Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting information for

Combined effects of graphene oxide and zinc oxide nanoparticle on human A549 cells: Bioavailability, toxicity and mechanisms

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Methods

Analysis of Zn^{2+} adsorption by GO

The 20 mg/L Zn^{2+} solution was prepared by zinc sulfate in RPMI-1640 media with 0, 1, 5 and 10 mg/L GO, respectively. The mixture was stirred and shaken for 24 h at 180 rpm and 37ºC. Then the samples were collected at 0, 10, 20, 60, 180, 480, and 1440 min with three replicates for the measurement of Zn^{2+} concentration. GO and adsorbed Zn^{2+} in the samples was removed by Nanosep. The concentrations of Zn^{2+} were measured by FAAS.

Figures

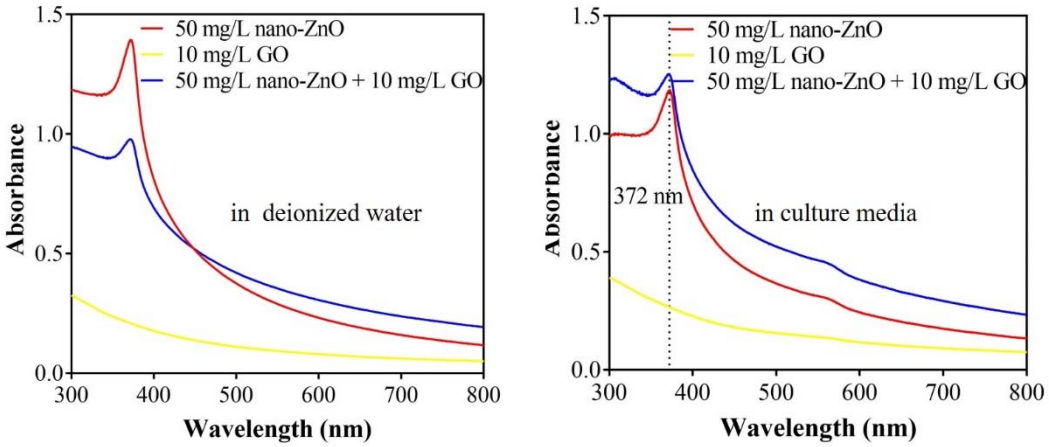

Figure S1 Characteristic peak of nano-ZnO with or without GO in the deionized water (left) and culture media (right) measured by UV-vis spectrophotometer.

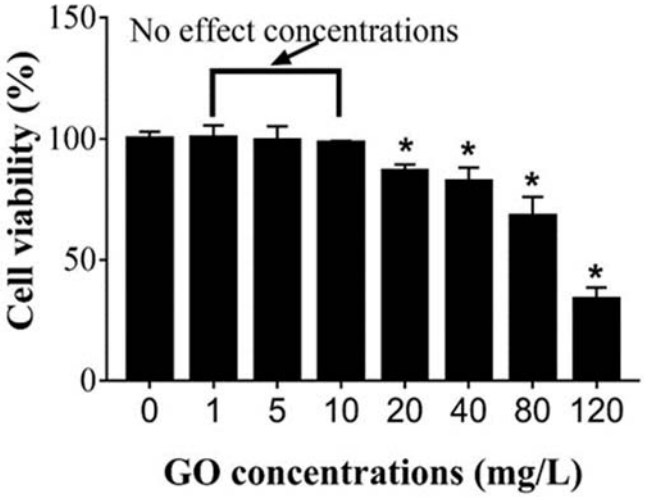

Figure S2 Cell viability of A549 cells exposed by GO alone. All data were expressed as the mean ± SD. All differences were identified by one-way ANOVA followed by Tukey post hoc test. * indicates *p*-value <0.05 compared with the control (0 mg/L) group.

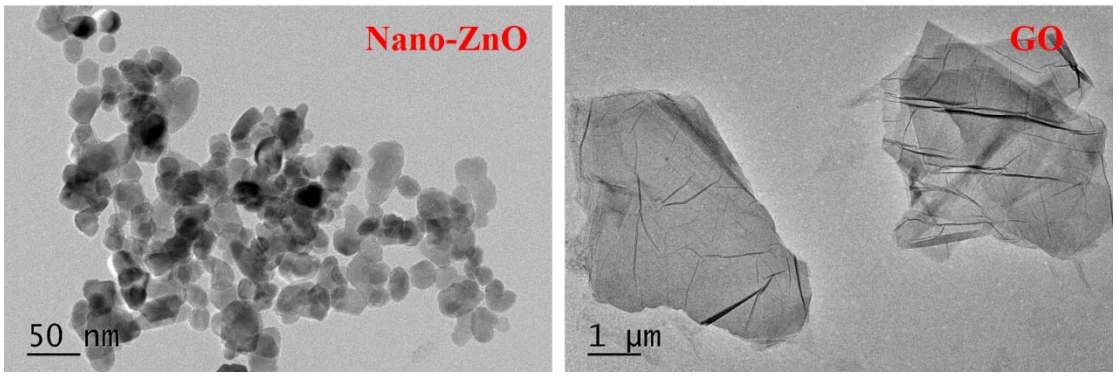

Figure S3 TEM images of nano-ZnO and GO used in this study.

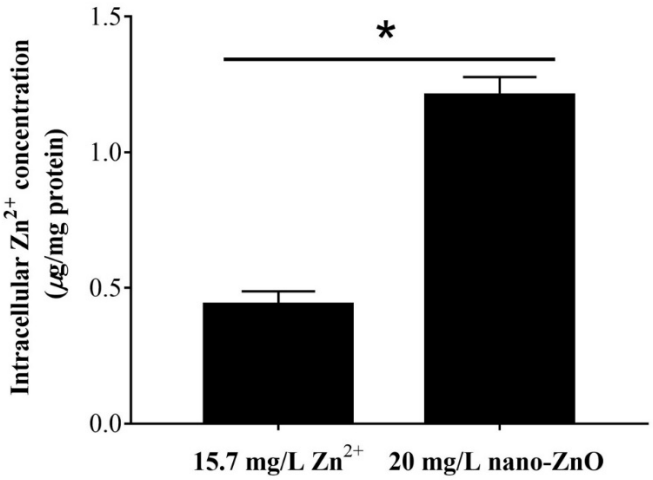

Figure S4 Accumulation of Zn^{2+} in A549 cells after nano-ZnO and zinc sulfate exposure. The Zn concentrations were normalized by the protein concentrations. The exposure concentrations of nano-ZnO and zinc sulfate were 20 mg/L and 15.7 mg/L, respectively, which had an equivalent of zinc contents. All data were expressed as the mean ± SD. Difference was identified by t-test. * indicates *p*-value <0.05.

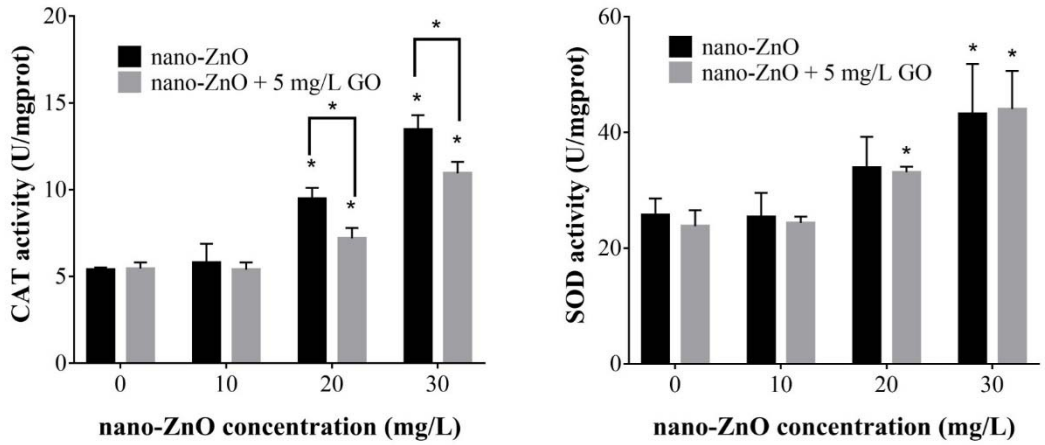

Figure S5 Influence of GO on activities of CAT and SOD in A549 cells. All data were expressed as the mean ± SD. All differences were identified by one-way ANOVA followed by Tukey post hoc test. ***** indicates *p*-value <0.05 compared with the control group (0 mg/L) or between two special groups.

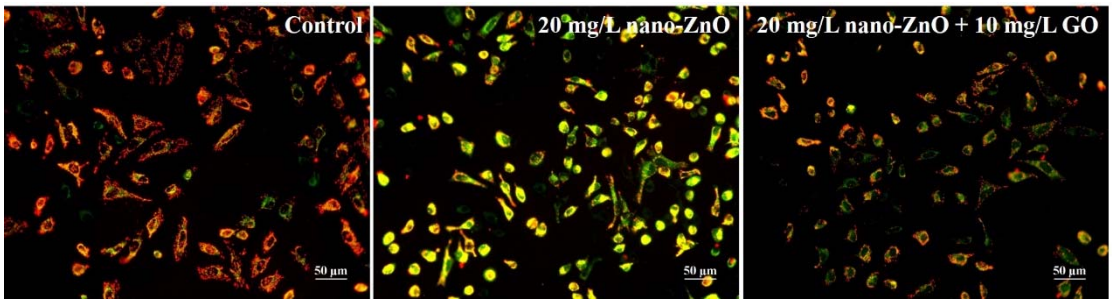

Figure S6 Fluorescence images of A549 cells taken by microscopy in the control, nano-ZnO-alone exposure and coexposure groups. The exposure concentrations of nano-ZnO and GO were 20 mg/L and 10 mg/L, respectively. Increase of green fluorescence indicates the mitochondrial depolarization.

Tables

Table S1 Z-average diameters of nano-ZnO and GO in deionized water and RPMI 1640 media determined by dynamic light scattering

	Z-average diameter in deionized water (nm)	Z-average diameter in RPMI 1640 media (nm)
nano-ZnO	290.4	67.75
GO	3714	658.8

Table S2 The 40 hydrophilic metabolites and 20 lipophilic metabolites identified in A549 cells

Hydrophilic metabolites				Lipophilic metabolites	
1	Ketoleucine	21	Creatinine	1	Cholesterol C-18
2	L-Isoleucine	22	L-Lysine	2	Cholesterol C-26
3	(R)-3-Hydroxybutyric acid	23	Phosphocreatine	3	Cholesterol C-27
4	Isobutyric acid	24	Phosphorylcholine	4	Fatty acyl chain terminal CH3
5	Lactate	25	Acetylcholine	5	Cholesterol C-21
6	L-Alanine	26	Trimethylamine N-oxide	6	Cholesterol C-19
7	Acetic acid	27	3,7-Dimethyluric acid	7	Fatty acyl chain (CH2)n
8	Glutamine	28	Taurine	8	Cholesterol ester
9	L-Methionine	29	Glycine	9	Fatty acyl chain –CH2CH=
10	Glutamate	30	Ethanol	10	Free fatty acids
11	(S)-Malate	31	Phenylacetylglycine	11	Fatty acyl chain =CHCH2CH=
12	Succinate	32	Dimethylglycine	12	Sphingomyelin N(CH3)3
13	2-Oxoglutarate	33	trans-Aconitic acid	13	Phosphatidylcholine N(CH3)3
14	Pyruvate	34	Glutathione	14	Phosphatidylethanolamine Glyceryl CH2 sn3
15	Citrate	35	ATP/AMP/ADP	15	Phosphatidylcholine Glyceryl CH2 sn3
16	Spermidine	36	L-Gulonolactone	16	Glycerol backbone C-1 H2/C-3 H2
17	L-Aspartic acid	37	D-Glucose	17	Phospholipid glycerol backone C-2 H2
18	Sarcosine	38	Uridine 5'-monophosphate	18	Triglycerides glycerol backbone C-2 H2
19	L-Asparagine	39	3-Methyladenine	19	Fatty acyl chain –HC=CH–
20	Trimethylamine	40	Formate	20	PTE plasmalogen

Table S3 Significantly changed metabolites and their fold changes in A549 cells after nano-ZnO and/or GO exposure groups

Metabolites	10 mg/L GO	20 mg/L nano-ZnO	20 mg/L nano-ZnO + 10 mg/L GO
Citrate	\	0.656533	0.677874
Acetylcholine	\	0.680969	0.730527
Spermidine	\	0.564072	0.594175
L-Gulonolactone	\	0.417023	0.5821
Pyruvate	0.76698	1.565182	1.517903
L-Isoleucine	\	2.487334	1.965886
Phosphorylcholine	\	0.709965	0.803116
Glutathione	\	0.78852	\
Trimethylamine	\	0.56313	0.632374
Creatinine	\	0.65399	0.724582
L-Lysine	\	0.662265	0.739519
Trimethylamine N-oxide	\	0.754242	0.85486
Ketoleucine	\	0.754242	\
L-Methionine	\	0.804503	0.818963
Glutamate	\	0.777401	0.825202
Glutamine	\	0.837244	0.852399
3-Methyladenine	\	0.776539	\
Glycine	\	\	1.308351
ATP/ADP/AMP	\	0.67038	\
Dimethylglycine	\	\	1.710787

Table S4 The KEGG pathway annotation of altered metabolites in the nano-ZnO-alone exposure and the coexposure groups

Class	Pathway	Match rate	*p*-value
Translation	Aminoacyl-tRNA biosynthesis	6/75	2.80E-05
Metabolism of other amino acids	D-Glutamine and D-glutamate metabolism	2/11	0.0038039
Metabolism of other amino acids	Glutathione metabolism	4/38	2.61E-04
Energy metabolism	Methane metabolism	3/34	0.0028797
Energy metabolism	Nitrogen metabolism	3/39	0.0042761
Carbohydrate metabolism	Citrate cycle (TCA cycle)	2/20	0.012534
Carbohydrate metabolism	Glycolysis or Gluconeogenesis	2/31	0.028958
Carbohydrate metabolism	Butanoate metabolism	2/40	0.046347
Carbohydrate metabolism	Ascorbate and aldarate metabolism	2/45	0.057315
Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	2/50	0.069103
Carbohydrate metabolism	Pentose phosphate pathway	2/32	0.030727
Amino acid metabolism	Alanine, aspartate and glutamate metabolism	3/24	0.0010304
Amino acid metabolism	Glycine, serine and threonine metabolism	3/48	0.0076938
Amino acid metabolism	Cysteine and methionine metabolism	3/56	0.011789
Amino acid metabolism	Lysine degradation	2/47	0.061937
Amino acid metabolism	Arginine and proline metabolism	5/77	4.01E-04
Amino acid metabolism	Valine, leucine and isoleucine biosynthesis	3/27	0.0014641
Amino acid metabolism	Valine, leucine and isoleucine degradation	2/40	0.046347
Nucleotide metabolism	Purine metabolism	3/92	0.043656
Lipid metabolism	Glycerophospholipid metabolism	2/39	0.044259