Electronic Supplementary Material (ESI) for Toxicology Research

A comparative study of cellular uptake and cytotoxicity of multi-walled carbon nanotube, graphene oxide, and nanodiamond

Xiaoyong Zhang,*[a,b] Wenbing Hu,[a] Jing Li,[a] Lei tao,[b] and Yen wei[b]

Preparation and characterization of CNMs

ND with individual sizes ranging from 2–10 nm (Gansu Gold Stone Nano.Material Co., Ltd.,), It was purified in a concentrated H_2SO_4–HNO_3 (3:1) solution at 80 °C for 7 days. The product was separated by centrifugation and thoroughly washed with deionized water. MWCNT (95% purity, contained 1-2% metal catalysts) commercially prepared by the CVD method was obtained from Shenzhen Nano Port. Co. Ltd. The CNT was several micrometers to tens of micrometers in length with diameter in the range of 20–40 nm. The CNT for biological assays was prepared according to our previous report [1], as-received CNT was first oxidized by concentration HNO_3 for 24 h at the temperature of 80 °C, and then the purified CNT was irradiated at the dosage of 250 KGay in the present of concentration H_2SO_4 to induce more defects on the surface of CNT. And then the irradiated CNT was further oxidized a 1:3 V/V mixture of HNO_3 (65%) and H_2SO_4 (98%) at 80 °C for 48 h with water bath. The resulting solid was collected by filtration and then dialyses with deionized water until the pH value approached to 6. The dark product was dried at 70 °C in a vacuum oven overnight. GO was prepared by using a modified Hummers method [2]. The obtained CNMs products were characterized by a series of characterization techniques; detailed characterization procedure was discribed below.

Transmission electron microscopy (TEM) images were recorded on a JEM-1200EX microscope operated at 100 kV; the TEM specimens were made by placing a drop of the nanoparticle suspension on a carbon-coated copper grid. The FT-IR spectra were obtained in a transmission mode on a Perkin-Elmer Spectrum 100 spectrometer (Waltham, MA, USA). Typically, 16 scans at a resolution of 1 cm^{-1} were accumulated to obtain one spectrum. Thermal gravimetric analysis (TGA) was conducted on a TA instrument Q50 with a heating rate of 20 °C/min. Samples weighing between 10 and 20 mg

were heated from 25 to 700 °C in air flow (60 mL/min), N_2 as the balance gas (40 mL/min). The zeta-potential and size distribution of ND and ND-CDs in water and PBS were determined using a zeta Plus particle size analyzer (ZetaPlus, Brookhaven Instruments, Holtsville, NY).

Cell line and experiment design

Human epithelial cervical cancer cells (Hela cells) were purchased from the Institute of Biochemistry and Cell Biology, Shanghai, China, which were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} of streptomycin. Cell culture was maintained at 37 °C in a humidified condition of 95% air and 5% CO_2 in culture medium. Culture media was changed every three days for maintaining the exponential growth of the cells.

Cell morphology changes

The effects of CNMs on the cell morphology changes were observed by optical microscopy. Briefly, cells were seeded in 6-well microplates at a density of 1×10^5 cells mL^{-1} in 2 mL of respective media containing 10% FBS. After cell attachment, plates were washed with PBS and the cells were treated with complete cell culture medium, or 40 μg mL^{-1} of ND, CNT, and GO prepared in 10% FBS containing media for 2 h, Then all samples were washed with PBS three times to remove the uninternalized carbon nanomaterials. Then continued to incubate with cell culture medium for up to 24 h, and cell morphology changes were observed by using an optical microscope (Motorized inverted system microscope IX81/IX81–ZDC, Japan), the overall magnification was × 100.

Results and discussion

The aggregation state of CNMs in PBS was investigated by optical microscopy, the concentration of CNMs solution was 200 μg mL^{-1}. Compared with CNT and GO, only a few aggregates were found

by microscopy at the magnification of 400 (Figure S1). These results indicated that the aggregates of ND were much smaller than that of CNT and GO. These results could also be used to interprete why the cell uptake ratios of ND is significant greater than that of CNT and GO.

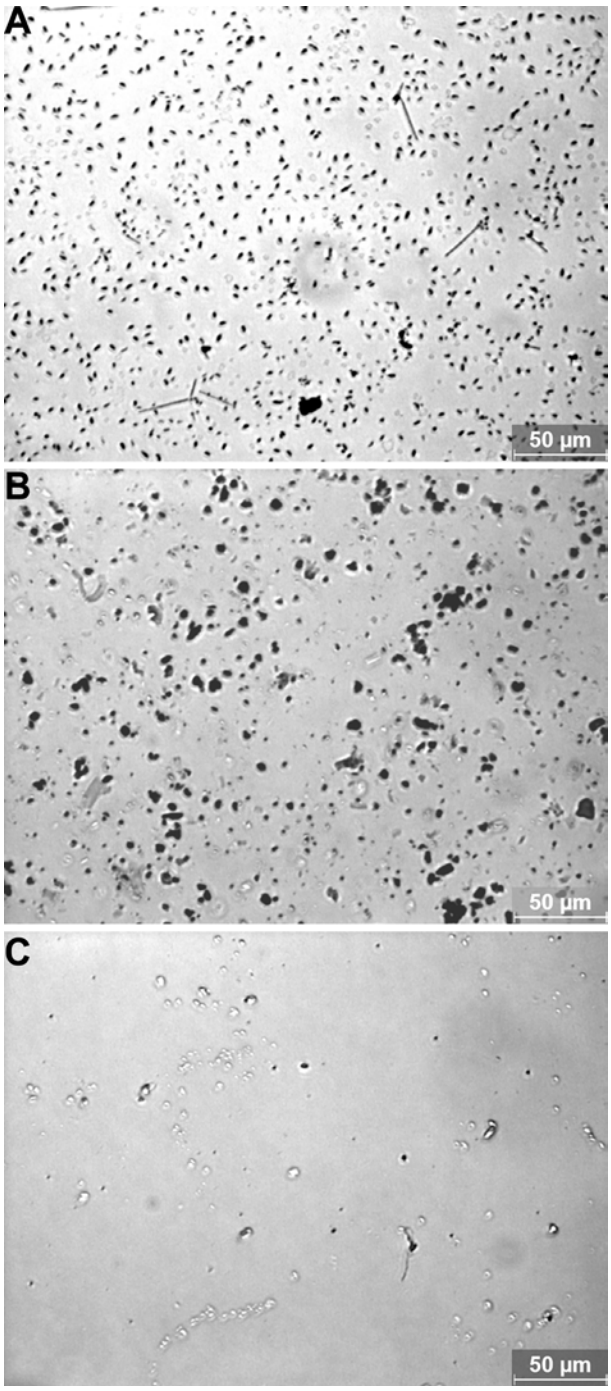

Figure S1. Optical microscopy images of CNMs, (A) CNT, (B) Go and (C) ND.

Cells morphology changes

The influence of CNMs on Hela cells were directly observed by optical microscopy. As shown in Figure S2, cells showed significant different biological responses when exposed to 40 μg mL^{-1} of ND, CNT, and GO for 24 h. We observed that ND showed relative low cytotoxicity compared with that of CNT and GO. It is worth to note that a lot of cell floating was observed after incubated with 40 μg mL^{-1} of GO for 24 h (Figure S2D). The microscopy observations given us the preliminary impression that GO is high toxicity to Hela cells.

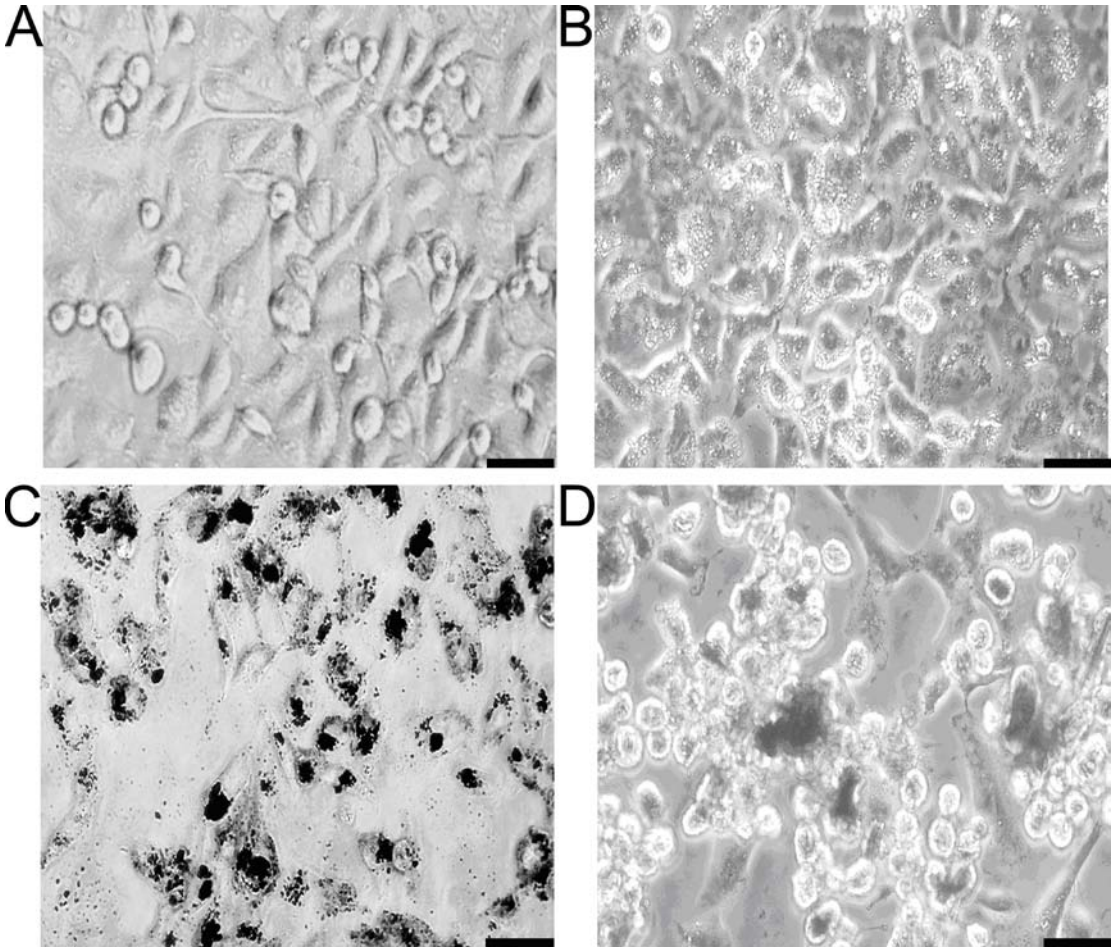

Figure S2. Cells morphology changes when cells were exposed to 40 μg mL^{-1} of CNMs. (A) control, (B) ND (C) CNT, (D) GO. Scale bar = 30 μm.

Reference

1. Zhang, X.; Zhu, Y.; Li, J.; Zhu, Z.; Li, W.; Huang, Q., Tuning the cellular uptake and cytotoxicity

of carbon nanotubes by surface hydroxylation. *J Nanopart Res* **2011,** *13* (12), 6941-6952.

2. Hummers Jr, W. S.; Offeman, R. E., Preparation of graphitic oxide. *J. Am. Chem. Soc* **1958,** *80* (6), 1339-1339.