Amine-Modified Graphene: Thrombo-Protective Safer Alternative to Graphene Oxide for Biomedical Applications

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Materials

Mouse monoclonal antibody against phosphotyrosine (clone 4G10) and horseradish peroxidase (HRP)-labeled anti-mouse secondary antibody were procured from Upstate biotechnology and Bangalore Genei, respectively. Fura-2 AM and Super Signal West Pico chemiluminescent substrate were the products of Molecular probes (Invitrogen India) and Pierce (Thermo Fischer Scientific India Ltd.), respectively. PVDF membrane was from Millipore India. H_2DCF-DA**,** human thrombin, apyrase, EGTA, sodium orthovanadate, acetylsalicylic acid, bovine serum albumin (fraction V), protease inhibitors, and other reagents were procured from Sigma Aldrich India (P) Ltd. Reagents for electrophoresis were purchased from Merck India. All other reagents were of analytical grade. Milli-Q grade deionized water (Millipore) was used for preparation of solutions.

Method

Characterization of Graphene

The crystallinity and quality of as-synthesized G-NH_2 sheets were analyzed by high resolution transmission electron microscope (HR-TEM) (JEOL 2200F TEM/STEM). FT-IR spectra were recorded from KBr pellets (Aldrich, 99%, FT-IR grade) using a Mattson 7000 FT-IR spectrometer with resolution 8 and 256 interferograms. Fluorescence measurements of graphene derivatives were performed on Hitachi fluorescence spectrophotometer (model F-2500) using FL Solutions software. The Raman spectra of GO and G-NH_2 were recorded using a Renishaw Raman spectrometer (laser excitation at 514.5 nm) (University of Aveiro) and results were compared with pristine single layer graphene sheets prepared by chemical vapor deposition (CVD) technique.

Zeta potential measurements were performed using a Zeta Sizer Nano Series (Malvern) equipment (University of Aveiro) to monitor the charge characteristics of colloidal aqueous suspensions of GO, RGO, and G-NH_2 as function of pH. The optical spectra were recorded by using a Jasco V-560 UV-Vis spectrometer (University of Aveiro).

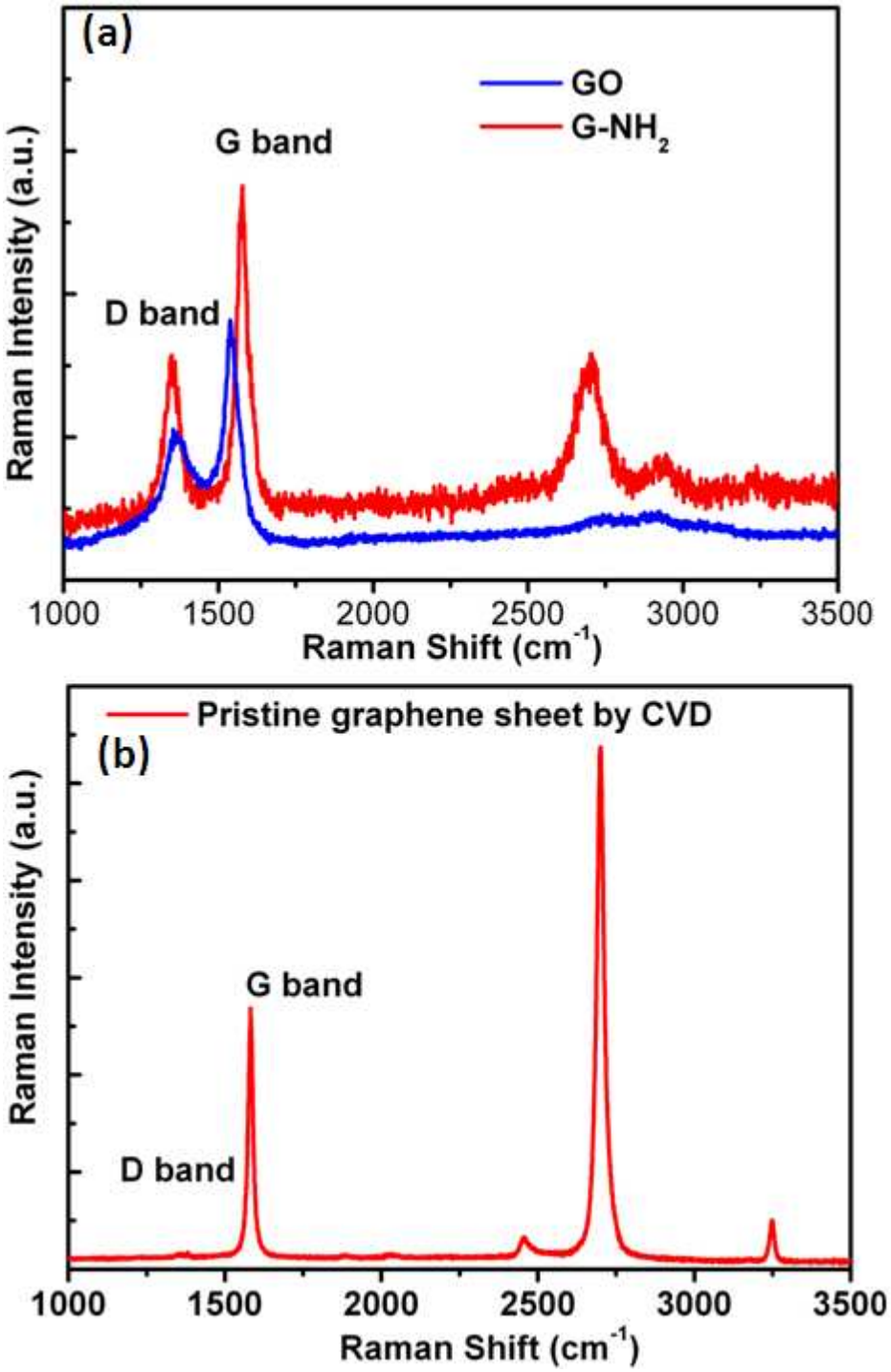


Figure S1: Evaluation of Raman spectra for GO and G-NH_2 (a) and pristine single layer graphene sheets (b).

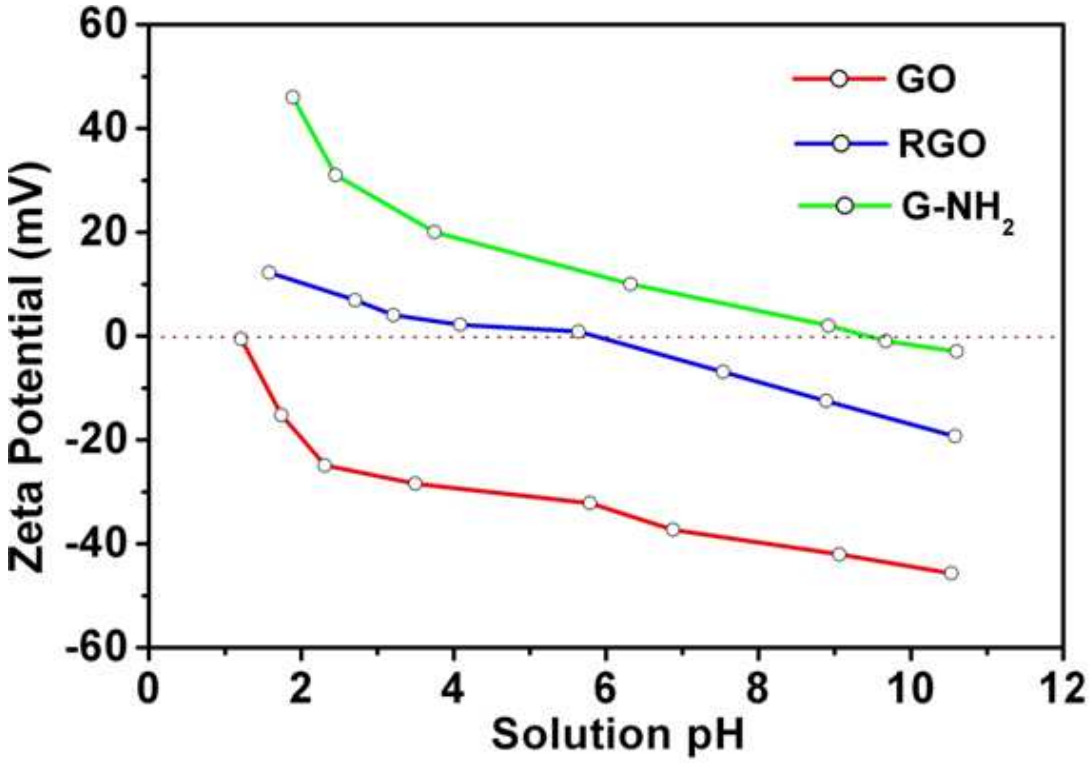


Figure S2: Zeta potential of graphene colloidal suspensions of GO, RGO and G-NH_2 as function of pH.

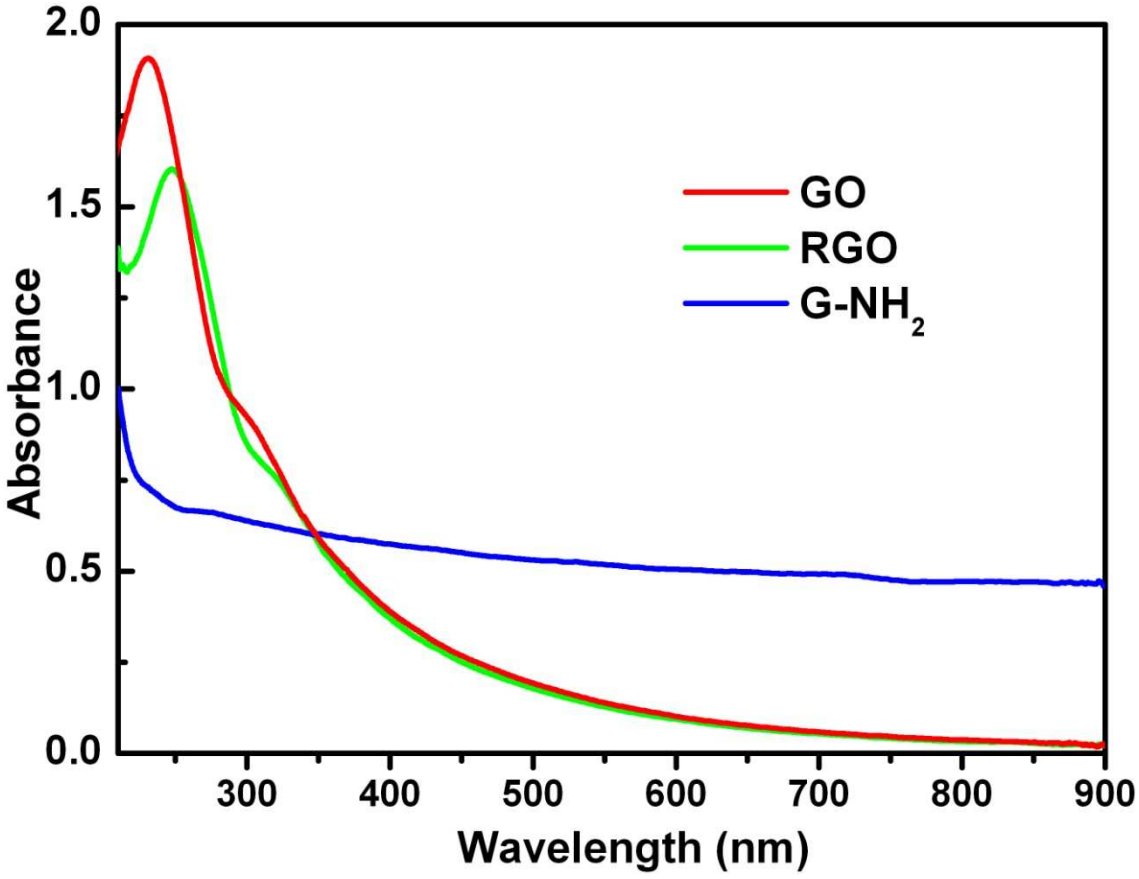


Figure S3: UV-Vis-NIR absorption spectra of GO, RGO and G-NH_2 (at individual concentrations of 0.01 mg/ml each)

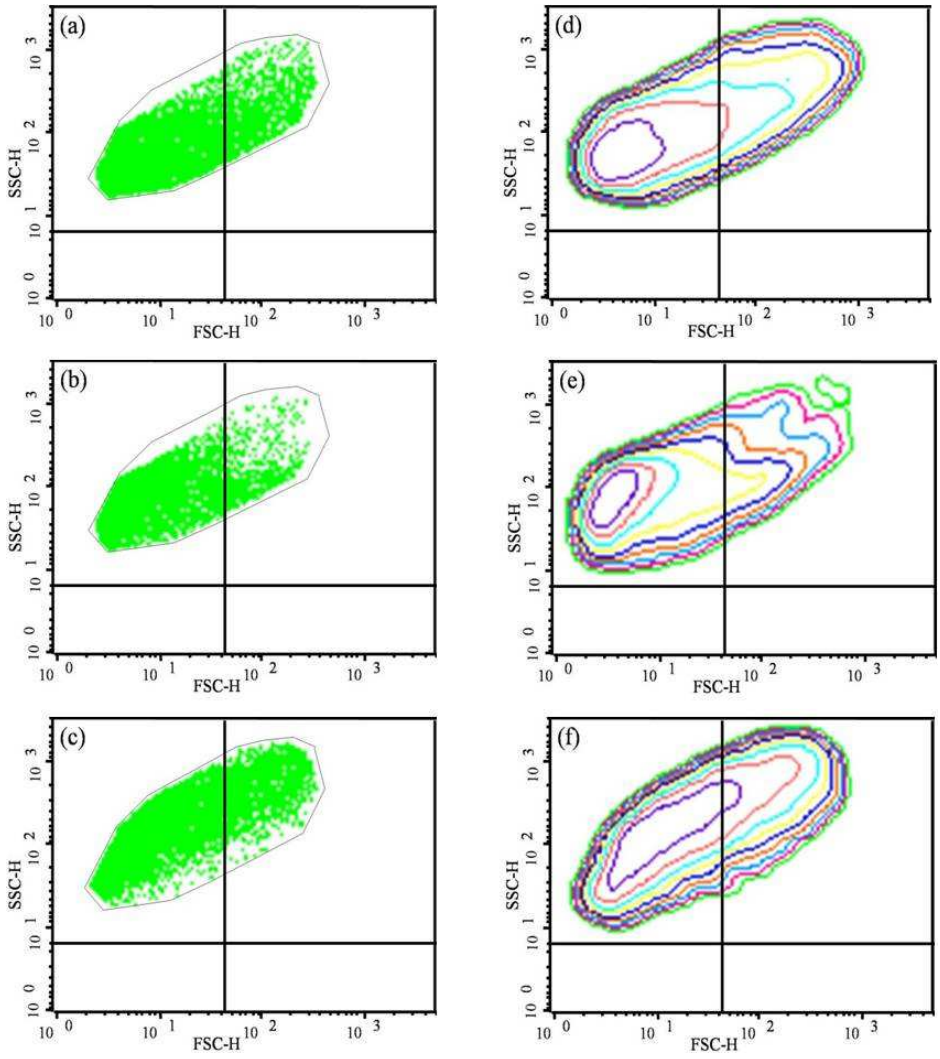


Figure S4. (a), (b) and (c) represent dot plots of GO, RGO and G-NH_2, respectively, under identical gating. (d), (e) and (f), respectively, represent corresponding contour plots. The number of events analyzed was 10,000. The results were representative of five independent experiments.

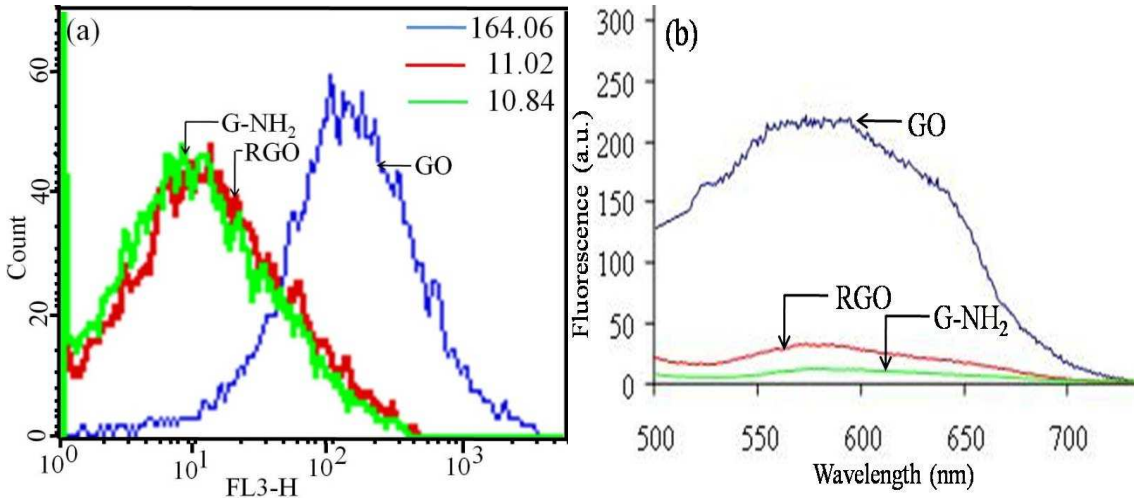
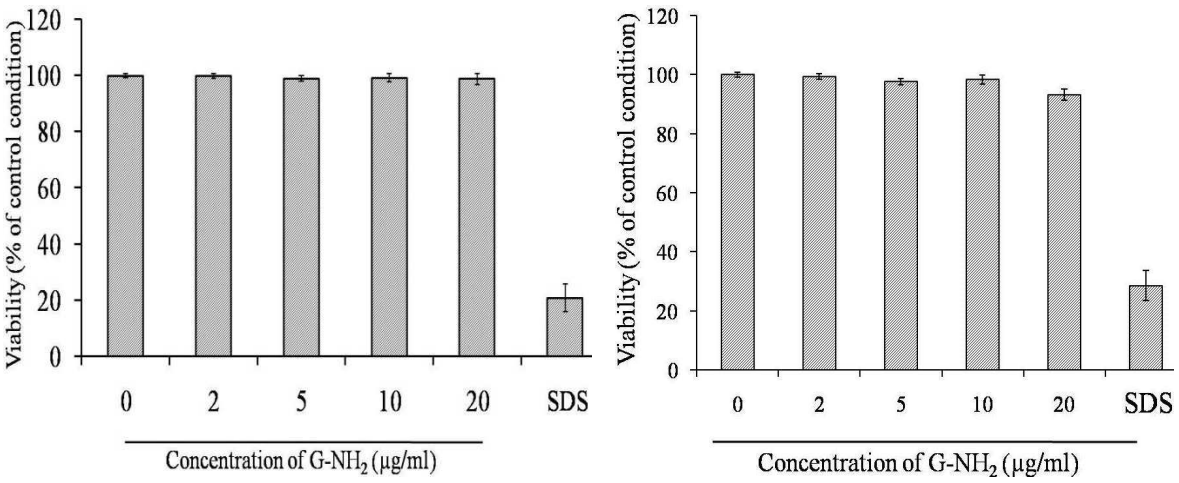


Figure S5. (a) Overlaid histograms representing level of fluorescence of GO (blue), RGO (red) and G-NH_2 (green) populations in FL3 fluorescence channel. The number of events analyzed was 10,000. Median values for GO, RGO and G-NH_2 are stated within the box. The results are representative of five independent experiments. (b) Fluorescence spectrum of different graphene derivatives at equal concentration (5 µg/ml) in the visible range. Excitation, 400 nm.



(a) **(b)**

Figure S6. MTT assay of G-NH_2 treated platelets (a) and THP-1 (Human monocyte cell line) (b). All data are representative of 3 independent experiments and are presented as mean ± SEM.