Supplementary Information

Polydopamine Decorated Ordered Mesoporous Carbon for Efficient Removal of Bilirubin under Albumin-rich Situation

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This supplementary data includes:

1. **Synthesis of ordered mesoporous carbon (OMC)**
2. **Polymerization time optimization**
3. **Thermogravimetric analysis of synthesized materials**
4. **Adsorption kinetic characterization**
5. **Adsorption isotherms fitting**
6. **Comparison with commercial sorbent materials**
7. **Competitive adsorption experiment**
8. **Scatchard analysis**
9. **Equilibrium adsorption isotherms of resin to bilirubin**

Figure S1 to **S9**;

Table S1 and **S2**.

1. Synthesis of ordered mesoporous carbon (OMC)

The synthesis diagram of OMC material could be found in Fig. S1. Phenol (6.1 g) was weighted and desolved in the mixture of formaldehyde (10.4 g, 37 wt%) and NaOH solution (1.3 mL, 5M) at 70 ℃ with continues stirring. After the mixture solution was cooled to room temperature, HCl solution (5M) was used to adjust the pH value of the mixture to 7.0. Then all the mixed solution was transferred into a volumetric flask and ehtanol was added until the total volumn reached 100 mL to obtain the resol precursor. Subsequently, F127 (1.6 g) was dissolved in ethanol (8 g) and HCl solution (0.2 M, 1 mL) was added for a better disolution. Then, TEOS (2 mL) and the prepared resol precursor solution (10 mL) was mixed with the aforementioned F127 solution under stirring for 2 h. After that, the reaction solution was transferred into a flat dish and ethnol was volatiled at room temperature. Then, the thermopolymerization reaction was conducted at 100 ℃ in oven for 24 h. Yellowish polymer was obtained and then carbonized under continuous N_2 flow under the following temperature program: (1) from 50 to 600 ℃ at a heating rate of 1℃ min^{-1}, (2) from 600 to 900 ℃ at a heating rate of 5 ℃ min^{-1}, (3) kept at 900 ℃ for 2 h. After the thermal decomposition of F127, the carbon-silica nanocomposite with ordered mesopores was obtaiend, of which the specific surface area and main pore size distribution were identified to be 300.75 m^2 g^{-1} and 6.77 nm respectively (Fig. S2). After HF etching (in 10 wt% HF solution for 48 h) and washing, OMC mterial was dried in vacumn and ready to be used. Detailed characterization resluts of OMC material could be found in the main body.

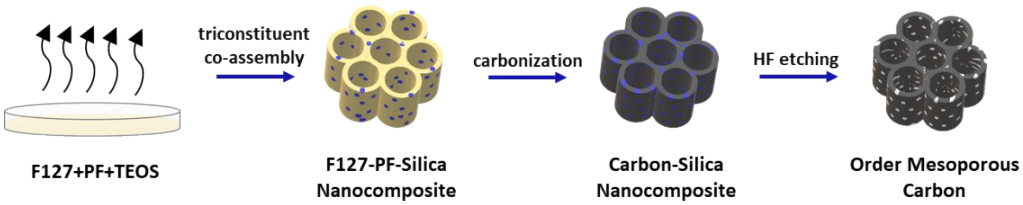

Fig. S1 The synthesis diagram of OMC material.

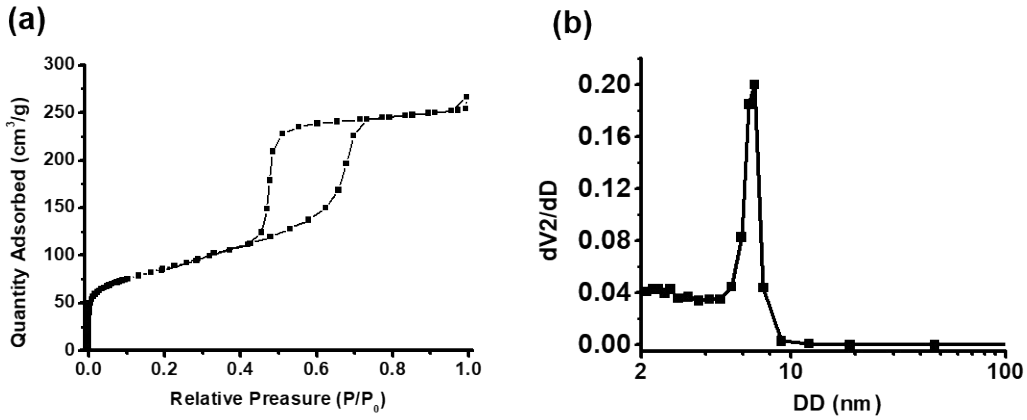

Fig. S2 The N_2 adsorption-desorption isotherm (a) and the BJH pore size distribution curve (b) of carbon-silica nanocomposite.

2. Polymerization time optimization

10 mg OMC was dispersed in bilirubin solution (5mL, 200 mg L^{-1}) in centrifuge tubes and the mixture was vibrated at 600 rpm for 10 min for pre-adsorption. Then, fresh dopamine solution (5mL, 20 mg mL^{-1} in Tris buffer) was added in the system, and after a gentle mix, the mixture was placed under dark for polymerization with a slight shaking every 5 min. Here 5, 10, 15, 20 30 min were chosed as the polymeriztion time duration. After polymerization, the material was collected and then ethanol and 0.01 mM NaOH solution were used in turns to wash the bilirubin form the material until the supernatant is colorless. After completely removed the templates, the OMC@PDA material was dried and stored in vacuum until use. The performances of the synthesized materials were evaluated by conducting the adsorption experiemnts in bilirubin solution (10 mg L^{-1}) for 60 min to get the equilibrium, and the result was presented in Fig. S3.

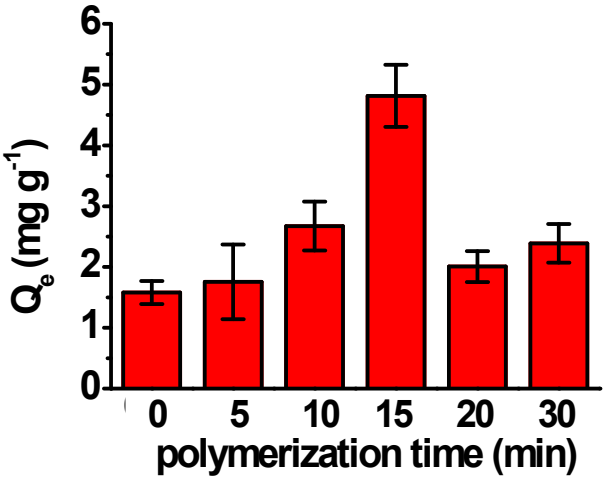

Fig. S3 The equilibrium adsorption amount on the materials undergoing different polymerization time (n=3).

3. Thermogravimetric analysis of synthesized materials

The thermogravimetric analysis experiments were conducted on a Pyris1 TGA analyzer form PerkinElmer Inc. and the results could be found in Fig. S4. Mass changes of both PDA and OMC@PDA at around 100 ℃ might be caused by the loss of water, therefore we mainly focused on the mass from 150 to 800 ℃ for further evaluation. By comparing the mass loss of three synthesized materials, the mass ration of PDA in OMC@PDA materials was calculated to be about 20%.

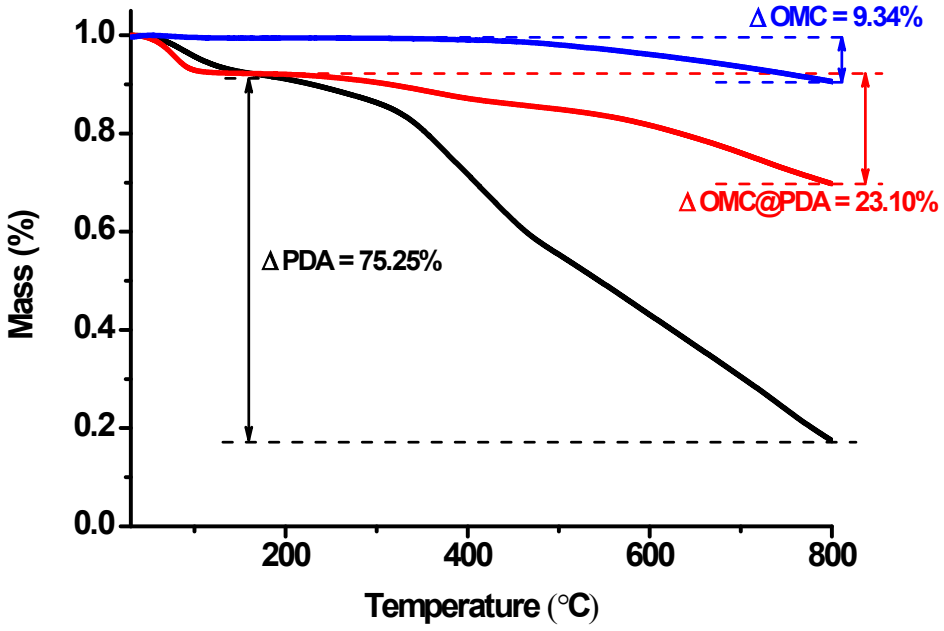

Fig. S4 The thermogravimetric analysis results of OMC (blue), PDA(black) and OMC@PDA (red) and their corresponding mass loss ratio between 150 and 800 ℃.

4. Adsorption kinetic characterization

The adsorption kinetic experiments were conducted on both bare OMC and OMC@PDA materials in albumin-rich situation to evaluate their adsorption efficiency. 21 centrifuge tubes containing 1 mg material and 1 mL mixing solution, in which the concentrations of bilirubin and BSA were 10 mg L^{-1} and 40 mg mL^{-1} respectively, were divided into 7 groups. The concentration of bilirubin was detected at 5, 10, 20, 30, 40, 60, 90 min after the adsorption had been started. Results could be found in Fig. S5.

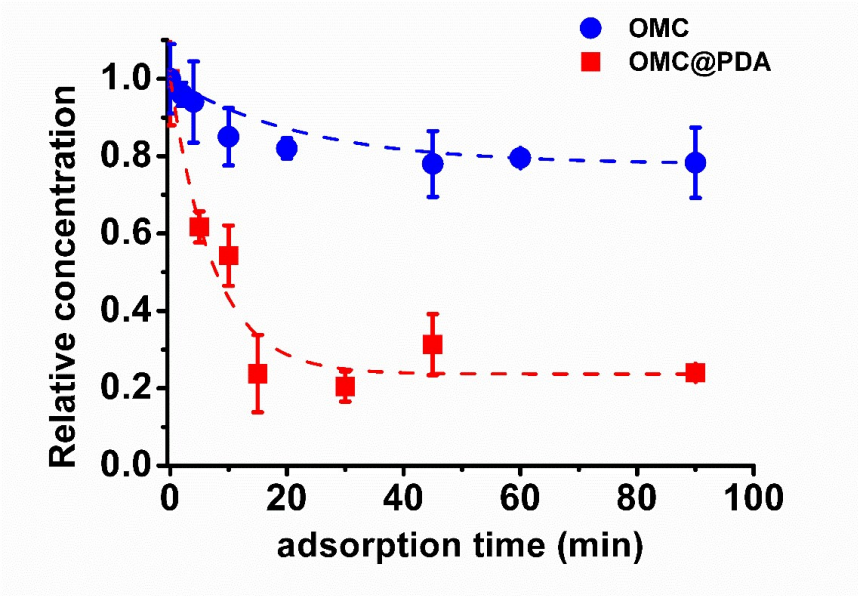

Fig. S5 The concentration of bilirubin in the system since adsorption started, and the data were normalized by the original concentration of bilirubin (n=3).

5. Adsorption isotherms fitting

After getting adsorption isotherm data (Fig. 3c), we fitted the isotherm curves into two typical adsorption models, including the Langmuir model and Freundlich model for further explanation.

The Langmuir adsorption model could be expressed as the following equation:

$$Q_e = \frac{K_L Q_m C_e}{1 + K_L C_e} \quad \text{(Eq. S1)}$$

where Q_e and Q_m (mg g^{-1}) represent the equilibrium adsorption and the theoretic maximum (or saturation) adsorption amount of bilirubin per unit weigh of adsorbent, C_e (mg L^{-1}) refers to the equilibrium concentration of adsorbate, and K_L (L mg^{-1}) is the Langmuir isotherm constant that related to the energy of adsorption. Fig. S6 showed the resulting plots of Langmuir model, from which it could be found that the theoretic maximum adsorption capacity of OMC@PDA to bilirubin reached 513.54 mg g^{-1} with a satisfied fitting coefficient (R^2=0.98), demonstrating the high potential of using this material in bilirubin removal.

The Freundlich adsorption model could be presented as:

$$\ln Q_e = \ln K_F + \frac{1}{n} \ln C_e \quad \text{(Eq. S2)}$$

where K_F (mg g^{-1}) and n are Freundlich constants related to adsorption capacity and intensity. With a satisfying correlation coefficient (R^2 = 0.97), the Freundlich parameter *1/n* was calculated to be smaller than 1, indicating a favorable adsorption[1] took place on the OMC@PDA material (Fig. S7). Detailed fitting parameters were summarized in Table S1.

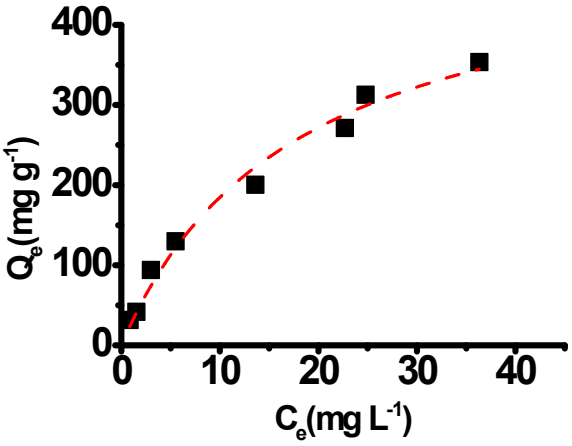

Fig. S6 The Langmuir adsorption isotherm of OMC@PDA to bilirubin.

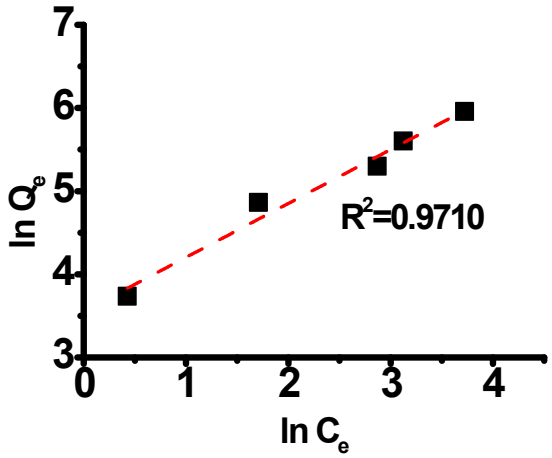

Fig. S7 The Freudlich adsorption isotherm of OMC@PDA to bilirubin.

Table S1 Langmuir and Freudlich constants obtained from model fitting for adsorption of bilirubin on OMC@PDA in PBS solution.

Langmuir model			**Freudlich model**		
Q_{max} (mg g^{-1})	K_L (L mg^{-1})	R^2	K_F (mg g^{-1})	1/n	R^2
513.54	0.0562	0.9831	35.02	0.6484	0.9710

6. Comparison with commercial sorbent materials

Bilirubin adsorption experiments were also conducted on other commercial sorbent materials for comparison. 1 mg material weighted in Eppendorf centrifuge tube and 1 mL mixing solution, in which the concentrations of bilirubin and BSA were 500mg L^{-1} and 40 mg mL^{-1}, was added. Then, the mixture was shook for 60 min in dark to ensure that adsorption on each material reached equilibrium. Although OMC material showed similar adsorption amount to other two commercial carbon material, the decoration of the PDA imprinted layer efficiently improved its affinity to bilirubin, therefore the equilibrium adsorption amount of bilirubin on OMC@PDA was over 2 times higher than those the selected commercial materials (Fig. S8a), and higher than those reported materials at the same situations (Table S2). The adsorption capacity comparison among OMC@PDA, bare OMC and bare PDA particles also indicated that the imprinted binding sites on OMC@PDA played a vital role in bilirubin adsorption (Fig. S8b).

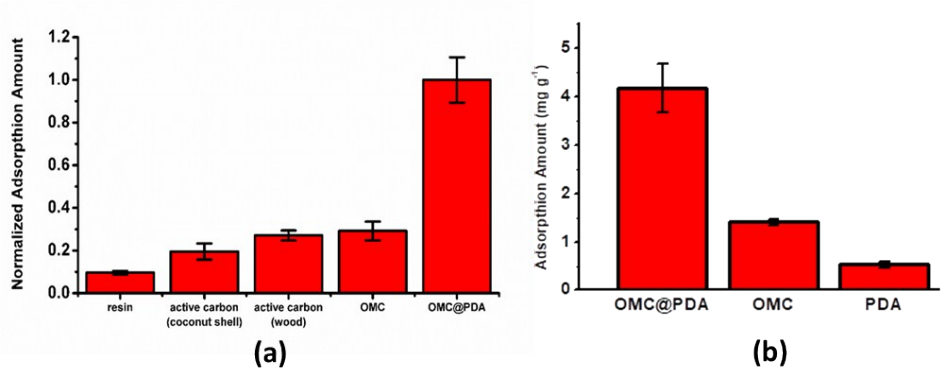

Fig. S8 The equilibrium adsorption amount of bilirubin on different materials in albumin rich condition (n=3).

Table S2 The adsorption capacity comparison between the synthesized OMC@PDA and other reported materials.

Adsorbent material	Maximum Adsorption capacity (mg g^{-1}, 25 ℃)		Reference
	Albumin-free	Albumin-rich	
NH_2-modified SBA-15	44	NR	[2]
PES beads	38.03	NR	[3]
heparin-CS/GH	92.59 (37℃)	8.96 (37℃)	[4]
millimeter-sized MCSs	148.4	NR	[5]
magnetic MCNTs	263.16 (30℃)	NR	[6]
HMCSs	304	31.28	[7]
Lysine modified-Ch/CNTs	107.2 (37℃)	NR	[8]
magnetic NpC	NR	31.0	[9]
3D porous graphene	NR	100.9 126.1 (37℃)	[10]
OMC@PDA	513.54	122.7	this work

*Abbreviations: Ch, chitin; CS, chitosan; GH, graphene oxide hybrid hydrogel; GO, Graphene oxide; MCNTs, multi-wall carbon nanotubes; NpC, nitrogen-doped porous carbon;

7. Competitive adsorption experiment

To investigate the possible explanation for the poor adsorption performance to bilirubin of bare OMC in high-content albumin solution, a competitive adsorption experiment was conducted. Besides BSA (40 mg mL^{-1}) and bilirubin (100 mg L^{-1}), sulfadiazine, a drug sharing the same primary binding site on BSA with bilirubin [11], was also added in the system with the concentration of 150 mg L^{-1}. The bilirubin concentration in the system was determined after 30 min adsorption and compared with that obtained in the competitive drug-free system. After adding sulfadiazine in the system, part of the bilirubin was released from the bilirubin-albumin composite and adsorbed by OMC material, therefore the concentration of bilirubin showed an obvious decrease in the dosing group (Fig. 4c). The result indicated that the declination on adsorption performance of OMC was mainly due to its poor affinity to bilirubin compared to albumin, rather than the binding sites being blocked by protein fouling phenomenon [12].

8. Scatchard analysis

To estimate the binding strength of the OMC@PDA material, the equilibrium adsorption amounts of bilirubin on OMC@PDA in pure bilirubin solutions were plotted according to the Scatchard equation,

$$\frac{Q_e}{C_s} = \frac{Q_{max} - Q_e}{K_d} \quad \text{(Eq. S3)}$$

where Q_{max} and Q_e (μmol/g) refer to the maximum and equilibrium adsorption amounts of bilirubin on OMC@PDA material respectively, C_s (μmol/mL)is the free concentration of bilirubin in the solution after adsorption and K_d is the dissociation constant. By plotting Q_e/C_s versus Q_e (Fig. 4d), the $1/K_d$ value, which is also presented as the association constant (K_a) between the binding sites on OMC@PDA and bilirubin, could be easily obtained according to the slope of the fitting line, and was finally calculated to be 4.52×10^4 M^{-1}. The apparent maximum binding capacity (Q_{max}) was calculated to be 451.82 mg/g, which is similar to the one obtained through Langmuir adsorption model fitting (Table S1). Moreover, the satisfying linear correlation (R^2=0.88) of the Scatchard plots indicated that there was only one kind of binding site formed on the OMC@PDA, demonstration that the successful fabrication of imprinted layer on the OMC material.

9. Equilibrium adsorption isotherms of resin to bilirubin

For comparison, the equilibrium adsorption experiments were also conducted on a commercial cation exchange resin applied in clinical. Detail procedures were the same as those conducted on OMC@PDA material (Section 2.6), except that 2 mg resin was added into the system in each tube. The obtained equilibrium adsorption isotherms could be found in Fig. S9. The adsorption amount significantly dropped when the albumin concentration was raised up to 40 mg mL^{-1}, and the equilibrium amount was less than 17mg g^{-1}, which was much lower than the one of OMC@PDA.

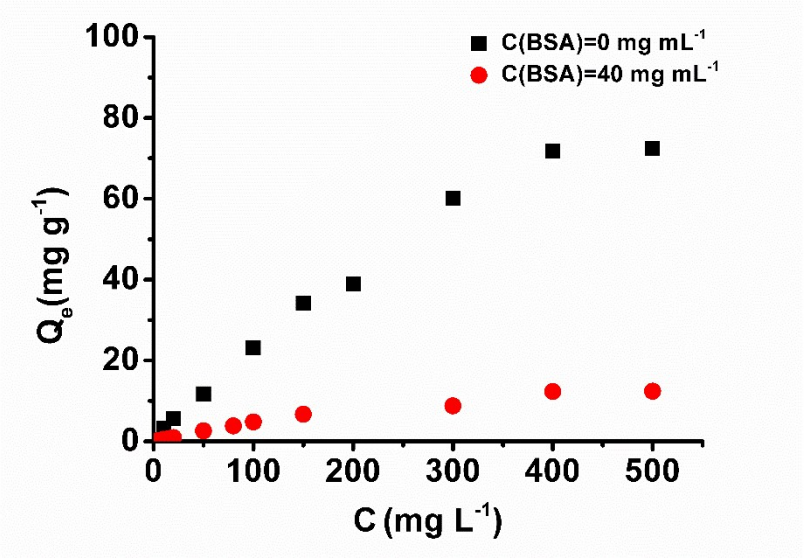

Fig. S9 Equilibrium adsorption isotherms of resin to bilirubin in albumin-free and albumin-rich environment.

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