Supplementary information

Adsorption of Surfactant Lipids by Single-Walled Carbon Nanotubes in Mouse Lung upon Pharyngeal Aspiration: Role in Uptake by Macrophages.

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Supplementary Methods:

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}.

Synthesis of FITC-SWCNTs was performed using the following steps (Figure S3):

1. *SWCNT cutting* (**1**). Hipco SWCNTs (10 mg) were dispersed in a 5 mL mixture of concentrated H_2SO_4/HNO_3 (3:1 v/v). The mixture was sonicated at 40° C in an ultrasonic bath for 3h 20min during which the bath water was changed every hour with cold water. After cooling to room temperature, the acidic dispersion was added to 100 mL of iced water. Then, the suspension was subjected to centrifugation at 3400 rpm for 15 min, and the condensed sample at the bottom of the tube was collected. Finally, the nanotube sample was filtered through a membrane with 0.22 μm pore size, followed by washing with distilled water until no residual acid was present (*i.e.* the pH of the filtrate was neutral). The collected sample was subsequently dried in a vacuum oven at 110° C, yielding 7 mg of short-cut SWCNTs.

2. *Synthesis of {2-[2-(2-Amino-ethoxy)-ethoxy]-ethyl}-carbamic acid tert-butyl ester* (**3**) [1]. A solution of Boc_2O (735 mg, 3.4 mmol) in 1,4-dioxane (10 mL) was added to a solution of 2,2'-(ethylene-dioxy)bis(ethylamine) (5 g, 34 mmol) in 1,4-dioxane (20 mL), dropwise for of 1 hr. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and the residue was dissolved in 4 mL of DCM. The organic phase was

washed with water (3 × 20 mL), dried by Na_2SO_4, and evaporated. The product, a yellow oil (632 mg, 75%), was obtained. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.41 (s, 9H) 2.8-3.7 (m, 12H).

3. *Derivatization of SWCNTs* [2]. 2 mg of short-cut SWCNTs (**1**) were suspended in 4 mL of oxalyl chloride and stirred at refluxing for 24 hr. The excess of oxalyl chloride was evaporated under vacuum, affording acyl chloride-SWCNTs (**2)**. Then, 200 mg of (**3**) was added to 6 mL of dry THF, and the mixture was stirred with refluxing for 48 hr to yield a brown solution. After cooling to room temperature, the mixture was filtered through a membrane with a 0.22 μm pore size. The filtrate was washed with methanol several times. The resulting functionalized SWCNTs (**4**) were dried at room temperature under vacuum. Then, (**4**) was suspended in 2 mL of 4 M HCl in dioxane and stirred at room temperature for 5h to cleave the Boc protecting group. The solution was filtered through a membrane with 0.22 μm pore size, washed with ethyl acetate several times, and dried under vacuum to afford amine-SWCNTs (**5**).

4. *Fluorescent-labeling of SWCNTs* (**6**). To a suspension of (**5**) in 1 mL of DMF, a solution of fluorescein isothiocyanate (FITC) (3 mg) in 500 μL of DMF was added. The mixture was stirred overnight at room temperature. Then, the solution was filtered through a membrane with 0.22 μm pore size, washed with methanol and ethyl acetate several times, and dried under vacuum.

Supplementary Figures

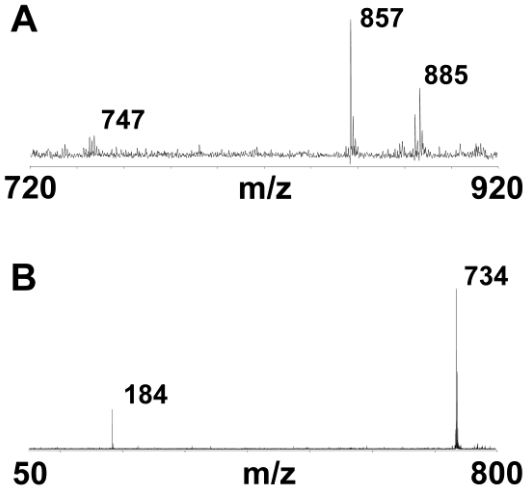

Figure S1. MALDI-MS characterization the total lipid extract from the SWCNT coating. A. Negative mode MALDI-MS analysis detects the major species of PI (857m/z, 885m/z) and PG (747m/z) but does not detect PC. B. Positive mode MALDI-MS/MS of the 734m/z peak confirms its identity as a PC species by the formation of the choline headgroup fragment (184m/z).

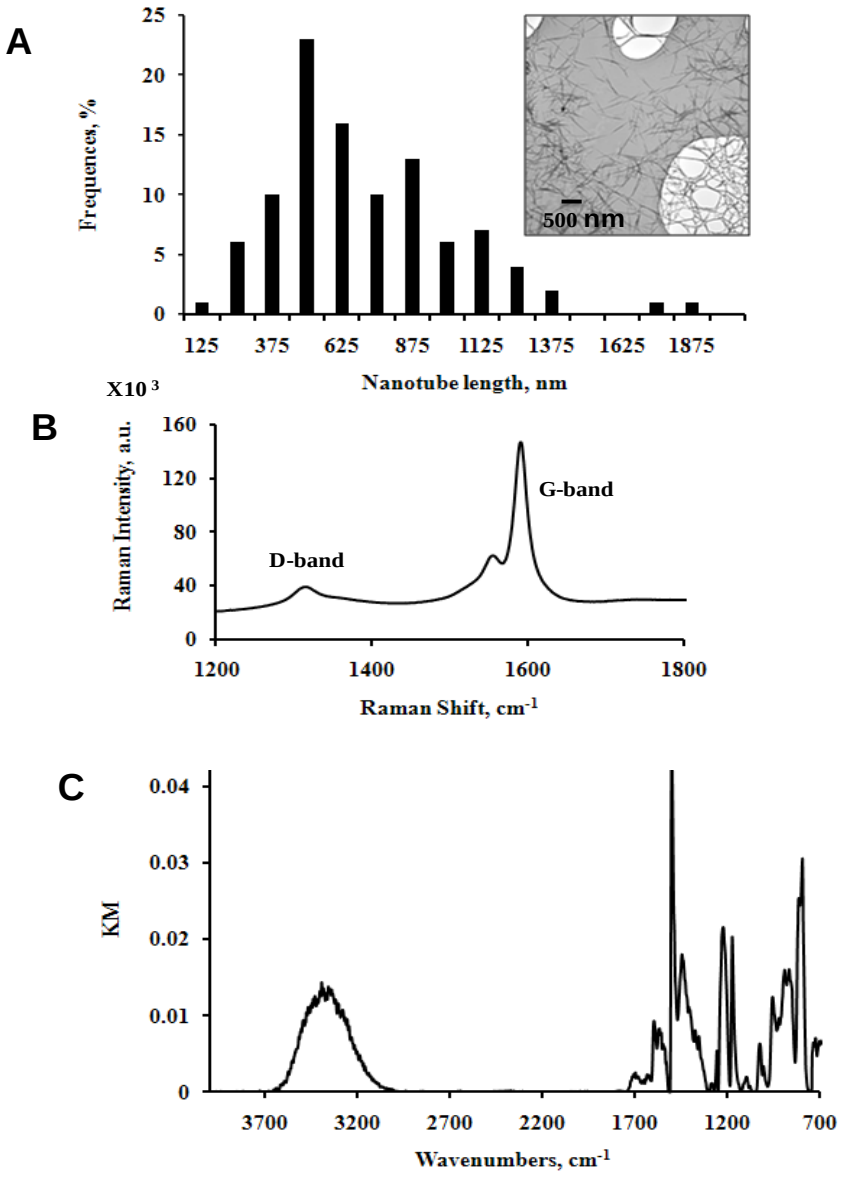

Figure S2. Characterization of SWCNT employed in the study.

A. Histogram detailing the length distribution of SWCNTs. The mean length was determined to be 676 ± 329 nm employing a sample size of 100. The insert depicts a TEM micrograph (500 nm scale bar) for the SWCNT sample. B. Raman spectrum for SWCNT; 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).

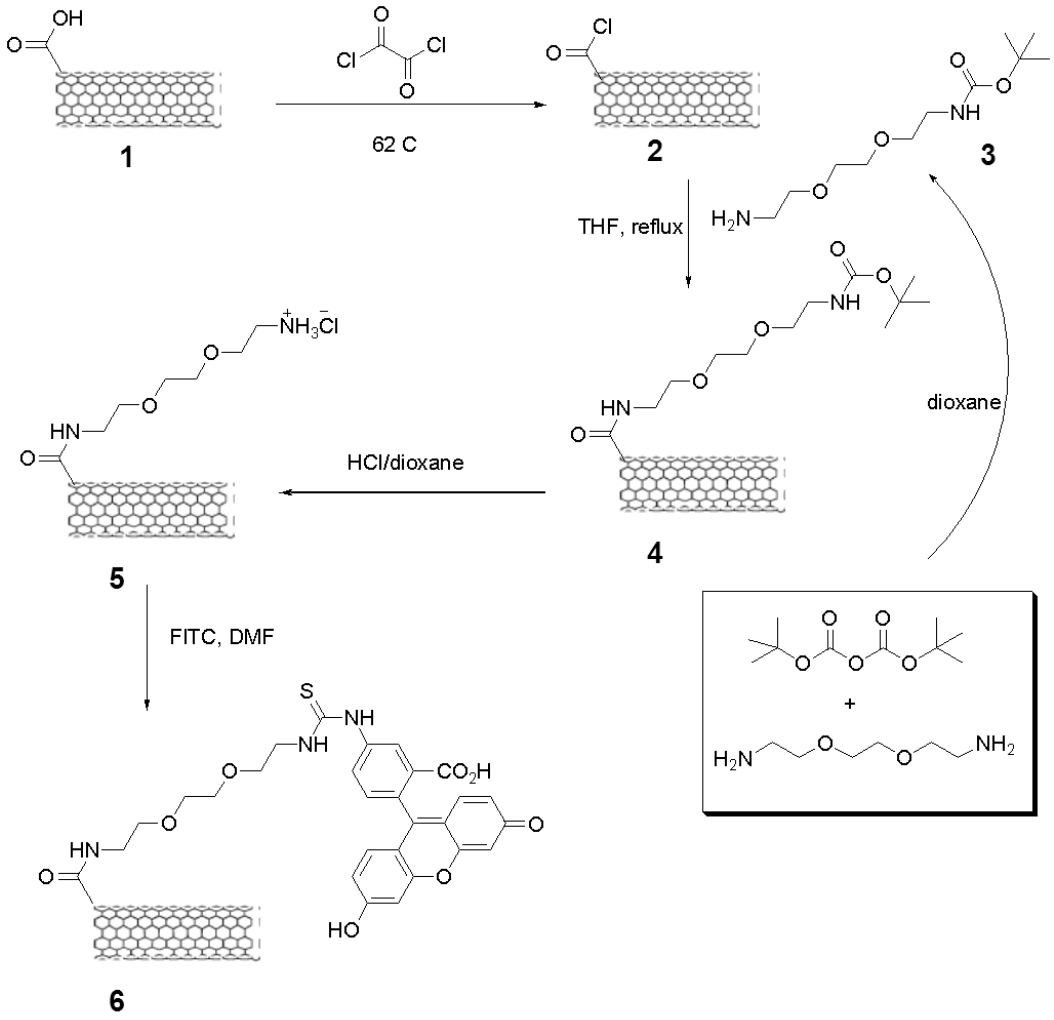

Figure S3. Scheme describing synthesis of FITC-SWCNT (**6**).

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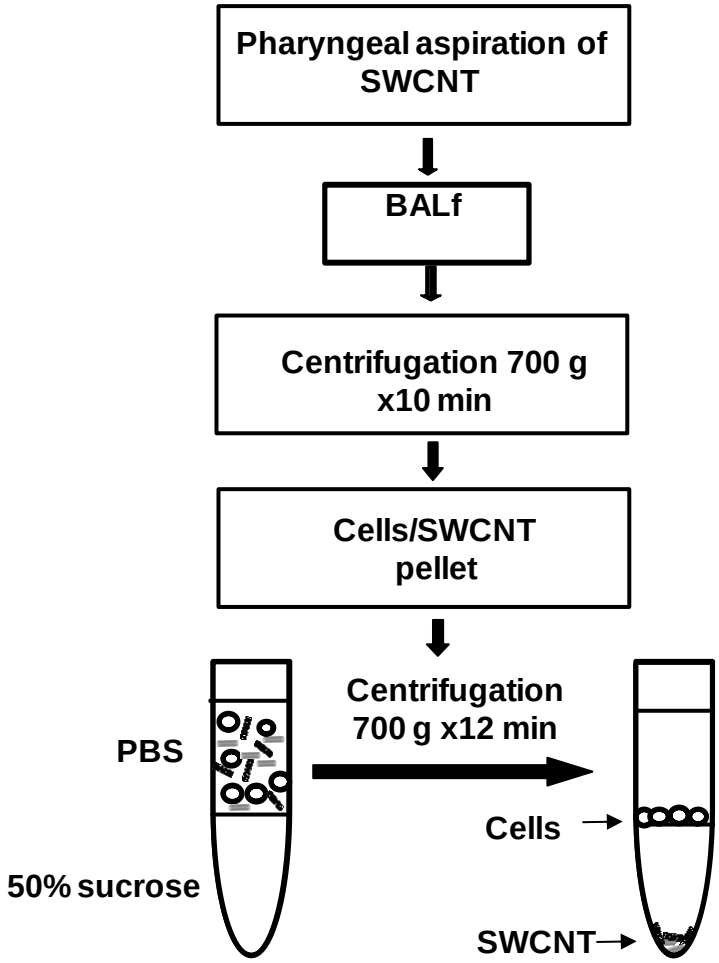

Figure S4. Schema of isolation and purification of SWCNT from BALf.

References:

1. G. Pastorin, W. Wu, S. Wieckowski, J.-P. Briand, K. Kostarelos, M. Prato and A. Bianco, Chem. Commun. 2006, 1182-1184.

2. W. Wu, S. Wieckowski, G. Pastorin, M. Benincasa, C. Klumpp, J.-P. Briand, R. Gennaro, M. Prato and A. Bianco, Angew. Chem. Int. Ed. 2005, 44, 6358-6362.