Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information

Limitations of MTT and CCK-8 assay for evaluation of graphene cytotoxicity

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Experimental section

1. Materials and apparatus

HepG2 cells and Chang liver cell lines were obtained from Harbin Medical University. MTT and Pluronic F68 were purchased from Sigma (Sigma-Aldrich Co. Ltd.). CCK-8 was obtained from Dojindo (Dojindo Co. Ltd., Japan). Trypsin-EDTA solution, Penicillin and Streptomycin were supplied by Beyotime Institute of Biotechnology. DMEM (Dulbecco's Modified Eagle Medium) and FCS (Fetal Calf Serum) were purchased from Thermo Fisher (Thermo Fisher, Co. Ltd.). The sterile water, PBS was made in our lab. Graphene (G) was purchased from Instituted of Coal Chemistry, Chinese Academy of Sciences (Tai Yuan. China).The cells were sedded in Cell flasks (Corning Co. Ltd. USA).The cells viability was assayed by Multiscan MK3 ELIASA (Thermo fisher scientific, USA). The images of cells were obtained by Inverted Olympus IX71 microscope (Japan) and Inverted Olympus IX71 fluorescence microscope (Japan). The other regents are analytical.

2. Sample preparation

In order to avoid the disturbance of the impurities from formation of graphene, purification procedures were carried out. Firstly, we increased the rinse times with deionized water to get rid of the trace acid and alkaline substance. Secondly, the graphene stock solution was suffered from sterilization under high temperature or through sterile membrane. Thirdly, the stock solution was prepared enough and avoided repeatedly preparation.

100 mg graphene was added in the 100 mL PBS solution in flask containing 1% Pluronic F68, and ultrasonic was then carried out for 2 h to improve dispersion, holding the bottom without sediment. At last, the aqueous solution was sterilized under the high temperature.

In cell flask, HepG2 cell line and Chang liver cell line were cultured in DMEM supplement with 10% FBS and 1% Penicillin and streptomycin at 37 °C, 5% CO_2. The cells are maintained in logarithmic phase by serial sub-cultivation for further analysis. The adherence cells were gathered by Trypsin solution for counting.

In order to avoid possibility deviation of cell multiple batches, the same passage number cells were simultaneously executed to MTT/CCK-8 assay. For each cell plate, to refrain the exponential phase cell from invoking significance discrepancy, the cell adherence time was strictly kept with overnight (14 h).

3. MTT assay and CCK-8 assay

In logarithmic phase HepG2 cell/Chang liver cell (the fifth generation) was pooled by trypsin to counting. At the density of 3×10^4 mL^{-1} (Chang liver cell 4×10^4 mL^{-1}) , 100 μL containing HepG2 (Chang liver cell) cell solution was added into the 96 cell plate to culture overnight for sufficiently adherence at 37 °C, 5% CO_2 incubator, then under the difference concentrations (2.5 $\mu g \cdot mL^{-1}$ ~ 100 $\mu g \cdot mL^{-1}$), the graphene solution were added into the cell plate as the treated group, and control group treated with equal volume PBS, after 24 h, the cell culture plate was added yellow color MTT solution 10 μL in each cell, and kept the plate from light in the cell incubator for 4 h to form the crystal, and then dropped out the culture medium to add 150 μL DMSO, centrifugalized at 1000 rpm for 10 mins to transfer another blank plate to reduce the interference from graphene. Finally, the plate was measured under the wave length of 570 nm by Multiscan MK3 ELIASA. Each treated group was repeatedly three times at different logarithmic phase. Before addition the CCK-8 reagents, the procedure of cell seeding was as same as the

MTT assay method. The cell culture medium was transferred to discard and then added new cell culture without graphene. 10 μL CCK-8 solution was added in to the plate, the plate was read by the equipment Multiscan MK3 ELIASA at the length of 450 nm after 1.5 h. Each treated group was also repeated three times at different logarithmic phase.

4. Adsorption interference

MTT was dissolved in PBS solution to arrive at the concentration of 5 $mg \cdot mL^{-1}$. MTT solution (90 μL) was added into the reaction tube containing 2910 μL PBS solution as the control group, the treated group contained 150 μg graphene with MTT solution (90 μL), and then four treated tubes were placed in oven with 37 °C. After the interval phase 0.5, 1, 1.5 and 2 h, respectively, the tubes were suffered from centrifugation 10mins at 15000 rpm, next, transferred the supernatant to the colorimetric device for analysis at the 377 nm with UV/vis spectrometer (Lab Tech, Beijing China). Each interval phase was repeated three times. Similarly, CCK-8 solution 120 μL was added into the reaction tube containing 2880 μL PBS solution and 150 μg graphene, the 4 tubes were setting in oven at 37 °C for various time interval phase (0.5, 1, 1.5, and 2 h), then the tube was centrifuged at 15000 rpm, 10mins, a aliquot supernatant was placing the colorimetric device for measure at the 267 nm with UV/vis spectrometer (Lab Tech, Beijing China). Each interval phase was repeated three times.

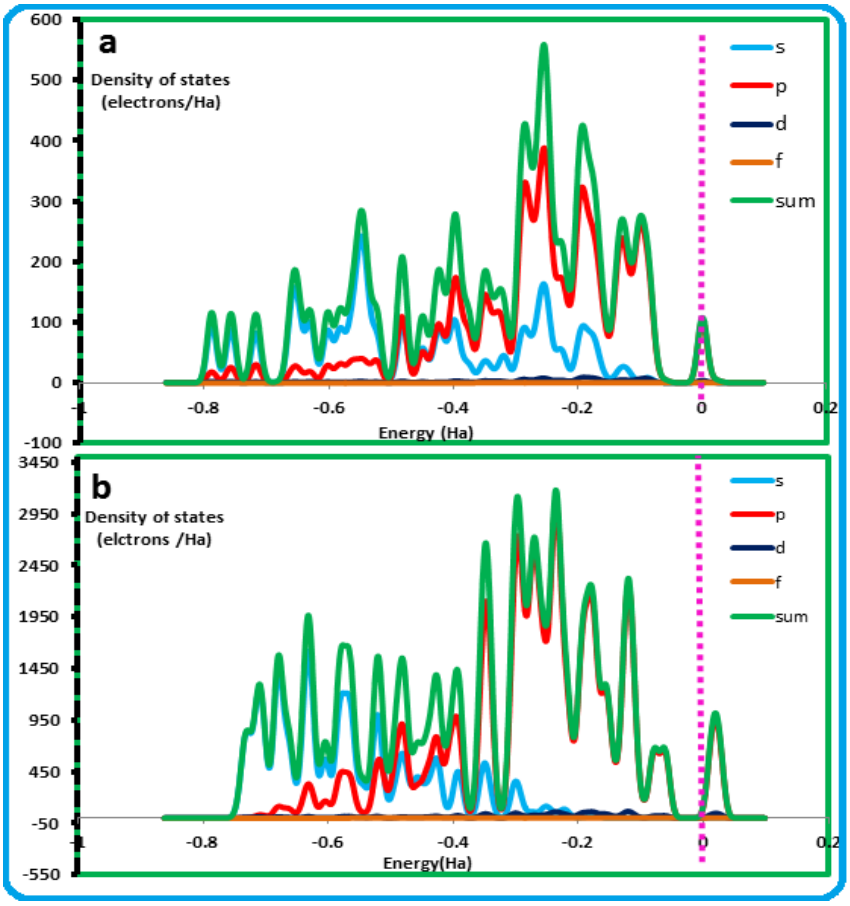

Fig. S1. The charge distribution and density of states of MTT (a) and graphene (b).