Supporting Information

Extracellular entrapment and degradation of single-walled carbon nanotubes

Consol Farrera,[a] Kunal Bhattacharya,[a] Beatrice Lazzaretto,[a] Fernando T. Andón,[a] Kjell Hultenby,[b] Gregg P. Kotchey,[c] Alexander Star[c] and Bengt Fadeel[a]

[a]Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, 171 77 Stockholm, Sweden.

[b]Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden.

[c]Department of Chemistry, University of Pittsburgh, Pittsburgh, 15260 PA, United States.

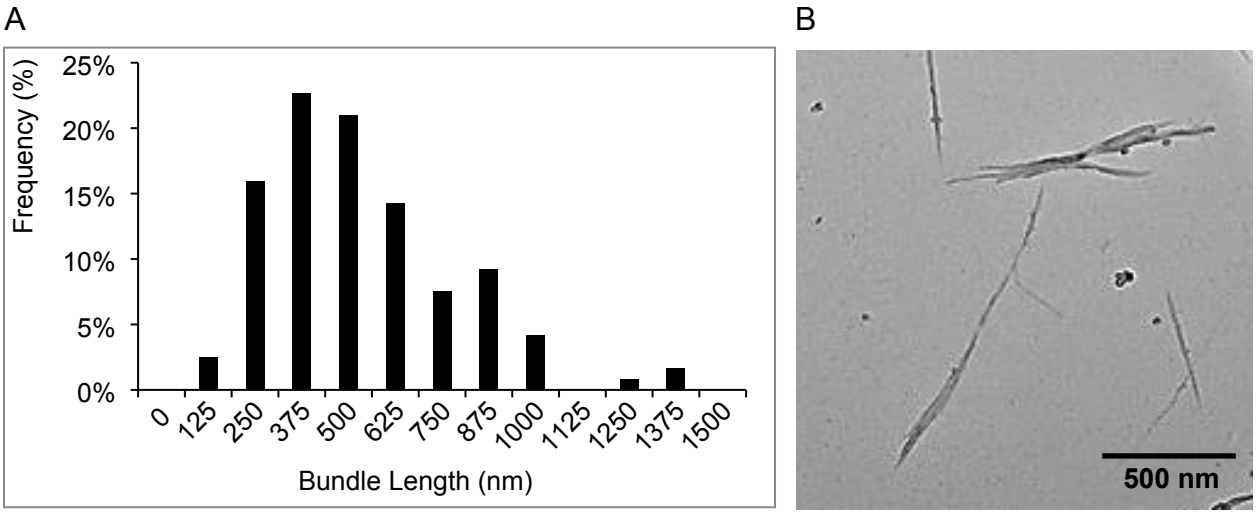


Figure S1. The length distribution of SWCNTs after 4 h acidic oxidation was determined by TEM. (A) Histogram representing the frequency percentage of the SWCNT bundles length (nm). The measurements were derived from TEM images. The 4 h oxidized SWCNTs had a mean bundle length of 486 ± 25 (n = 120). (B) Representative TEM image of the 4 h oxidized SWCNTs.

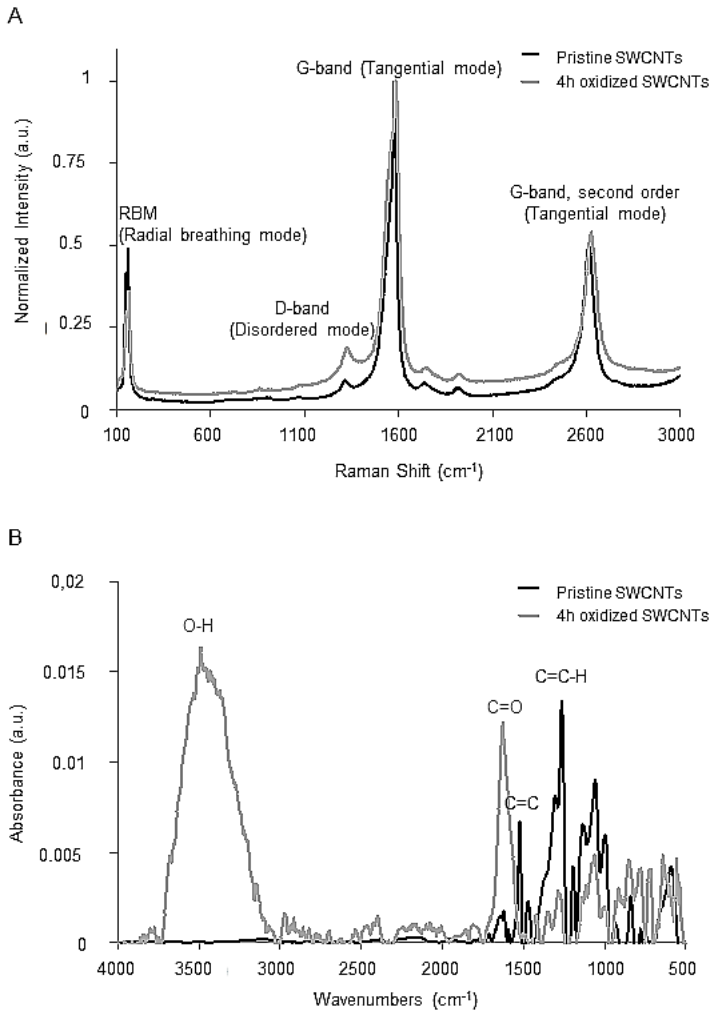


Figure S2. Characterization of the pristine *vs*. oxidized SWCNTs. (A) Mean Raman spectra for pristine SWCNTs (black line) and 4 h oxidized SWCNTs (gray line). The G, D, and RBM features of the spectra are indicated. As a result of the oxidation process, the D/G ratio for oxidized SWCNTs was 2.7 times greater than that of the pristine SWCNTs (i.e., 0.248 vs. 0.0911, respectively). (B) Spectra for pristine SWCNTs (black line) and 4 h oxidized SWCNTs (gray line) obtained using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). The 4 h oxidized CNTs present the characteristic carbonyl and hydroxyl bands at around 1700 and 3500 cm^{-1}.

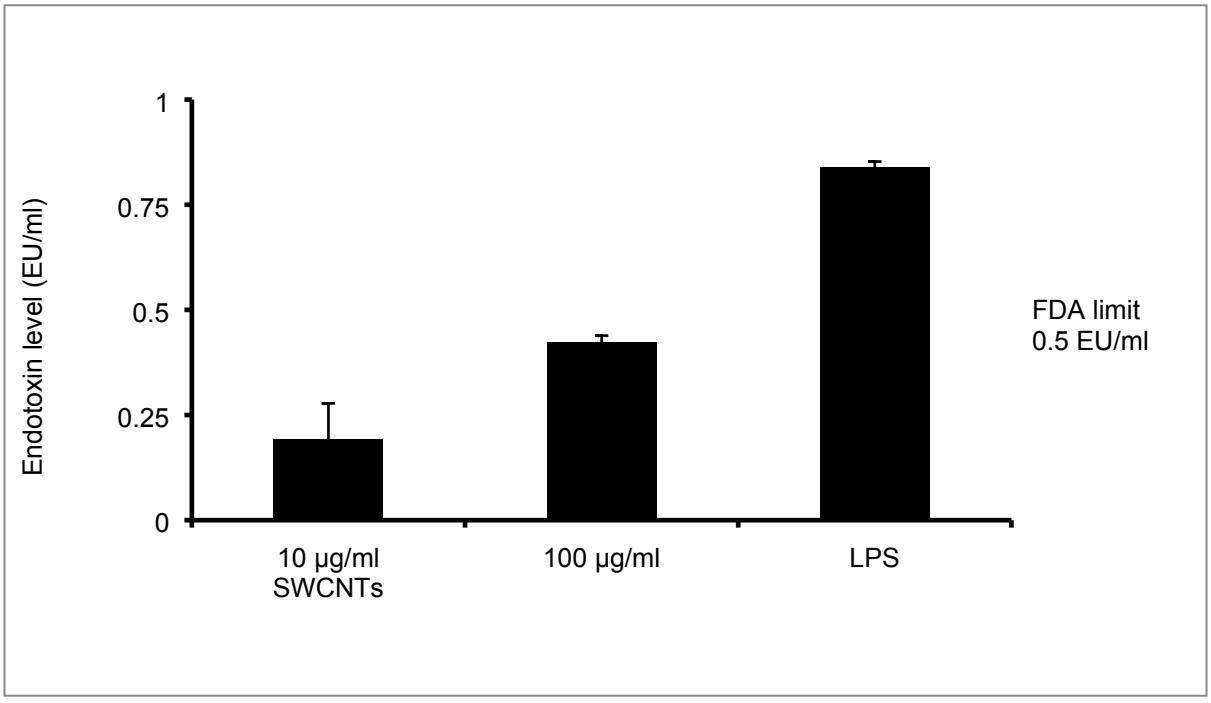


Figure S3. The presence of endotoxin in the SWCNT samples was determined using the LAL test. Endotoxin levels were found to be below the FDA-mandated limit. LPS (100 ng/ml) was included as a positive control.

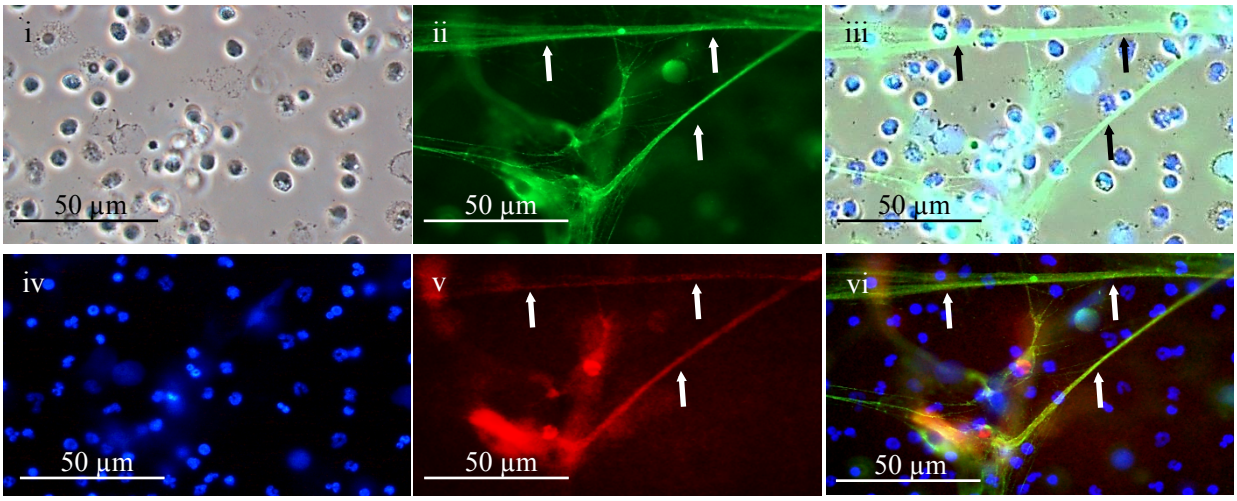
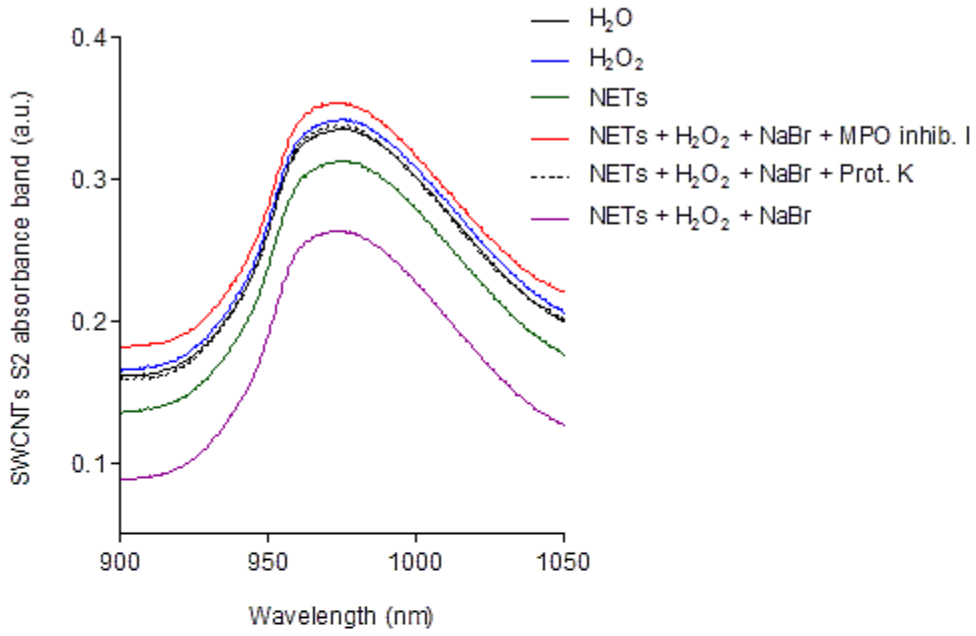
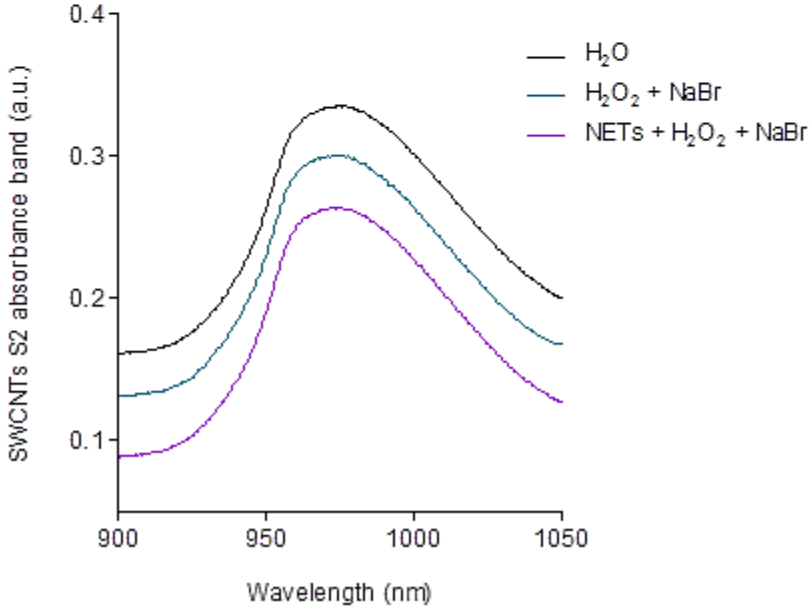


Figure S4. Neutrophil extracellular traps (NETs). Neutrophils were cultured for 2 h at 37°C in phenol red-free RPMI-1640 medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin and staining of spontaneously formed NETs was performed. (i) Light microscope image of untreated neutrophils. (ii) Fluorescence microscope image showing spontaneous NETs formation using NE-specific primary antibody followed by staining with FITC-labelled secondary antibody (green). White arrows indicate presence of NE along typical NET fibers. (iii) Merged image created using panels (i) and (ii). Sample was counterstained with DAPI to visualize cell nuclei. (iv) DAPI staining alone. (v) MPO staining using primary MPO antibody and secondary goat anti-mouse Alexa-594 labeled antibody (red). White arrows indicate presence of MPO along typical NET fibers. (vi) Merged image showing co-localization of NE and MPO (yellow) on NETs. The slide was counterstained with DAPI to visualize cell nuclei.

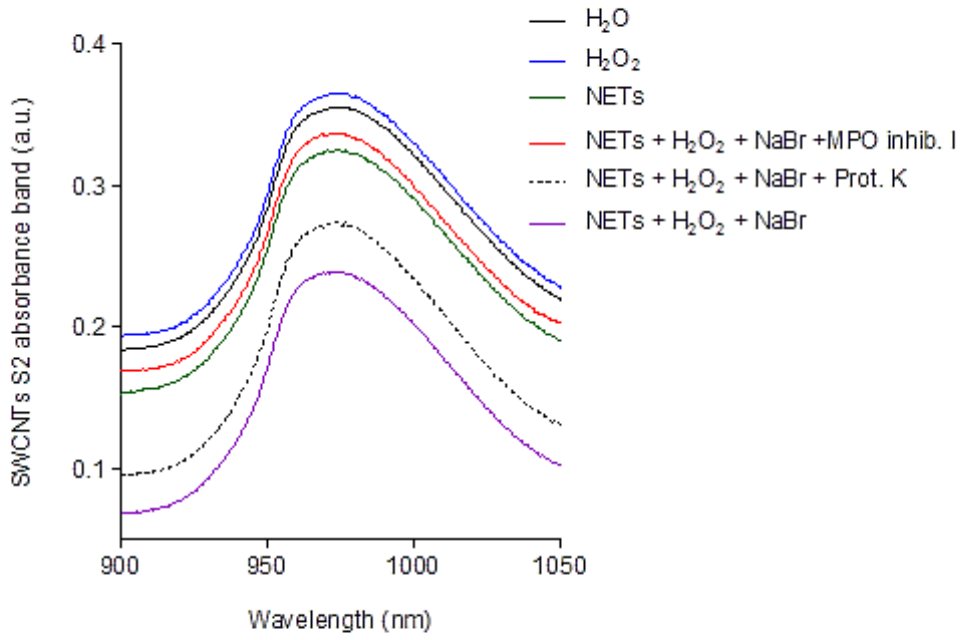
A


SWCNTs S2 absorbance band (a.u.)
0.4
0.3
0.2
0.1
900
950
1000
1050
Wavelength (nm)
H2O
H2O2
NETs
NETs + H2O2 + NaBr + MPO inhib. I
NETs + H2O2 + NaBr + Prot. K
NETs + H2O2 + NaBr

B


SWCNTs S2 absorbance band (a.u.)
0.4
0.3
0.2
0.1
900
950
1000
1050
Wavelength (nm)
H2O
H2O2 + NaBr
NETs + H2O2 + NaBr

C


H_2O
H_2O_2
NETs
NETs + H_2O_2 + NaBr +MPO inhib. I
NETs + H_2O_2 + NaBr + Prot. K
NETs + H_2O_2 + NaBr
SWCNTs S2 absorbance band (a.u.)
0.4
0.3
0.2
0.1
900
950
1000
1050
Wavelength (nm)

D

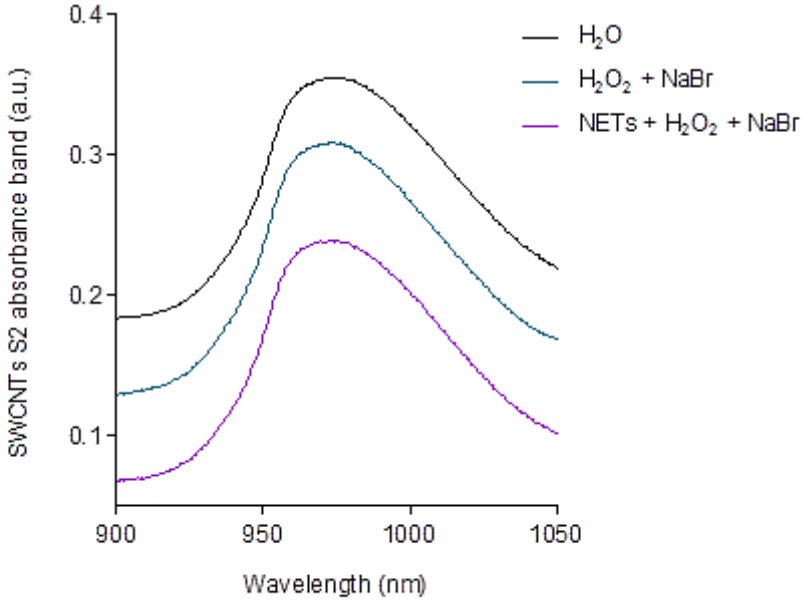

H_2O
H_2O_2 + NaBr
NETs + H_2O_2 + NaBr
SWCNTs S2 absorbance band (a.u.)
0.4
0.3
0.2
0.1
900
950
1000
1050
Wavelength (nm)

Figure S5. Biodegradation of SWCNTs assessed by UV-Vis/NIR spectroscopy. (A, B) S2 absorbance spectra (S2 band: 980 nm) of SWCNTs after 4 h incubation at the indicated conditions. S2 absorbance spectra of SWCNTs suspended in H_2O (negative control) / in NETs supplemented with 100 μM H_2O_2 + 100 μM NaBr are the same in panels A and B, but the spectra are displayed as two figures for clarity. (C, D) S2 absorbance spectra of SWCNTs after 12 h incubation at the indicated conditions. S2 absorbance spectra of SWCNTs suspended in H_2O (negative control) / in NETs supplemented with 100 μM H_2O_2 + 100 μM NaBr are the same in panel C and D. For further experimental details including concentrations of each reagent, see Methods.

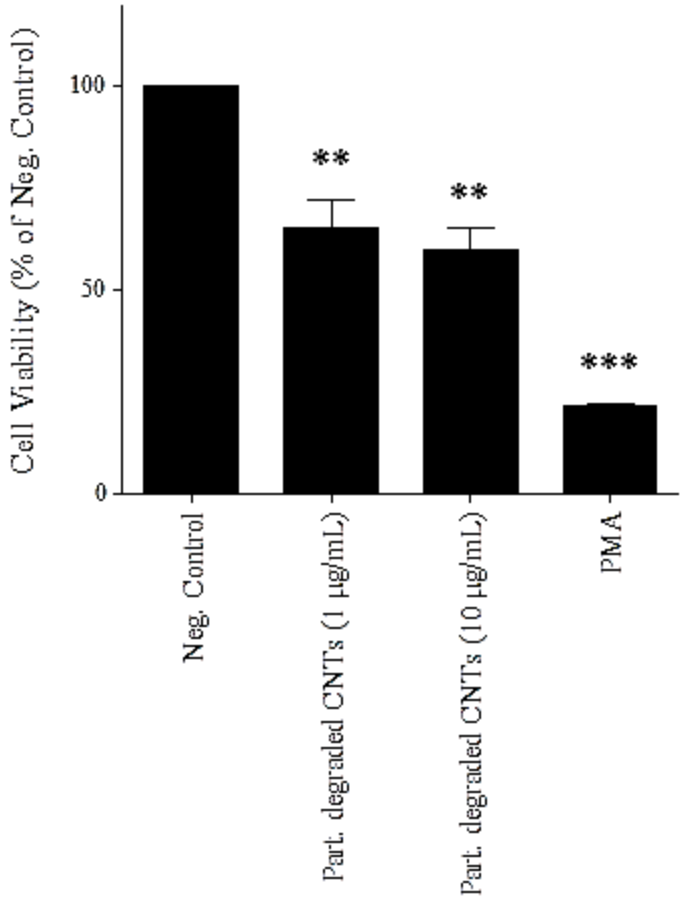


Figure S6. Cell viability of neutrophils exposed to partially degraded SWCNTs. Cellular ATP levels were determined after 6 h exposure to 1 or 10 µg/mL of partially degraded SWCNTs (biodegraded for 18h according to the protocol of Kagan *et al*., 2010). Neutrophils were exposed to 25 nM PMA as a positive control while unexposed neutrophils were taken as negative control. Cell viability of the neutrophils was significantly reduced upon incubation with partially degraded SWCNTs (**, $p \leq 0.01$; ***, $p \leq 0.001$, by one-way ANOVA with a posthoc Tukey's multiple comparison test). A similar trend, albeit non-significant, was seen at 2 h of exposure to the same materials (data not shown).