Supporting Information (SI)

Molecularly-imprinted Hydrogel Beads via Self-sacrificing Micro-reactors as Safe and Selective Bilirubin Adsorbents

Shiqi Yin,† Yinghui Xu,† Zhoujun Wang,† Zhiwei Wei,† Tao Xu,† Weifeng Zhao,†, and Changsheng Zhao †,‡,**

† *College of Polymer Science and Engineering, State Key Laboratory of Polymer Materials Engineering, Sichuan University, Chengdu 610065, China*

‡ *College of Chemical Engineering, Sichuan University, Chengdu, 610065, China*

∗Corresponding authors.

E-mail addresses: zhaoscukth@163.com (Weifeng Zhao);

zhaochsh70@scu.edu.cn or zhaochsh70@163.com (Changsheng Zhao).

Tel.: +86-28-85400453; Fax: +86-28-85405402.

Experimental section

Blood compatibility

Plasma collection

Healthy human fresh blood (from three 24-year-old male donors) was collected by using the vacuum tubes (5 mL, Jiangsu Kangjian Inc., China) with sodium citrate (anticoagulant, anticoagulant-to-blood ratio, 1:9 (v/v)). The platelet-rich plasma (PRP)

and platelet-poor plasma (PPP) were obtained by centrifuging blood at 1000 and 4000 rpm, respectively, for 15 min. The same donor blood samples were used all through the blood tests. The experiments were approved and performed by West China Hospital, Sichuan University, and all the experiments were performed in compliance with the relevant laws and national guidelines (GB/T 16886.4-2003/ISO 10993-4:2002, General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, Standardization Administration of the People's Republic of China). Informed consent was obtained for any experimentation with human subjects, and all regulations (e.g. IRB) were fulfilled for using human blood.

Hemolysis ratio

To evaluate the red blood cells (RBCs) compatibility of the beads, the hemolysis experiment has been carried out. The whole blood (5 mL) was first added to calcium- and magnesium-free PBS solution (10 mL), and then the plasma was centrifuged at 2000 rpm for 10 min to obtain the RBCs (the centrifugation procedure was repeated at least 5 times). To detect the hemolysis ratios, the diluted RBCs suspension (1 mL, approx. 10^8 cells/mL) was added to incubated with the beads (a certain amount of beads was previously immersed in PBS overnight) at 37 °C for 3 h. PBS was selected as a negative control while deionized water was chosen as a positive control. Then, the suspensions were centrifuged at 8000 rpm for 3 min, and the absorbance of the released hemoglobin in the suspensions was measured at 540 nm via a UV-vis spectrometer (UV-1750, Shimadzu Co., Ltd, Japan). The hemolysis ratios of the beads could be calculated by the following formula:

$$Hemolysis\ ratio\ (\%) = \frac{A_s - A_n}{A_p - A_n} \times 100 \quad (1)$$

where A_s is the absorbance of the suspensions, A_p and A_n are the absorbances the positive control and the negative control, respectively.

Platelet adhesion

In order to eliminate the interference of other components in blood, such as

erythrocyte and leucocyte, PRP was used for studying platelet adhesion. The beads were first immersed into PBS and equilibrated at 37 °C for 1 h. After removing PBS resolution (pH=7.4), fresh PRP (1 mL) was added and the beads were incubated with the PRP at 37 °C for 2 h. Then, the PRP was removed and the beads were rinsed three times with PBS solution. The beads and platelet were fixed by 2.5 wt. % glutaraldehyde in PBS solution at 4 °C for 24 h. Subsequently, the beads were washed with PBS solution and subjected to a gradient dehydration process with alcohol-PBS solutions (30, 50, 70, 80, 90, 95 and 100 %). The adhered PLT was eventually observed via an FE-SEM (JSM-7500F, JEOL, Japan).

Contact activation

Commercial enzyme-linked immunosorbent assays (Elisa) were used to investigate the platelet activation (Human Platelet Factor 4 (PF-4), Cusabio Biotech, China), coagulation activation (thrombin–antithrombin III complex (TAT), Enzygnost TAT micro, Assay Pro, USA), and complement activation (Human Complement Fragment 3a (C3a) and Human Complement Fragment 5a (C5a), Cusabio Biotech, China). The beads were immersed in PBS in a 24-well cell culture plate overnight. Then, the PBS was removed and 250 μL of human whole blood was introduced. After 1-h incubation, the whole blood was centrifuged for 10 min at 2500 g centrifugal force (2-8 °C) to obtain plasma. For TAT test, 50 μL of the obtained plasma was added into an Antibody Coated Well (provided by the TAT kit). For PF4 test, 40 μL of the obtained plasma was diluted for 10 times with PF4-Sample Diluent; then 200 μL of the diluted plasma was added into another Antibody Coated Well (provided by the PF4 kit). For C3a test, 5 μL of the obtained plasma was diluted for 500 times with C3a-Sample Diluent, and 100 μL of the diluted plasma was added into an Antibody Coated Well (provided by C3a kit). For C5a test, 10 μL of the obtained plasma was diluted for 10 times with C5a-Sample Diluent, and the diluted plasma was added into another Antibody Coated Well (provided by C5a kit).

The detections were eventually proceeded according to the respective instruction manuals. The whole blood was used as a control sample. Each sample was tested at

least 5 parallel sample groups to get a reliable value, and the result was expressed as mean ± SD (n = 5).

Blood routine test

To study the effect of the beads on the blood environment, the whole blood was utilized. The beads were first immersed in PBS overnight, and incubated with the whole blood at 37 °C for 30 min. Then, the blood was analyzed by an Auto Hematology Analyzer (5-diff, BC-5100, Mindray, China) to obtain the blood routine data. Each sample was tested at least 5 parallel sample groups to get a reliable value, and the result was expressed as mean ± SD (n = 5).

Anticoagulation ability

Activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT), were measured to investigate the anticoagulant properties of the beads by a semiautomatic blood coagulation analyzer CA-50 (Sysmex Corporation, Kobe, Japan). The testing process was as follows: the beads were first immersed in PBS overnight and then incubated at 37 °C for 1 h. After that, the PBS was removed, and 200 μL of fresh PPP was introduced. After incubating at 37 °C for 30 min, 50 μL of the incubated PPP was moved to a test cup and mixed with 50 μL of APTT agent (Dade Actin Activated Cephaloplastin Reagent, Siemens; incubated 10 min at 37 °C before use), followed with adding 50 μL of 0.025 M $CaCl_2$ solution, and then the APTT was measured. For TT test, 50 μL of the incubated PPP was added in a test cup and mixed with 100 μL of thrombin agent (Sysmex; incubated 10 min at 37 °C before use), and then the TT was measured. For PT test, 50 μL of the incubated PPP was added in a test cup, followed by the addition of 100 ml Thromborel S (Siemens; incubated 10 min before use), and incubated at 37 °C for 2 min, and then the PT was measured. At least 5 parallel samples were applied to get a reliable value, and the result was expressed as mean ± SD (n = 5).

Results and discussions

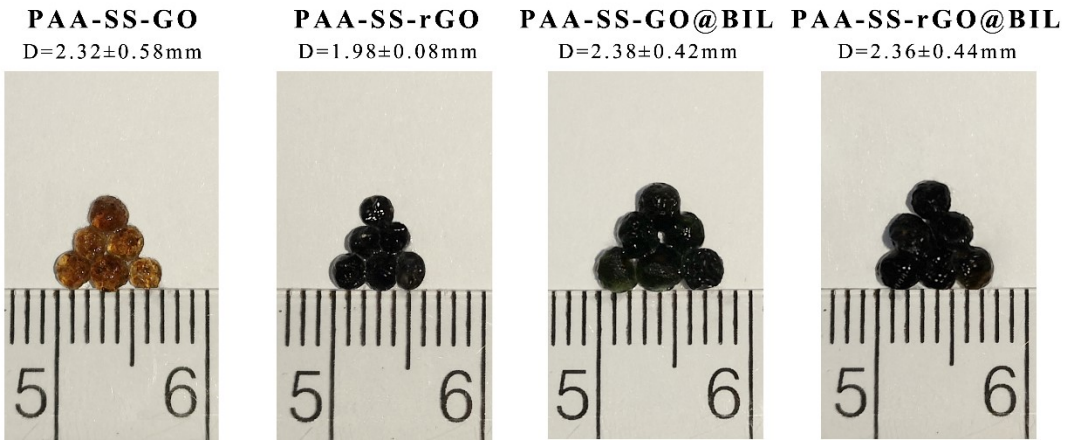

Figure S1. Digital photographs and diameters of the beads. All values are expressed as mean ± SD, n=6.

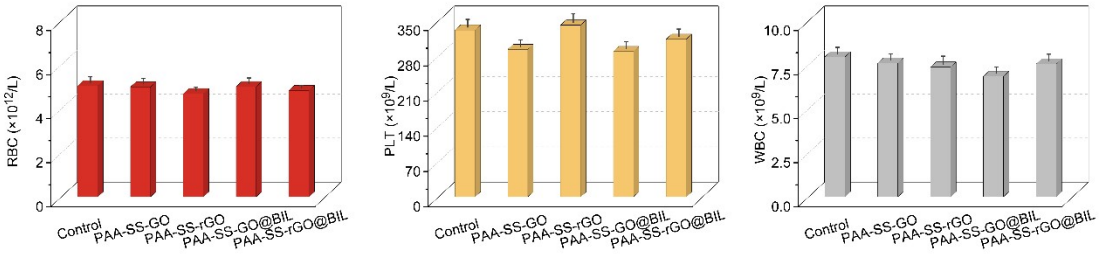

Figure S2. The concentrations of RBC, PLT, and WBC in whole blood after incubating with PAA-SS-GO, PAA-SS-rGO, PAA-SS-GO@BIL, and PAA-SS-rGO@BIL beads. All values are expressed as mean ± SD, n=5.