Electronic Supplementary Material (ESI) for Toxicology Research.

Supplementary Information

Toxicity Mechanism in Fetal Lung Fibroblast Cells for Multi-Walled Carbon Nanotubes defined by Chemical Impurities and Dispersibility

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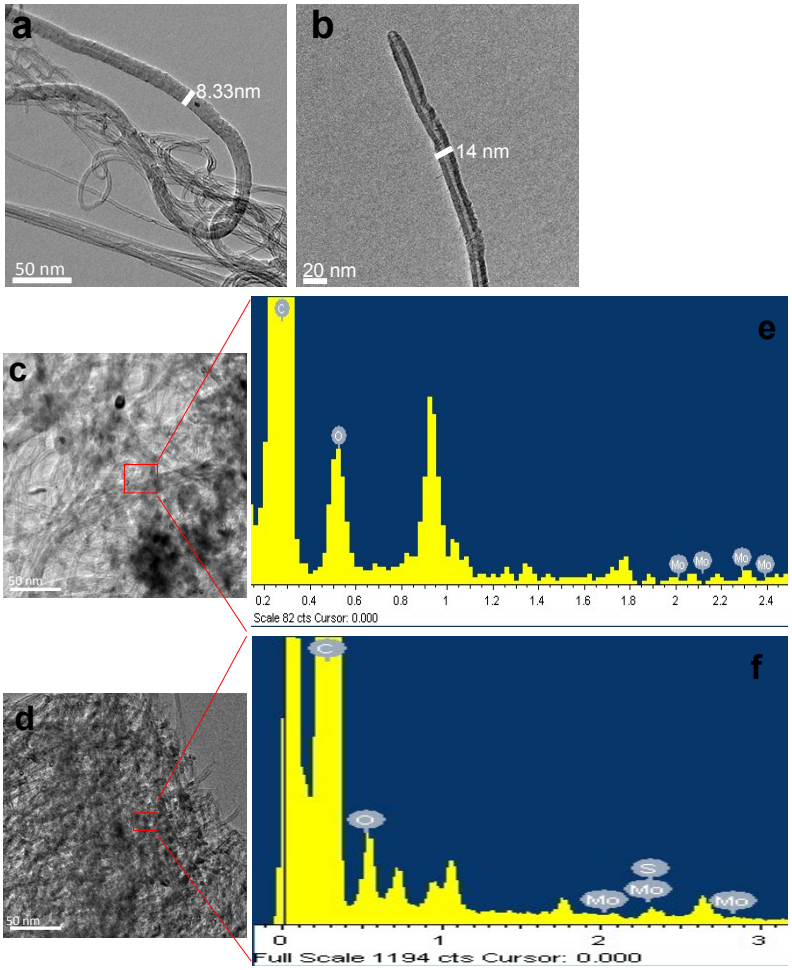
A. Characterization of Multi-walled carbon nanotubes (MWCNTs):

MWCNTs were studied for fiber size, structure and elemental composition using TEM and Nanosight. The diameter of IG-MWCNTs was approximately 10 nm (Figure S1a) and for RG-MWCNTs was approximately 15 nm (Figure S1b). IG-MWCNTs formed agglomerates in distilled water and had reduced dispersibility (Figure S1c and S1g). RG-MWCNTs formed large agglomerates in which the individual fibers extended from the agglomerates before sonication and dispersed more uniformly after sonication

(Figure S1d and S1g). We used Energy Dispersive X-ray spectroscopy (EDX) analysis with TEM to analyze the elemental composition of MWCNTs. TEM EDX spectrum of IG-MWCNTs EDX spectra revealed presence of carbon, oxygen and molybdenum (Figure S1e) whereas RG-MWCNT agglomerates (Figure S1f) contained carbon, oxygen, molybdenum and sulfur. IG-MWCNT aggregates were found to be deficient in sulfur. Figure S1g illustrates the dispersion of IG-MWCNTs and RG-MWCNTs in distilled water before sonication. Figure S1h shows the size distribution of RG-MWCNTs' and IG-MWCNTs' agglomerates in distilled water (Figure S1h). The concentration of nanometer-sized particles in distilled water was found to be zero.



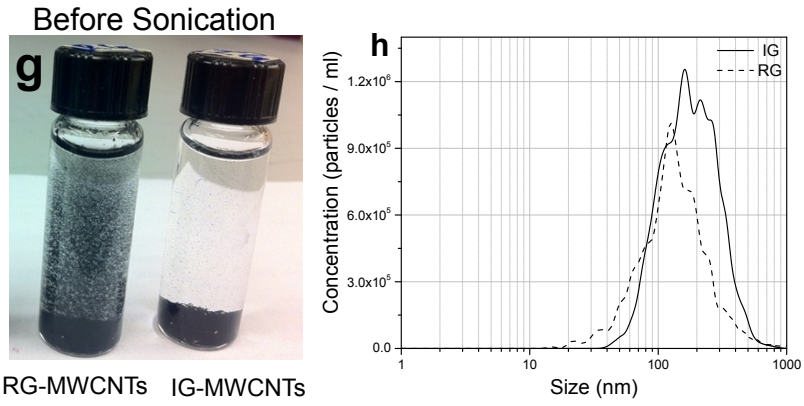


Figure S1. Characterization of MWCNTs. Standard scale bar: 50 nm. (a) TEM micrograph for IG-MWCNTs displaying single nanotube, (b) TEM micrograph for RG-MWCNTs displaying single nanotube (c & e) illustrates the image and EDX spectra respectively for IG-MWCNTs. (d & f) illustrates the image and EDX spectra respectively for RG-MWCNTs. (e) Dispersion of 1mg/ml of MWCNTs (IG-MWCNTs and RG-MWCNTs) in water before sonication. (f) Size distribution analysis of IG-MWCNTs and RG-MWCNTs in water using Malvern Nanosight™ LM10. Further, in Table 1 we have listed the elemental and impurities composition in percentage for both IG-MWCNTs and RG-MWCNTs.

B. Elemental Composition Analysis using X-ray Photoelectron Spectroscopy (XPS):

XPS analysis was performed to confirm the difference in the elemental composition of two types of MWCNTs. We have shown comparative XPS spectrum of C1s and O1s between IG-MWCNTs and RG-MWCNTs in Figure S2. The C1s core peak levels of carbon atoms for both IG-MWCNTs and RG-MWCNTs was observed at approximately 284.5 eV. Three C1s peaks respectively located at 284.5, 285 and 286.5 eV were seen in the C1s XPS spectra (Figure S2a) for both IG-MWCNTs and RG-MWCNTs. The peak at 284.5 and 285 eV were attributed to sp2 hybridized and sp3 hybridized graphitic carbon atom. The peak located at 286.5 eV was bestowed by carbon atoms from carbonyl (C-O) species. The satellite shake up peak (π-π^*) was observed at 291.1 eV. The peak at 287.7 for carboxyl (C=O) was almost invisible due to negligible concentrations. Oxygen binding energy O1s was deconvulated into two peaks at 532.3 and 532.8 eV, which could be labelled as C-OH and C=O respectively (Figure S2b). O1s peak area and

height observed in IG-MWCNTs was comparatively lesser than peak area and height of RG-MWCNTs. Further, the peak 532.1 was more shifted to left at 531.7 as compared to RG-MWCNTs.

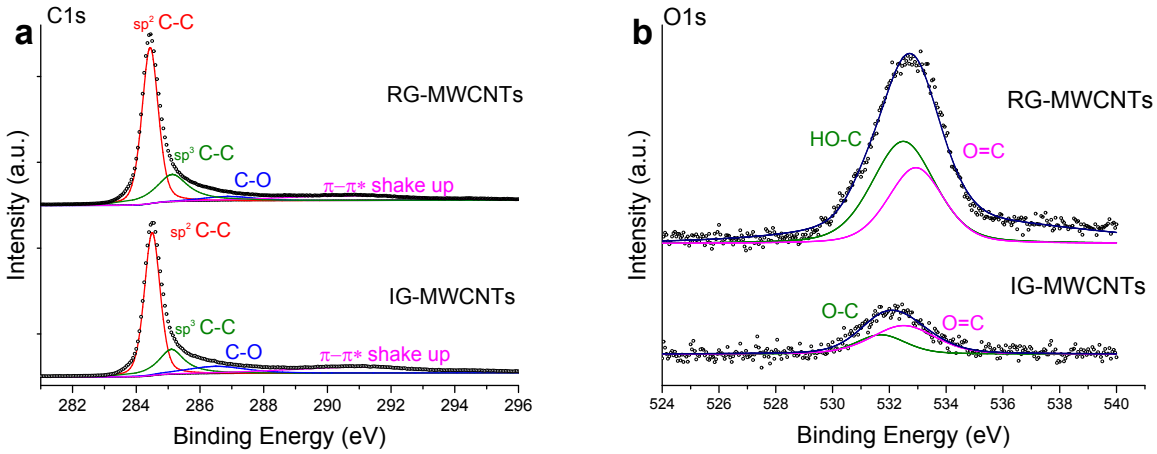


Figure S2. Comparative XPS spectra for IG-MWCNTs and RG-MWCNTs of (a) C1s and (b) O1s. Note the difference in the peak area between IG-MWCNTs and RG-MWCNTs in O1s spectra.

	IG-MWCNTs	RG-MWCNTs
Element	Weight (%)	Weight (%)
C	96.19	99.97
O	2.88	1.54
Mo	0.93	0.43
S		0.06
	100	100

Table S1: Elemental Composition of IG-MWCNTs and RG-MWCNTs following analysis using TEM EDX.