

# Hemocompatible poly(lactic acid) membranes prepared by immobilizing carboxylated graphene oxide via mussel-inspired method for hemodialysis

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## Supporting Information

### Materials

Poly(lactic acid) (PLA, Revode 190, Mn 61.1 k) was supplied by Zhejiang Hisun Biomaterials Co., Ltd., China. 1,4-dioxane, 1-methyl-2-pyrrolidinone (NMP), concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>, 95%), concentrated hydrochloric acid (HCl, 38%), concentrated nitric acid (HNO<sub>3</sub>, 65%), sodium nitrate (NaNO<sub>3</sub>) and potassium permanganate (KMnO<sub>4</sub>) were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd., China. Graphite (powder, 99.95% metals basis, 1200 mesh), tris(hydroxymethyl)aminomethane hydrochloride (Tris), dopamine (DA), bovine serum albumin (BSA, Mw 67,000), lysozyme (Mw 14,000), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), N-hydroxysuccinimide (NHS) and p-dimethylaminobenzaldehyde (PDAB) were used directly and purchased from Aladdin, China. The anticoagulant sheep whole blood was supplied by Beijing Pingrui Biotechnology Company, China. The deionized water (DI water) was home-made.

### Preparation of the original PLA membranes

The PLA membranes were prepared via the classical nonsolvent induced phase separation (NIPS) process. In brief, PLA (15 g) were dissolved in NMP (68 g) and 1,4-dioxane (17 g). The mixture was stirred at 80~90 °C until forming homogeneous solution. The casting solution was kept in vacuum oven at 45 °C to release bubbles, and then cast onto a clean glass plate using Elcometer 4340 automatic coating machine (England) with a 250 μm casting knife. Immediately they were immersed into a water bath at 10 °C. The phase separation occurred to form the porous membrane accompanying with the exchange of solvent (NMP and 1,4-dioxane) and non-solvent (water). After 10 min, PLA membrane was taken out from the water bath and thoroughly washed with deionized water to remove any residual solvent.

### Preparation of graphene oxide (GO), reduced graphene oxide (rGO) and carboxylic graphene oxide (GO-COOH)

The graphene oxide was prepared by modified Hummers' method.<sup>1</sup> Briefly, graphite powder (5 g) was placed in concentrated H<sub>2</sub>SO<sub>4</sub> (115 mL) at 0 °C. KMnO<sub>4</sub> (15 g) was added gradually by stirring while also keeping the temperature of the mixture below 20 °C. The mixture was then stirred at 35 °C for 2 h, followed by the addition of DI water (230 mL), and stirring was continued for 15 min. DI water (700 mL) was then added to terminate the reaction. Subsequently, 30% H<sub>2</sub>O<sub>2</sub> (10 mL) was added and the colour of the mixture changed to bright yellow. The mixture was washed with 10% HCl solution to remove residual metal ions. Then,

the mixture was washed with DI water and centrifuged until the solution became neutral. To exfoliate the oxidized graphite, the product was treated with an ultrasonic wave at 400 W for 15 min per hour and repeated 4 times. The obtained exfoliated GO was freeze dried by vacuum freeze dryer and stored in vacuum box.

The reduced graphene oxide was prepared by microwave treatment.<sup>2</sup> In short, 20 mL GO/water solution (containing 75 mg GO) was added into a flask. Then 100 mL DMAc was added and treated with an ultrasonic wave for few minutes. The prepared suspension in DMAc/H<sub>2</sub>O was put into a microwave chemistry reactor (MCR-3, China) under dry nitrogen gas and treated at 800 W (130 °C) for 20、40 and 60 minutes, respectively. Then the suspension was filtered washed by ethanol for three times. Next the filter cake was added into 20 mL ultra-pure water under the mild ultrasound.

The carboxylic GO was prepared by slightly modified mixture acid.<sup>3</sup> Briefly, 2 g GO were treated with mixture acid with the volume ratio of 3:1 of concentrated sulfuric and nitric acid by sonicating at 60 °C for 12 h. Then, the mixture was washed with DI water and centrifuged repeatedly until the suspension became neutral.

#### **rGO coating on the PLA membrane surface**

20 mL suspension of RGO with different reduced time (containing 60 µg RGO) was filtered on PLA membrane surface. After freeze drying by vacuum freeze dryer for 12h, rGO coated PLA membranes were obtained and named by RG 20, RG 40 and RG 60.

#### **The -COOH group densities of rGO, DA-g-GO and DA-g-GOCOOH**

The -COOH densities of rGO, DA-g-GO and DA-g-GOCOOH were determined by acid-base titration.<sup>4-6</sup> Briefly, 15 mg DA-g-GOCOOH was added to NaCl solution (2 mL, 0.1 M). After the solution was stirred for 24 h, the solid was separated by filtration. Then the filtrate was titrated to an end point at pH 7.0 with 0.01 M NaOH. The total acid densities were estimated by calculation the amount of NaOH consumed.

**Table S1** The -COOH group densities of rGO, DA-g-GO and DA-g-GOCOOH. Data were mean ± SD (n=3)

Samples	n <sub>-COOH</sub> (mM/g)
DA-g-GOCOOH	5.470
DA-g-GO	2.430
rGO 20	0.453
rGO 40	0.439
rGO 60	0.310

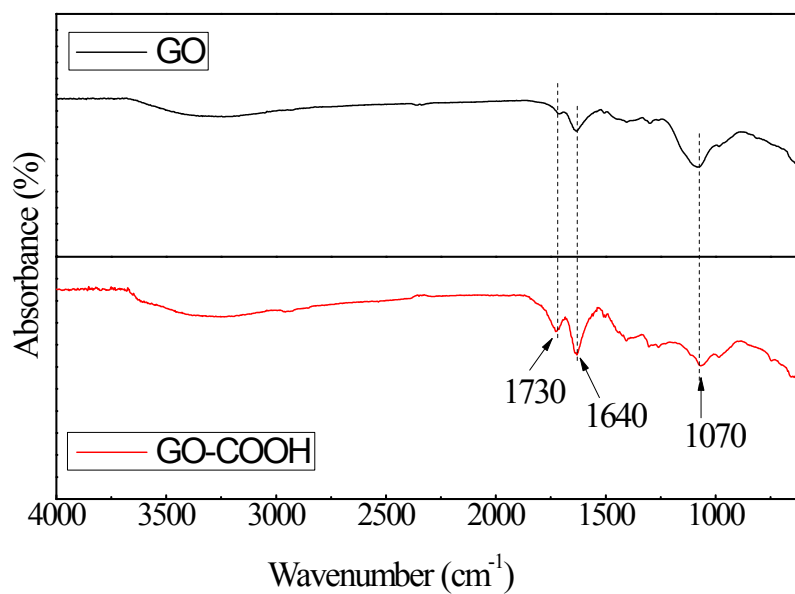


Fig. S1. FTIR spectra for graphene oxide (GO) and carboxylic graphene oxide (GO-COOH).

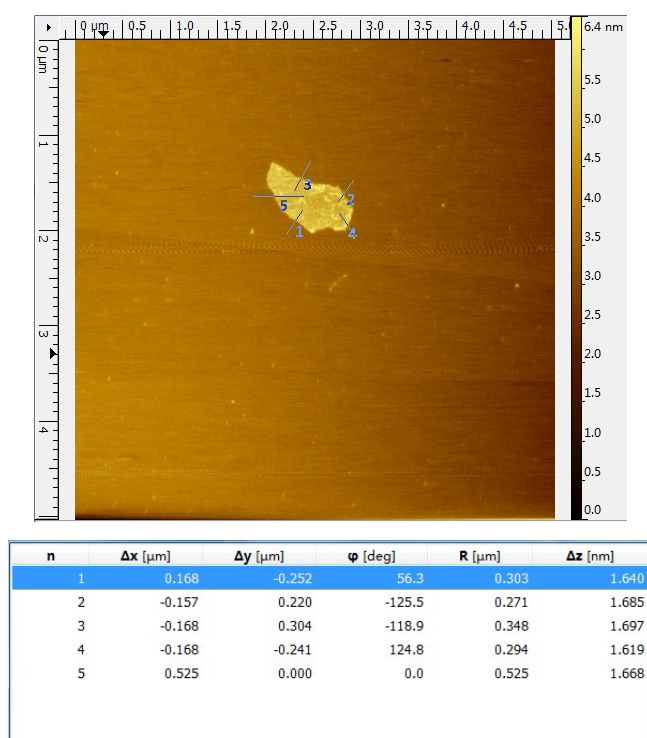
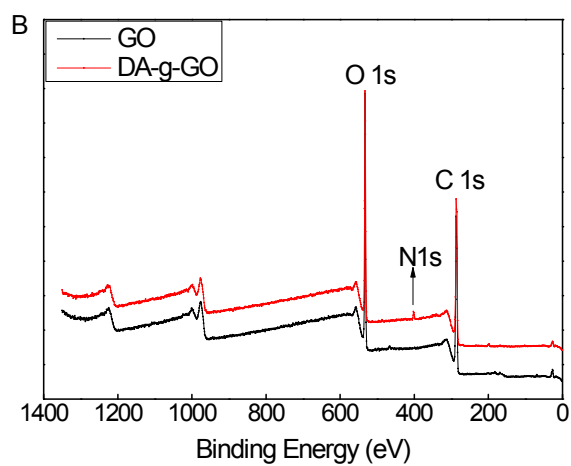
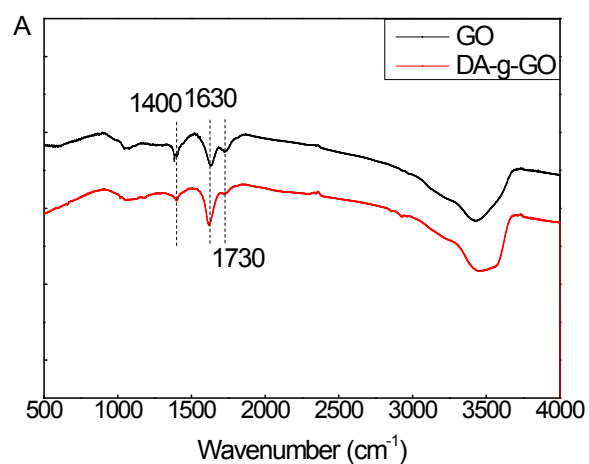


Fig. S2. The AFM image for GO-COOH nanosheet (up) and the thickness of GO-COOH (down).



**Table S2** The elemental molar percentages determined by XPS.

Samples	Percentage of components (mol%)		
	C	O	N
GO	67.99	32.02	-
DA-g-GO	68.45	28.94	2.02

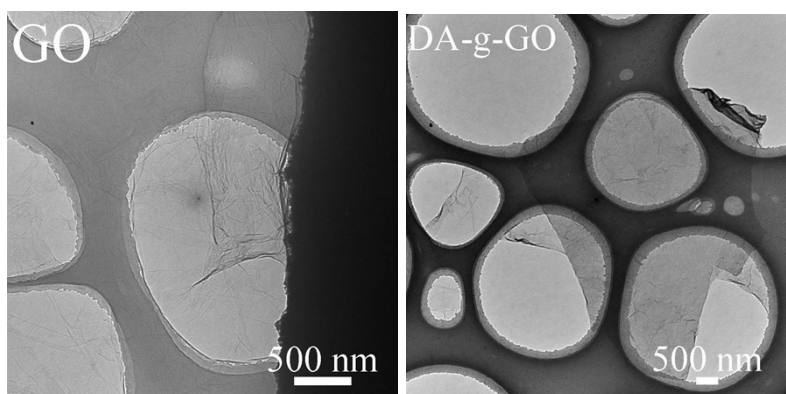


Fig. S3. FTIR spectra (A), XPS wide scans (B) and TEM images of the GO and DA-g-GO

nanosheets. The peaks at 1730 , 1630 and 1380  $\text{cm}^{-1}$  appeared in the FTIR spectra of GOCOOH and DA-*g*-GO were attributed to the stretching vibration of C=O in carboxy group, asymmetric deformation of  $\text{COO}^-$  in carboxy group, and C-OH blending vibration in carboxy group respectively. Compared with GO, the peaks at 1730 and 1400  $\text{cm}^{-1}$  became weaker. The new peak of N 1s, which was certainly attributed to the C-N group, could be observed at the XPS spectra and the molar percentage of N 1s increased from 0 to 2.02%. The wrinkle-like thin sheets, which were the characteristic structure of GO nanosheet, were observed in both GOCOOH and DA-*g*-GO. The TEM image of the as-prepared DA-*g*-GO exhibited a 2D surface morphology.

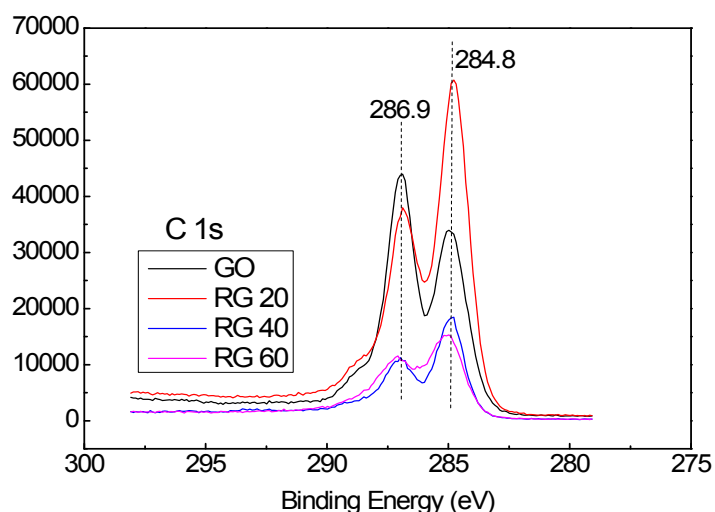
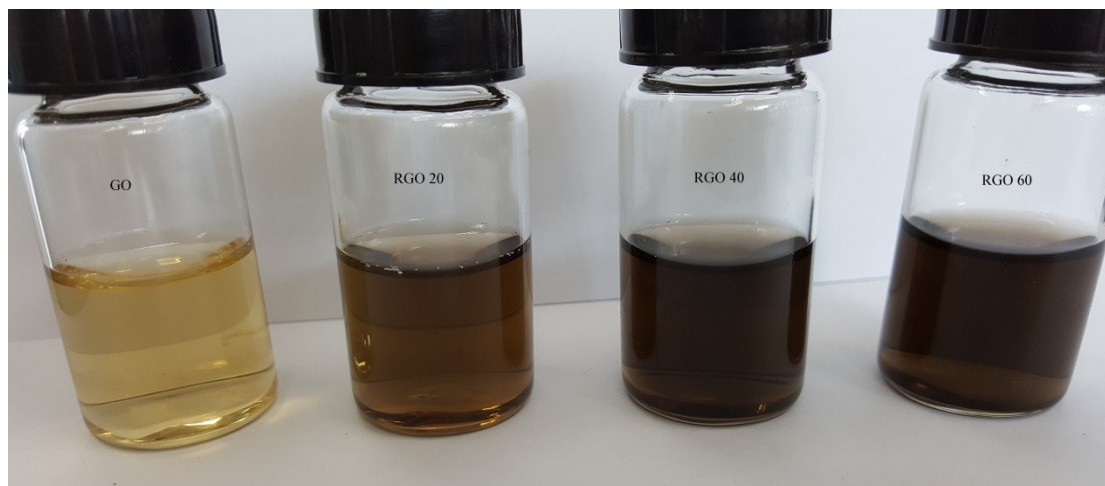


Fig. S4. Photographs of GO suspension with concentration of 0.1 mg/mL in DMAc/H<sub>2</sub>O (5:1) after microwave treatment for 0, 20, 40, 60 min respectively, and XPS wide scans of C 1s. The color of GO suspension became deeper after microwave treatment. Two bands at 286.9 and 284.8 eV are observed which could be attributed to the graphitic C-O species and C=C species.<sup>2</sup> For GO, the peak at 286.9 was higher than the peak at 284.8, which indicated that C-O species were more than C=C species in GO. However, the peaks at 286.9 were lower than the peak at 284.8 after microwave treatment. It indicated that GO were reduced during the microwave treatment.

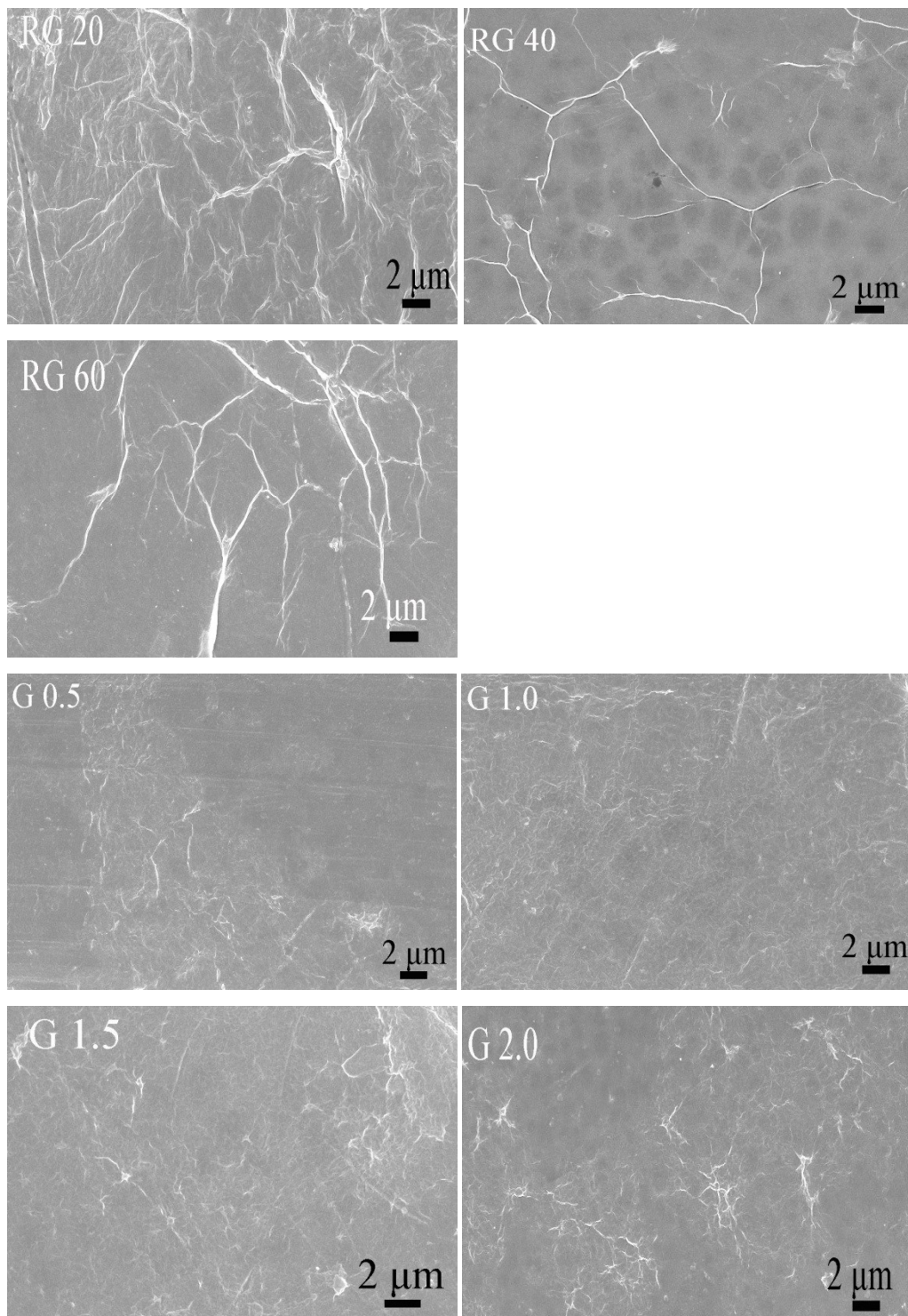
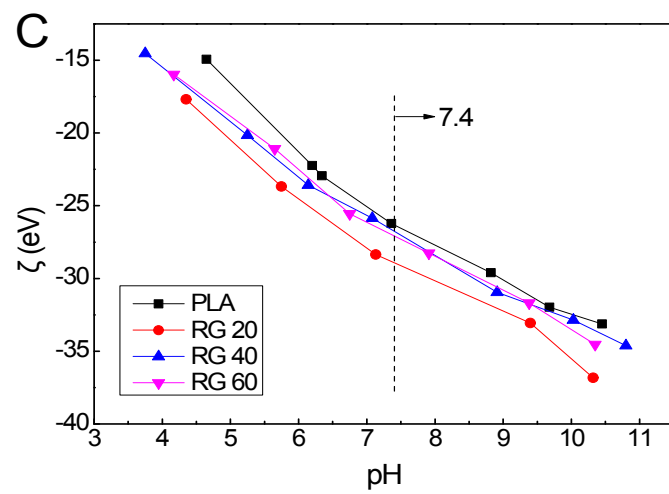
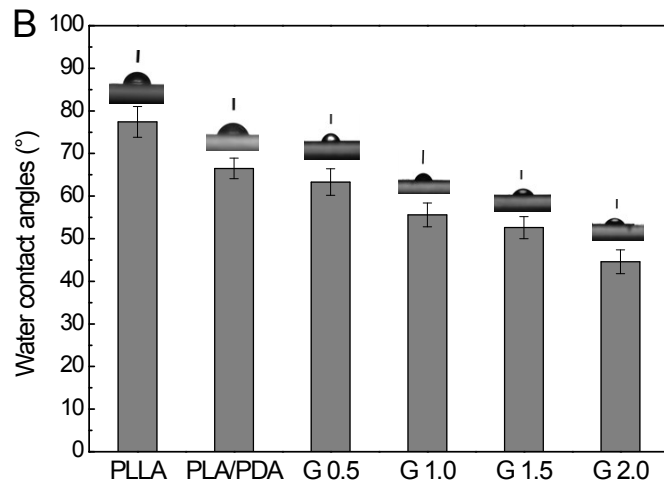
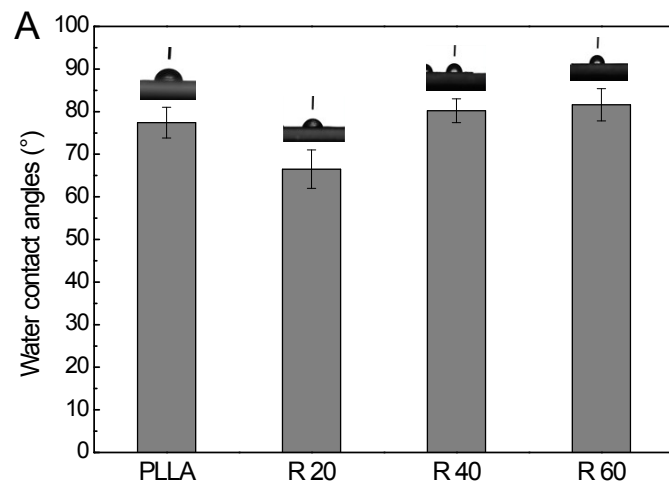


Fig. S5. The SEM image for rGO coated PLA membranes (RG 20, RG 40, RG 60 mean rGO with different microwave treatment time 20, 40, 60 min ) and DA-g-GO coated PLA membranes (G 0.5, G 1.0, G 1.5, G 2.0 mean different immersing concentration of DA-g-GO).



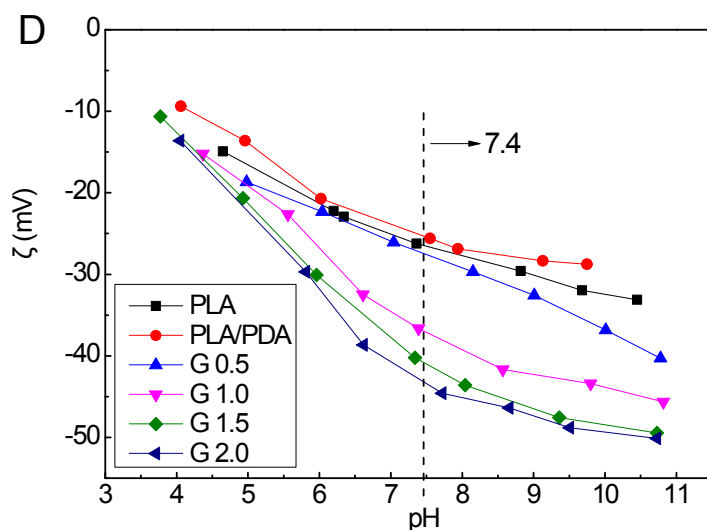
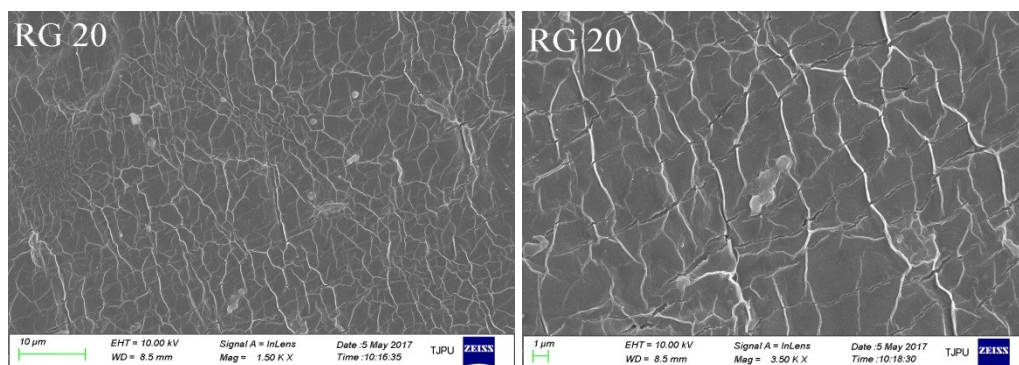
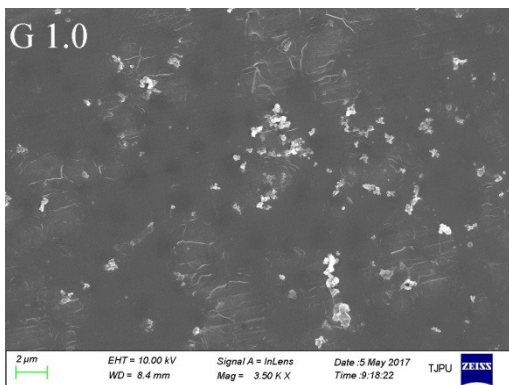
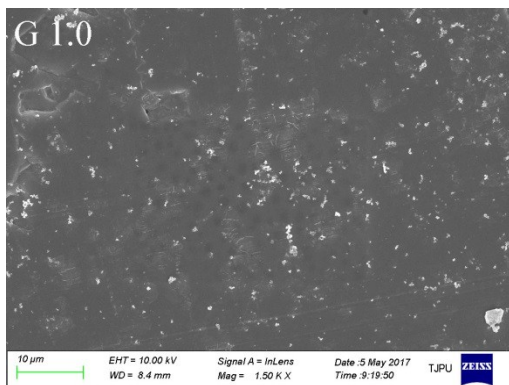
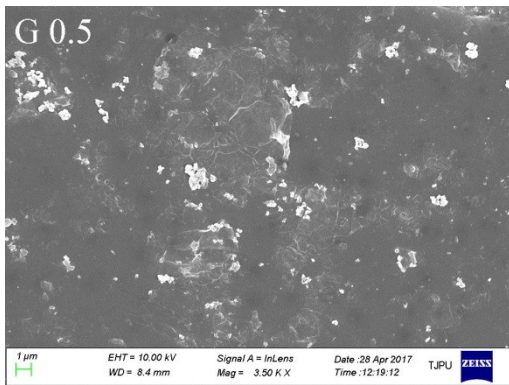
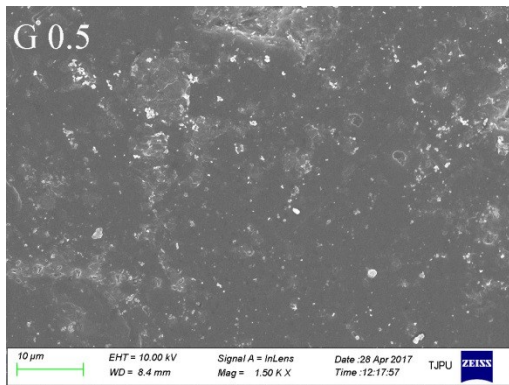
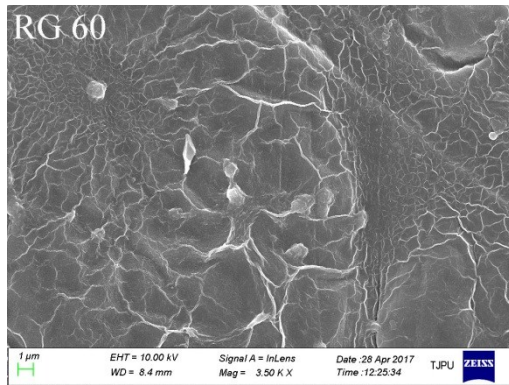
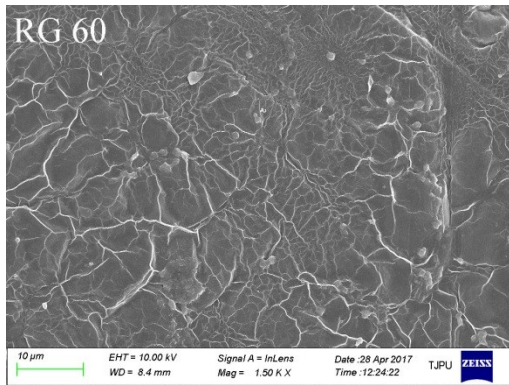
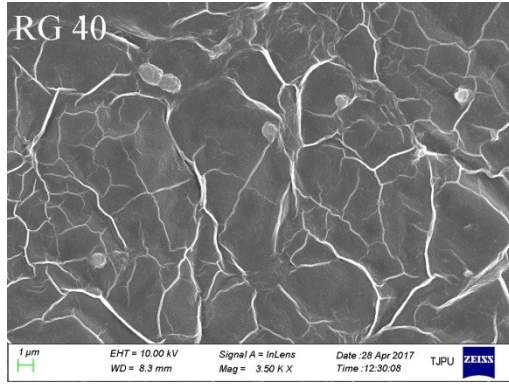
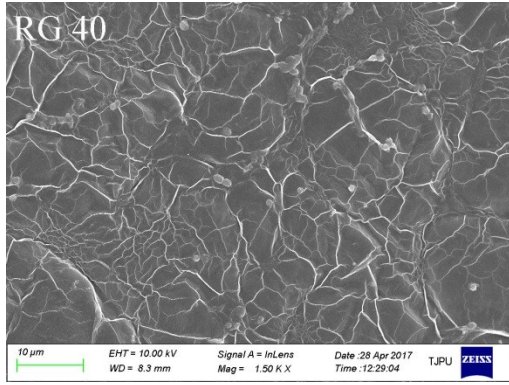


Fig. S6. Water contact angle (A: PLA, RG 20, RG 40, RG 60; B: PLA, PLA/PDA, G 0.5, G 1.0, G 1.5, G 2.0) and zeta potential (C: PLA, RG 20, RG 40, RG 60; D: PLA, PLA/PDA, G 0.5, G 1.0, G 1.5, G 2.0) of membranes. Compared with the pure PLA membrane ( $77.4^\circ$ ), the water contact angle of RG 20 membrane decreased by  $10.9^\circ$ . However, the water contact angle of RG 40 and RG 60 membranes increased to  $80.2$  and  $81.6$ , respectively. And the zeta potentials of PLA, RG 20, RG 40 and RG 60 were  $-26.3$ ,  $-29.0$ ,  $-26.7$  and  $-27.0$  mV, respectively, at  $\text{pH}=7.4$ . This phenomenon might be related to the reduction degree of GO by microwave treatment. There were relatively more oxygen-containing functional groups after 20 min microwave treatment (RG 20) than those after 40 and 60 min microwave treatment (RG 40 and RG 60). The water contact angles of DA-g-GO coated membranes (Fig. S6. B) decreased from  $63.3^\circ$  to  $44.6^\circ$  with the increment of DA-g-GO immersing concentration (from  $0.5$  mg/mL to  $2.0$  mg/mL). Compared with the original PLA membrane ( $-26.3$  mV), the zeta potentials ( $\text{pH } 7.4$ ) of DA-g-GO coated membranes decreased from  $-27.1$  mV (G .05) to  $-42.8$  mV (G 2.0). The enhancement of hydrophilicity and electronegativity of membranes modified by DA-g-GO were attributed to the  $-\text{COOH}$  and  $-\text{OH}$  groups of DA-g-GO.







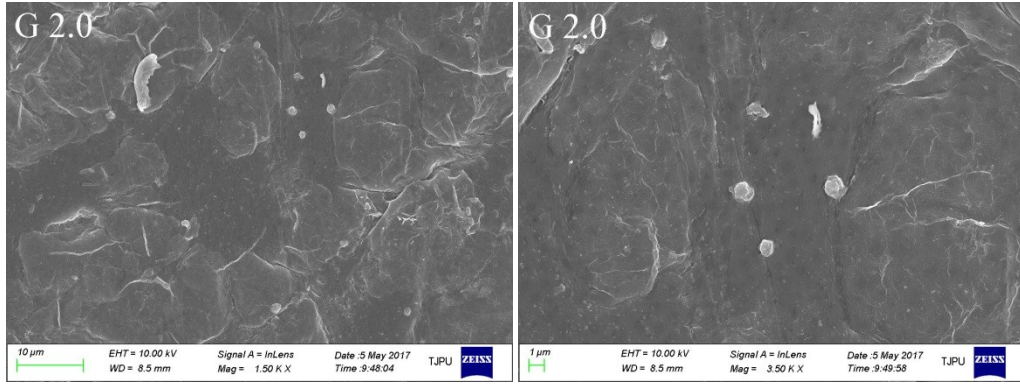
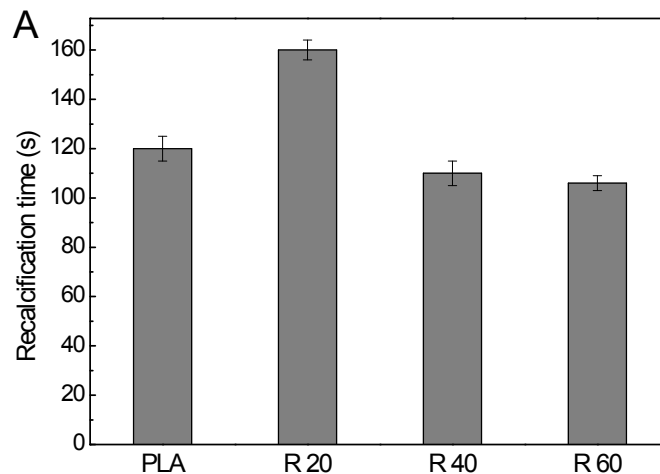


Fig. S7. The representative SEM images of platelets adhered on membrane surfaces: (RG 20, RG 40, RG 60) PLA/rGO membranes modified by 20, 40, 60 min microwave treatment GO; (G 0.5, G 1.0, G 2.0) PLA/(DA-g-GO) membranes with DA-g-GOCOOH immersing concentration of 0.5, 1.0, and 2.0 mg/mL, respectively. It was found that more platelets adhered on the RG 40 and RG 60 membranes surface than RG 20 membrane. Because the RG 20 membrane was more hydrophilic (Fig. S6. A) and electronegative (Fig. S6. C) than the RG 40 and RG 60 membranes, more platelets adhered on the RG 40 and RG 60 membranes surface than RG 20 membrane. It was found that aggregation of the platelets on the RG 20 membrane occurred. Besides, the platelets spread in irregular shapes on the surface of RG 20 membrane. For the DA-g-GO modified PLA membranes, remarkable aggregation and badly irregular spread of the platelets on the G 0.5 and G 1.0 membranes occurred. When the immersing concentration of DA-g-GO was 2.0 mg/mL, less platelets adhered on the G 2.0 membrane surface and the platelets spread relatively regularly. It indicated that rGO and DA-g-GO modified PLA membranes were less hemocompatible than DA-g-GOCOOH modified PLA membranes. This might be related to the higher oxidation of GOCOOH than rGO and GO.<sup>7</sup>



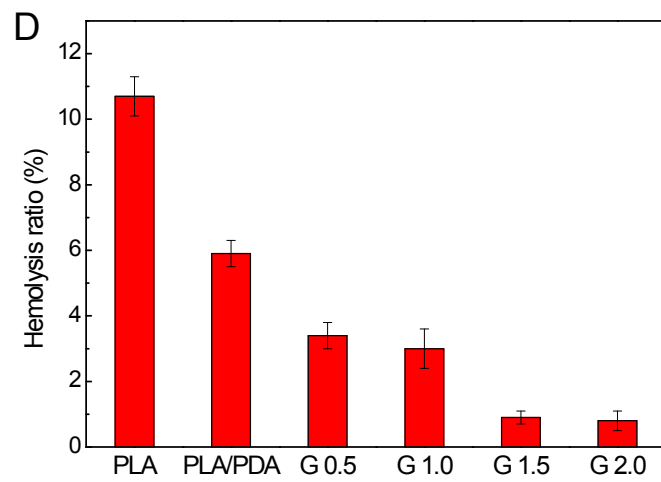
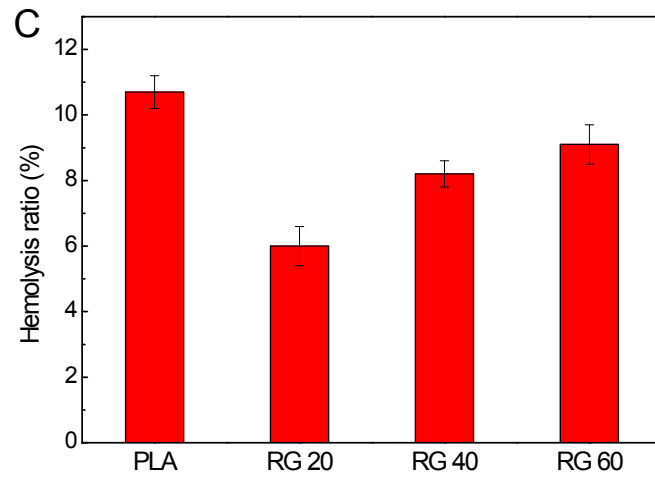
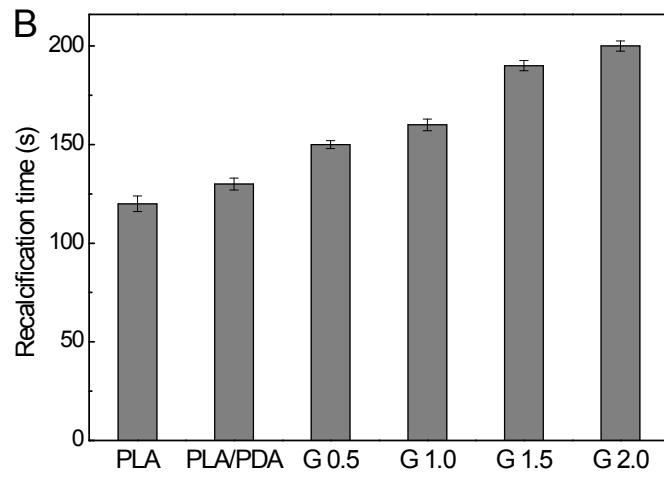
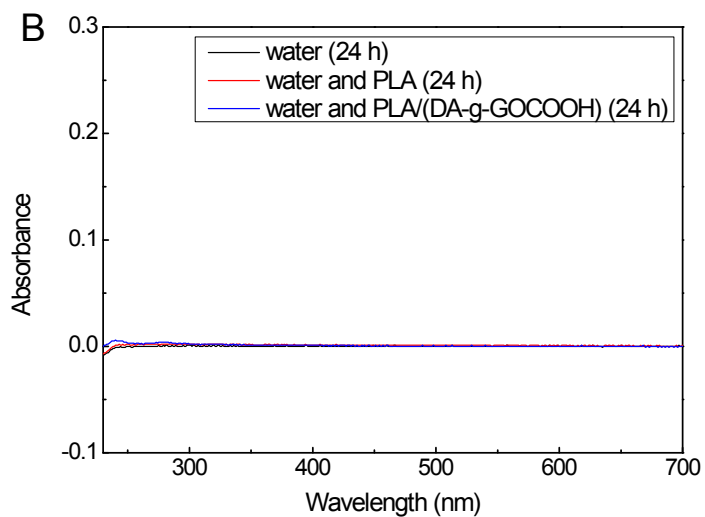
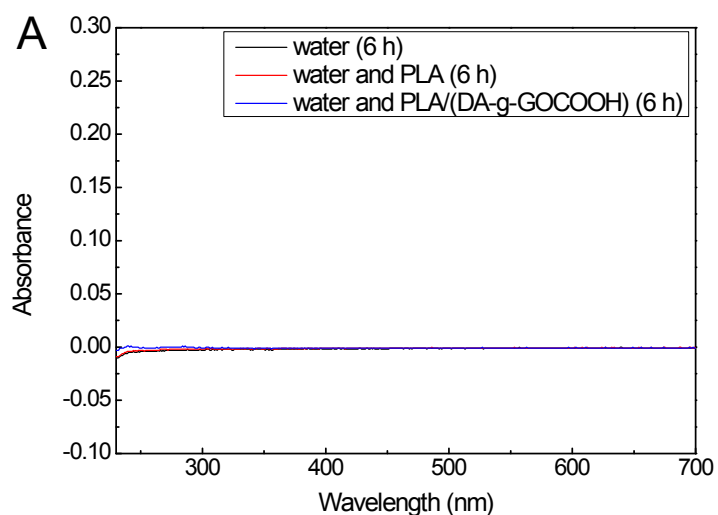


Fig. S8. The plasma recalcification time and hemolysis ratio for rGO (A, C) and DA-g-GO (B, D) modified PLA membranes. The recalcification time of PLA, RG 40 and RG 60 membranes were almost similar (120 s, 130 s and 126 s, respectively). The recalcification time of RG20 membrane was 160 s. It might be related to the more hydrophilicity and electronegativity of RG 20 membranes than PLA, RG 40 and RG 60 membranes. The recalcification time increased with the increment of DA-g-GO immersing concentration (G 0.5, 150 s; G 2.0, 200 s). This was also contributed to the increment of hydrophilicity and electronegativity.



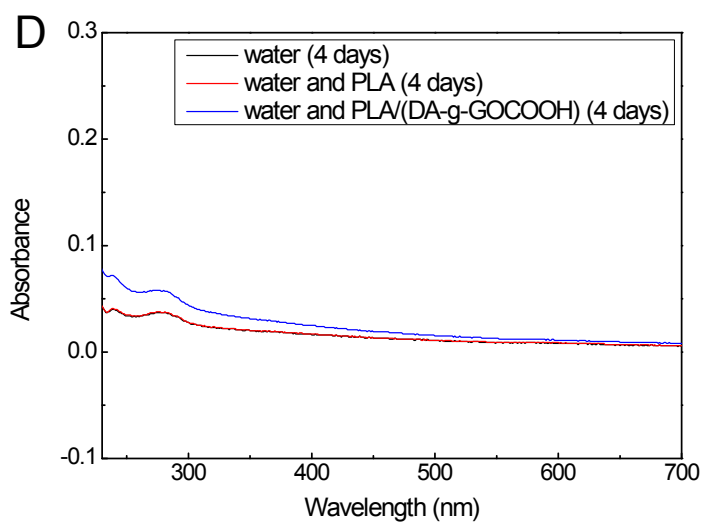
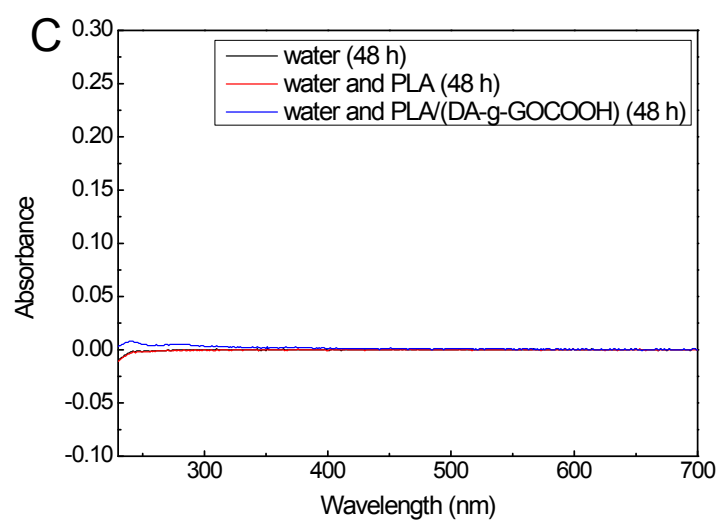
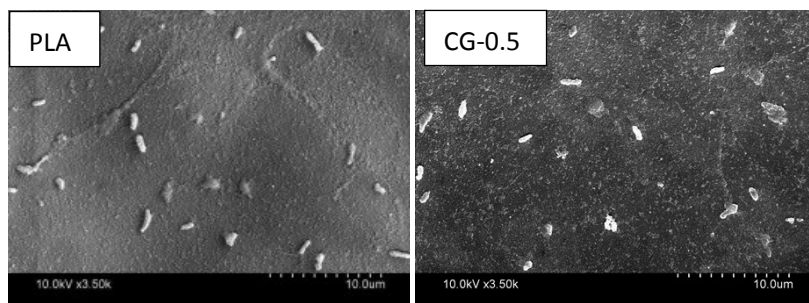


Fig. S9. UV-vis absorbance of DA-g-GOCOOH for PLA and modified membranes after incubating different time in water: (A) 6 h, (B) 24 h, (C) 48 h, (D) 4 days.



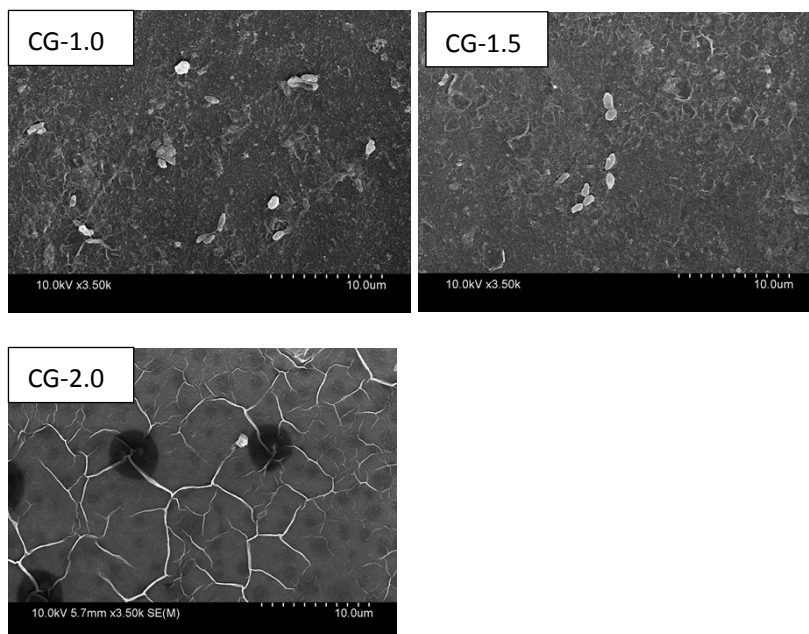


Fig. S10. SEM images of platelet adhesion on PLA and modified PLA membranes after incubating 48 h in water.

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