Supplementary information

Lung Macrophages Digest Carbon Nanotubes Using Superoxide/Peroxynitrite Oxidative Pathway

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Supplementary Figures

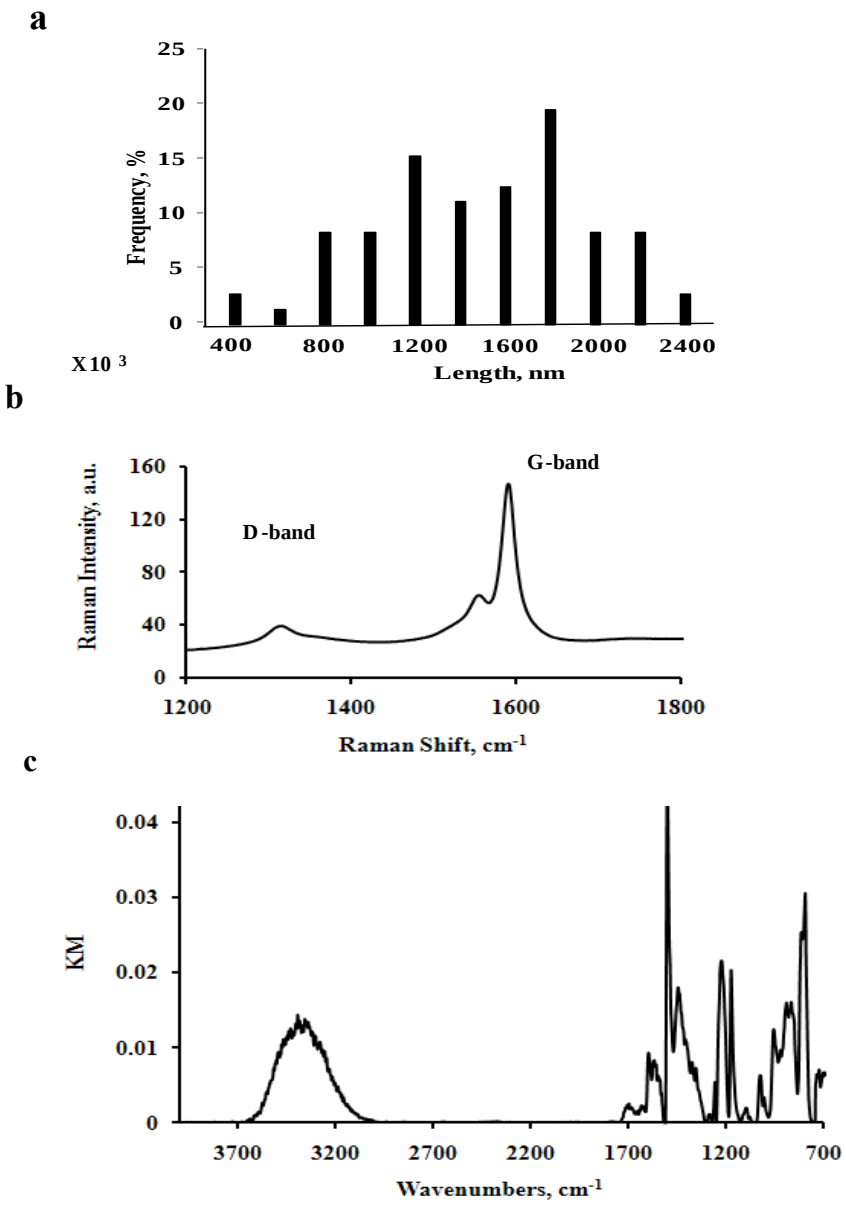
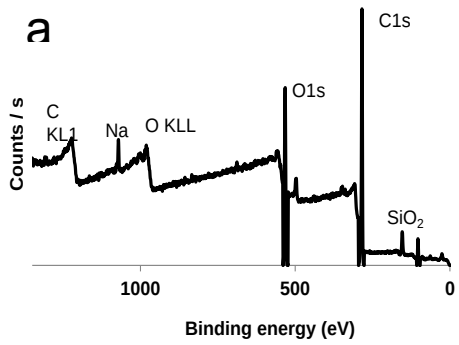
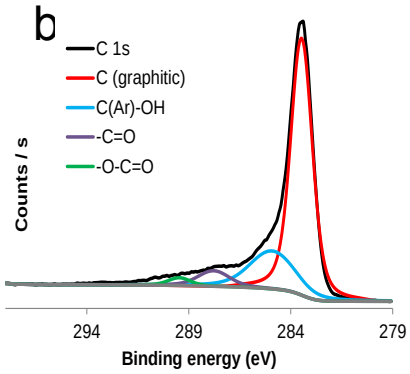


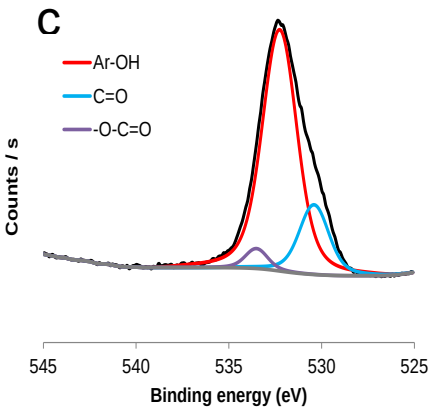
Figure S1. Characterization of SWCNTs employed in the study. a. Histogram detailing the length distribution of SWCNTs. b. Raman spectrum for SWCNTs; the D- and G- bands are marked on the spectrum, c. The spectrum obtained utilizing diffuse reflectance infrared Fourier Transform spectroscopy (DRIFTS). The unit for the ordinate axis is Kubelka-Munk (KM).



Element	Atomic (%)	Average ± SD (%)
C1s	75	69.7 ± 5.9
O1s	25	30.3 ± 5.9



Functional group	Peak (eV)	Atomic (%)	Average ± SD (%)
Graphitic Carbon	283.47	70.0	66.5 ± 9.4
Hydroxyl (**C**$_{Ar}$-OH)	284.92	21.6	20.7 ± 4.8
Ketone (-**C**=O-)	287.78	6.2	7.2 ± 1.3
Carboxylic Acid (-O-**C**=O-)	289.47	2.2	4.1 ± 1.9



Functional group	Peak (eV)	Atomic (%)	Average ± SD (%)
Ketone (-C=**O**-)	530.41	19.1	15.0 ± 4.2
Hydroxyl (-**O**H)	532.26	76.8	83.7 ± 6.3
Carboxylic Acid (-**O**-C=O-)	533.51	4.1	4.1 ± 1.3

Figure S2. X-ray photoelectron spectroscopy (XPS) characterization of SWCNTs. a.XPS survey spectrum of ox-SWCNTs, b. High-resolution C 1*s* XPS spectrum,. c. High-resolution O 1*s*XPS spectrum.

Supplementary Materials and Methods

Reagents. RPMI-1640 cell culture medium, fetal bovine serum (FBS), and hydroxyphenyl fluorescein (HPF) were purchased from Life Technologies (Carlsbad, CA), phorbol 12-myristate 13-acetate (PMA) xanthine oxidase (XO), and xanthine (X) were obtained from Sigma Aldrich (St. Louis, MO), 3-morpholinosyndnomine (*SIN-1*), 3-(2-hydroxy-2-nitroso-1-propylhydrazino)-1-propanamine (PAPA NONOate) and (Z)-1-{N-[3-Aminopropyl]-N-[4-(3-aminopropylammonio) butyl]-amino}-diazen-1-ium-1,2-diolate (spermine NONOate) were obtained from Cayman Chemical Company (Ann Arbor, MI).

X-ray photoelectron spectroscopy (XPS) was obtained *via* a Thermo Scientific ESCALAB 250xi photoelectron spectrometer using monochromated Al K Alpha X-rays as the source. The spot size of the sample (ox-SWCNTs, starting material) was 400 μm (microns). Charge compensation was provided by a low energy electron source and Ar^+ ions. Survey scans were collected using a pass energy of 150 eV, and high resolution scans were collected using a pass energy of 50 eV. The average percentage indicates means representing three different sample spots.

Zeta potential was recorded on Brookhaven ZetaPals at 25 ˚C under pH 7.00. The ox-SWCNTs (starting material) was dispersed in nanopure water (0.1 mg/mL), and a 10 mM KCl solution was added to adjust the conductance. The recorded zeta potential of the ox-SWCNTs was −42.92 ± 1.67 (mV) (means ± SD of five replicate measurements).

Hydrodynamic particle size of ox-SWCNTs (*i.e.*, starting material) was determined using dynamic light scattering (DLS). To this end, a Brookhaven ZetaPlus Particle Sizing equipped with a laser (532 nm) was employed. After five replicated measurements of the same sample, the effective diameters ranged from 1400 nm to 5000 nm, and the polydispersity was 0.3–0.7. Due to inconsistency between the replicate measurements, different concentrations (0.01–1.0 mg/mL) of the ox-SWNT were prepared and analyzed; this action, however, failed to resolve the issue.

Fourier Transform spectroscopy (DRIFTS) was performed employing an IR-Prestige spectrophotometer (Shimadzu Scientific, Kyoto, Japan) outfitted with an EasiDiff accessory (Pike Technologies). SWCNTs were homogeneously mixed with KBr. Using KBr as the background

and taking 32 scans per sample, a spectrum was obtained over the range of 700 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

Particulate instillation. Mouse pharyngeal aspiration was used for particulate administration. Briefly, after anesthetization with a mixture of ketamine (Phoenix, St. Joseph, MO) and xylazine (Phoenix, St. Joseph, MO) (62.5 and 2.5 mg/kg subcutaneous in the abdominal area), the mouse was placed on a board in a near vertical position and the animal's tongue extended with lined forceps. A suspension (approximately 50 µl) of particulates prepared in phosphate-buffered saline (PBS) at a dose of 40 µg/mouse were placed in the posterior of the throat and the tongue held until the suspension was aspirated into the lungs. All particles were sterilized prior to administration. Control mice were administered sterile Ca^{+2} + Mg^{+2}-free PBS vehicle. All mice in the particle and PBS groups survived this exposure procedure.

Obtaining bronchoalveolar lavage from mice. Mice were weighed and euthanized with an intraperitoneal injection of sodium pentobarbital (SPB, Fort Dodge Animal Health, Fort Dodge, Iowa) (>100 mg/kg) on days 7 and 28 post exposure. The trachea was cannulated with a blunted 22 gauge needle, and BAL was performed using cold sterile Ca^{2+} + Mg^{2+}-free PBS at a volume of 0.9 ml for the first lavage (kept separate) and 1.0 ml for subsequent lavages. Approximately 5 ml of BAL fluid per mouse was collected and pooled in sterile centrifuge tubes. Pooled BAL cells were washed in Ca^{+2} + Mg^{+2}-free PBS by alternate centrifugation (800 × *g* for 10 min at 4 °C) and resuspension. Slides for the counting of alveolar macrophages with SWCNTs from BAL fluids were prepared by centrifugation using Shandon Cytospin 4 (Thermo Electron Corporation, Cheshire, UK) and staining with Diffquick (Fisher Scientific, Pittsburgh, PA).

Lung fixation. Lung tissues were prepared using standard conditions. Animals were deeply anesthetized with an overdose of sodium pentobarbital. The trachea was exposed, cannulated, and secured with a suture. Prior to instillation of fixative, the diaphragm was ruptured to collapse the lungs. The lungs were subsequently fixed with 1% paraformaldehyde/0.1% glutaraldehyde in situ at 5 cm of pressure for 0.2 h. Thereafter, the trachea was ligated and the lungs were excised and submerged in fixative overnight before embedding. Lung tissue slices were prepared from both right and left lung lobes and embedded in paraffin. Sections (5 µm) were prepared using a HM

320 rotary microtome (Carl Zeiss, Thornwood, NY). Lung specimens were stained with hematoxylin and eosin (H&E) for quantitative assessment of alveolar macrophages with SWCNT.