I. Deliverable Name, Associated Task ID and Task Title

Year 1 Deliverable: Data on the characterization, fate, and toxicity (tested in model mammalian cells) of CNT nanoparticles.
Task Title: Predicting, Testing, and Neutralizing Nanoparticle Toxicity
Task ID: 425.027

II. Summary/Abstract

This report summarizes recent results that address concerns about the Environmental Safety and Health (ESH) of single-walled carbon nanotubes (SWNTs). Molecular simulation model parameters were developed and cell growth assay experiments were performed to assess the toxicity of SWNTs supplied by commercial vendors towards model mammalian cells. Evidence of toxicity was found in a carboxylated SWNT product. However, a simple filtration procedure detoxified this product. This provides preliminary evidence on the intrinsically benign nature of SWNT materials, with ESH concerns shifted to the process by-products and contaminants.

III. Technical Results and Data

In the present study, ESH concerns about SWNTs were addressed using: 1) chemical and physical characterization; 2) computational modeling; and 3) biological testing.

Four different SWNT products, including pristine (CG100, AP, P2) and carboxylated (P3) SWNTs, were purchased from major vendors. Thorough characterization was performed on these samples (Fig. 1). In particular, we found that the P3 carboxylated SWNT product was contaminated with what we hypothesize are amorphous carbon fragments. These fragments could be removed using a 0.22 μm filter as shown by atomic force microscopy (AFM) and Raman analysis (Fig. 2).

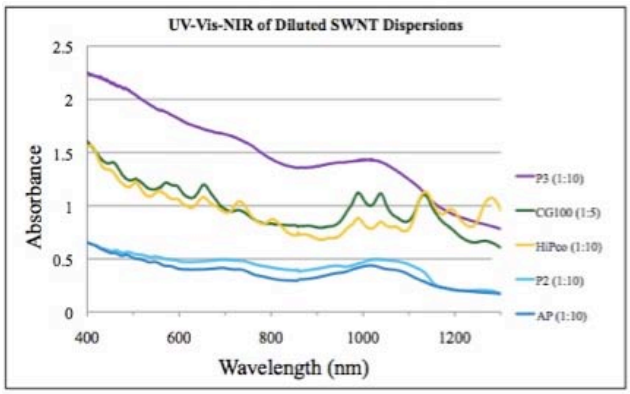
Molecular simulation parameters were developed and validated against experimental and prior simulation studies (data not shown). The interaction between a SWNT and a cellular membrane (Fig. 3) was characterized through structural and thermodynamic quantities. The free energy profile of a SWNT translocating through a cellular membrane shows that pristine SWNTs should interact with cellular membranes to a greater extent than carboxylated SWNTs (Fig. 4).

For biological testing, the SWNTs were dispersed using an aqueous HEPES buffer for maintaining a neutral pH and bovine serum albumin (BSA) for colloidal stability. Cultured NRK cells were incubated for 3 days with 100 μg/mL of various SWNT dispersions. Cellular uptake of SWNTs was monitored using Raman microscopy (Fig. 5). The toxicity of a SWNT product was assessed by comparing the number of cells present after 3 days with that of untreated control cells. Only the carboxylated SWNT commercial product was found to be cytotoxic (Fig. 6). This finding was at odds with the modeling results, which showed that carboxylated SWNTs have minimal cellular membrane interactions. However, the toxic activity could be removed by a filtration procedure. The recovered carboxylated SWNTs show little toxicity (Fig. 6).

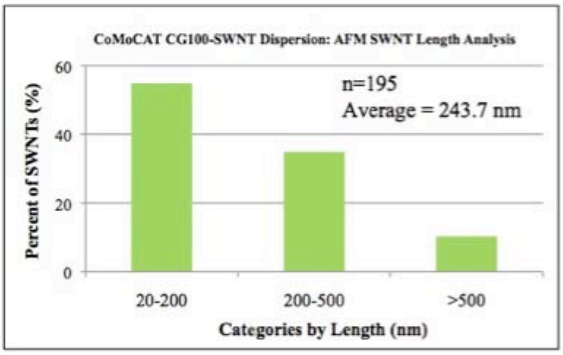
In summary, we conducted a comprehensive study of SWNT ESH concerns from which we can draw the following conclusions: 1) thorough materials characterization is needed to identify potential contaminants and process by-products, 2) molecular simulation gives insight into the effect of SWNT carboxylation on cellular membrane interactions, and 3) pristine and filtration-purified carboxylated SWNTs show little toxicity and can be used for various applications with minimal ESH complications.

IV. Supplementary Tools or Materials Available

-GRC Publication P055218



SWNT Dispersion	SWNT concentration (µg/mL)
CG100	197 ± 37
AP	246 ± 20
P2	301 ± 26
P3	752 ± 58
HiPco	NA



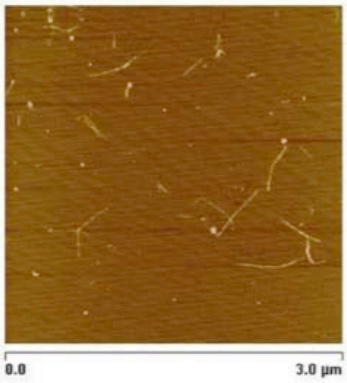


Fig. 1. SWNT dispersions were analyzed using UV-Vis-NIR spectroscopy to identify individual SWNT types and their relative abundances (top left panel), SDS-PAGE to quantify absolute SWNT amounts in the dispersions (top right panel; P3 is carboxylated, all other SWNTs are pristine), and AFM imaging for nanotube height and length analysis and uniformity assessment (bottom two panels).

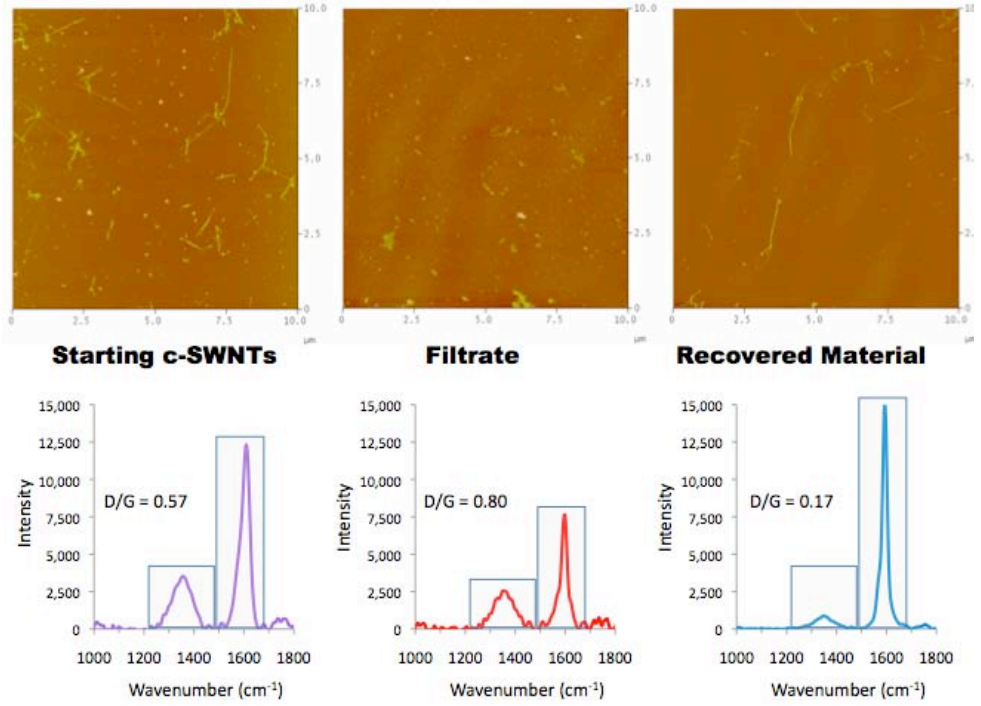


Fig. 2. AFM and Raman analysis of the P3 carboxylated SWNT material before and after filtration. For the corresponding toxicity analysis see the right hand panel of Fig. 6.

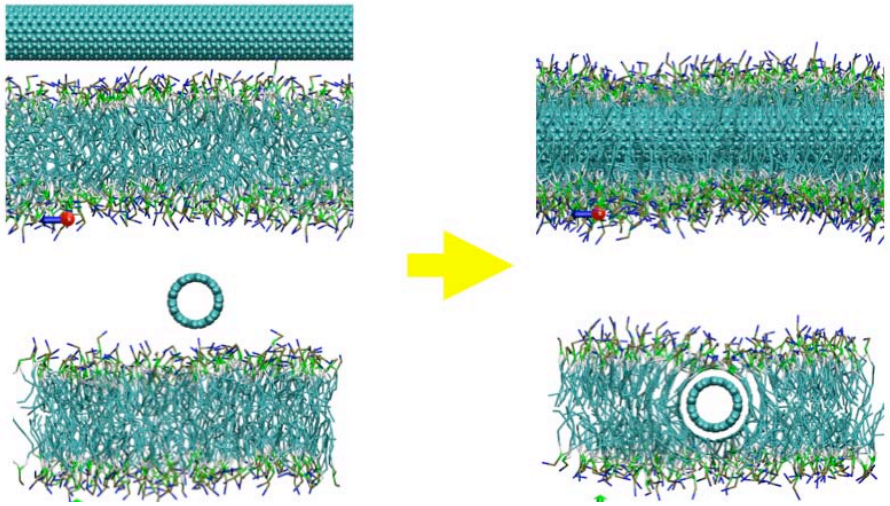


Fig. 3. Computer simulation procedure: a SWNT in aqueous media is translocated through a cellular membrane (top row: side view; bottom row: end view). This event is characterized through structural and thermodynamic quantities.

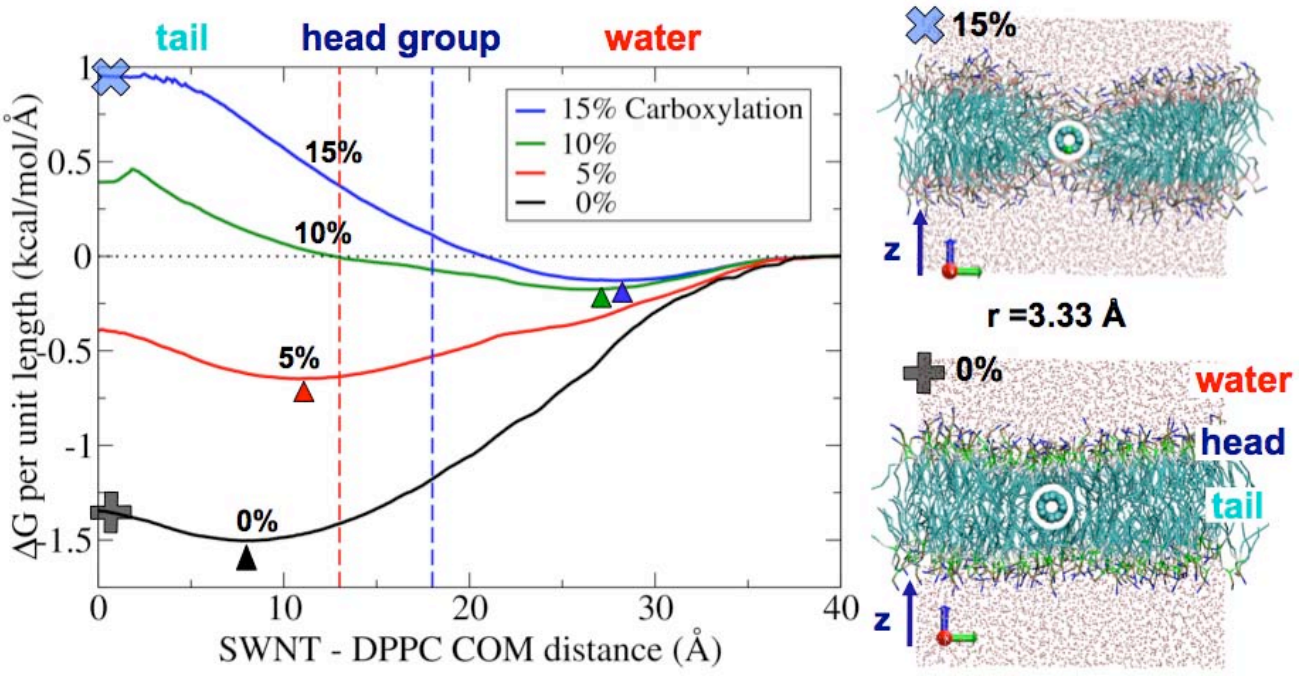


Fig. 4. Free energy profile of SWNTs translocating through a cellular membrane. It is favorable for pristine SWNTs to penetrate a cellular membrane (black curve, snapshot shown in lower right hand panel). Conversely, it is unfavorable for 10-15% carboxylated SWNTs to penetrate a cellular membane (blue curve). At the point marked with an X (and shown in the upper right hand panel), the SWNT has severely distorted the cellular membrane.

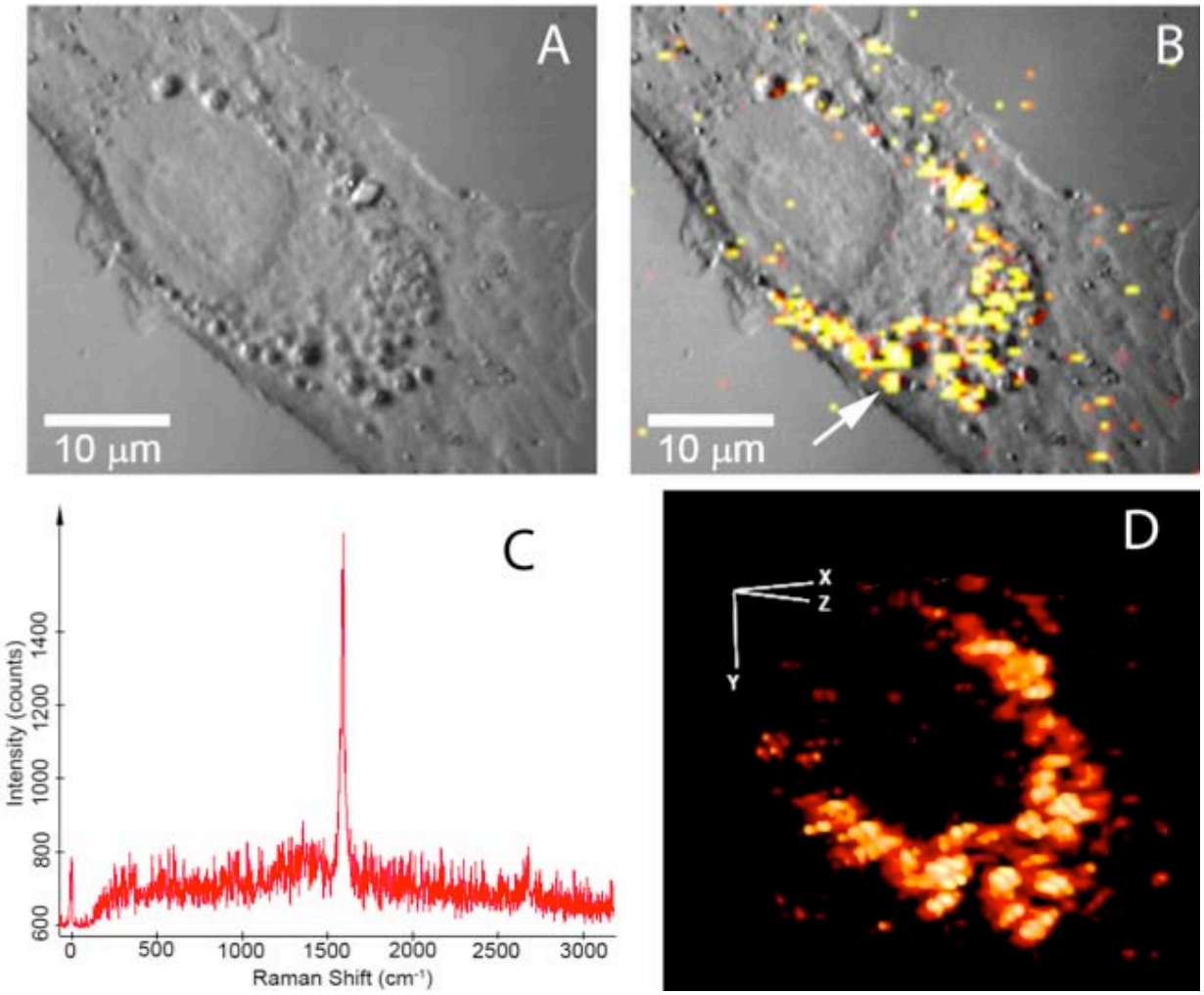
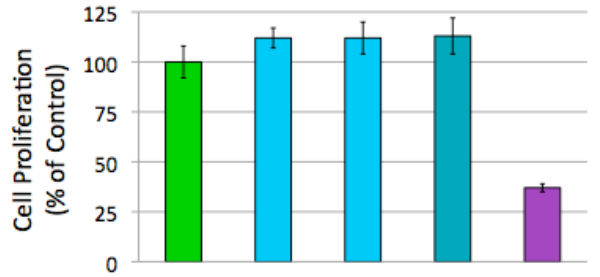


Fig. 5. Direct evidence of SWNT uptake by mammalian cells using confocal Raman microscopy. The Raman G-band (panel C) is used to identify SWNTs in a scan of a mammalian cell (panel D). When superimposed on a visible light microscope image of the cell (panel A), the cellular uptake is clearly seen (panel B).



SWNT Product	Control	AP	CG100	P2	P3
Vendor	-	A	B	A	A
Modification	-	Pristine	Pristine	Purified	Carboxylated

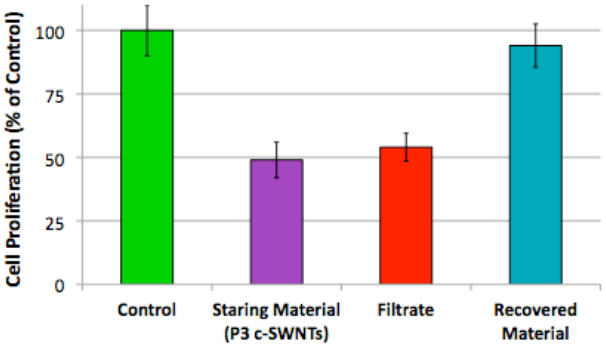


Fig. 6. Cytotoxicity of SWNT products using a cell growth assay. *Left panel*: SWNTs tested are: CoMoCAT CG100-SWNTs, electric arc as-prepared (AP), purified (P2), and carboxylated (P3)-SWNTs. *Right panel*: Toxicity analysis of the P3 carboxylated SWNT material before and after filtration (see Fig. 2).