Self-assembled 3D biocompatible and bioactive layer at macro-interface via graphene-based supermolecules

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Supporting Information

Water contact angle

The hydrophilicity of the membrane surface was characterized on the basis of static contact angle measurement using a contact angle goniometer (OCA20, Dataphysics, Germany) equipped with a video capture. A piece of 1×1 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 double distilled water was dropped on the airside surface of the membrane at room temperature, and the contact angle was measured after 10 s. At least eight measurements were averaged to get a reliable value. The measurement error was ±3°.

Protein adsorption

Protein adsorption experiments were carried out with BSA and BFG solutions under static condition. Firstly, the membrane with an area of 1 × 1 cm^2 was immersed in a phosphate buffer solution (PBS), containing BSA or BFG with the concentration of 1 mg/mL, and incubated at 37 °C for 1 hour; then the membrane was rinsed slightly with PBS solution and double distilled water. And then the membrane was placed in a washing solution (2 % sodium dodecyl sulfate (SDS), 0.05 M NaOH) at 37 °C, and shaken for 2 hours to remove the adsorbed protein. The adsorption and desorption times were carefully determined in preliminary experiments. The protein concentration in the washing solution was determined by using the Micro BCA™ Protein Assay Reagent Kit (PIERCE), and then the adsorbed protein amount was calculated.

Hemolysis test

The hemolytic potentials of the pristine PES substrate and the surface layer assembled PES substrates were measured by a universal method similar to an earlier report.[1] Human whole blood samples were prepared according to our earlier paper:[2] 10 mL of whole blood was added to 20 mL of calcium- and magnesium-free phosphate buffered saline (PBS, pH 7.4) and then centrifuged at 500 g for 10 min to

isolate red blood cells (RBCs) from human plasma for 5 times, and the RBCs were diluted into PBS solution with a final volume of 100 mL. Then, the membranes were added to 0.2 mL of the diluted RBC suspension (around 5×10^8 cells mL^{-1}), respectively. D.I. water dispersed RBCs was used as the positive control and the PBS (pH 7.4) dispersed RBCs was used as the negative control. All the suspensions were incubated in a rocking shaker at 37 °C for 3 h, and then centrifuged at 10016 g for 3 min. The absorbance of the released hemoglobin in the suspensions was measured at 540 nm using a spectrophotometry. The hemolysis ratio was calculated using the following formula.

$$Hemolysis\ ratio(\%) = \left\{ \frac{Suspensions_{abs} - Negative\ control_{abs}}{Positive\ control_{abs} - Negative\ control_{abs}} \right\} \times 100$$

Cell culture and sample preparation

Human umbilical vein endothelial cells (HUVECs) and human hepatocytes LO2 chosen as the model cells for the evaluation of cell proliferation and viability, respectively. Cells were grown in R1640 medium supplemented with 10% fetal bovine serum (FBS, Hyclone, USA), 2 mM L-glutamine and 1% (*V/V*) antibiotics mixture (10000 U penicillin and 10 mg streptomycin). Cultures were maintained in humidified atmosphere of 5% CO_2 at 37 °C (Queue Incubator, Paris, France). Confluent cells were detached from the culture flask with steriled PBS and 0.05% trypsin/EDTA solutions, and the culture medium was changed every day.

The pristine PES membrane and surface 3D graphene layer assembled membranes were cut into 1×1 cm^2 to suit the sizes for 24-well cell-culture polystyrene plates, and

pre-wetted by immersion in the culture medium for 3 h in a 37 °C incubator. And then the membranes were placed into the cell-culture plates, rinsed with PBS and sterilized by γ -ray.

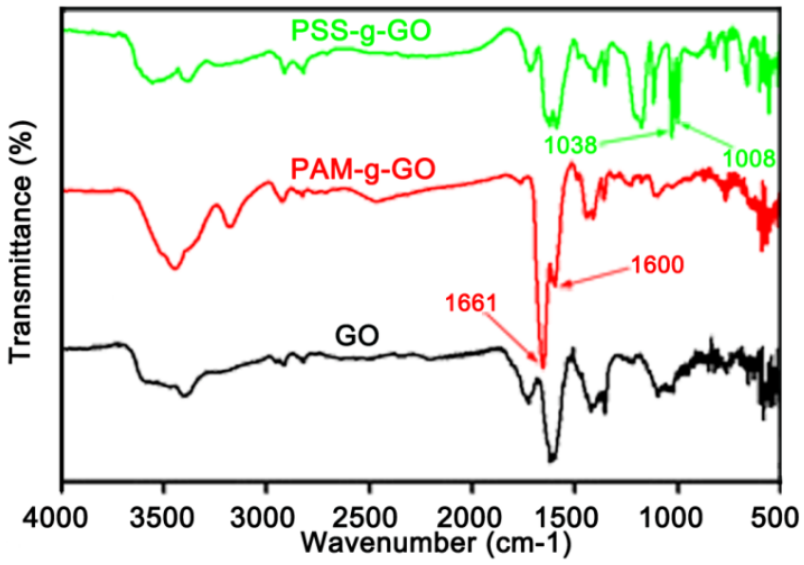

Fig. S1 FTIR spectra for GO, PSS-g-GO and PAM-g-GO nanosheets.

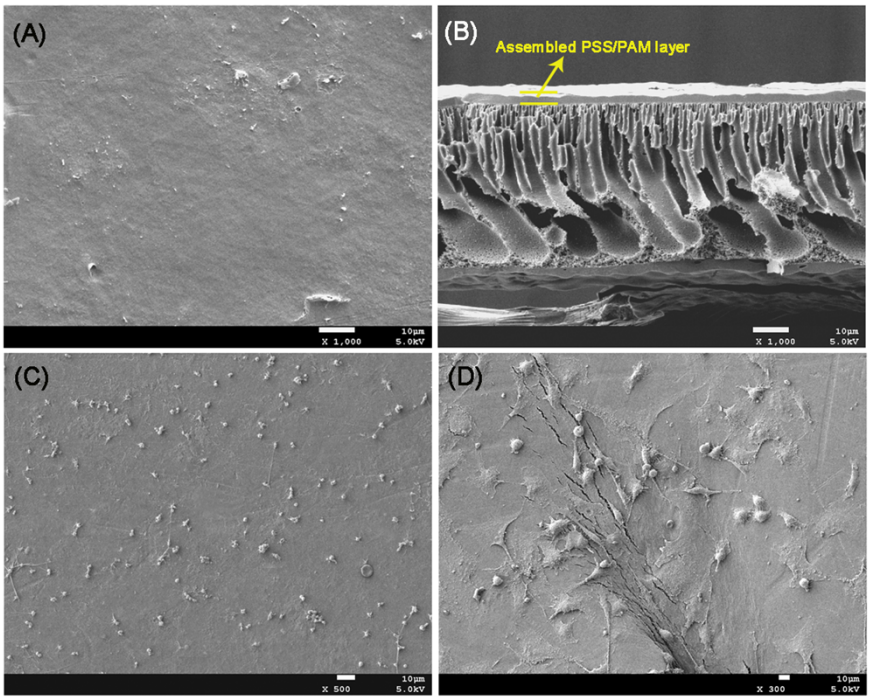

Fig. S2. SEM images for the surface morphology and the biological performances of the assembled PSS/PAM bilayers on the PES substrates. (A) General surface morphology and (B) cross-section view of the surface PSS/PAM layer coated membrane. (C) SEM image for the platelet adhesion. (D) Morphology of the HUVECs for PES/$(PSS/PAM)_5$. All the scale bars are 10 μm. Note: the PSS is purchased from Sigma-Aldrich, and the molecular weight is about 100,000; the PAM, with a low molecule weight of about 64,000, is synthesized via reversible addition−fragmentation chain-transfer polymeraization (RAFT) method as indicated in our earlier paper [Macromol. Biosci., 2012, 12, 116–125.]. The assembly condition is the same as the PSS-GO/PAM-GO.

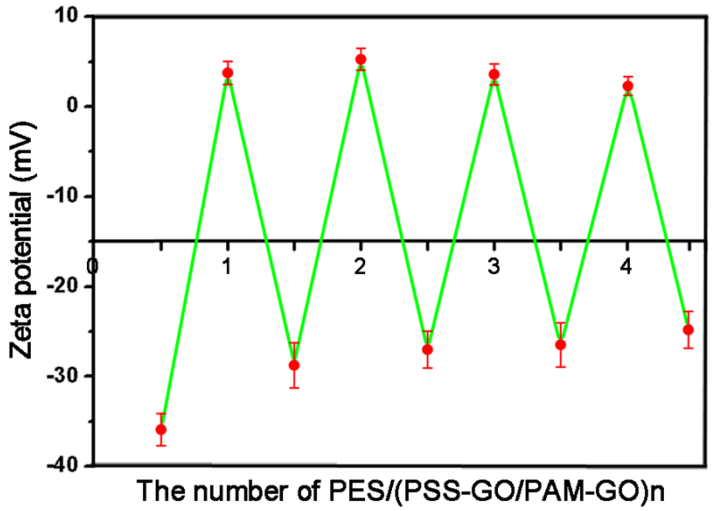

Fig. S3 Zeta potential data for the surface assembled $(PSS\text{-}g\text{-}GO/PAM\text{-}g\text{-}GO)_n$ films on PES substrates. 0.5, 1.5, 2.5, 3.5 and 4.5 are membranes with PSS-g-GO as the outside assembled layer.

Reference:

1. Y.-S. Lin and C. L. Haynes, *J. Am. Chem. Soc.* , 2010, **132**, 4834-4842.
2. A. He, B. Lei, C. Cheng, S. Li, L. Ma, S. Sun and C. Zhao, *RSC Adv.*, 2013, **3**, 22120-22129.