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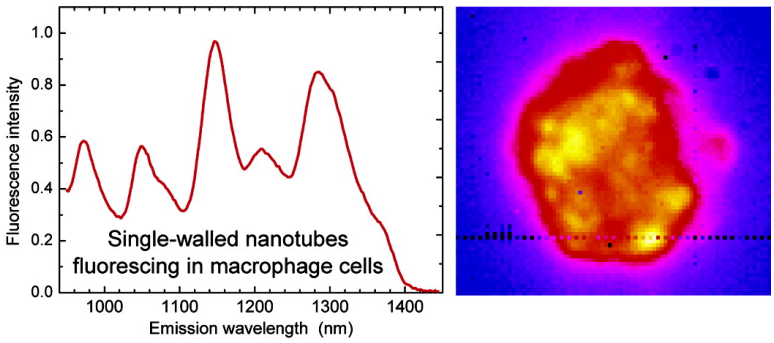
J. Am. Chem. Soc., **2004**, 126 (48), 15638-15639• DOI: 10.1021/ja0466311 • Publication Date (Web): 11 November 2004



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Published on Web 11/11/2004

Near-Infrared Fluorescence Microscopy of Single-Walled Carbon Nanotubes in Phagocytic Cells

Paul Cherukuri,[†,§] Sergei M. Bachilo,[‡] Silvio H. Litovsky,[†,||] and R. Bruce Weisman*[,‡]

Department of Internal Medicine, University of Texas Health Science Center, Houston, Texas 77030, and Department of Chemistry, Center for Biological and Environmental Nanotechnology, and Center for Nanoscale Science and Technology, Rice University, 6100 Main Street, Houston, Texas 77005

Received June 8, 2004; E-mail: weisman@rice.edu

The interactions between artificial nanomaterials and biological systems form an emerging research topic of broad importance. Work in this area is motivated both by the hope that nanomaterials will have useful applications in biology and medicine[1,2] and by the concern that they may exert harmful effects on organisms.[3−5] Despite the prominence of carbon nanotubes in nanotechnology, exploration of their interactions with biological materials remains at a very early stage.[6] In part, this reflects the challenge of observing nanotubes in biological environments. Single-walled carbon nanotubes (SWNTs) in tissues evade detection by elemental analysis (because they contain only carbon) and electron microscopy (because of low contrast and small diameters). Radiolabeling methods are currently impractical with SWNTs. Recently, SWNTs covalently linked to visible-wavelength fluorophores have been imaged in cells.[7,8] In this approach, however, the chemical linkage must resist enzymatic cleavage, emission from the visible-wavelength fluorophore must be detected above background endogenous fluorescence, and one must be aware that chemical processing of nanoparticles may dramatically change their biological fate.[9] We present here a method for observing pristine, hydrophobic SWNTs in biological media through their unique near-infrared intrinsic fluorescence.[10]

We have selectively detected low concentrations of nanotubes in biological specimens using a spectrofluorometer and a fluorescence microscope modified for near-IR imaging. The unusually long wavelengths of SWNT emission allow high contrast detection with strong discrimination against endogenous fluorescence. We illustrate our technique with near-IR fluorescence spectra, quantitative emission intensities, and microscopic images of mouse macrophage-like cells that have actively ingested nanotubes while incubated in SWNT suspensions. We also find that viability and population growth of these cells are not affected by SWNT exposure under the experimental conditions.

As detailed in the Supporting Information, cultured mouse peritoneal macrophage-like cells were incubated for varying periods in a growth medium containing various concentrations of SWNT (ca. 1.0 nm average diameter, ca. 1 μm average length) in Pluronic surfactant. Cell cultures with added surfactant or added surfactant plus 3.8 μg/mL of SWNTs showed equivalent population growth and normal adhesion, morphology, and confluence. A prior study found similarly low cytotoxicity from derivatized SWNTs at higher concentrations.[8] After careful washing and fixing, the incubated cells were analyzed for near-IR nanotube fluorescence using a spectrofluorometer and a fluorescence microscope adapted for imaging between 1125 and 1600 nm. In both cases, fluorescence was excited by light from a 660 nm diode laser.

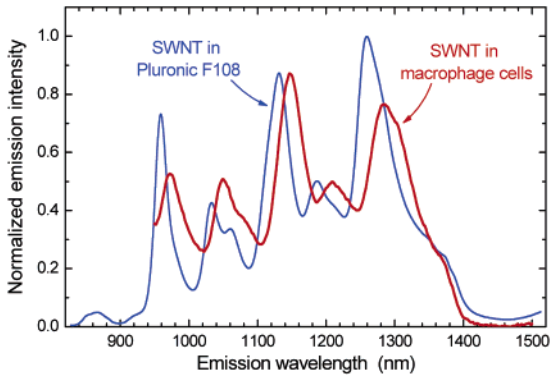

Figure 1. SWNT emission spectra in an aqueous Pluronic F108 suspension (blue trace) and in macrophage cells incubated in SWNT suspension and then washed (red trace). Samples were excited at 660 nm. Intensities have been scaled to aid comparison. Emission beyond 1350 nm is strongly attenuated by H_2O absorption.

Figure 1 displays the measured fluorescence spectrum of the aqueous Pluronic SWNT suspension that was added to the culture medium and the spectrum from incubated and washed macrophage cells. The distinct emission peaks arise from identified semiconducting SWNT species in our sample.[11] Significant broadening and red-shifting of these spectral peaks is observed for nanotubes in the culture medium and in incubated macrophage cells, as compared to Pluronic suspension. We believe that these spectral changes arise from displacement of the nanotubes' surfactant coating by proteins. Note that the wavelengths of SWNT emission are far longer than those of the longest-wavelength organic fluorochromes used for bioimaging.

Figure 2a shows the growth in nanotube fluorescence emission per cell vs incubation time. The smooth increase indicates a steady uptake rate. A comparison sample incubated at 27 °C showed only 40% of the nanotube uptake found at 37 °C. This finding differs sharply from the temperature-independent cell penetration by functionalized SWNTs reported by Pantarotto et al.,[7] but it is consistent with the temperature dependence of macrophage phagocytosis,[12] suggesting active ingestion of the nanotubes. Figure 2b shows that nanotube uptake into the cells is a smoothly increasing function of nanotube concentration in the medium. The uptake scale was calibrated from measured nanotube loss in the growth medium. Taking the mass of a typical 1 μm long nanotube as 2.2×10^{-18} g, we estimate an average ingestion rate (at our highest nanotube concentration) of approximately 1 nanotube per second per cell.

Using samples incubated to give an average uptake of ca. 70 000 nanotubes per cell, we recorded fluorescence microscopic images of the macrophages between 1125 and 1600 nm. Controls incubated

[†] University of Texas Health Science Center.
[‡] Rice University.
[§] Current address: Department of Chemistry, Rice University.
[||] Current address: Department of Pathology, School of Medicine, University of Alabama at Birmingham.

10.1021/ja0466311 CCC: $27.50

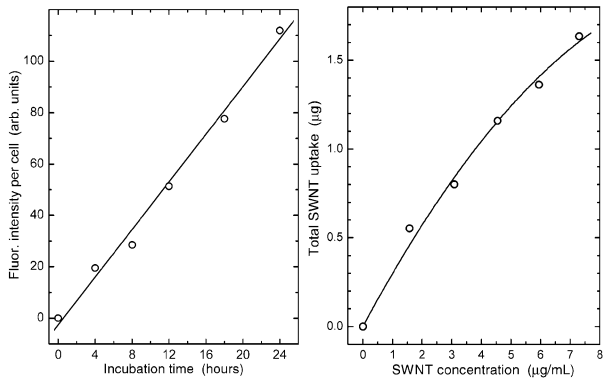

Figure 2. SWNT fluorescence intensity (integrated from 950 to 1500 nm) from incubated, washed samples of 10^7 macrophage cells: (a) as a function of incubation time with 7.3 μg/mL of SWNTs; (b) as a function of SWNT concentration after 24 h incubation. The solid lines show linear (a) and quadratic (b) fits to the data.

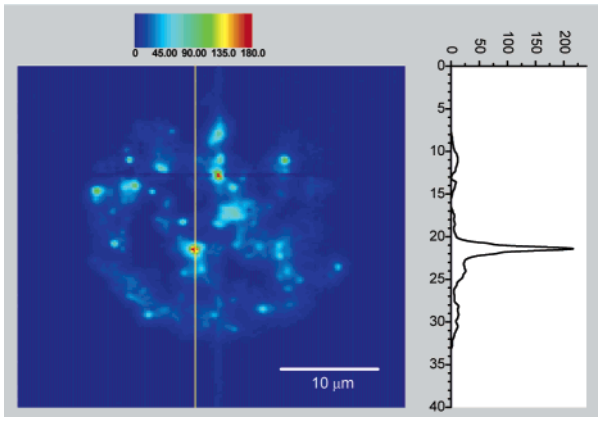

Figure 3. Fluorescence image of one macrophage-like cell incubated with SWNTs, showing emission detected from 1125 to 1600 nm with excitation at 660 nm. Intensities are coded with false color, and the image was obtained from a *z*-axis series by deconvolution processing. Intensity along the yellow vertical line is plotted on the graph to the right, showing high image contrast and localized emission sources.

without nanotubes showed no detectable emission, whereas all samples incubated with nanotubes gave emission. Figure 3 shows a false-color coded fluorescence image of one cell, obtained by deconvolving a *z*-series of images (100 s exposure each) to suppress out-of-focus components. This image was taken near the cell's vertical center and clearly shows many localized intracellular regions of near-infrared light emission, most with apparent diameters limited by our optical resolution of ca. 1 μm. We interpret these as nanotubes contained in small phagosomes and suggest that nanotubes were actively ingested through phagocytosis. Active ingestion is consistent with the observed temperature-dependent uptake. We note that the nanotubes retain some emissive character despite the harsh oxidizing environment in macrophage phagosomes.[13,14]

Two factors allow intracellular SWNT to be detected with high contrast despite their low fluorescence quantum yield. First, endogenous fluorescence from tissues is far weaker at wavelengths above 1125 nm than in the limited near-IR segment from 750 to 1100 nm commonly used for bioimaging. Second, semiconducting SWNT have very large shifts between excitation and emission wavelengths when (as here) they are excited at second van Hove absorption transitions and detected through first van Hove emission.[11] Background signals from endogenous Raman scattering can therefore be avoided, because the SWNT excitation−emission shifts exceed all vibrational Raman shifts. The high optical contrast may allow SWNTs to serve as a fluorophore in future families of biochemically targeted tissue markers for selective detection and imaging in bioresearch and medicine. Nanotubes apparently share the high photostability of quantum dots,[17,18] as no photobleaching was observed in our imaging experiments for irradiation times of up to 100 min. Although carbon nanotubes currently show fluorescent quantum efficiencies lower than those often found for inorganic quantum dots,[10,15,16] they carry no risk of heavy metal toxicity.

In summary, we find that macrophage cells can actively ingest significant quantities of single-walled carbon nanotubes without showing toxic effects. The ingested nanotubes remain fluorescent and can be imaged through near-infrared fluorescence microscopy at wavelengths beyond 1100 nm. High contrast imaging is aided by very low levels of endogenous fluorescence in this previously unexploited spectral region. Fluorescence detection and imaging of single-walled carbon nanotubes provide powerful tools for tracing the interactions of nanotubes with cells, tissues, and organisms. When sorted nanotube samples having selected, narrowed spectra become available, their use with tailored excitation and detection will further improve optical imaging contrast and sensitivity. Future applications may include studies of nanotube biodistributions in organisms and the development of new families of bioconjugated fluorescent markers and contrast agents for use in cell biology research and medical diagnosis.

Acknowledgment. We are grateful to the NSF (CHE-0314270), the NSF Center for Biological and Environmental Nanotechnology (EEC-0118007), the Welch Foundation (C-0807), and the U.S. Army (DREAMS, DAMD 17-98-1-8002) for funding. We thank the Rice CNST for instrumentation; R. E. Smalley, R. H. Hauge, and V. C. Moore for SWNT samples; S. W. Casscells, III for support; D. Engler for experimental help; and K. M. Beckingham and L. Lachman for helpful discussions.

Supporting Information Available: Experimental materials, methods, and further data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Penn, S. G.; He, L.; Natan, M. J. *Curr. Opin. Chem. Biol.* **2003**, *7*, 609−615.
(2) Bianco, A.; Prato, M. *Adv. Mater.* **2003**, *15*, 1765−1768.
(3) Huczko, A.; Lange, H.; Calko, E.; Grubek-Jaworska, H.; Droszcz, P. *Fullerene Sci. Technol.* **2001**, *9*, 251−254.
(4) Lam, C. W.; James, J. T.; McCluskey, R.; Hunter, R. L. *Toxicol. Sci.* **2004**, *77*, 126−134.
(5) Warheit, D. B.; Laurence, B. R.; Reed, K. L.; Roach, D. H.; Reynolds, G. A. M.; Webb, T. R. *Toxicol. Sci.* **2004**, *77*, 117−125.
(6) Martin, C. R.; Kohli, P. *Nat. Rev. Drug Discov.* **2003**, *2*, 29−37.
(7) Pantarotto, D.; Briand, J.-P.; Prato, M.; Bianco, A. *Chem. Commun.* **2004**, *2004*, 16−17.
(8) Kam, N. W. S.; Jessop, T. C.; Wender, P. A.; Dai, H. *J. Am. Chem. Soc.* **2004**, *126*, 6850−6851.
(9) Gupta, A. K.; Curtis, A. S. G. *Biomaterials* **2004**, *25*, 3029−3040.
(10) O'Connell, M.; Bachilo, S. M.; Huffman, C. B.; Moore, V.; Strano, M. S.; Haroz, E.; Rialon, K.; Boul, P. J.; Noon, W. H.; Kittrell, C.; Ma, J.; Hauge, R. H.; Weisman, R. B.; Smalley, R. E. *Science* **2002**, *297*, 593−596.
(11) Bachilo, S. M.; Strano, M. S.; Kittrell, C.; Hauge, R. H.; Smalley, R. E.; Weisman, R. B. *Science* **2002**, *298*, 2361−2366.
(12) Matsui, H.; Ito, T.; Ohnishi, S.-I. *J. Cell Sci.* **1983**, *59*, 133−143.
(13) Duerksen-Hughes, P. J.; Gooding, L. R. Macrophage-Mediated Cytotoxicity. In *Cytotoxic Cells*; Sitkovsky, M. V., Henkart, P. A., Eds.; Birkhaeuser: Boston, MA, 1993; pp 439−454.
(14) Burke, B., Lewis, C. E., Eds. *The Macrophage*, 2nd ed.; Oxford University Press: Oxford, UK, 2002.
(15) Alivisatos, A. P. *J. Phys. Chem.* **1996**, *100*, 13226−13239.
(16) Watson, A.; Wu, X.; Bruchez, M. *Biotechniques* **2003**, *34*, 296−303.
(17) Bruchez, M.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013−2016.
(18) Chan, W. C. W.; Nie, S. *Science* **1998**, *281*, 2016−2018.

JA0466311