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Supporting Information

A combined toxicity study of zinc oxide nanoparticles and vitamin C in food additives

Yanli Wang[a], Lulu Yuan[a], Chenjie Yao[a], Lin Ding[a], Chenchen Li[a], Jie Fang[a], Keke Sui[a], Yuanfang Liu[b]*, Minghong Wu[a]*

[a]Institute of Nanochemistry and Nanobiology, Shanghai University, Shanghai 200444, P.R. China, [b]Beijing National Laboratory for Molecular Sciences, Department of Chemical Biology, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China. *e-mail: yliu@pku.edu.cn; mhwu@shu.edu.cn

Characterization of ZnO NPs

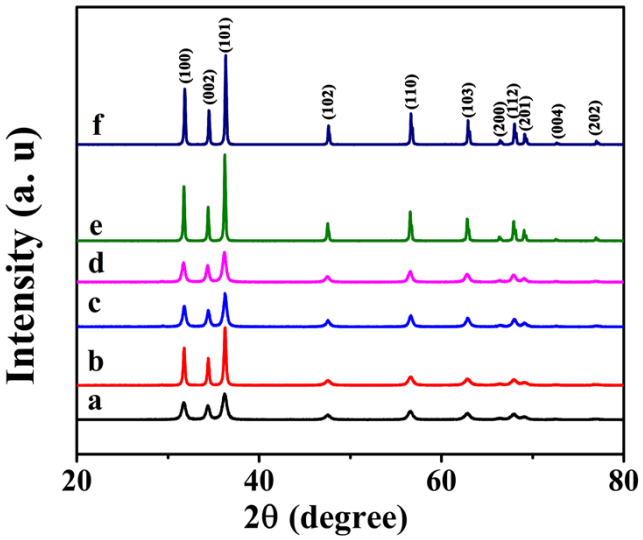

Fig. S1 XRD of ZnO NPs. (a) ZnO-1; (b) ZnO-2; (c) ZnO-3; (d) ZnO-4; (e) ZnO-5; (f) ZnO-6. All diffraction peaks can be perfectly indexed to hexagonal wurtzite structure of ZnO (JCPDS Card No. 36-1451).

Table S1. Physicochemical Characterizations of ZnO NPs.

Samples	Purity (%)	Size [nm] (in water)	Size [nm] (in culture medium)	Zeta potential [mV] (in water)	Zeta potential [mV] (in culture medium)
ZnO-1	99.59	149±51	185±75	-20.6±0.7	-6.2±0.8
ZnO-2	99.33	121±41	173±48	-18.8±1.1	-6.7±0.7
ZnO-3	99.97	112±42	196±74	-21.4±2.0	-5.9±1.2
ZnO-4	99.56	112±40	148±60	-17.4±1.7	-5.5±1.0
ZnO-5	99.96	125±48	162±76	-19.4±0.7	-6.4±2.3
ZnO-6	99.97	133±51	159±75	-18.3±0.6	-5.9±0.7

Cytotoxicity evaluation

There are some differences on the cytotoxicity induced by six kinds of ZnO NPs (Fig. S2). In GES-1, the biocompatibility of ZnO-1 and ZnO-2 is better than others. At the concentration of 25 mg/L, cell viability remained more than 90%. For ZnO-3, at a concentration of 20 mg/L, cell viability maintained above 90%. However, for ZnO-4, ZnO-5, ZnO-6, cell viability remained more than 90% only at the concentration lower than 15 mg/L. Cytotoxicity of six kinds of ZnO NPs are shown in Fig.S3 indicating that ZnO NPs inside the cells cause no cytotoxicity when concentrations are below 15 mg/L at 24 h incubation time. And cytotoxicity induced by ZnO NPs on the neural stem cells (NSCs) is more remarkable than on the gastric epithelial cell line (GES-1). With increasing concentration, the cell viability is sharply decreased showing a dose-dependent relationship.

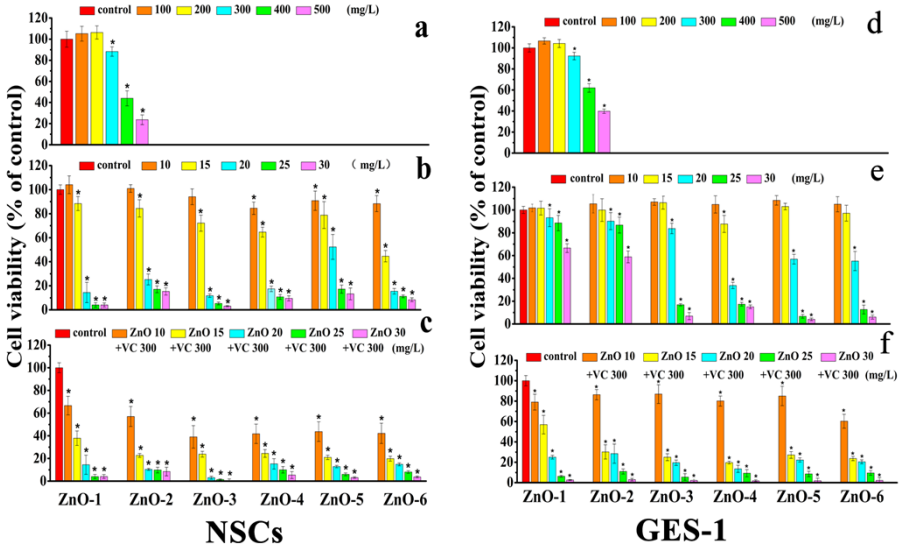

Fig. S2 Cytotoxicity evaluation of ZnO NPs, Vc and ZnO NPs plus Vc treatment for 24 h. (a, d) exposed to Vc only; (b, e) ZnO NPs only; (c, f) different concentrations of ZnO NPs plus 300 mg/L Vc.*$p<0.05$ compared with control.

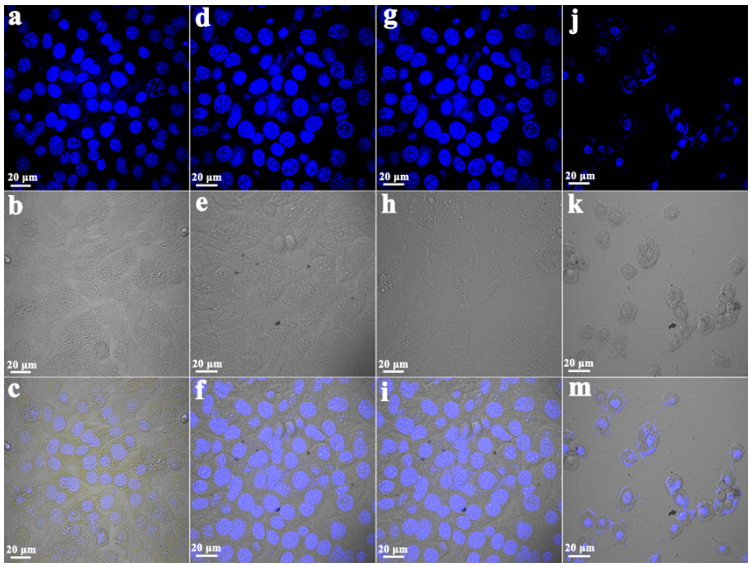

Fig. S3 Observation of the morphological changes of NSCs after treatment with ZnO NPs, Vc and ZnO NPs plus Vc. (a, b, c) control; (d, e, f) 15 mg/L ZnO NPs; (g, h, i) 300 mg/L Vc; (j, k, m) 15 mg/L ZnO NPs plus 300 mg/L Vc. (a, d, g, j) stained by DAPI; (b, e, h, k) in the bright-field; (c) merge of (a) and (b); (f) merge of (d) and (e); (i) merge of (g) and (h); (m) merge of (j) and (k).

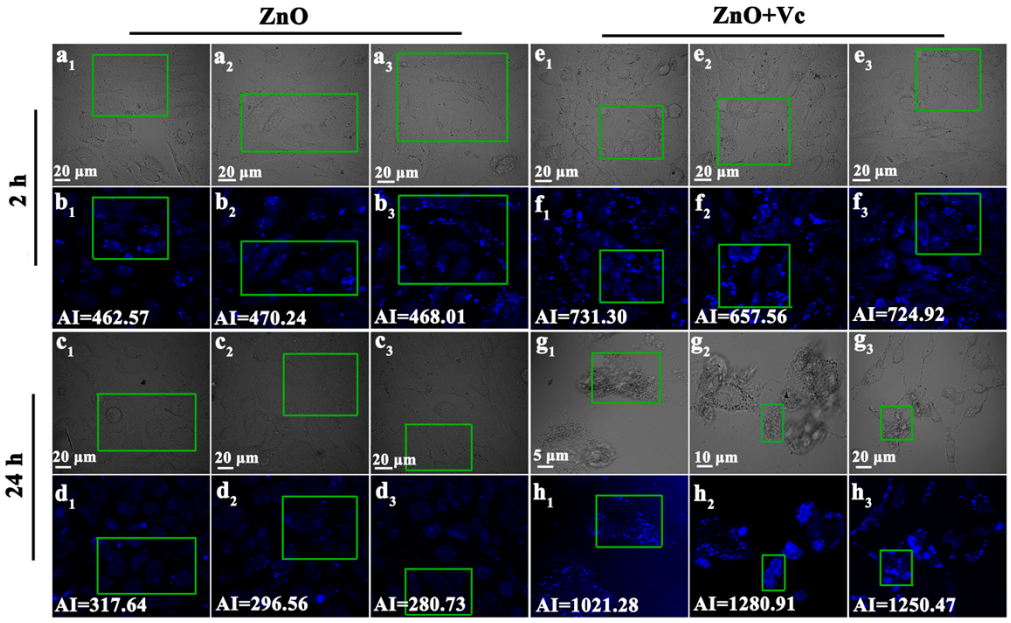

Fig. S4 Analysis of average fluorescent intensity of zinc ion in GES-1 after incubated with ZnO NPs and ZnO NPs plus Vc for 2 h and 24 h respectively. (a-h) average fluorescent analysis of confocal image: (a-d) ZnO NPs; (e-h) ZnO NPs plus Vc. AI=Average Fluorescent Intensity.

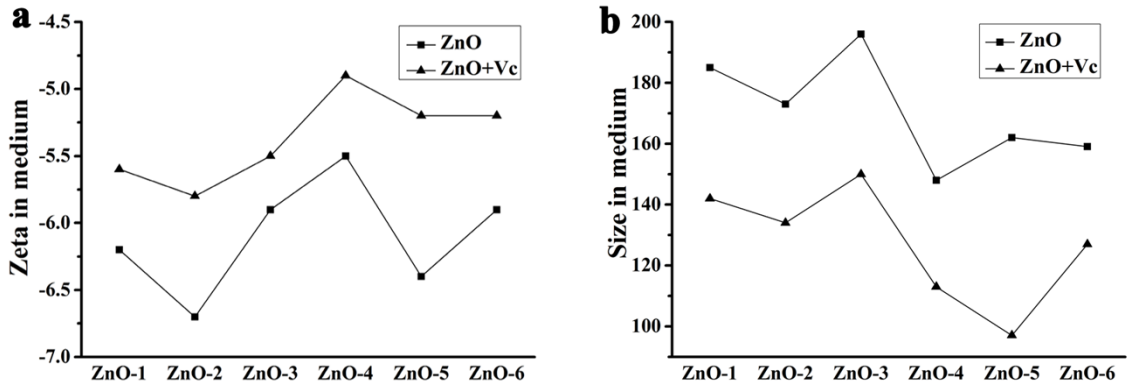

Fig. S5 Zeta potential and size of ZnO NPs with or without Vc in medium. (a) Zeta potential; (b) size.

Combined toxicity study *in vivo*

At the endpoint of experiment, we also investigated some other index. The results are shown in Table S2. Though the other related liver and kidney index keep unchanged (Table S2), the significant liver index change after oral repeated injection for 30 days show that the long time repeating take may have more serious potential harm to human beings health.

Table S2. Serum biochemical assays of three groups

	Groups	ALT (U/L)	AST (U/L)	TBIL (umol/L)	BUN (mmol/L)	Cr (umol/L	ALP (U/L)
First	Control	24.22±2.35	85.69±18.94	17.21±3.81	9.26±0.82	30.57±2.60	138.77±3.66
	ZnO	24.82±2.62	101.32±16.53	23.17±2.43	12.04±2.00	39.97±8.84	155.55±13.50
	Vc	25.20±3.50	103.28±18.15	23.39±6.14	11.07±1.86	33.80±8.16	147.23±18.50
	ZnO +Vc	25.47±2.87	101.56±11.56	20.47±8.03	10.31±1.47	37.24±3.97	163.29±8.83*
Second	Control	27.00±3.32	113.54±12.43	10.33±0.73	10.38±1.70	30.87±2.86	162.01±8.40
	ZnO	25.44±3.79	124.42±4.37	10.11±1.72	9.85±2.27	33.04±2.67	157.31±4.72
	Vc	24.48±0.81	112.48±15.44	10.45±1.79	11.53±1.47	33.75±3.23	159.42±13.33
	ZnO +Vc	28.17±2.07	112.85±9.51	11.14±1.47	9.67±1.85	31.69±3.18	164.1±11.51
Third	Control	27.28±2.35	111.50±8.70	9.36±0.76	9.85±1.57	28.87±1.42	152.63±11.88
	ZnO	27.60±3.40	129.03±9.18	11.22±1.87	12.28±0.56	36.52±5.26	152.24±2.86
	Vc	31.60±3.49*	119.58±11.09	13.10±1.60*	13.61±2.12	29.18±1.42	170.46±26.12
	ZnO +Vc	27.66±2.82	123.30±3.40	13.28±2.23*	14.40±1.62*	35.96±5.30	175.40±10.36

Data are presented as mean±SD of 5 mice, *P<0.05 compared with control.

Combined cytotoxicity study of TiO_2-NPs and Vc

Four kinds of TiO_2-NPs were used in this study, namely TiO_2-1 (nanoparticles with an average diameter of 20 nm, hydrophilic), TiO_2-2 (nanoparticles with an average diameter of 20 nm, oleophylic), TiO_2-3 (nanoparticles with an average diameter of 50 nm, highly dispersed food grade) and TiO_2-4 (nanoparticles with an average diameter of 50 nm, food grade). TiO_2-1, TiO_2-2 (Wan jingxin materials Co., Ltd. Hangzhou, China), TiO_2-3, TiO_2-4 (Jianghu industrial Co., Ltd. Shanghai, China).The TEM images show the size and morphology of TiO_2-NPs (Fig.S6). From TEM images show that the sizes of TiO_2-NPs are diverse. However, there is no big difference of the sizes of TiO_2-NPs in water or in medium. The purities of these NPs are higher than 95% according to X-ray fluorescence (XRF) analyses. Based on the X-Ray diffraction (XRD) patterns the TiO_2-NPs are in anatase phase.

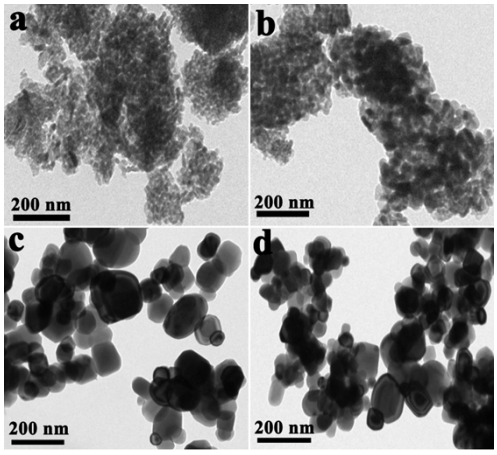

Fig. S6 TEM images of TiO_2 NPs. (a) TiO_2-1; (b) TiO_2-2; (c) TiO_2-3; (d) TiO_2-4.

Table S3. Physicochemical Characterizations of TiO_2- NPs

Samples	Purity (%)	Size [nm] (in water)	Size [nm] (in culturemedium)	Zeta potential[mV] (in water)	Zeta potential[mV] (in cuture medium)
TiO_2-1	95.51	244.7±37	280.8±32	-45.2±1.9	-27.4±1.1
TiO_2-2	95.44	196.1±46	248±26	7.8±1.3	2.7±0.9
TiO_2-3	98.11	260.6±43	291.4±45	-28.9±0.7	-21.8±0.7
TiO_2-4	95.89	248.5±23	247.1±34	16.6±0.9	12.3±0.6

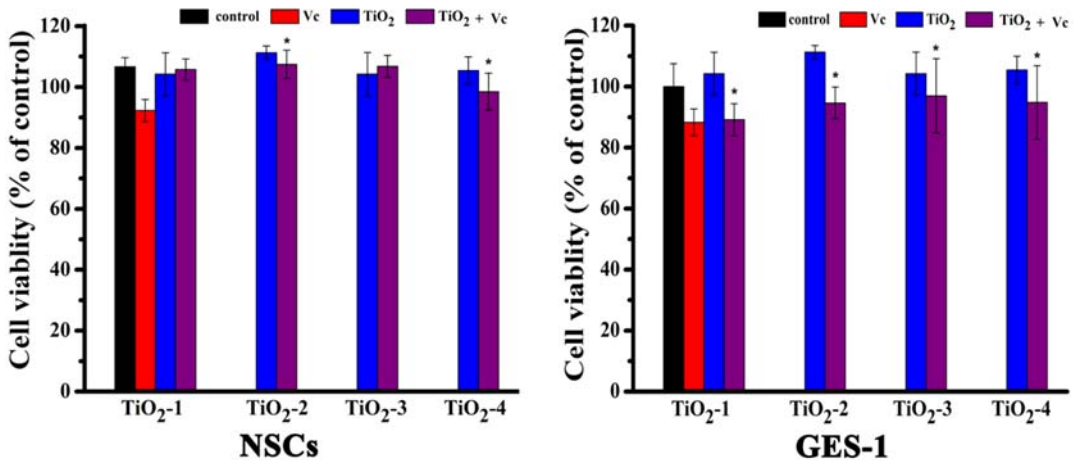

Fig. S7 Cytotoxicity evaluation of exposure to TiO_2 NPs and TiO_2 NPs plus Vc for 24 h. (a, d) exposed to TiO_2 only; (b, e) different concentrations of TiO_2 NPs plus 300 mg/L Vc; (d, f) 50 mg/L TiO_2 NPs plus different concentrations of Vc. *p<0.05 compared with TiO_2 treated alone.

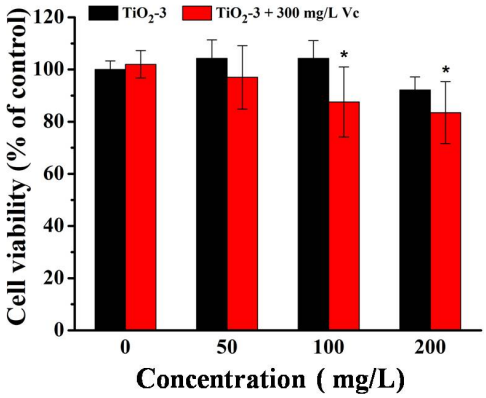

Fig. S8 Combined toxicity of TiO_2-3 at diffrent concentration plus 300 mg/L Vc in GES-1for 24 h. *p<0.05 compared with exposure to TiO_2-3 alone.