# Supporting Information

# Surface engineered 3D nanogels assembly with integrated blood compatibility, cell proliferation and antibacterial property: towards multifunctional biomedical membrane

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## 1. Water contact angle

The membrane hydrophilicity was characterized via the data of static contact angle using a contact angle goniometer (OCA20, Dataphysics, Germany) equipped with a video capture. A piece of 2×2 $cm^2$ membrane was attached on a glass slide and mounted on the goniometer. For the static contact angle

measurements, a total of 3 μL DI water was dropped on the membrane surface at room temperature, and the contact angle was measured after 10 s. At least 8 measurements were averaged to get a reliable value. The measurement error was ± 3°.

**2. Protein adsorption**

Protein adsorption experiments were carried out with BSA solutions and BFG solution under the static condition, respectively. Firstly, the membranes, 1×1 $cm^2$, was immersed in PBS (containing BSA or BFG with the concentration of 1 mg/mL) and incubated at 37 °C for 1 hour; then the membranes were rinsed slightly with PBS and DI water. Then the membranes were transferred into a washing solution (containing 2 % sodium dodecyl sulfate (SDS) and 0.05 M NaOH) at 37 °C; and then shaken for 2 hours to remove the adsorbed BSA or BFG protein. The above adsorption and desorption times were pre-determined based on preliminary experiments. The protein concentration in the washing solution was used to calculate the adsorbed protein amounts and determined by using the Micro BCA™ Protein Assay Reagent Kit (PIERCE). More than 95 % of the adsorbed protein could be eluted into the SDS solution.

**3. Anticoagulant property of the membranes**

To evaluate the antithrombotic activity of the heparin-mimicking membranes, activated partial thromboplastin time (APTT) and thrombin time (TT) were measured to evaluate the blood clotting time and thrombotic potential.[2]

The APTT and TT were tested by automated blood coagulation analyzer CA-50 (Sysmex Corporation, Kobe, Japan), and the method for each measurement was described as follows: synchronously, the membranes (0.5 cm × 0.5 cm, three pieces) were immersed in PBS (200 μL, pH = 7.4) for 1 hour. Then the PBS was removed and 100 μL of fresh collected platelet-poor plasma (PPP) was introduced. After incubating at 37 ºC for 30 minutes, 50 μL of the incubated PPP was added into the test cup, followed by adding 50 μL APTT agent (pre-incubated for 10 minutes before use) and then incubated at 37 ºC for another 3 minutes. Thereafter, 50 μL of 0.025 M $CaCl_2$ solution was added, and then the APTT was measured. At least 3 tests were conducted and the results were analyzed by a statistical method.

The TT test was carried out in a similar process as the APTT test, after membranes were firstly incubated with PPP, the pre-incubated 100 μL of TT agent was added into the test cup (containing 50 μL of the incubated PPP), and then the TT was measured.

**4. Platelet adhesion**

In order to eliminate the interference of other components of blood, such as erythrocyte and leucocyte, the platelet-rich-plasma (PRP) was used for the study of platelet adhesion on the nanogels and heparin-like polymers assembled membranes using healthy fresh human blood.[1]

The pristine PES and surface assembled PES membranes were immersed in PBS and equilibrated at 37 °C for 1 h. Firstly, the PBS was removed and then 1

ml of fresh PRP was introduced, the membranes were incubated with PRP at 37 °C for 2 h. Then the PRP was decanted off and the membranes were rinsed for 3 times with PBS. Finally, the membranes were treated with 2.5 wt. % glutaraldehyde in PBS at 4 °C for 1 day. Drying process: the samples were first washed with PBS, then passed through a series of graded alcohol-PBS solutions (30, 50, 70, 80, 90, 95 and 100%, 10 minutes for each ) and isoamyl acetate-alcohol solutions (25%, 50%, 75%, and 100%, 15 minutes for each),[1] Followed with the liquid $CO_2$ critical point drying of the samples. For SEM observation: the specimens were sputter-coated with a gold layer and examined by using a FE-SEM (JSM-7500F, JEOL, Japan). The adhered platelets numbers of the membranes was calculated from 6 SEM pictures at 500× magnification from different places on each membrane, respectively.

**5. Cell culture**

Human umbilical vein endothelial cells (HUVECs) were grown in R1640 medium, which was supplemented with 10% fetal bovine serum (FBS, Hyclone, USA) and 2 mM L-glutamine and 1% (*V/V*) antibiotics mixture (10000 U penicillin and 10 mg streptomycin). Cell cultures were maintained in humidified atmosphere of 5% $CO_2$ at 37 °C. Confluent cells were detached from the cell culture flask with sterilized PBS and 0.05% trypsin/EDTA solutions, and the cell culture medium was changed every day.

The sterilized PES and surface assembled PES membranes with an area of 1×1 $cm^2$ were pre-wetted in 24-well cell-culture polystyrene plates by cell culture medium for 3 h in a 37 °C incubator.

**6. Cell morphology on the membranes**

For the SEM observation, the HUVECs were seeded onto the pristine and modified membranes at a density of approx. $2.5 \times 10^4$ cells/$cm^2$. After 5 d, the seeded membranes were rinsed with PBS immediately and then fixed by 2.5 % (wt. %) glutaraldehyde in PBS solution at 4 °C for 12 h. For cell morphology observation, the fixed membranes were subjected to a drying process by passing them through graded alcohol-PBS solutions (30, 50, 70, 80, 90, 95 and 100 %, 10 minutes for each ) and then dehydrated through isoamyl acetate-alcohol solutions (25, 50, 75, and 100 %, 15 minutes for each), and followed by using the liquid $CO_2$ critical point drying . The samples were then sputter-coated with a gold layer and examined by a FE-SEM (JSM-7500F, JEOL, Japan).

1. C. Cheng, S. Nie, S. Li, H. Peng, H. Yang, L. Ma, S. Sun and C. Zhao, *J. Mater. Chem. B*, 2013, **1**, 265-275.

2. P.-C. Chen, L.-S. Wan and Z.-K. Xu, *J. Mater. Chem.*, 2012, **22**, 22727-22733.