Spectroscopic Characterization of Protein-Wrapped Single-Wall Carbon Nanotubes and Quantification of Their Uptake in Macrophages

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Abstract

Biomedicine is one area in which single wall carbon nanotubes (SWNTs) have potential for great impact.[1][2][3] However, the toxicological profile of SWNTs remains a significant concern.[4][5][6] In our study, we perform quantitative evaluation of the time-evolution of SWNT uptake in mouse macrophages over a period of three cell cycles. Macrophages are white blood cells important for the innate and adaptive immune systems of vertebrates[7] and hence represent especially relevant systems to study. Macrophages phagocyte debris and pathogens, and contribute to the initiation of other defense mechanisms, thus playing an important role in the body's response towards foreign objects. SWNTs grown via two different processes (CoMoCAT and P2) are investigated to ensure the results are not specific to a single type of SWNT. Both the SWNT types are conjugated with BSA to enhance their biocompatibility. Raman spectroscopy is utilized to monitor the SWNT concentration in the bulk of cell suspension to obtain statistically significant sampling. In the process, we also apply optical absorption and photoluminescence spectroscopy methods to characterize the properties of the BSA-SWNT dispersions. Optical absorption spectroscopy offers the first quantifiable characterization of the BSA-functionalized SWNTs. Figure 1(left) shows the absorption spectra for an aqueous solution of BSA and for aqueous dispersions of BSA-CoMoCAT and BSA-P2. The BSA spectrum exhibits peak absorption at ~277 nm. The same peak is observed in the BSA-CoMoCAT and BSA-P2 spectra, but with shifted positions and at much reduced intensities. Since this BSA absorption peak is primarily caused by amino acids with aromatic rings,[8] the observed shift suggests that a change in the protein structure occurs when the BSA molecule binds to the SWNT surface. Semiconducting SWNTs (s-SWNTs) have a direct band gap. Therefore, photoluminescence emission from isolated s-SWNTs due to exciton recombination is expected (Figure1 (right)). The optical signatures associated with the (6,5) species are red-shifted by ~8 nm (150 eV), while the larger diameter chirality (7,5) nanotubes do not exhibit any noticeable shifts. The shifts in the emission wavelengths are likely due to increased doping of the SWNTs through SWNT-biomolecule charge transfer.[9] The larger shifts and resultant increased doping of smaller diameter nanotubes are also due to preferential wrapping and isolation of the smaller diameter SWNTs by BSA. For the BSA-SWNTs, the intensity of the G^+ peak is monitored at different SWNT concentrations to produce a calibration relationship between intensity and concentration for Raman signals. We found that the G-band Raman intensity follows a well-defined power law for SWNT concentrations of up to 30 µg/ml in aqueous solution. RAW 264.7 mice macrophages are incubated with BSA-SWNT hybrids. The viability of the macrophages incubated with BSA-SWNTs is studied by monitoring cellular growth and using the redox-based Alamar Blue assay. Our experiments demonstrate that incubation of BSA-SWNT complexes with macrophages neither affects the cellular growth, nor the cellular viability over multiple cell generations (Figure2). We then determine the uptake of BSA-functionalized SWNTs by macrophages, specifically as a function of time. As mentioned before, the G^+ intensity in SWNT Raman spectra can be directly correlated to the nanotube concentration in the sampled region. The presence of the characteristic SWNT peaks in the Raman spectrum obtained from the cells incubated with BSA-CoMoCAT nanotubes (Figure3a) is a simple confirmation that this functionalized nanotube species is uptaken by the macrophages. The average number of nanotubes internalized per cell was found remaining relatively constant over consecutive cell generations (Figure3b). The number of internalized macrophages is found to be ~ 40 x 10^6 SWNTs/cell for a 60 mm^{-2} seeding density and ~ 140 x 10^6 SWNTs/cell for a 200 mm^{-2} seeding density. Our results show that BSA-functionalized SWNTs are an efficient molecular transport system with low cytotoxicity maintained over multiple cell generations.

References

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Figures

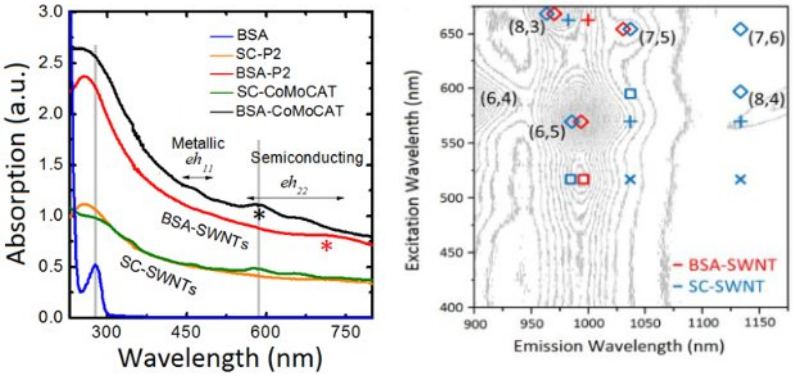

Figure1: (left) Absorption spectra. (right) PLE spectrum. Each resonance is labeled with the chiral index of the corresponding SWNT and is denoted by ◊. The symbols □, + and X represent phonon sidebands, EET, and EET between sidebands of donors and excitons of acceptor nanotubes in small bundles, respectively.

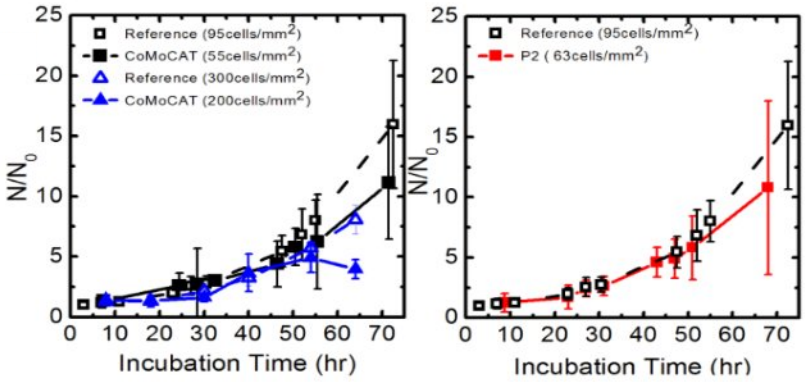

Figure2: macrophage viability.

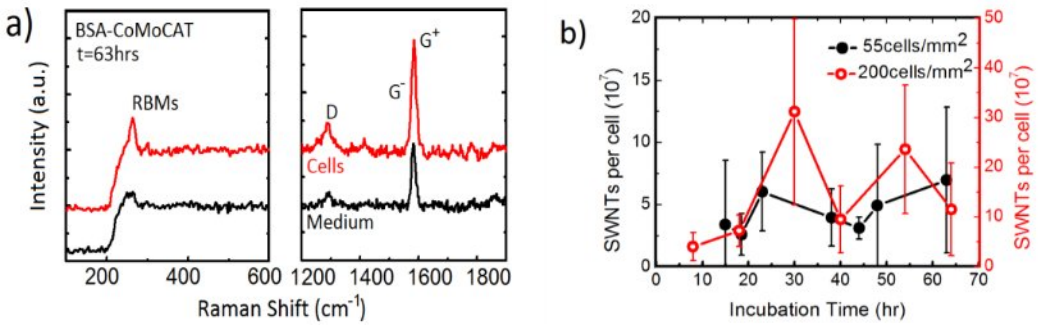

Figure3: a) Raman spectra for macrophages incubated with BSA-CoMOCAT. b) Amount of BSA-CoMoCAT complexes uptaken.