

Supplemental Information

Reduction of Graphene Oxide Alters Its Cyto-Compatibility Towards Primary and Immortalized Macrophages

Yakun Wu^{1,2}, Fanfan Wang³, Shunhao Wang^{1,2}, Juan Ma^{1,2}, Ming Xu^{1,2}, Ming Gao^{1,2},
Rui Liu^{1,2}, Wei Chen^{3,*}, Sijin Liu^{1,2,*}

1. State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China.
2. University of Chinese Academy of Sciences, Beijing 100049, China.
3. College of Environmental Science and Engineering, Ministry of Education Key Laboratory of Pollution Processes and Environmental Criteria, Tianjin Key Laboratory of Environmental Remediation and Pollution Control, Nankai University, Tianjin 300350, China.

Correspondence to Profs. Wei Chen, Ph.D. (Email: chenwei@nankai.edu.cn) and Sijin Liu, Ph.D. (Email: sjliu@rcees.ac.cn)

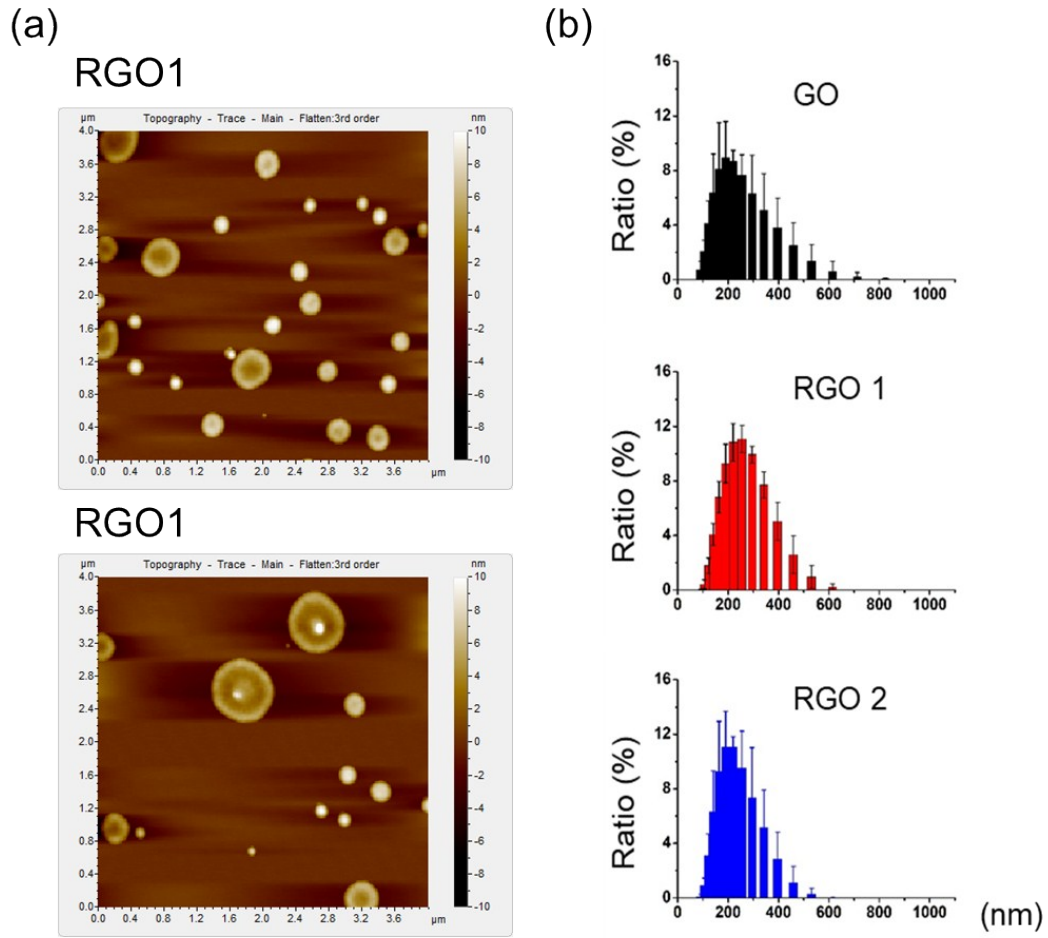


Figure S1. Characterization of GO materials. (a) AFM images of RGO materials. (b) Hydrodynamic diameter of the materials in DMEM supplemented with 10% FBS.

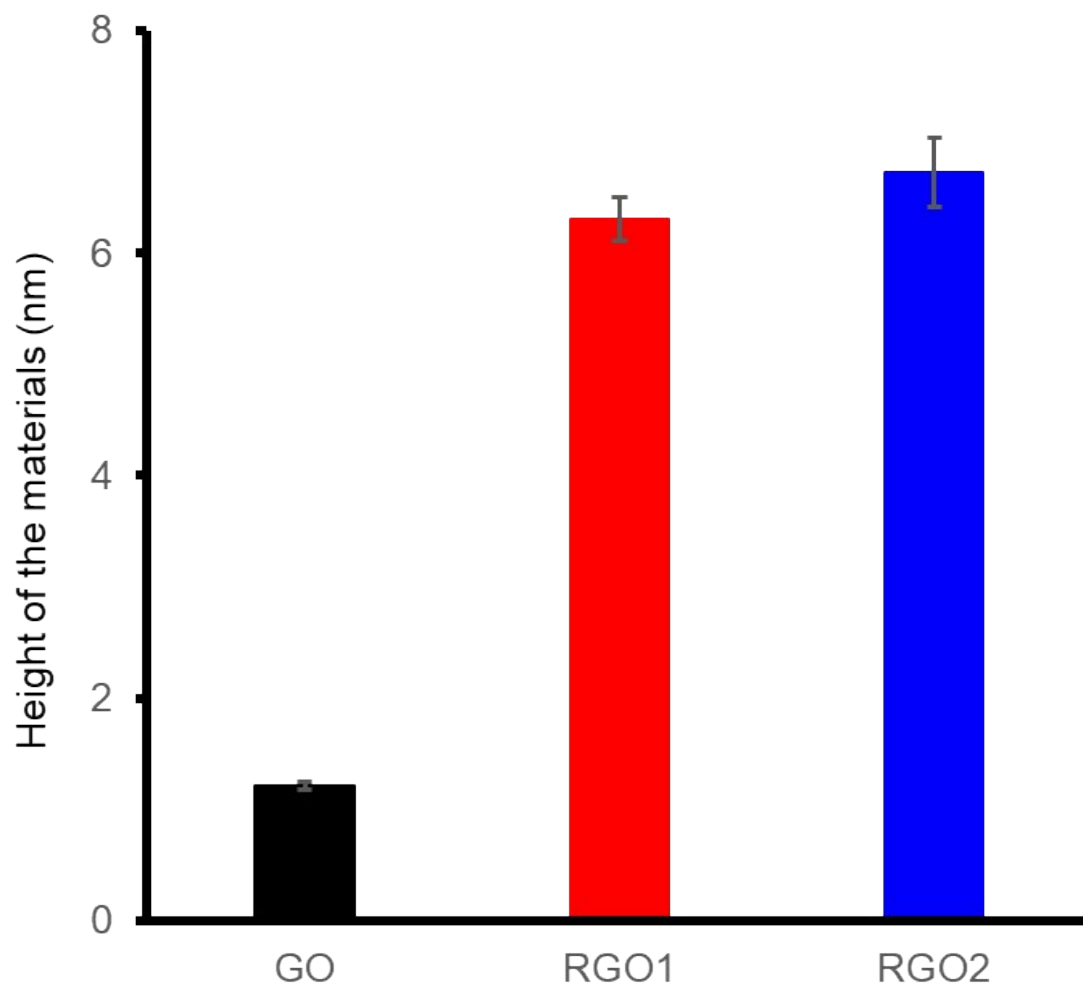


Figure S2. Representative height of GO, RGO1 and RGO2. Quantified data of height profiles for the materials (n=13). #: $P < 0.001$, relative to GO.

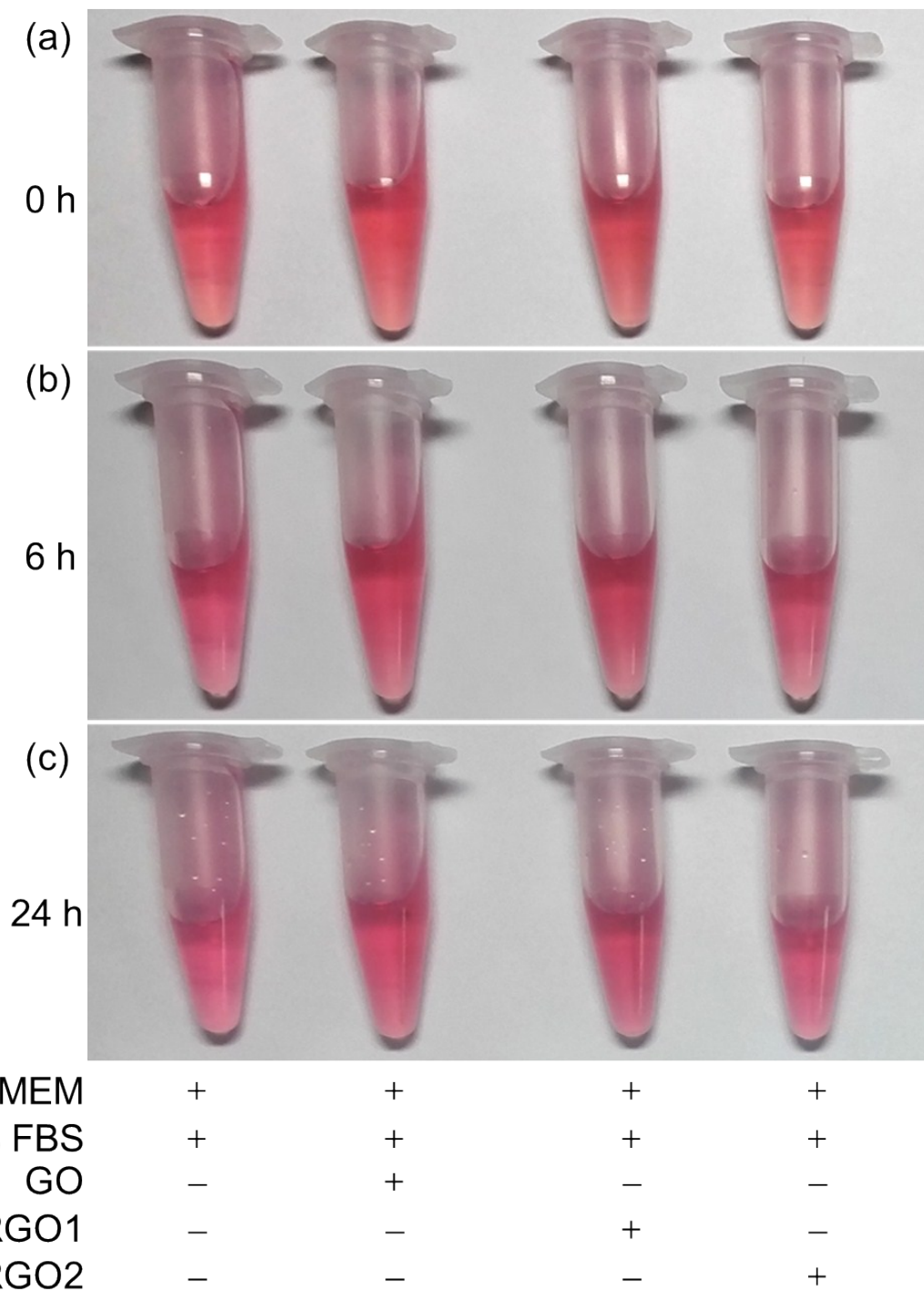


Figure S3. Dispersivity assessment of the materials. Dispersivity of the materials (at 10 $\mu\text{g}/\text{mL}$) in culture medium with 10% FBS at (a) 0 h, (b) 6 h and (c) 24 h.

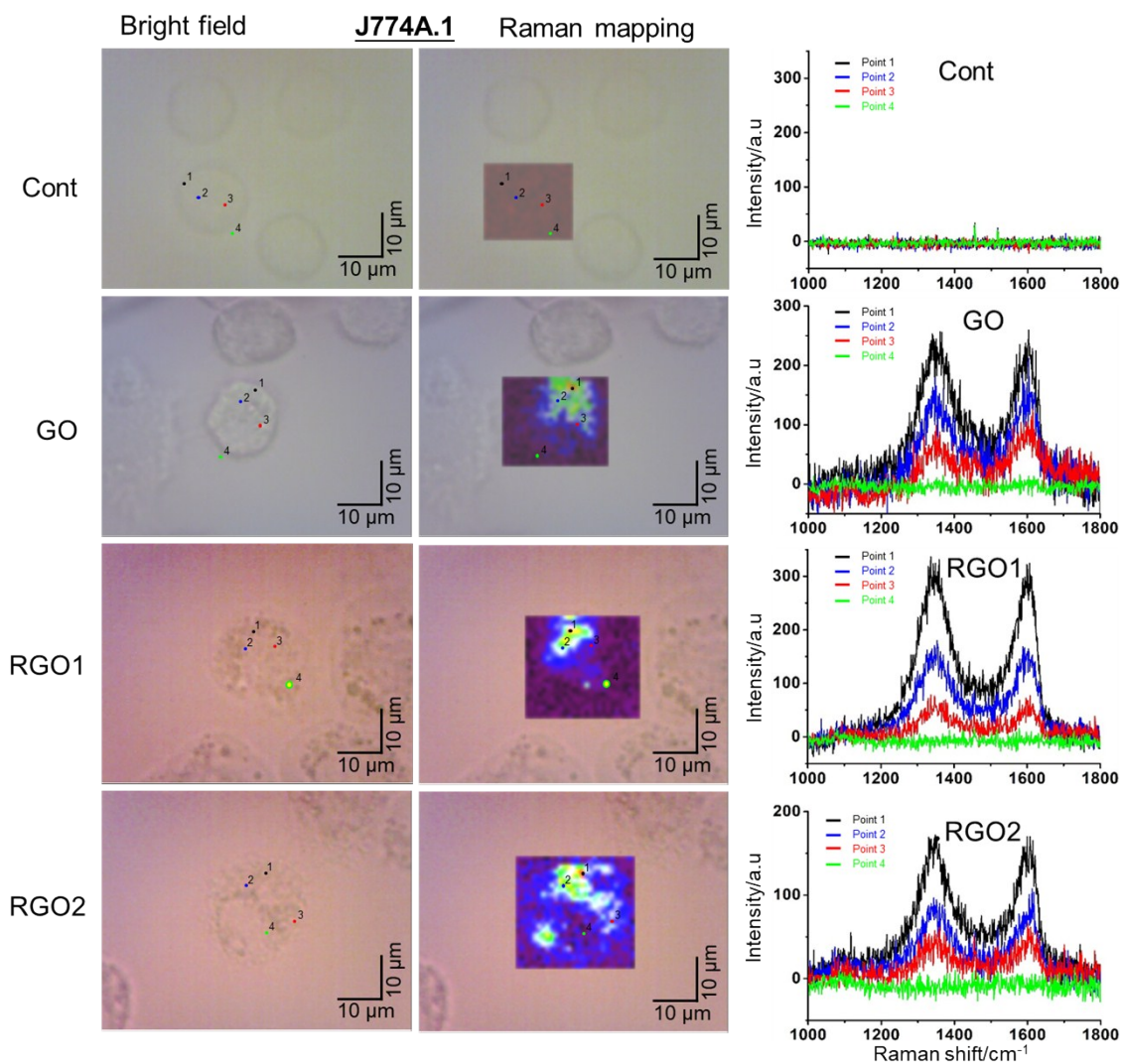


Figure S4. Intracellular localization characterization of GO and RGO materials in J774A.1 cells. Raman mapping spectra of J774A.1 cells treated with GO, RGO1 and RGO2 at 10 $\mu\text{g}/\text{mL}$ for 3 h. The rainbow color represents typical graphene signal. Point 1 (black) shows the highest intensity of Raman signal in each image. Point 2 (blue) and Point 3 (red) manifest gradually decreasing intensity of Raman signal in each image within the cytosolic proportion, respectively. Point 4 (yellow) indicates no Raman signal outside of cells.

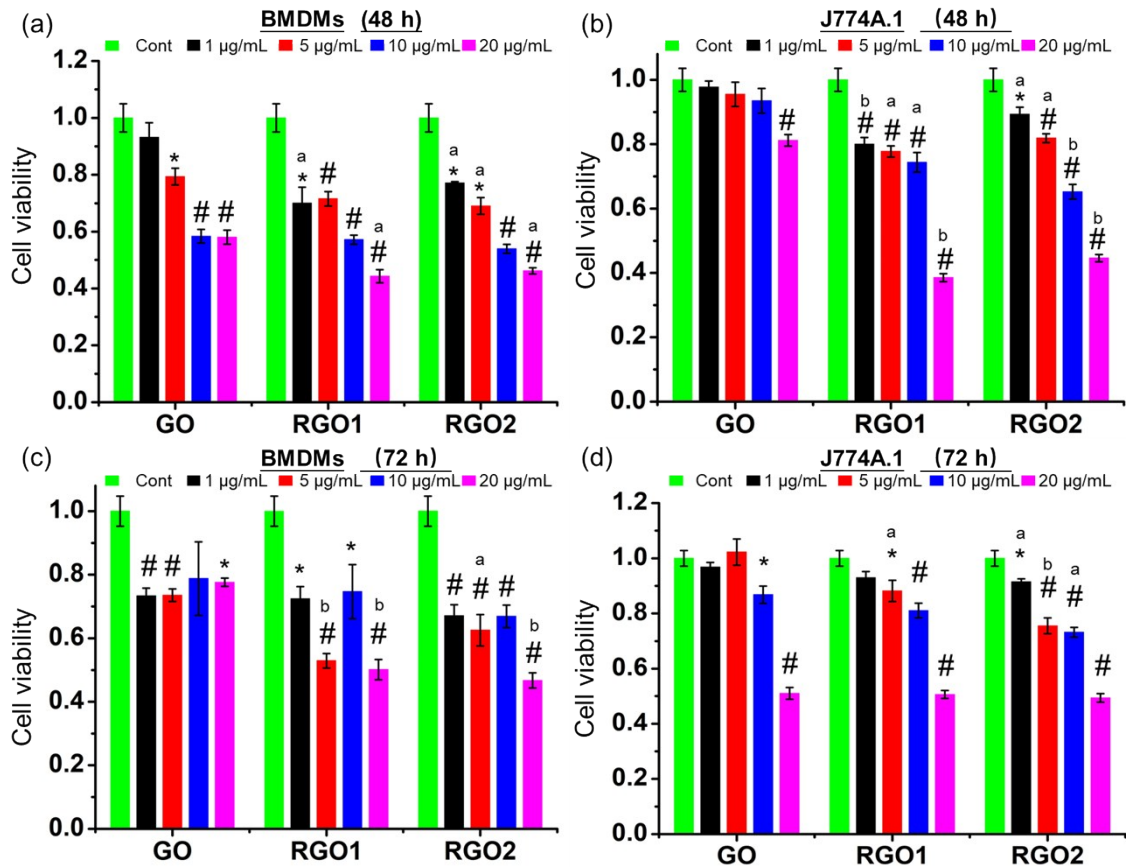


Figure S5. Cell viability of BMDMs and J774A.1 macrophages treated with GO and RGO for 48 h and 72 h. Cells were treated with GO and RGO materials at various concentrations as indicated. *: $p < 0.05$, #: $p < 0.001$, relative to untreated control; a: $p < 0.05$, b: $p < 0.001$, relative to GO-treated cells at the same concentration.

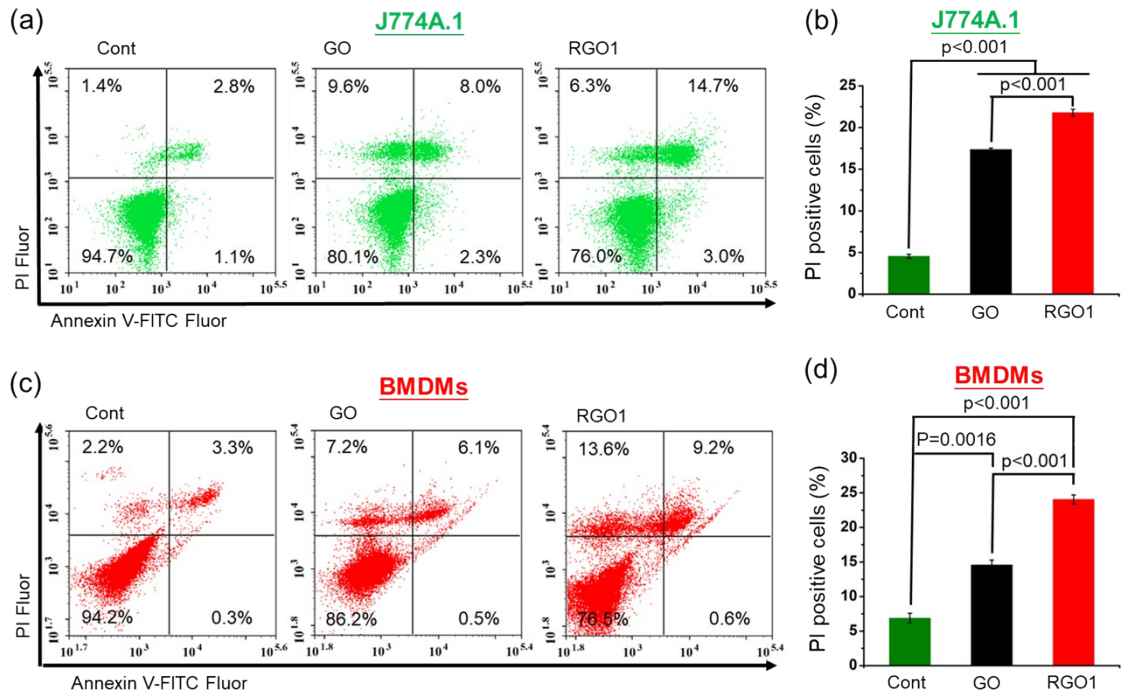


Figure S6. Annexin V/PI analysis of GO materials treated of J774A.1 and BMDMs cells. (a) J774A.1 treated with 20 $\mu\text{g}/\text{mL}$ GO and RGO1 for 24 h, (b) Proportion of PI positive J774A.1 cells based on the flow cytometry data (n=3), (c) BMDMs treated with 20 $\mu\text{g}/\text{mL}$ GO and RGO1 for 48 h, (d) Proportion of PI positive BMDMs from the flow cytometry data (n=3).

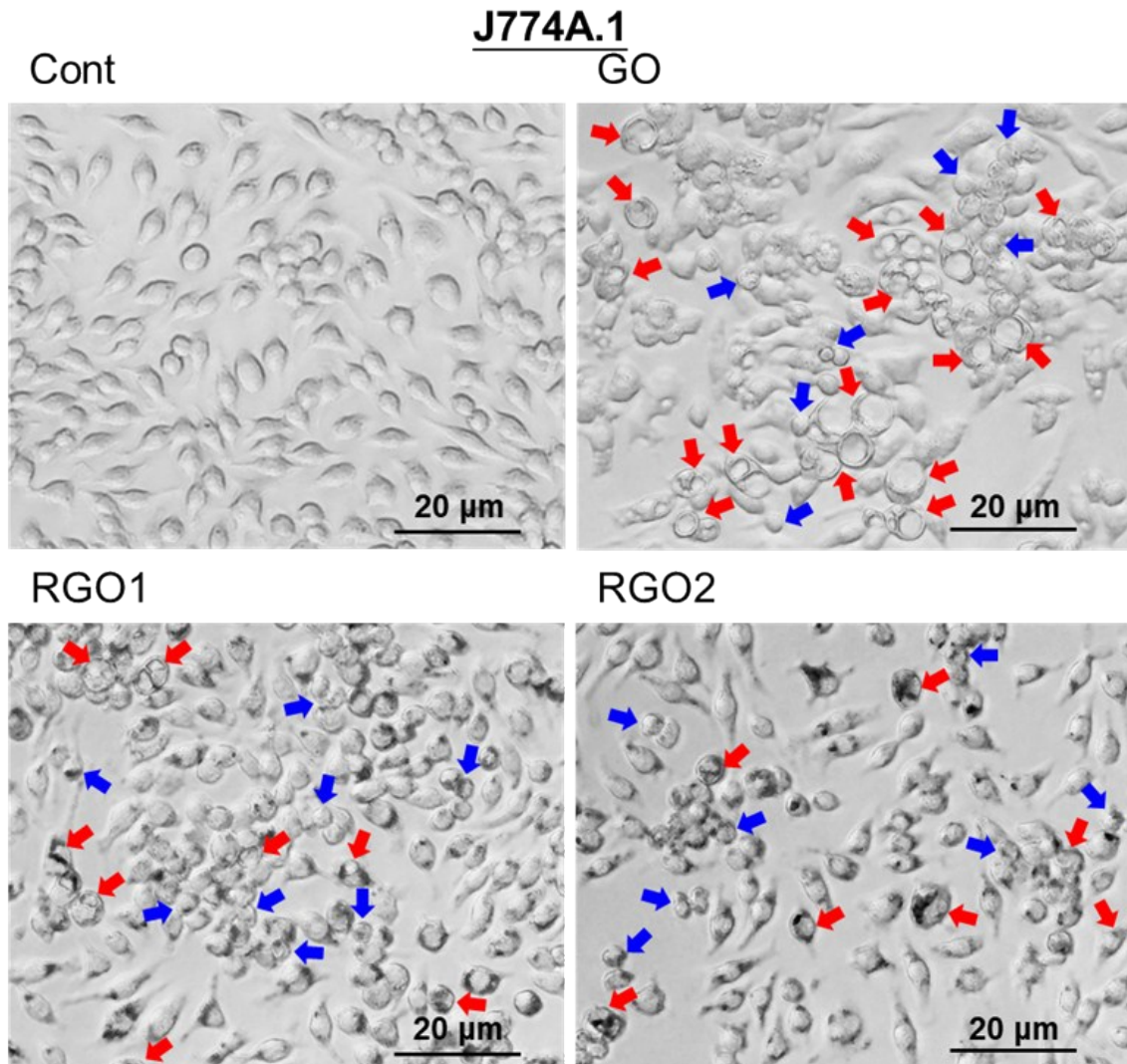


Figure S7. Morphological delineation of J774A.1 cells after exposure to 20 $\mu\text{g/mL}$ GO and RGO for 48h. Red arrows indicate the collapse of cell membrane. Blue arrows point at condensed cells. Scale bar: 20 μm .

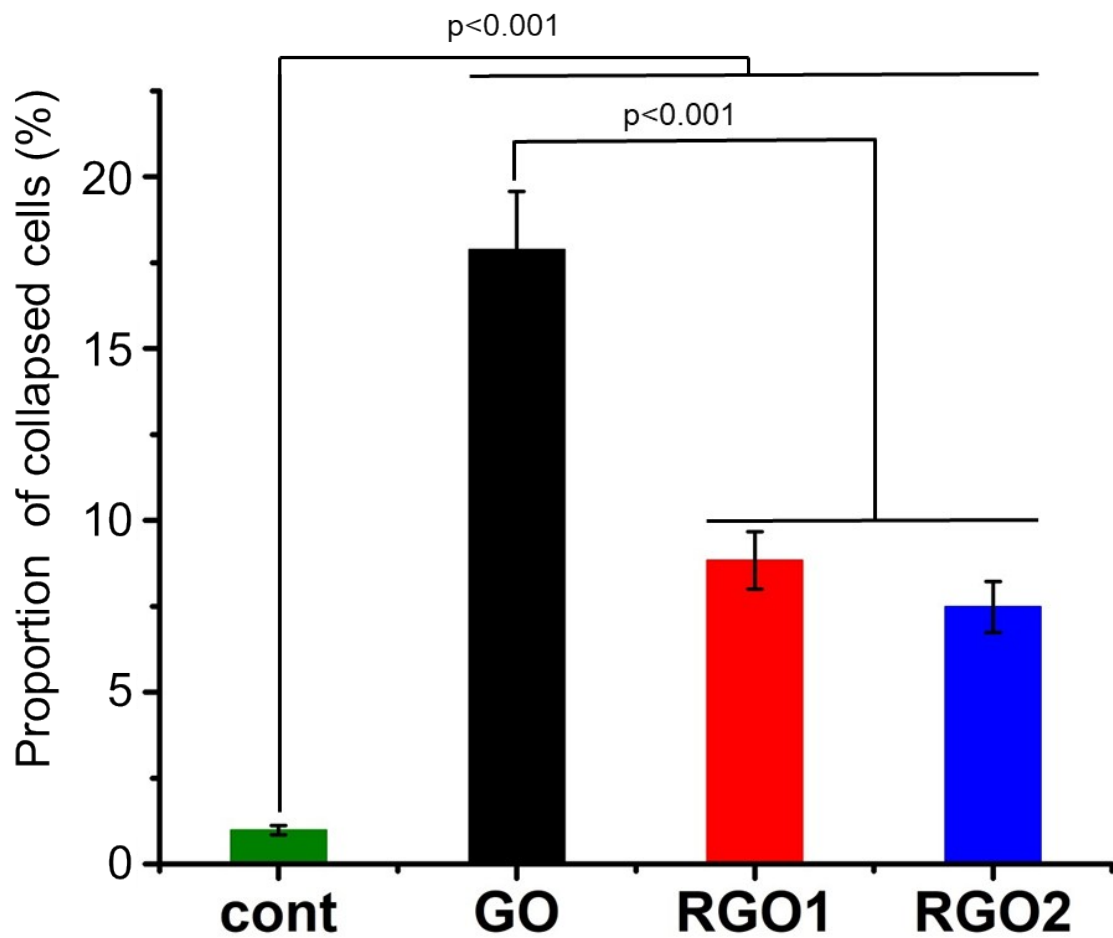


Figure S8. Proportion of collapsed cells. J774A.1 cells were treated with 20 $\mu\text{g}/\text{mL}$ GO and RGO for 24 h. Eight fields were randomly selected, and the total number (approximately 300-500 cells) and the number of collapsed cells were counted in each field. Proportion of collapsed cells was calculated out of the total number of cells.

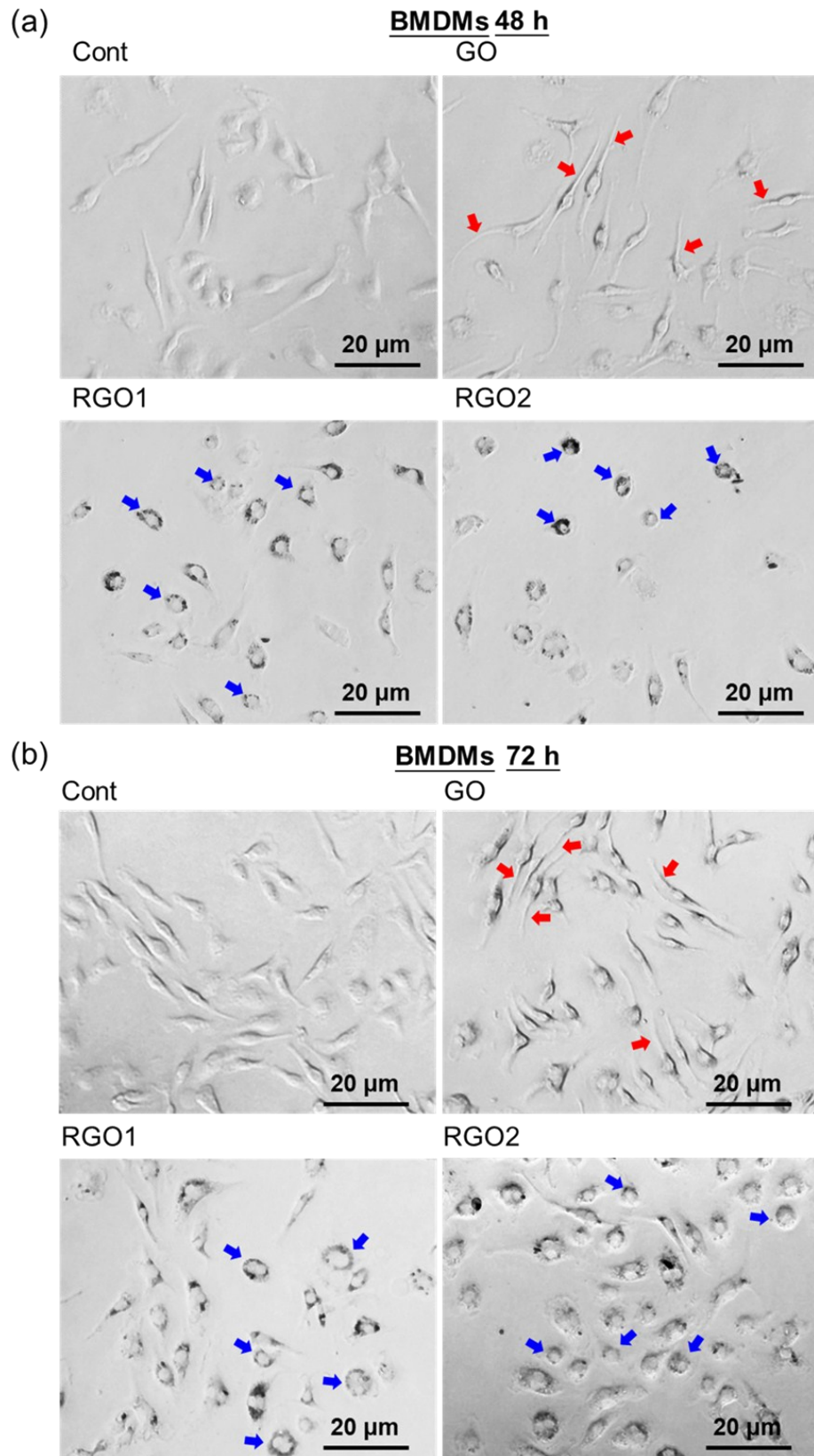


Figure S9. Morphological determination of BMDMs exposed to 10 $\mu\text{g}/\text{mL}$ GO and RGO for 48 h (a) and 72 h (b). Red arrows indicate elongated protrusions. Blue arrows show shrunk BMDMs with a rounder shape. Scale bar: 20 μm .

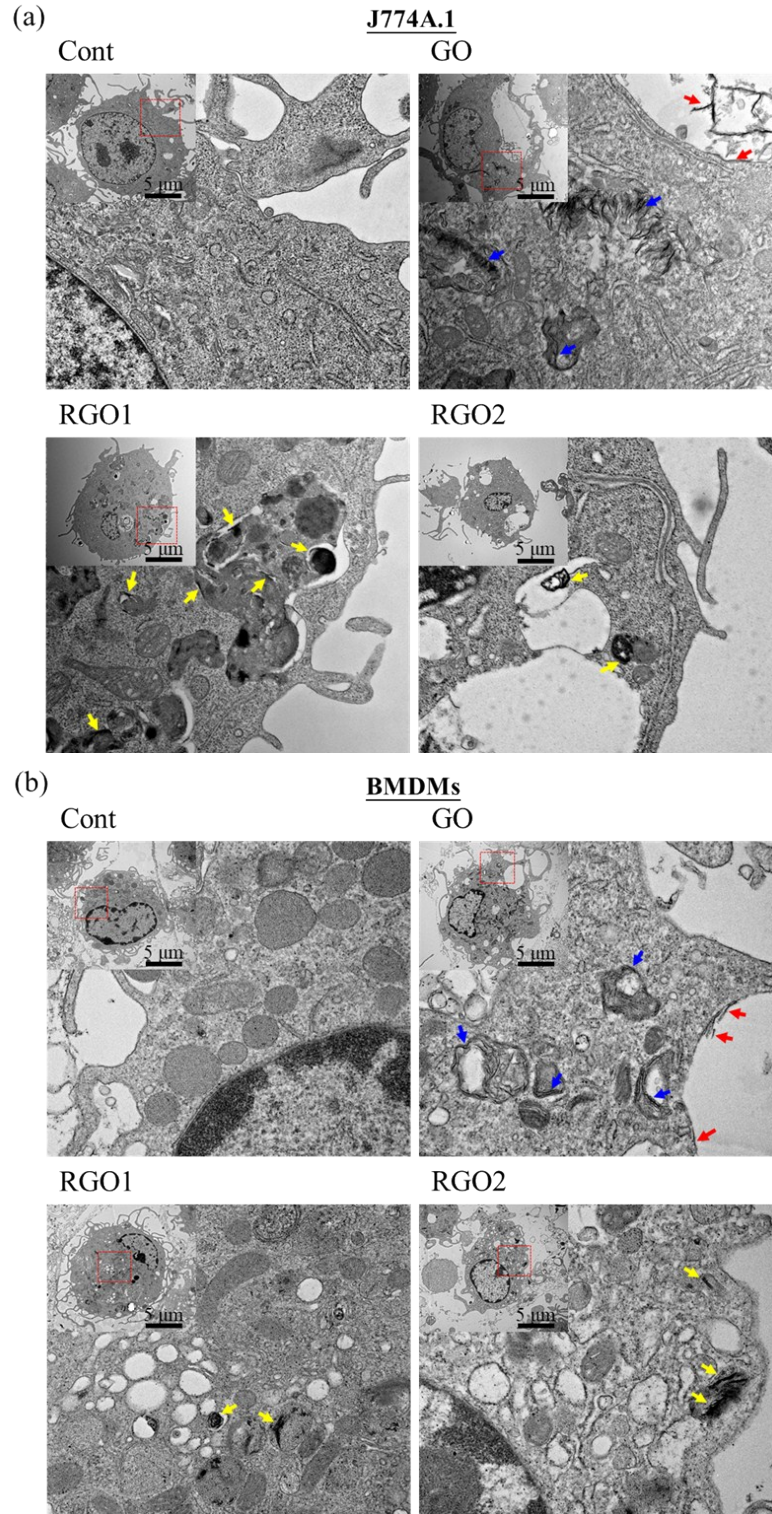


Figure S10. Intracellular localization of GO materials. Representative high resolution TEM images of J774A.1 (a) cells and BMDMs (b) treated with 10 $\mu\text{g}/\text{mL}$ GO and RGO materials for 24 h. Red arrows indicate GO materials on plasma membrane, blue arrows denote GO materials in phagosomes within the cytosol, and yellow arrows point at the stacked sheets of RGO localized in phagosomes within the cytoplasm. Scale bar: 5 μm .

| DMEM+ 10% FBS | PDI | ζ -potential (mV) |
|------------------|-------------|-------------------------|
| GO | 0.854±0.028 | -4.9±1.2 |
| RGO1 | 0.726±0.064 | -5.8±0.4 |
| RGO2 | 0.745±0.067 | -5.7±0.7 |

Table S1. PDI and ζ -potential of the materials. PDI and ζ -potential of the materials (at 10 $\mu\text{g/mL}$) in culture medium with 10% FBS were measured on a Zeta sizer (n=8).