

# Supporting Information

## Single-Walled Carbon Nanotubes Exhibit Strong Antimicrobial Activity

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### Materials and Methods

**Single-Walled Carbon Nanotubes (SWNT).** SWNT were prepared using a 2.8 nm diameter pore 3% Co incorporated MCM-41 catalyst<sup>1, 2</sup>. The catalyst was first reduced in hydrogen at 975 K and then SWNT were grown by CO disproportionation at 6 atm and 1125 K. The sample was then purified in four steps: (1) reflux in 1 M NaOH twice, for 1 hr each, to remove silica, followed by filtration with a 5  $\mu\text{m}$  Omnipore membrane (Millipore, USA); (2) reflux to remove Co in 37% HCl for 8 hr followed by filtration with a 5  $\mu\text{m}$  Omnipore membrane; (3) oxidation by 4% O<sub>2</sub> at 400°C for 30 min to remove amorphous carbon; and (4) a second reflux in 37 % HCl for 8 hr followed by filtration with a 5  $\mu\text{m}$  Omnipore membrane. The carbon products were then washed with excess deionized water, and collected on the Omnipore membrane filter. Based on TEM analysis, the tube diameter ranged from 0.75 to 1.2 nm and over half of the tubes were between 0.8 and 0.9 nm. The length of the prepared SWNT was estimated to be between 1 and 3  $\mu\text{m}$ . Prior to use, SWNT were dispersed in an aqueous saline solution (0.9 % NaCl) and sonicated for 30 minutes (Aquasonic 150T, VWR, USA).

**SWNT-coated Filter.** 6 mg of SWNT were dispersed in 20 mL of dimethyl sulfoxide (DMSO) and sonicated for 15 minutes. The suspension was then filtered through a 5  $\mu\text{m}$  Omnipore PVDF membrane (Millipore). This allowed the formation of a SWNT-coated filter with a smooth SWNT layer (4.6  $\mu\text{m}$  thick) on top of the PVDF membrane. 100 mL ethanol was then filtered through the SWNT-coated filter to remove residual DMSO, followed by filtering of 200 mL deionized water to remove residual ethanol.

**Cell Culture.** *E. coli* K12 grown in LB medium at 37°C was harvested at exponential growth phase. Cells were washed twice and resuspended in saline solution (0.9 % NaCl) before tests involving interaction with SWNT.

**Loss of Viability for Cells Interacting with Suspended SWNT.** Inoculation of *E. coli* (final cell concentration of  $5 \times 10^7$  per mL) was performed in a saline solution containing 5  $\mu\text{g}$  SWNT/mL and a control with no SWNT. Cultures were incubated under gentle shaking for 60 min at  $37^\circ\text{C}$ . For the fluorescence-based assay, *E. coli* cells were stained with propidium iodide (PI, 50  $\mu\text{g}/\text{mL}$ ) for 15 minutes, and counter-stained with 4'-6-diamidino-2-phenylindole (DAPI, 3  $\mu\text{g}/\text{mL}$ ) for 5 minutes in the dark. The stained solution (50  $\mu\text{L}$ ) was added to the cell counting chamber (Fisher, USA), and 10 representative images were taken during a single batch experiment at 200x magnification at various locations in the chamber. The images were viewed under an epifluorescence microscope (Olympus) with U filter (364nm/440nm) for detecting cells stained with both PI and DAPI, and IB filter (464nm/604nm) for detecting cells stained with PI. Cells stained with PI and DAPI were determined either by direct cell counting for free swimming cells, or by area-based estimation (Figure A below) for cells attached to SWNT aggregates. The percent of inactivated cells (i.e. stained with PI) was determined from the ratio of the number of cells (or pixels) stained with PI divided by the number of cells (or pixels) stained with DAPI plus PI.

**Loss of Viability for Cells Interacting with a SWNT Deposit Layer.** A SWNT-coated filter was formed by filtering 6 mg of SWNT through a 0.45  $\mu\text{m}$  PVDF membrane as described above. 50 mL saline solutions (0.9 % NaCl) with  $2 \times 10^6$  *E. coli* cells were filtered through the SWNT-coated filter and a bare 0.45  $\mu\text{m}$  PVDF membrane filter (serving as a control). The membrane coupons were then incubated for 30 minutes in the saline solution, and cell viability was determined via the fluorescence-based assay discussed above. The percent of inactivated cells was determined by direct cell counting (of 10 representative images) from the ratio of the number of cells stained with PI divided by the number of cells stained with DAPI plus PI.

**Verification of Cell Viability by Determining Cell Metabolic Activity.** A metabolic activity marker, 5-cyano-2,3-ditolyl-tetrazolium chloride (CTC) (Sigma-Aldrich, USA), was used for direct epifluorescent microscopic enumeration of viable *E. coli* cells<sup>3</sup>. The CTC is a redox dye indicating respiratory activity of cells. Cells were deposited on a SWNT-coated membrane and a bare membrane (as described above) and incubated for 30 minutes in a saline solution. Membrane coupons were then transferred to a growth medium (180 mg/L glucose with 0.94 mM  $\text{NH}_4\text{Cl}$ , 0.45 mM  $\text{KH}_2\text{PO}_4$ , 0.5 mM  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.5 mM  $\text{NaHCO}_3$ , 2.0 mM NaCl, and

0.6mM MgSO<sub>4</sub>·7H<sub>2</sub>O) and incubated again for 30 minutes. CTC (1.5 mL, final concentration 1 mM) was then added onto the membrane coupons, followed by incubation (37 °C) in the dark for 60 minutes. Cells were counter-stained with DAPI for 5 minutes. The percent of active cells was determined by direct cell counting (of 10 representative images) from the ratio of the number of cells stained with CTC (red fluorescent formazan) divided by the number of cells stained with DAPI plus CTC.

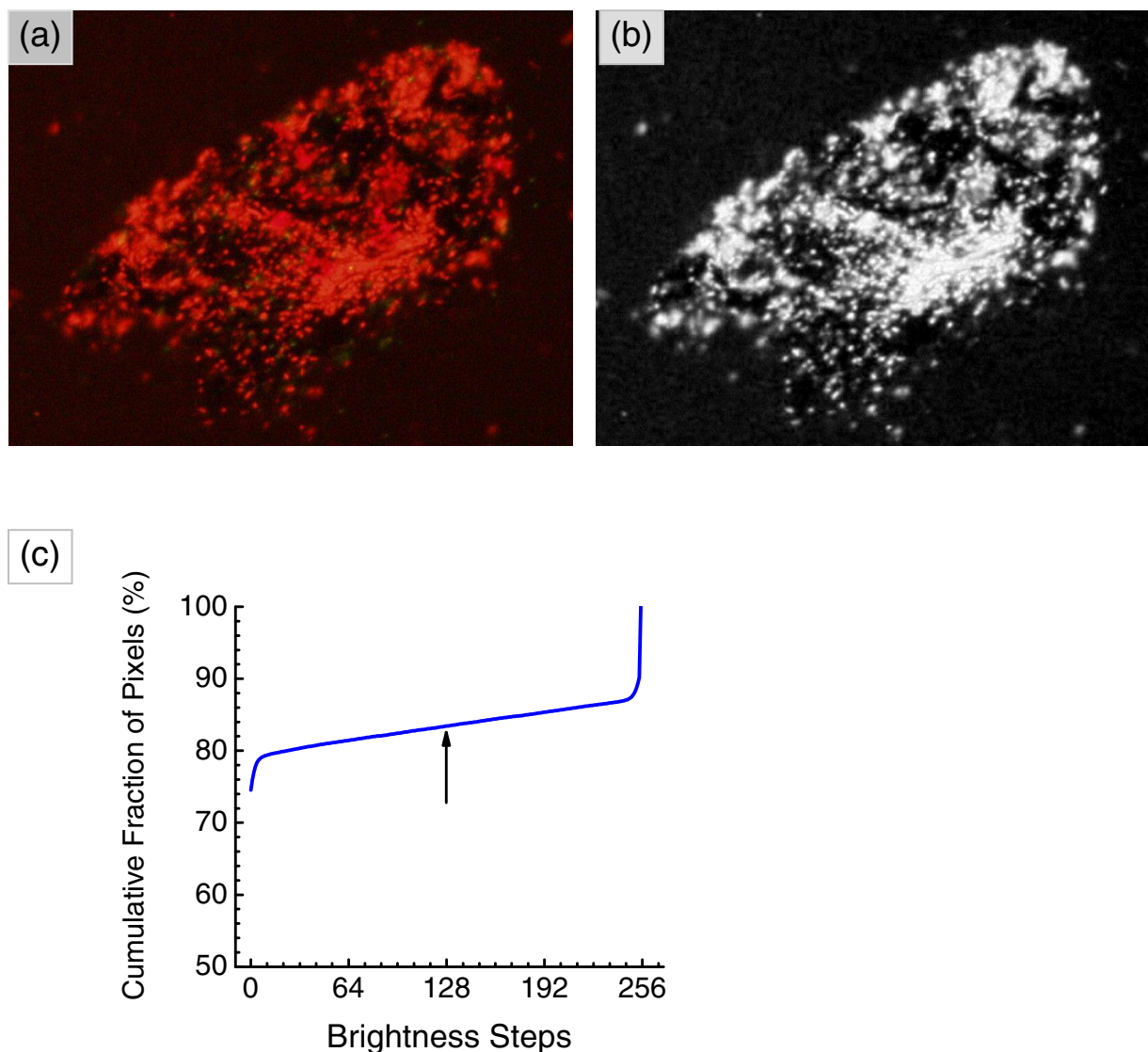
**TEM.** For the measurement of SWNT diameter distribution, transmission electron microscopy (TEM) was performed on a Tecnai F20 microscope (Philips). The SWNT were dispersed in ethanol and dried on 200-mesh copper grids coated with carbon-Formvar (Electron Microscopy Science). Images were obtained at 200 kV accelerating voltage.

**SEM.** Samples were filtered through a 0.22 µm PVDF membrane (Millipore) and fixed with 2.5 % glutaraldehyde and 1 % osmium tetroxide<sup>4</sup>. The cells were sputter-coated with gold (30 s, 30 mA), and then viewed under an XL30 microscope (FEI, USA) to determine the morphology of cells for samples with and without SWNT.

**Raman Spectroscopy.** Raman spectra were recorded on a Jasco Raman spectrometer. The instrument is equipped with an Olympus confocal microscope. Excitation wavelengths of 488, 532, and 785 nm were used.

**Efflux of DNA.** Plasmid vector pGEM-Teasy (3015 bp) was electroporated into *E. coli* XL1 blue. The strain was then cultured at exponential growth phase and incubated with and without SWNT for 60 min as described before. After filtration through a 0.22 µm low protein binding Millex membrane (Millipore), the concentration of plasmid DNA in the filtrate was analyzed by fluorescence spectroscopy (Horiba, Japan) using DAPI as a fluorescent dye (excitation 370, emission 470 nm). Salmon sperm DNA (Invitrogen) was used as a standard (0 - 168 ng/mL).

**Efflux of RNA.** Cells were harvested at exponential growth phase and incubated with and without SWNT in 50 mL of 5-times diluted LB medium for 2 hours. After the incubation, cell suspensions were immediately centrifuged at 25,000 g for 15 minutes. 10 mL of the supernatant were placed in vial tubes with 50 µL of β-mercaptoethanol. RNA was then isolated using a RNA purification kit (Qiagen, USA), and measured with a ND1000 spectrophotometer (NanoDrop, USA). All samples were kept at 4°C during RNA sampling and isolation.



**Figure A.** Area-based estimation of viable and dead cells. (a) A sample of raw image obtained from direct microscopic observation. (b) An image converted into gray scale. (c) Cumulative fraction of pixels from black (0) to white (255). Original images (1360x1024 pixels) acquired through the microscope (Olympus BX51) were downloaded to a PC and analyzed with image analysis software (Image J, NIH). In a raw grey-scale image, each pixel holds 256 steps of an integer grey-level value which describes the intensity of the light (0 = black, 255 = white). The distribution of the grey level was then analyzed and the threshold value was chosen at the middle of the light intensity (from the curve of the cumulative fraction of pixels).

## References

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