Supporting Information

Carbon Nanotube Uptake Changes the Biomechanical Properties of Human Lung Epithelial Cells in a Time-dependent Manner

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Table S1: Energy dispersive X-ray (EDX) spectroscopy allowed characterization of elemental composition of pristine (as purchased) and 1h acid washed multi-walled carbon nanotubes (simply called user-characterized MWCNTs). Following the acid treatment, the content of oxygen (O) increased while the contents of iron (Fe), copper (Cu), silica (Si), sulfur (S), and carbon (C) decreased for the acid washed samples.

Element	**Pristine (wt %)**	**MWCNTs (wt %)**
C	91.99	90.25
O	3.92	7.56
Si	0.82	0.42
S	0.30	0.27
Cu	1.69	0.41
Fe	1.28	1.09

Table S2: Dispersity analysis of MWCNTs in different solutions, i.e., maximum amount of MWCNTs suspended in solution.

Type	**DI Water (mg/ml)**	**PBS (mg/ml)**	**DMEM (mg/ml)**	**DMEM+FBS (mg/ml)**
Pristine	0.19	0.25	0.44	1.69
MWCNTs	0.38	0.50	0.94	2.50

Table S3: Length distribution of MWCNTs evaluated using atomic force microscopy (AFM).

Length (μm)	Mean	Standard Deviation
Pristine	4261	2354
MWCNTs	1040	553

Table S4: Raman analysis of MWCNTs and Alexa-BSA- MWCNT conjugates.

Type	**D band (cm^{-1})**	**G band (cm^{-1})**	**I_D/I_G ratio**
MWCNTs	1348	1588	0.769
Alexa-BSA-MWCNTs conjugates	1343	1591	0.967

Reactive oxygen species (ROS) generation

Intracellular ROS generation was determined using the sensitive fluorescent probe 2′,7′-dichlorofluorescein diacetate (DCF-DA; Sigma, USA). Upon formation of ROS, cellular esterase hydrolyzes DCF-DA to the highly fluorescent 2', 7'–dichlorofluorescein (DCF).[1] Briefly, BEAS-2B cells were seeded overnight in a 96-well plate (Corning, USA) at a density of $1.5x10^4$ cell per well, and incubated with 5 µM DCF-DA at 37 °C for 30 min. Subsequently, the cells were treated with 24 µg/cm^2 MWCNTs suspended in Hank's Balanced Salt Solution (HBSS; Life Technologies, USA) for different exposure duration. Control samples of unexposed cells and cells exposed to pristine nanotubes were further investigated using the same experimental set up. Quantification of the intracellular levels of ROS was performed using a multi-well plate reader (FLUOstar OPTIMA, BMG LABTECH Inc., USA) and evaluating the DCF fluorescence at 485 nm excitation and 520 nm emission.

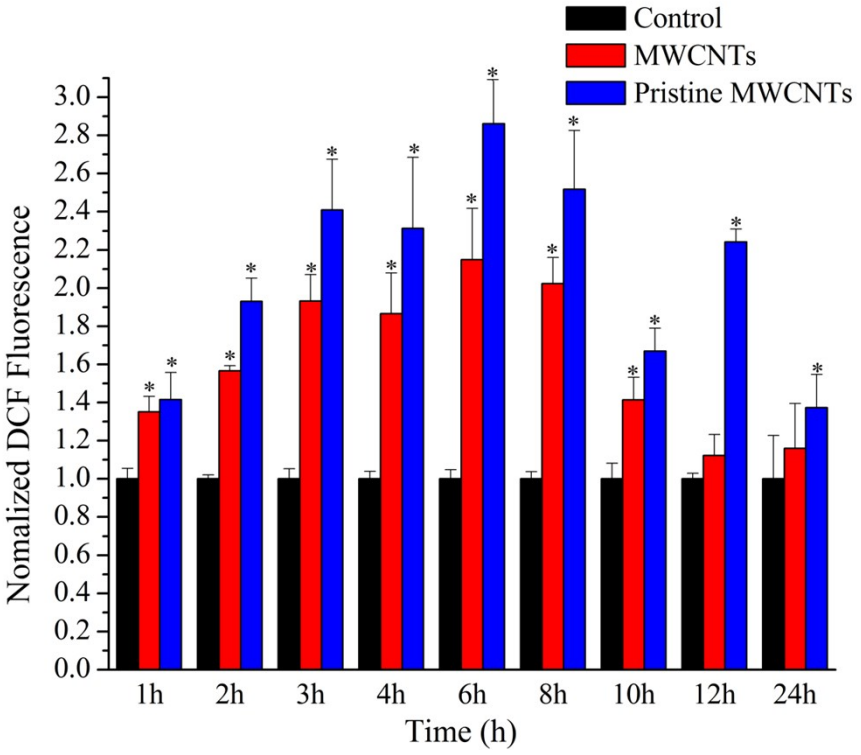

Figure S1: Quantification of the intracellular levels of ROS generated post exposure of BEAS-2B cells to 24 µg/cm^2 of pristine and purified MWCNTs; different exposure periods were assessed. Results are considered significant for p*<0.05

References:

1. Gomes, A.; Fernandes, E.; Lima, J. L. F. C., Fluorescence probes used for detection of reactive oxygen species. J Biochem Bioph Meth 2005, 65 (2-3), 45-80.