Electronic Supplementary Information

for

Green and easy synthesis of biocompatible graphene for use as an anticoagulant

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Synthesis of GO

GO sheets were prepared by the oxidation of graphite according to a modified Hummers method. [1, 2] Graphite powder (3.0 g) was put into an 80 ^{o}C solution of concentrated H_2SO_4 (12.0 mL), $K_2S_2O_8$ (2.5 g), and P_2O_5 (2.5 g). The mixture was kept at 80 ^{o}C for 4.5 h. Subsequently, the mixture was cooled to room temperature and diluted with 0.5 L of Mili-Q purified water and left overnight. Then, the mixture was filtered and washed with water using a 0.45-μm microporous membrane to remove the residual acid. The product was dried under room temperature overnight.

This pre-oxidized graphite was then subjected to oxidation described as follows. Pretreated graphite powder was put into cold (0 ^{o}C) concentrated H_2SO_4 (120.0 mL). Then, $KMnO_4$ (15.0 g) was added gradually under stirring and the temperature of the mixture was kept to be below 20 ^{o}C by cooling. Successively, the mixture was stirred at 35 ^{o}C for 2 h, and then diluted with water (250 mL) in an ice bath to keep the temperature below 50 ^{o}C. The mixture was stirred for 2 h, and then additional 0.7 L of water and 20 mL of 30% H_2O_2 was added.

The mixture was filtered and washed with 1:10 HCl aqueous solution (1 L) to remove metal ions followed by 1 L of water to remove the acid. The resulting solid was dried at 50 ^{o}C. Finally, it was purified by dialysis for one week to remove the remaining metal species.

Synthesis of Hydrazine-rGO

Hydrazine-rGO was prepared according to a previous report. [3] The details are described as follows. 50 mL (0.25 mg/mL) of purified GO dispersion was mixed with 14 μL of hydrazine monohydrate and 150 μL of ammonia solution (28 wt% in water). The mixture was stirred at 95 °C for 1 h. After reduction, a homogeneous black dispersion with a small amount of black precipitate was obtained. Then, this dispersion was purified by centrifugation and washing, and finally a stable black aqueous dispersion of hydrazine-rGO was obtained.

Morphology and Structure of Graphene

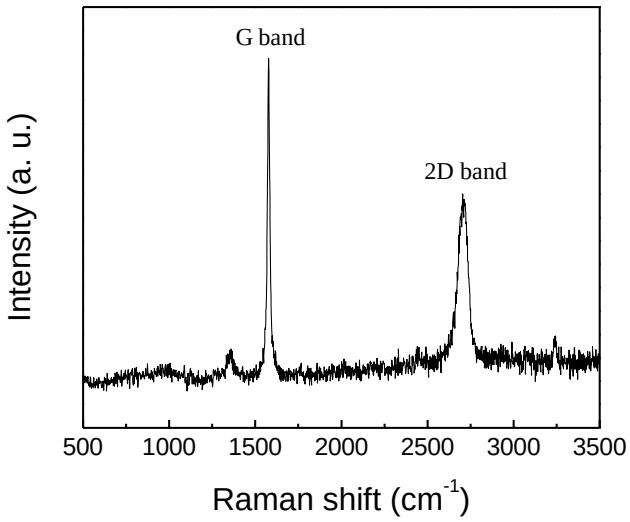

Fig. S1 Raman spectrum of pristine graphite.

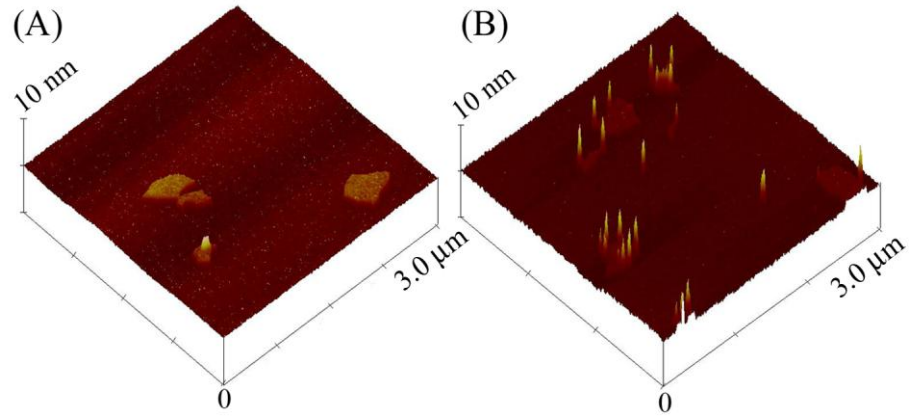

Fig. S2 AFM images of GO (A) and heparin-rGO (B) in 3-D mode (3.0 × 3.0 μm).

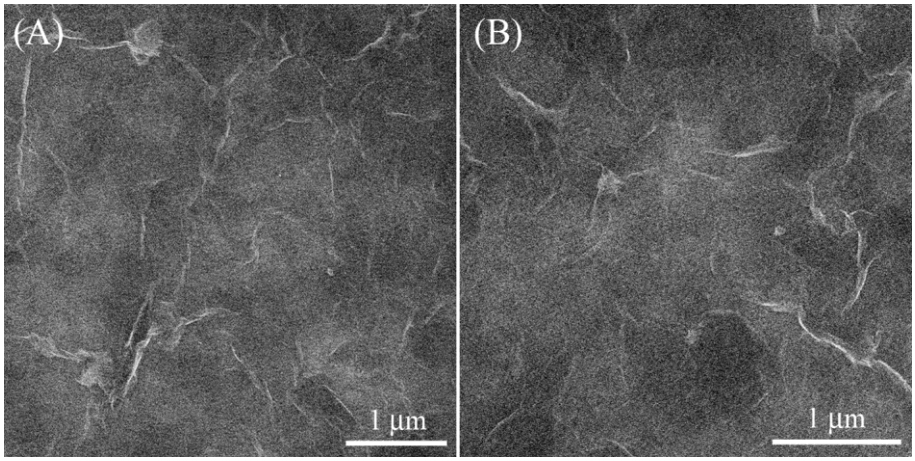

Fig. S3 SEM images of GO (A) and heparin-rGO (B).

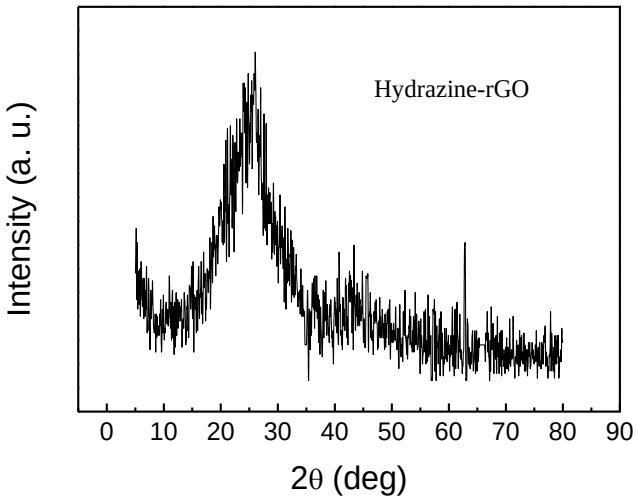

Fig. S4 XRD pattern of hydrazine-reduced rGO.

Drug Loading of Heparin-rGO

Loading of doxorubicin (DOX) on the heparin-rGO was carried out by adding DOX to the heparin-rGO aqueous suspension with stirring for 24 h. The rGO/DOX complex was centrifuged and washed with water to remove free drug. The loading ratio of DOX (~70%) on heparin-rGO was estimated from the absorbance at 490 nm in the UV-vis spectra.

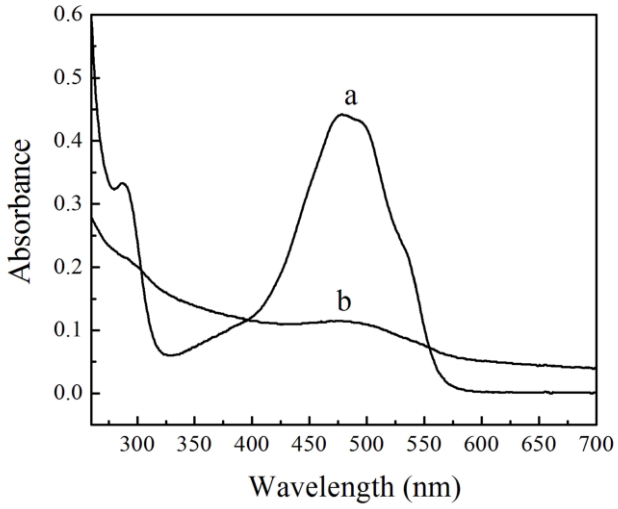

Fig. S5 UV-vis absorption spectra show the efficient loading of DOX on the heparin-rGO sheets. (a) Initial DOX, and (b) free DOX after the loading. The concentrations of DOX and heparin-rGO used are 0.05 mg/mL and 0.25 mg/mL, respectively.

Anticoagulant Activity Investigations of Graphene

Table 1 Experimental data show the influence of heparin-rGO, GO and hydrazine-rGO on the blood coagulation tests, respectively.

		Final concentration			
		0 μg/mL	7 μg/mL	34 μg/mL	67 μg/mL
APTT (s)	Heparin-rGO	27.7 ± 2.5	89.4 ± 2.4	> 300	> 300
	GO	27.7 ± 2.5	46.7 ± 0.6	54.7 ± 0.8	85.2 ± 2.4
	Hydrazine-rGO	27.7 ± 2.5	40.8 ± 0.7	55.3 ± 0.4	95.8 ± 1.7
PT (s)	Heparin-rGO	12.1 ± 0.3	13.8 ± 0.6	16.7 ± 0.7	34.2 ± 0.6
	GO	12.1 ± 0.3	11.8 ± 0.4	12.0 ± 0.4	11.4 ± 0.8
	Hydrazine-rGO	12.1 ± 0.3	11.7 ± 0.4	12.4 ± 0.6	12.5 ± 0.7

References

1 W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.*, 1958, **80**, 1339–1339.
2 Y. Xu, H. Bai, G. Lu, C. Li and G. Shi, *J. Am. Chem. Soc.*, 2008, **130**, 5856–5857.
3 Y. Xu, L. Zhao, H. Bai, W. Hong, C. Li and G. Shi, *J. Am. Chem. Soc.*, 2009, **131**, 13490–13497.