Supporting Information

In Vitro Hemocompatibility and Toxic Mechanism of Graphene Oxide on Human Peripheral Blood T Lymphocytes and Serum Albumin

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Experimental details, Figure S1, Figure S2, Figure S3, Figure S4, Figure S5, Figure S6, , Figure S7, and Figure S8

Experimental details

Isolation of primary human peripheral blood T lymphocytes. Peripheral blood samples were obtained from healthy volunteers. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation (Ficoll-PaqueTM PREMIUM, GE healthcare) from anticoagulated blood samples. The isolated PBMCs were washed thrice with Hanks balanced salt solution (HBSS) and cultured in RPMI 1640 medium (HyClone). Differential adherence properties of T cells, B cells and accessory cells can be employed to enrich for T cells by nylon fiber columns. Nylon fibers (polysciences) were soaked overnight in 0.2 M HCl, rinsed with deionized (DI) water and dried. Plastic 20 mL syringes were loaded with 2 g of treated nylon fibers. The columns were rinsed with 50 ml of PBS, followed by 50 mL of 10 % Fetal Bovine serum (FBS, HyClone) in PBS prewarmed to 37℃. Both ends of the syringe barrel columns were sealed with Parafilm and the columns are incubated for 30 min at 37℃. Following incubation, the seals were removed, the columns were placed vertically, and 1-2 mL of lymphocytes suspension prepared as above were run into the column, while excess PBS escaped through the needle end. The ends of the syringe were then sealed and the column incubated for 30min at 37℃. After incubation, a needle was fixed at the lower end of the syringe, which allowed an outflow of approximately 2 mL of liquid per min from the column. The columns were washed with 10 mL of PBS preheated to 37℃ and the effluent containing the non-adherent cells (mainly T lymphocytes) was collected in a centrifuge tube. The isolated T lymphocytes were washed thrice with HBSS and cultured in RPMI 1640 medium at a final concentration of 1×10^6 cells per mL.

TCR binding. TCR antibody was used to judge the binding of GO with membrane protein receptors on T lymphocytes. After respective incubation with *p*-GO or *f*-GO for 6 h, the cells were washed and blocked with 10% skim milk. FITC conjugated Mouse Anti-Human TCR αβ (BD Biosciences) was added and incubated with cells for 2 h. The stained cells were resuspended in binding buffer and directly analyzed in Flow cytometry (FACSAria II, Becton, Dickinson Co.) by measuring the fluorescence at 530 nm. Untreated T lymphocytes stained with FITC conjugated TCR antibody were taken as a control.

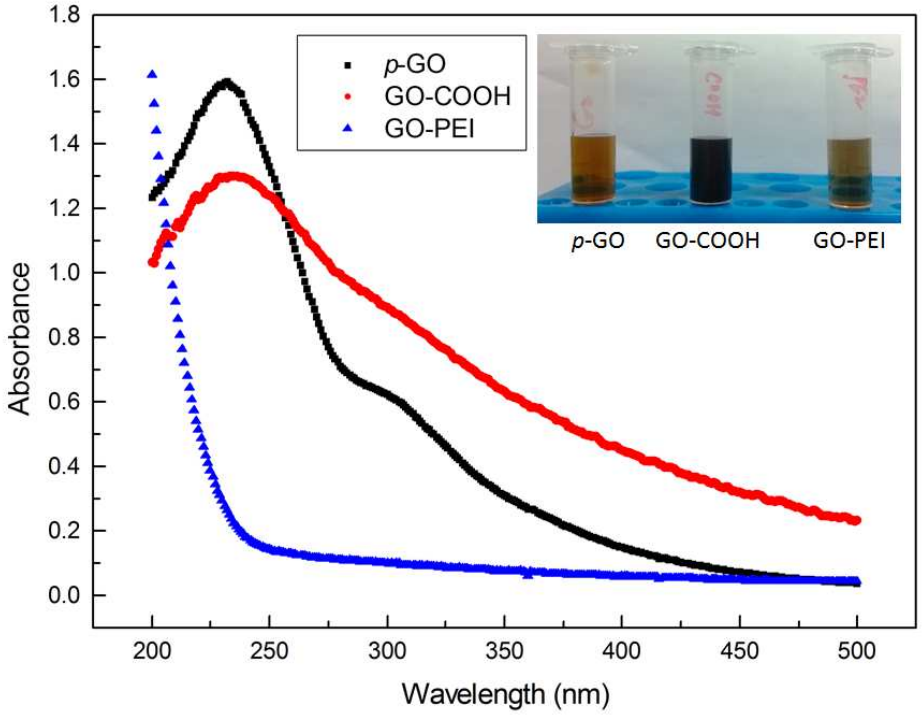

Figure S1. The UV-vis spectra and photographs of GO: The UV-vis spectrum of *p*-GO shows that *p*-GO has a characteristic peak at near 230 nm and a shoulder at about 300 nm. The UV absorption peak of GO-COOH is shown at around 235 nm without a shoulder, and GO-PEI has no obvious characteristic absorption peak in near ultraviolet band. Insert shows the photographs of aqueous dispersions of GO at 100 mg mL^{-1}. The aqueous dispersion of *p*-GO is brown, and it (GO-COOH) becomes black after carboxylation. After modified with PEI, the aqueous dispersion of GO-PEI becomes pale brown.

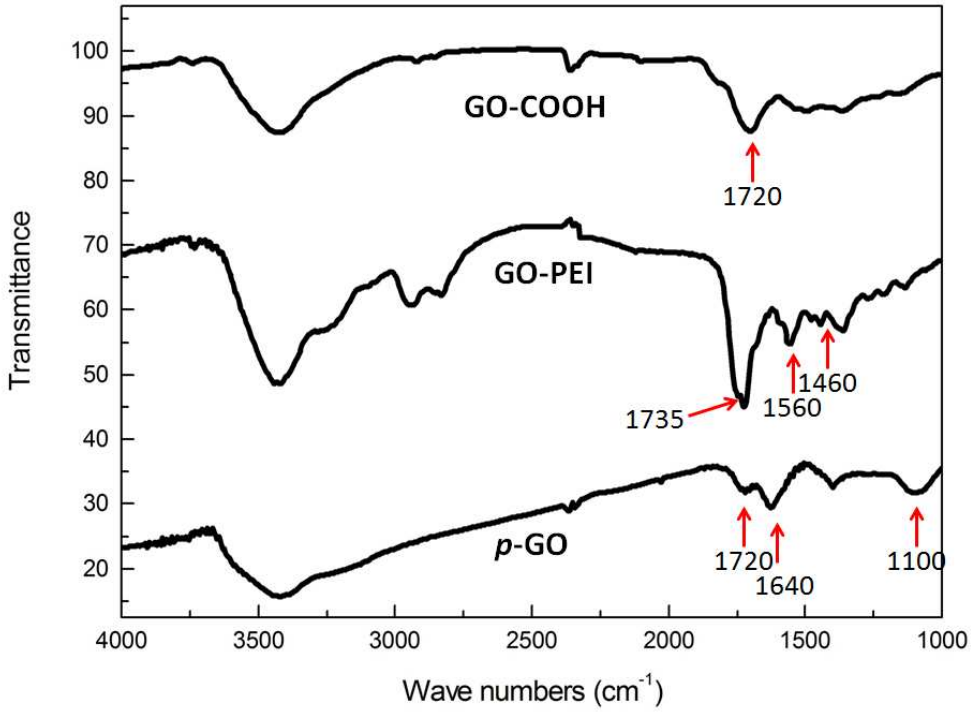

Figure S2. FT-IR spectra of GO: The peaks at 1720, 1640 and 1100 cm^{-1} in the FT-IR spectrum are characteristic of the C=O and C-O stretches of the ketone groups and epoxy groups, respectively, on *p*-GO. Compared with *p*-GO，the FT-IR spectrum of GO-COOH shows a stronger -COOH peak and a bigger peak width. This implies that partial epoxy and hydroxyl are activated into carboxyl. In the spectrum of the GO-PEI, also a strong band emerges at 1735 cm^{-1}, corresponding to the C=O characteristic stretching band of the amide groups, a -NH2 bending peak at 1560 cm^{-1} and a C-N stretching vibration at 1460 cm^{-1} from PEI are also observed.

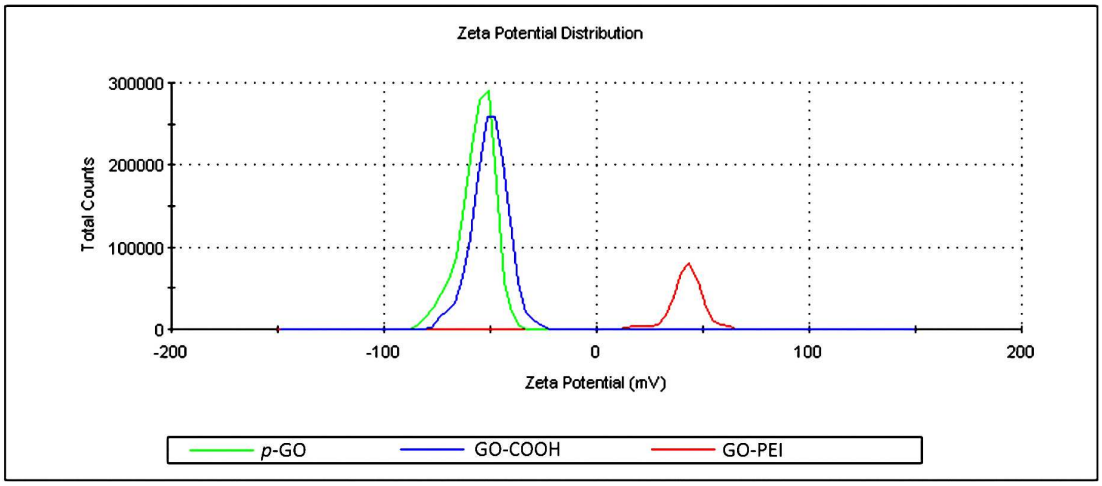

Figure S3. Zeta potential of GO. The potential distribution curve of *p*-GO has a negatively charged peak with position centered at about -56.3 mV. For GO-COOH, the peak position of its zeta potential shifts to -50.3 mV. After modified with the polymer PEI, the peak position of its zeta potential shifts to positive charge of 42.4 mV.

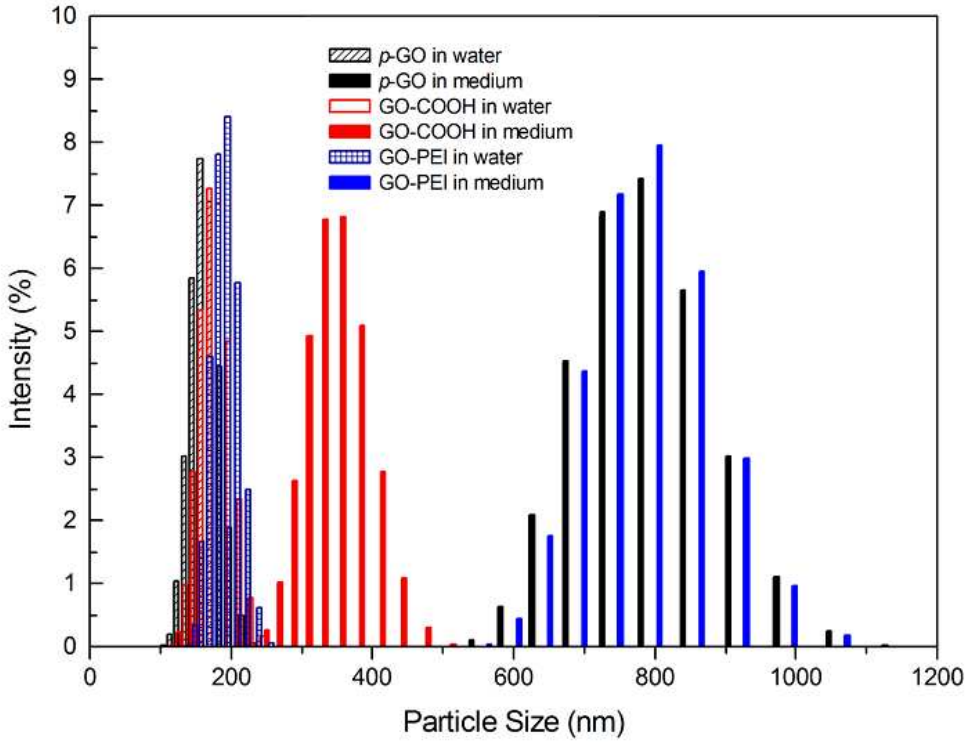

Figure S4. Hydrodynamic size distributions of GO before and after incubation in culture medium for 24 h. The hydrodynamic radius of *p*-GO, GO-COOH and GO-PEI solution are 175.50, 177.35 and 181.00 nm. After incubation in culture medium for 24 h, the hydrodynamic radius of GO increase to 774.25, 344.04 and 777.15 nm, respectively.

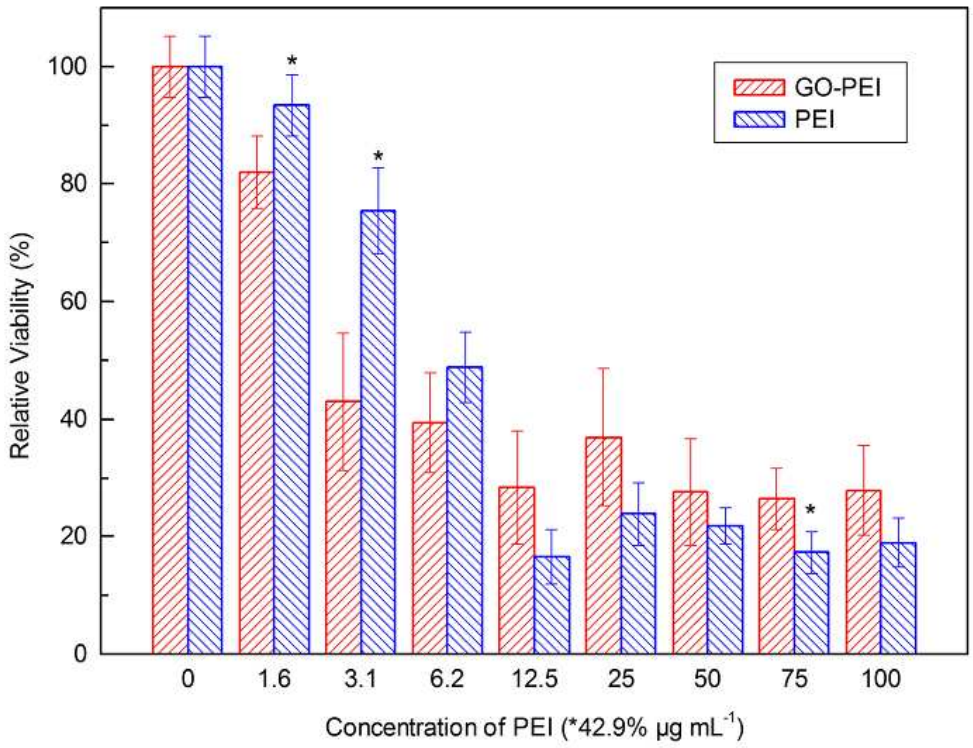

Figure S5. Cell viability of T lymphocytes incubated with GO-PEI and free PEI (equal to conjugated PEI in GO-PEI, the amount of conjugated PEI in GO-PEI is 42.9%) at various concentrations for 24 h using WST-8 assay. * denotes $p < 0.05$ compared with GO-PEI. Obvious difference in toxic effect between GO-PEI and PEI implies that PEI is not the only factor to influence the toxicity of GO-PEI. Graphene oxide also acts as a supporter of two-dimensional nanomaterial to impact on the properties of GO-PEI, such as the size, shape, etc and further affects the cytotoxicity of GO-PEI.

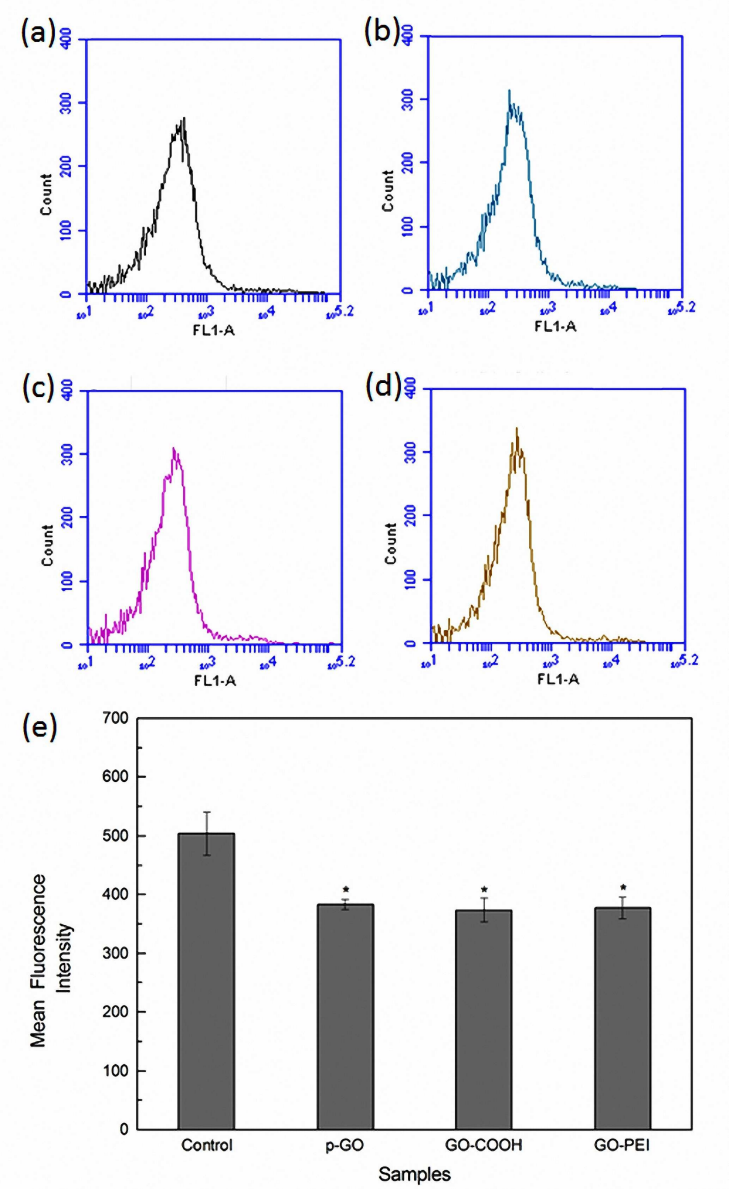

Figure S6. The interaction of GO with TCR on T lymphocytes. After incubation with GO (100 μg mL^{-1}) for 6 h, the T lymphocytes were labeled with FITC conjugated anti-TCR and analyzed by flow cytometry. (a-d) Histogram of the mean fluorescence intensity of (a) untreated T lymphocytes as a control and T lymphocytes treated with (b) *p*-GO, (c) GO-COOH, (d) GO-PEI. (e) The summary of the mean fluorescence intensity of T lymphocytes labeled with FITC conjugated anti-TCR. * denotes $p < 0.05$ compared with control.

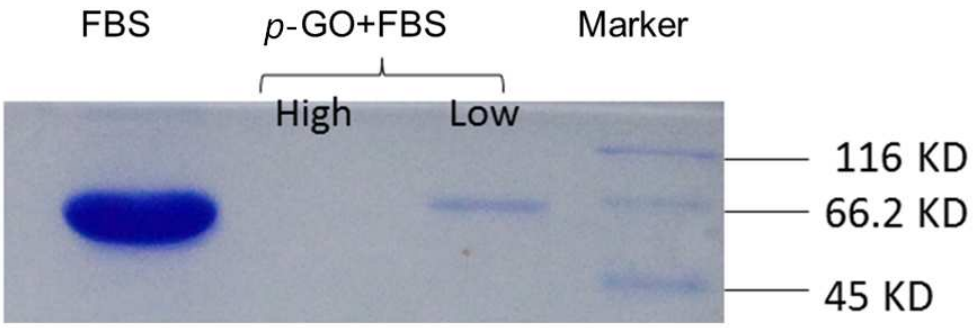

Figure S7. SDS-PAGE electrophoresis of FBS proteins in the supernatant after centrifugation. 10% FBS solution was incubated with high or low concentration of *p*-GO (lane 1: blank; lane 2: 1 mg mL^{-1} *p*-GO; lane 3: 200 μg mL^{-1} *p*-GO; lane 4: marker) respectively for 2 h at 37℃.

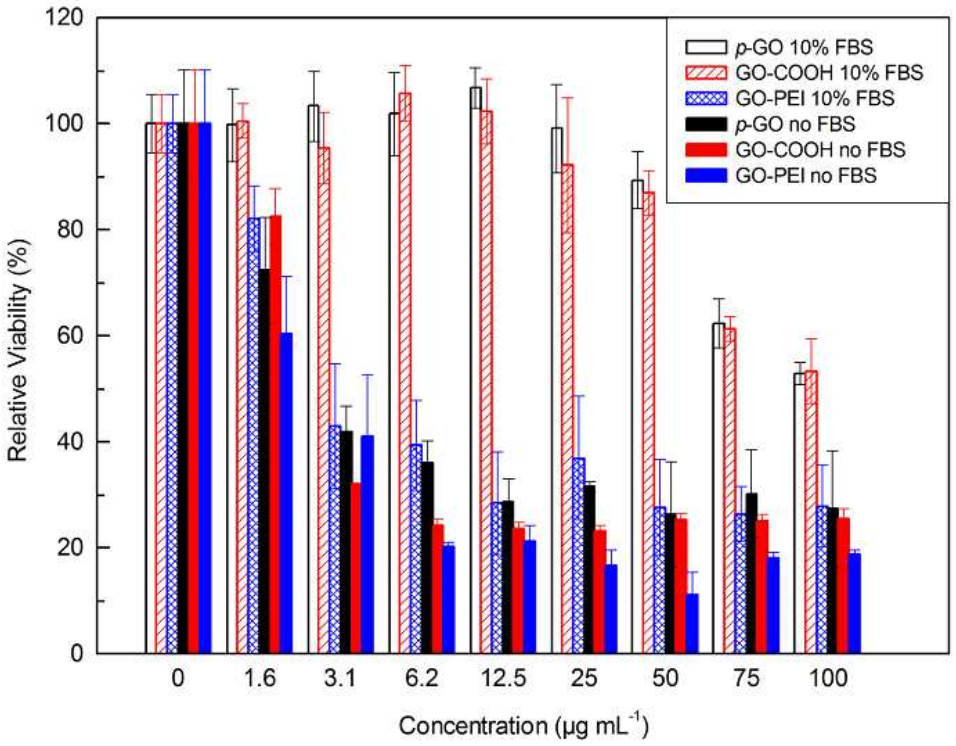

Figure S8. Cell viability of T lymphocytes incubated GO at various concentrations for 24 h, cultured in RPMI 1640 culture medium supplemented with/ without 10% FBS.