Supporting information

Carbon nanotubes physicochemical properties influence the overall cellular behavior and fate

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Materials and Methods:

1. Single walled carbon nanotubes (SWCNTs) acid treatment

User-tailored SWCNTs were obtained by liquid phase oxidation of commercial or pristine SWCNTs (Unidym Inc.). Specifically, pristine SWCNTs were incubated in a mixture of 3:1 (v/v) concentrated sulfuric acid (Fisher Scientific, 96.4%) and nitric acid (Fisher Scientific, 69.6%) for different periods of time (i.e., 3 and 6 h respectively) to obtain SWCNTs with different degrees of O-related functionalities and lengths (denoted user-tailored SWCNTs). Upon time elapsed, the nanotube/acid mixture was diluted in deionized water and filtered through a GTTP filter membrane, (0.2 μm pore size, Fisher Scientific). The nanotubes on the filter were subsequently washed extensively with deionized water, dried under vacuum and stored until further use.

2. Material characterization

Raman spectroscopy was used to investigate the physical and chemical properties of the samples. For this, dry samples (pristine, 3 and 6h treated samples) were deposited onto clean glass slides and scanned using a Raman spectrometer (CL532-100, 100 mW, USA) and a 532 nm green laser with a spot size of <0.01 mm^2 directed though a 50 X objective. Detailed scans were taken in the 100 to 3200 cm^{-1} range and at low laser energy (i.e., < 0.5 mV), while short exposure times (10 sec) were maintained to prevent unexpected heating effects of the samples.

Energy dispersive X-ray spectroscopy (EDX) was employed for quantitative elemental analysis of the SWCNTs (pristine and user-tailored). For this, dry samples were mounted onto silica wafers and their elemental composition was evaluated using a Hitachi S-4700 field emission scanning electron microscope with a S-4700 detector integrating secondary (SE) and backscattered (BSE) electron detection (in a single unit).

Analyses of sample-induced agglomerate size were performed using a dynamic light scattering device (DLS, DelsaTM). For this, suspensions of 50 μg/mL samples (pristine or user-tailored, both 3 and 6 h treated) were prepared in Dulbecco Minimum Essential Media (DMEM) supplemented with 5% fetal bovine serum (FBS), and scanned at 20 ^{o}C. For each sample, 150 measurements were recorded and the mean sample diameter was calculated by evaluating the intensity, volume, and number distribution data being collected.

The dispersity of SWCNT samples in both distilled water and DMEM supplemented with 5% FBS was investigated using standard protocols. Briefly, SWCNTs were prepared in the corresponding solvent to yield 3 mg/mL solution and the suspension was sonicated for 2 min and centrifuged for 5 min at 3000 rpm. Subsequently, 0.8 mL supernatant was removed and filtered through a 0.2 μm GTTP filter membrane. The membrane was then dried under vacuum and the weight of SWCNTs was evaluated. The solubility of the SWCNT samples in different solvents was calculated based on the initial starting weight and final weight of the filter paper, as well as volume used for suspension.

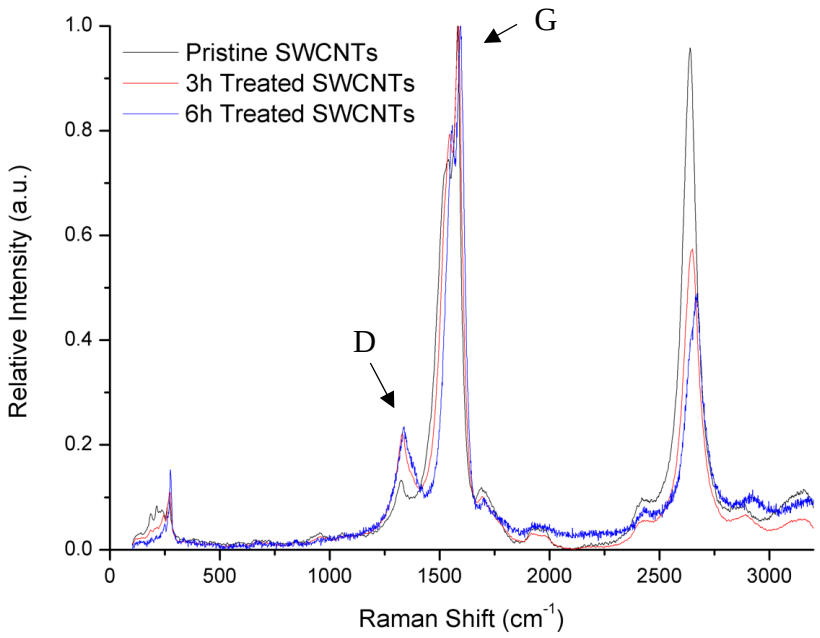
3. Cell nuclear deformation

BEAS-2B cells were seeded overnight onto a 12-well plate at a density of $2.5x10^5$ cell/ml and exposed to cellular media containing suspensions of 50 μg/ml pristine or acid treated SWCNTs for 24 h. Subsequently, the cells were incubated with 10 μg/ml of Hoechst 33342 (Molecular Probes) for 30 min at 37 ºC, washed twice with Phosphate Buffered Saline (PBS, Fisher), and analyzed using fluorescence microscopy (Leica Microsystems) to assess the percentage of cells showing deformation in nuclear morphology, intensely condensed chromatin, and/or fragmented nuclei.

Results and Discussion:

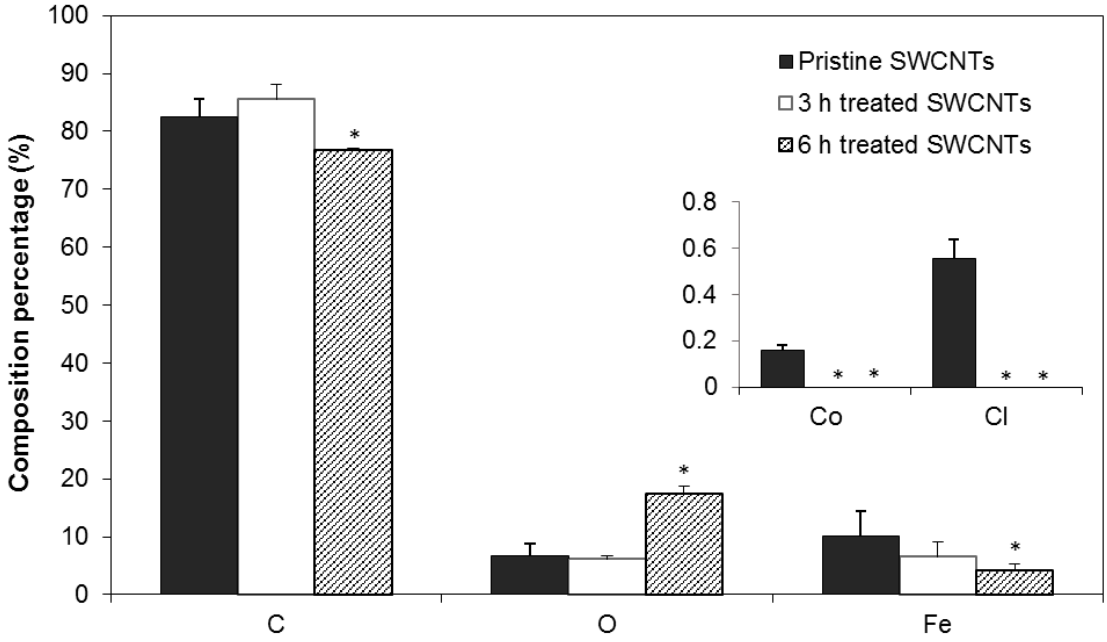
1. Effects of acid treatment on the physical and chemical properties of pristine SWCNTs

Raman analysis showed that the intensity ratio between the D (treatment-induced disorder) and G (treatment-induced change in purity) peaks (I_D/I_G) were 0.210 ± 0.1 for the 3 h and 0.47 ± 0.05 for the 6 h acid treated samples, which were correspondingly 16 % lower and 85 % higher respectively than the I_D/I_G of pristine SWCNTs (**Supporting Figure 1**).



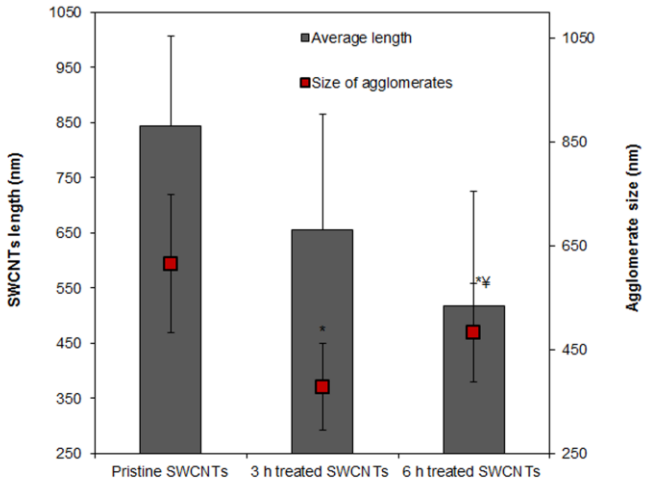
Supporting Figure 1: Raman spectra of pristine, 3 and 6 h treated SWCNTs.

Energy dispersive elemental (EDX) analyses showed a reduction in the metal impurities (i.e., Fe, Co, Cl) normally present in the pristine samples, all with the acid treatment incubation time (**Supporting Figure 2**).



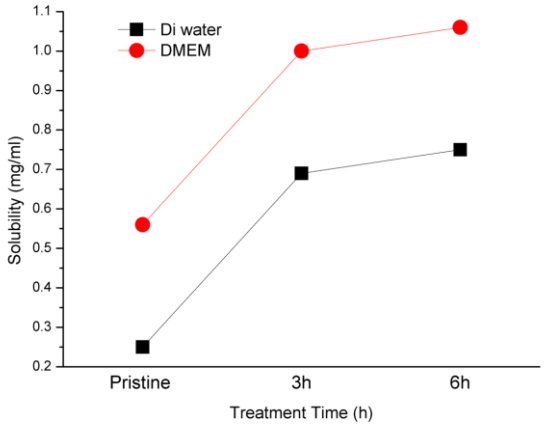
Supporting Figure 2: Elemental composition analysis of pristine, 3 and 6 h treated SWCNTs. (*indicates signicant difference when compared to pristine SWCNTs, $p<0.05$).

The average indvidual length of SWCNTs as evaluated by atomic force microscopy was reduced by 22 % and 43 % for the 3 and 6 h treated samples respectively, all relative to the pristine one. Further, analysis of the average agglomerate size in cellular media as investigated by dynamic light scattering technique (DLS) revealed a 40 % and 22 % reduction for the 3 h and 6 h acid treated samples respectively, all relative to the pristine SWCNTs (**Supporting Figure 3**).



Supporting Figure 3: Length and agglomerate size analysis of pristine, 3 and 6 h treated SWCNTs. (*indicates significant difference when compared to pristine SWCNTs, ¥ indicates significant difference relative to cells exposed to 3 h acid treated SWCNTs, $p<0.05$).

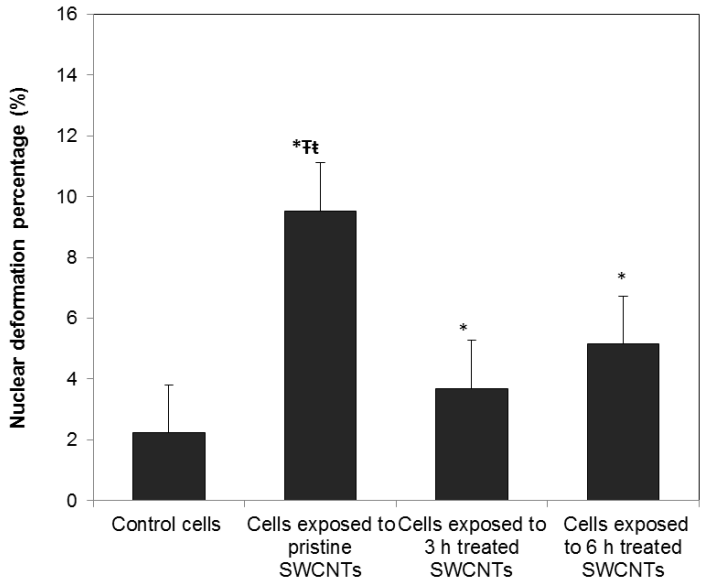
The difference in the agglomerates size also led to an increase in dispersity by 176 % and 200 % in deionized water and 70 % and 90 % in cellular media respectively, for the 3 h and 6 h acid treated samples when compared to the pristine one (**Supporting Figure 4)**.



Supporting Figure 4: Solubility analysis of pristine, 3 h and 6 h acid treated SWCNTs dispersed in distilled water (di water) or cellular media.

2. Effects of SWCNTs exposure on nuclear deformation

Investigation of the effects of the user-tailored SWCNTs exposure on the nuclear deformation was performed by scoring the percentages of cells showing abnormalities in their nuclear morphology after the exposure and reporting relative to control cells. Results showed that exposure to pristine SWCNTs led to significant increase in the percentage of cells with nuclear deformation when compared to control cells, and cells exposed to 3 and 6 h treated SWCNTs respectively.



Supporting Figure 5: Quantification of the percentage of cells showing nuclear damage following 24 h exposure to pristine, 3 and 6 h treated SWCNTs. (*indicates significant difference when compared to control cells, Ŧ and ŧ indicate significant differences when compared to cells exposed to 3 h and 6 h acid treated SWCNTs respectively, $p<0.05$).