring and the mixture was kept stirring for 1 h to yield uniform silica spheres. Afterwards a mixture containing 5 mL of TEOS, 2 mL C18TMS and 1 mL 0.45 M $Al_2(SO_4)_3 \cdot 18H_2O$ were added into the above-prepared colloidal solution containing silica spheres, and then further stirred for 1 h. The resulting octadecyltrimethoxy-incorporated silica shell/solid core nanocomposite was retrieved by centrifugation, washed with deionized water, dried at 100 ℃, and further calcined at 550 ℃ for 6 h under air to obtain the SCMS templates. The TEM image of as-prepared SCMS is shown in Fig.S1. Synthesis of HCMSs: Incipient-wetness impregnation was employed to introduce carbon source into SCMS templates. Briefly, 0.5 g SCMSs were impregnated with 0.7 mL furfuryl alcohol, and the resulting mixture was dried at 60 ℃ for 3 h, followed by drying under vacuum at 60 ℃ for 10 h to generate the polymerization of furfuryl

alcohol, which was in-situ catalyzed by the aluminosilicate framework on the shell. Subsequently, the polymerized furfuryl alcohol was converted into carbon inside the mesopores in the shells of SCMSs by pyrolysis at 600 ℃ for 4 h under N_2 flow with a heating rate of 2 ℃/min. HMCSs were then recovered by removing silica framework using 2M NaOH aqueous solution at 85 ℃, then washed several times using deionized water and dried at 100 ℃ overnight.

Bilirubin adsorption experiments: Take HMCSs as an example. The measurements of the changes of bilirubin concentration as a function of time: 0.1g HMCSs 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. Here, the liquid was filtered off HMCSs by using a syringe filter. The measurements of bilirubin equilibrium adsorption isotherms: 0.05 g HMCSs was added into a brown bottle containing 20 mL bilirubin PBS solution with different concentrations. After a certain time period (30 min for HMCSs), 1 mL liquid from the bottle was taken to be analyzed by UV-vis absorption spectroscopy after being diluted by 10 times and filtered off HMCSs. The bilirubin adsorption tests were almost the same for CMK-3 and activated carbon except for the longer adsorption time periods (40 min for CMK-3 and 300 min for active carbon) for the equilibrium adsorption tests

Calibration curve of bilirubin in PBS solution was determined by taking absorbance at 438 nm vs bilirubin concentrations between 0 and 120 mg/L as parameters. For this interval, the calibration curve fits the Lambert and Beer' Law with $A = 0.06288C$, where A is the absorbance at 438 nm and C is the bilirubin concentration (mg/L).

Bilirubin versus albumin adsorption: 0.1 g HMCSs was added into 20 mL bilirubin (200 mg/L)

and albumin (40 mg/mL) PBS solution under stirring at 100 rpm at room temperature. After 2 h, 1 mL of the liquid was taken to be analyzed by UV-vis absorption spectroscopy after being diluted by 10 times and filtered off HMCSs.

Calibration curves of bilirubin and albumin in bilirubin and albumin PBS solution were determined by taking absorbance vs bilirubin or albumin concentration at 438 nm for bilirubin and 278 nm for albumin. Here, 25 mL PBS solution containing 0.05 g bilirubin (200 mg/L) and 1 g albumin (40 mg/mL) was prepared first. Then, the five standard samples were obtained by diluting the as-prepared 1 mL bilirubin and albumin PBS solution by 10, 20, 40, 50 and 100 times. For bilirubin, the calibration curve fitted the Lambert and Beer' Law with $A = 0.05709C + 0.0015$, where A is the absorbance at 438 nm and C is the bilirubin concentration (mg/L). For albumin, the calibration curve fitted the Lambert and Beer' Law with $A = 0.88592C + 0.02225$, where A is the absorbance at 278 nm and C is the albumin concentration (mg/mL).

Hemolysis assay: The experiment was carried out according to the former report.[3] 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 (RBS) 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 RBS 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) 1.2 mL of HMCS suspensions at concentrations ranging from 25 to 200 μg/mL. The mixtures were then vortexed and then let to rest for 2 h at room temperature. After that time, the samples were centrifuged, 1 mL of the supernatants was diluted by 3 times and the absorbance of the supernatants at 541 nm was

measured on a UV-visible station.

Characterization: TEM images were obtained on a JEM-2010 electron microscope operated at 200kV. SEM images were obtained on a JSM-6700F operated at 10 kV. Nitrogen sorption isotherms at -196 ℃ were measured on a Micromeritics Tristar 3000 system. Before measurements, samples were pre-treated at 100 ℃ for 12 h under nitrogen. The specific surface area and the pore size distributions were calculated from the BET and BJH data. The UV/Vis absorbance spectra were measured using a Shimadzu UV-3600PC spectroscope.

1 D. Zhao, J. Feng, Q. Huo, N. Melosh, G. Fredrickson, B. Chmelka and G. Stucky, Science, 1998, **279**, 548.
2 X. Dong, H. Chen, W. Zhao, X. Li and J. Shi, Chem. Mater. 2007, **19**, 3484.
3 I. Slowing, C. Wu, J. Vivero-Escoto and V. Lin, Small, 2009, **5**, 57.

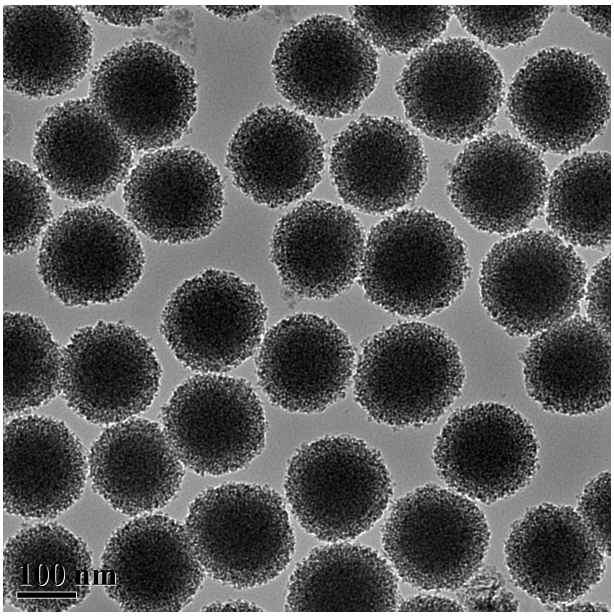

Fig. S1 The TEM image of as-prepared SCMSs templates

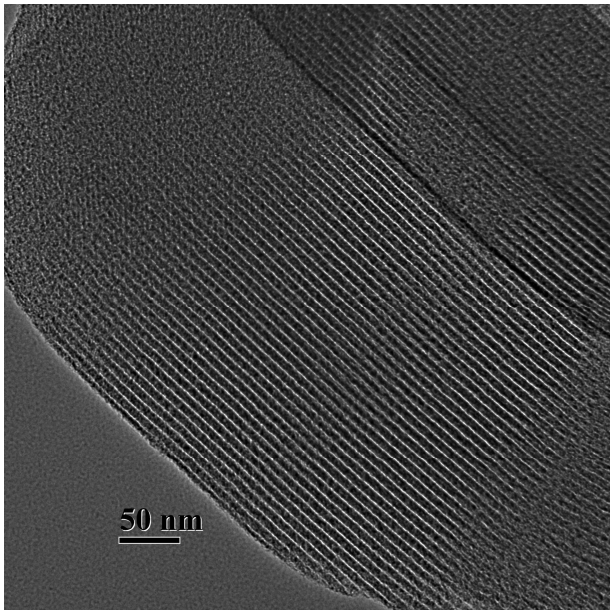

Fig. S2 The TEM image of mesoporous carbon (CMK-3).

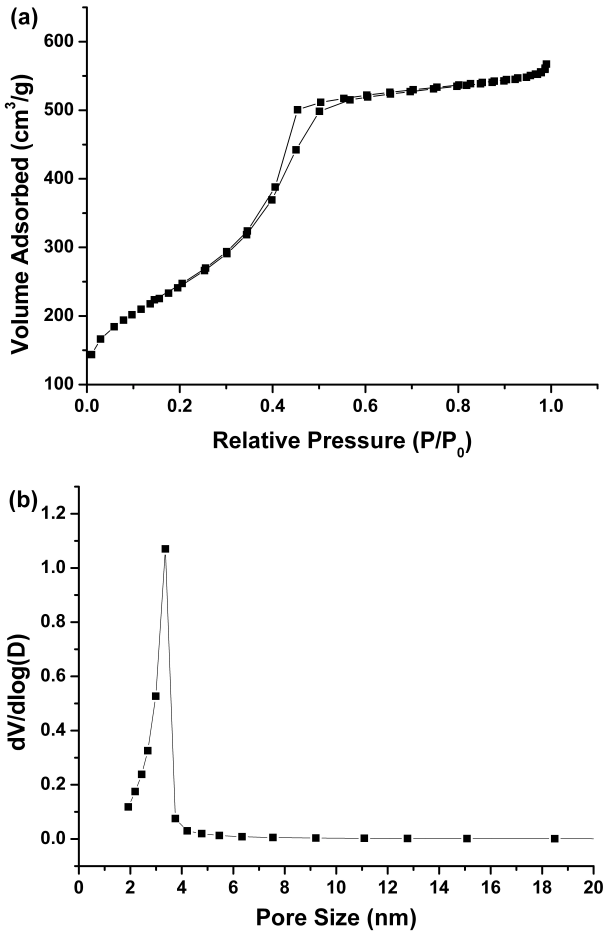

Fig. S3 Nitrogen sorption isotherm (a) and corresponding pore size distribution (b) curves of CMK-3.

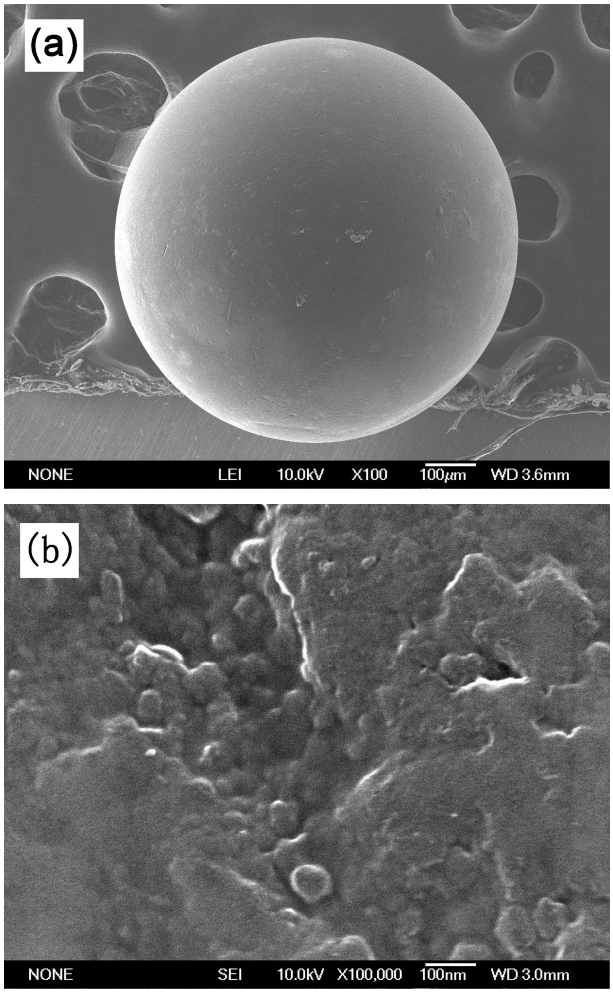

Fig. S4 The SEM images of activated carbon: the macroscopical spherical morphology (the substrate is conductive adhesive) (a) and the microscopical structure of spherical surface (b).

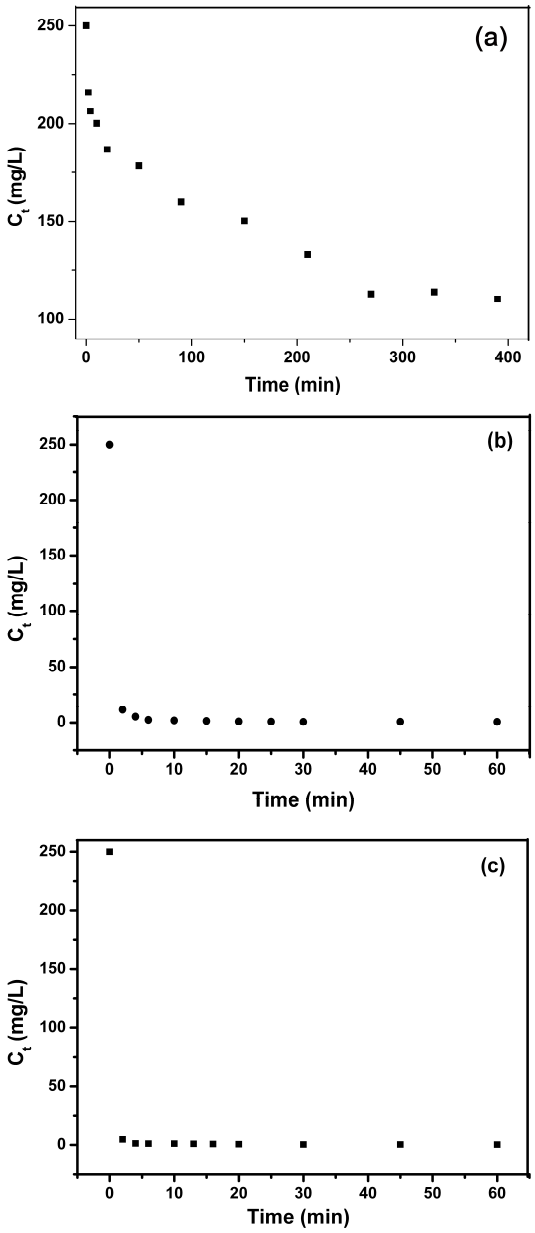

Fig. S5 The change of bilirubin concentration as a function of time using activated carbon (a), CMK-3 (b) and HMCSs (c) as adsorbent.

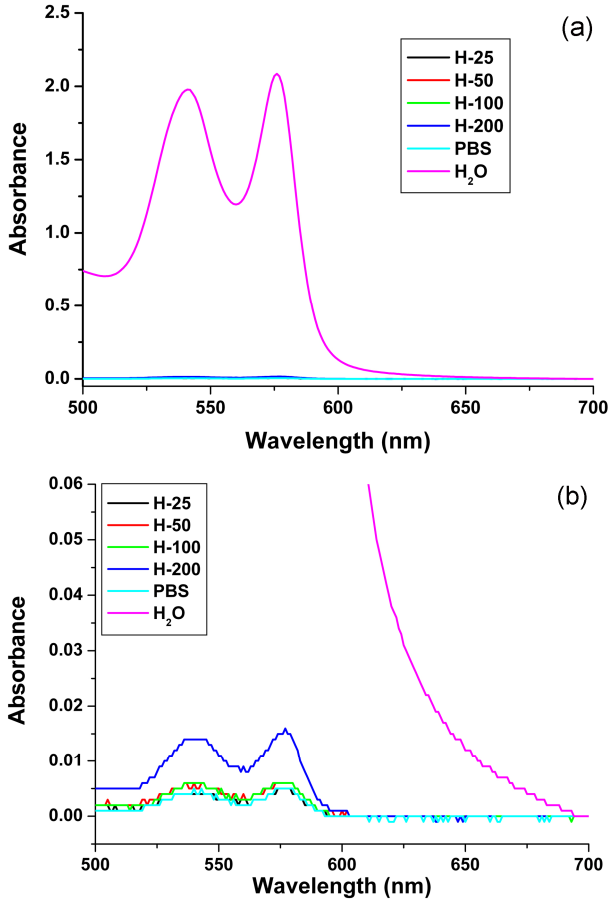

Fig. S6 The UV/Vis absorption spectra to detect the presence of hemoglobin at 541 nm. H-25, H-50, H-100 and H-200 stand for HMCSs suspensions in PBS at concentrations of 25, 50, 100 and 200 μg/mL. H_2O and PBS were used as a positive and a negative control. (a) is in normal scale, (b) is in greatly magnified scale.