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STUDY OF THE NEAR INFRARED-MEDIATED HEATING OF DISPERSIONS OF PROTEIN-COATED PRISTINE AND CARBOXYLATED SINGLE-WALLED CARBON NANOTUBES

Alex T. Sheardy*, Jeremy J. Taylor*, Jennifer L. Chilek*, Synyoung Li†, Ruhung Wang*†, Rockford K. Draper*†‡, and Paul Pantano*†§

*Department of Chemistry, The University of Texas at Dallas, Richardson, TX 75080-3021, USA

†Department of Molecular & Cell Biology, The University of Texas at Dallas, Richardson, TX 75080-3021, USA

‡The Alan G. MacDiarmid NanoTech Institute, The University of Texas at Dallas, Richardson, TX 75080-3021, USA

Abstract

Previously, we demonstrated the selective NIR-mediated ablation of tumor cells *in vitro* using pristine single-walled carbon nanotubes (SWNTs) with adsorbed tumor-targeting ligands and carboxylated SWNTs with covalently-attached ligands. The covalent approach is advantageous in ensuring that protein ligands remain associated with the NIR-absorbing SWNTs in biological matrices and the noncovalent approach has the advantage of enabling SWNT functionalization without perturbation of the SWNT lattice and photothermal properties. Herein, we compare the ability of moderately-carboxylated (~4 at.% carboxylic acid groups) and pristine SWNT materials to absorb 808 nm radiation and convert it to heat. Under conditions of a constant 808 nm laser power density, the approach involved measuring the temperature of aqueous dispersions of protein-coated SWNTs as a function of the irradiation time. Nearly identical temperature profiles were observed for dispersions of moderately-carboxylated and pristine SWNTs possessing matched 808 nm optical densities and equivalent concentrations of carbonaceous species (i.e., SWNTs and amorphous carbon impurities). The results indicate that the amount of carbonaceous species in purified dispersions of protein-coated SWNTs is more important for converting absorbed 808 nm radiation into heat than whether or not the SWNTs were moderately carboxylated, and that moderately-carboxylated SWNTs could be the SWNT-material of choice for the targeted photothermal ablation of tumor cells.

Keywords

Photothermal therapy; nanoparticle-mediated hyperthermia; tumor cell ablation; oxidized carbon nanotubes

1. Introduction

The application of nanotechnology to medicine has opened new areas of cancer treatment including the use of nanomaterials whose properties are suited for the photothermal mediation of hyperthermia. Hyperthermia refers to the treatment of tumors by the administration of heat and is generally defined by a temperature rise beyond 40°C, which can compromise cell membranes, denature proteins and disrupt normal cellular processes.^{1,2} However, since heat can harm both healthy and cancerous cells, the main challenge with administering hyperthermal therapies has been applying heat only to a specified, localized region.^{3,4} One solution to this obstacle involves the use of nanoparticles that can be selectively targeted to a tissue of interest and heated by an external electromagnetic source, thereby eliminating the use of implantable metal rods or other invasive heating devices. The most common electromagnetic source has been the 780–820 nm near infrared (NIR) spectral region where tissue components such as hemoglobin, water and lipids collectively have their lowest NIR absorption coefficients,⁵ and the most prevalent nanoparticles have been gold-coated silica nanoshells,⁶ gold nanorods⁷ and carbon nanotubes,⁸ which are readily taken-up by living cells and are strong NIR absorbers.

Single-walled carbon nanotubes (SWNTs) are ideal nanomaterials for mediating the photothermal ablation of tumors because they can effectively convert absorbed electromagnetic energy into thermal energy. In brief, the transfer of energy from incident photons to quantized lattice vibrations (phonons) is very efficient in SWNTs and depends on the optical absorption cross section, phonon dispersion relations, and phonon densities of states.^{9,10} Ultimately, the heating of a solution (or a living cell) occurs after the absorbed photon energy is converted to thermal energy, which rapidly diffuses along the sp²-hybridized carbon lattice and to the medium inside and surrounding the SWNT.^{11,12}

Targeting SWNTs to tumors is accomplished by coating them with tumor-specific cell binding ligands such as vitamins, peptides, and monoclonal antibodies. To date, the use of targeted SWNTs to thermally ablate tumor cells either *in vitro* or *in vivo* using 808 nm laser excitation has been demonstrated using both pristine SWNTs with adsorbed targeting ligands,^{8,13–15} and oxidized SWNTs with covalently-attached ligands.^{16–18} However, since disruption of the sp²-hybridized carbon lattice will affect the propagation of phonons in a SWNT, it stands to reason that the photothermal properties of a SWNT could be affected by the manner in which the tumor-targeting ligand is attached. For example, the covalent attachment of ligands to SWNTs typically involves oxidizing the SWNTs to some degree to generate surface carboxyl groups that can be reacted with free amines on a targeting ligand to form stable amide bonds. Oxidizing SWNTs, however, can disrupt the sp²-hybridized carbon lattice through the formation of defect sites and sp³ bonds, which could alter phonon propagation, and hence, photothermal conversion efficiencies. On the other hand, the noncovalent adsorption of ligands to SWNTs has the advantage of enabling SWNT functionalization while still preserving the SWNT's electronic structure since the sp²-hybridized bonding is not altered.¹⁹ The downside is that adsorbed ligands are likely to be displaced by other macromolecules in biological fluids resulting in the disassociation of the targeting moiety from the NIR-absorbing nanomaterial.

In previous works, we demonstrated the selective NIR-mediated ablation of cancer cells *in vitro* using pristine SWNTs with adsorbed antibodies¹⁵ and carboxylated SWNTs with covalently-attached antibodies.¹⁶ Herein, we compare the NIR-mediated heating of the pristine and moderately-carboxylated (~4 at.% carboxylic acid groups) SWNT starting materials used in these *in vitro* experiments by measuring the NIR-mediated heating of aqueous dispersions of each material. Under conditions of a constant 808 nm laser power density, the temperatures of different concentrations of protein-coated pristine or

carboxylated SWNTs were measured as a function of the irradiation time. The results showed that dispersions of pristine and moderately-carboxylated SWNTs with matched 808 nm optical densities and equivalent concentrations of carbonaceous species (i.e., SWNTs and amorphous carbon impurities) were indistinguishable in their ability to absorb 808 nm radiation and heat a solution. For example, after a 2 min exposure to 2.9 W/cm² of 808 nm radiation, the temperatures of pristine and carboxylated SWNT dispersions, each containing ~16 µg/mL of carbonaceous species, were both 32°C higher than control solutions that did not contain SWNTs. The results indicate that the amount of carbonaceous species in purified dispersions of protein-coated SWNTs is more important for converting absorbed 808 nm radiation into heat than whether or not the SWNTs were moderately carboxylated. Since pristine SWNTs with adsorbed antibodies and carboxylated SWNTs with covalently-attached antibodies were shown previously to be equally effective in targeting and killing cancer cells via NIR-mediated thermal ablation,^{15,16} and since the covalent approach is advantageous in ensuring that targeting ligands remain associated with the NIR-absorbing nanomaterial in biological fluids, the combined results suggest that moderately-carboxylated SWNTs could be the SWNT-material of choice for the targeted photothermal ablation of tumors.

2. Experimental Procedure

2.1. Materials and solutions

Pristine SWNTs (Lot No. Raw-0559) were purchased from Unidym Inc. and SWNTs carboxylated by reaction with nitric acid (Batch No. P3-348; 1.5–6.0 at.% carboxylic acid groups) were purchased from Carbon Solutions Inc. **Caution:** *a fine particulates respirator should be worn when handling dry SWNT powders.* Aqueous 100 mg/mL bovine serum albumin (BSA; Sigma-Aldrich) solutions were prepared by dissolving 10 g BSA in 100 mL of deionized water and adjusting the pH to 7.4 with NaOH before filtering the solution through a 0.22 µm membrane. Deionized water (18.2 MΩ-cm) was obtained using a nanopure infinity water purification system (Barnstead); all other chemicals were of the highest quality available and were used as-received.

2.2. Thermogravimetric analyses

Thermogravimetric analysis (TGA) measurements of SWNT powders were performed using a Perkin Elmer Pyris-1 thermogravimetric analyzer as detailed previously.²⁰ In brief, 1.0–3.0 mg of a thoroughly-mixed pristine or carboxylated SWNT powder was transferred into the platinum pan of the analyzer and heated from 100–1000°C at 5°C/min in 21% O₂/79% N₂ at a flow rate of 20 mL/min. A baseline was generated for each scan and baseline-subtracted thermograms were converted to weight percents. The determination of a component's mass was performed by subtracting the weight percent lost between peak onset and end.

2.3. Preparation of stock BSA-SWNT dispersions and standard BSA-SWNT suspensions

A sonication/centrifugation protocol was used to process dry SWNT powders into stock BSA-SWNT dispersions.²¹ In brief, 1.0 mL of an aqueous 100 mg/mL BSA solution and 1.0 mg of either a pristine- or carboxylated-SWNT powder were mixed in a 1.5 mL microcentrifuge tube and then probe-sonicated at 0°C for 10 min using a Branson 250 Sonifier operated at 9 W. Probe sonication was performed with the ice-cooled, 3 mm diameter probe tip centered in the tube 5 mm from the bottom. The resulting black suspension was centrifuged in an Eppendorf 5417C centrifuge for 2 min at 16000 × *g* (14000 RPM). The upper ~950 µL of the supernatant was recovered without disturbing the sediment and placed in a clean tube before a second 2 min centrifugation at 16000 × *g* was performed. The upper ~900 µL of the second supernatant was carefully recovered to afford

an individual BSA-SWNT dispersion. Individual dispersions were pooled to minimize sample preparation variabilities, and the pooled stock dispersions could be stored at 4°C for at least one month without any SWNTs precipitating out of solution. BSA controls (without SWNTs) were obtained by centrifuging an aqueous BSA solution for 4 min at $16000 \times g$. The preparation of standard BSA-SWNT suspensions was identical to the preparation of stock BSA-SWNT dispersions except that standard suspensions were not centrifuged.

2.4. Absorption spectroscopy

UV-Visible-NIR absorption spectra of BSA-SWNT dispersions were obtained using a Shimadzu UV-1601PC spectrometer. All spectra were background corrected against a BSA control using a scan speed of 280 nm/min and 1.0 cm quartz cuvettes.

2.5. X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) of carboxylated SWNT material was performed by Evans Analytical Group using a PHI Quantum 2000 spectrometer with monochromatic X-rays (1486.6 eV) operating at 20 W. The electron take-off angle was 45° and the analysis area was a 1 mm² spot. Aqueous dispersions of carboxylated SWNTs were filtered and dried as described previously.²² The binding energies were corrected by reference to the C1s line at 284.5 eV and spectra were fitted using Multipak software from Physical Electronics.

2.6. Elemental analyses

Elemental analyses of BSA-SWNT dispersions were performed using a ThermoElectron X-Series inductively coupled plasma mass spectrometer. Samples (100 µL of pristine or carboxylated BSA-SWNT dispersions, BSA controls, and Fe, Ni and Y standards) were acid digested using a protocol developed in association with PreciLab Inc. as described previously.²⁰

2.7. Dynamic light scattering

Dynamic light scattering (DLS) analyses of pristine and carboxylated BSA-SWNT dispersions were performed using a Brookhaven ZetaPALS dynamic light scattering instrument running ZetaPlus particle sizing software and calibrated with 0.1–1.0 µm microsphere standards (Polysciences Inc.). Triplicate 1 min trials were acquired from diluted samples displaying mean count rates $>2 \times 10^5$ counts/s.

2.8. Gel electrophoresis

The amount of carbonaceous species (i.e., SWNTs and amorphous carbon) in BSA-SWNT dispersions were measured using sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) with optical detection as described previously.²¹ In brief, 20 µL aliquots of pristine or carboxylated BSA-SWNT dispersions, standard pristine or carboxylated BSA-SWNT suspensions, and BSA controls were electrophoresed in separate gel lanes at 100 V for 2.5 h. Band intensities from samples and standards containing carbonaceous species were background subtracted using the intensities from the BSA-control lane. Optical images of all gel bands were digitized using a 16-bit flatbed scanner and quantification of the opacity of the bands was performed using IP-Lab software. The carbonaceous species content in pristine and carboxylated BSA-SWNTs was determined from calibration curves prepared from the respective BSA-SWNT standards; in both cases, the weight percent of carbonaceous species in standard BSA-SWNT suspensions was adjusted by the TGA-determined mass of carbonaceous species in each respective pristine and carboxylated SWNT powder.

2.9. NIR diode laser

All samples were contained in 1.6 cm diameter $\times$ 1.0 cm deep cell culture dishes and were exposed to 808 nm radiation provided by a 30-W Newport/Spectra-Physics Integra-MP (model 140-8S-30KS) diode laser system. **Caution:** *NIR-protective eyewear should be worn at all times.* The laser output was guided through a 1.2 mm diameter fiber-optic bundle whose distal tip was equipped with safety shutter and a flattop beam diffuser lens. The lens was positioned 20 cm above the dish and adjusted to produce a circular 1.8 cm² spot. Power densities were determined using a Newport 818P-040-25 thermopile detector coupled to a Newport 1928-C power meter. In all experiments, the laser diode current was set to 27.25%, the number of pulses was 8, the pulse duration was 15 s, and the delay between pulses was 5 s.

2.10. Temperature measurements

A waterproof Extech Instruments thermistor (model 39240) was chosen to measure solution temperatures because its 2.3 mm diameter $\times$ 3 mm long probe tip could be completely submerged in the small volume (0.5–1.0 mL) of liquid in the dish. The thermistor displayed an accuracy of 1.1°C between 20°C and 80°C (as determined by calibration in deionized water against a standard mercury thermometer). In contrast to type-K thermocouples, the thermistor was also accurate when operated in aqueous 200 mM KCl solutions. Temperature recordings of stirred, 850 μ L samples of pristine or carboxylated BSA-SWNT dispersions and BSA controls were acquired during the laser delay period with the submerged thermistor tip positioned next to the inner wall of the dish, adjacent to the irradiated central region of the dish. While the thermistor probe was never placed directly in the laser beam path, the metal tip of the probe was covered with white heat shrink tubing to minimize inadvertent heating by the laser beam. With this arrangement, discrepancies between observed and expected NIR-mediated temperature increases were attributed to poor thermal isolation, thermistor accuracy, incomplete irradiation of the entire dish, and the positioning of the thermistor outside of the laser irradiation zone at the bottom of the dish. The thermistor probe tip was cleaned by immersing it in an ultrasonic water bath for 30 s after every run.

3. Results

3.1. Characterization of BSA-SWNT dispersions

The pristine- and carboxylated-SWNT starting materials chosen for this work were the same as those used in our previous *in vitro* ablation studies.^{15,16} The average diameters of the pristine- and carboxylated-SWNTs were $\sim$ 1.0 nm and $\sim$ 1.4 nm, respectively, and both comprised 60–70% semi-conducting SWNTs as reported by the manufacturers. Carboxylated SWNTs were shown to possess 4 at.% carboxylic acid groups as determined by XPS. The dispersant chosen for this work was BSA, a water-soluble protein well known to disperse both pristine and oxidized SWNTs.^{23–27} Stock dispersions of BSA-coated SWNTs were prepared using a well-established sonication/centrifugation protocol. The main advantages of this purification protocol for our work have been the facile production of individually-dispersed SWNTs with short lengths and the effective removal of heavier metal-containing carbonaceous species and SWNT bundles.^{15–17,20–22} ICP-MS analyses of stock BSA-SWNT dispersions revealed < 2 ppm Fe and Ni in pristine BSA-SWNT dispersions and < 4 ppm Ni and Y in carboxylated BSA-SWNT dispersions. DLS analyses confirmed the lengths of BSA-SWNTs were within the ranges (i.e., 0.2–1.4 μ m for pristine SWNTs and 0.2–1.5 μ m for carboxylated SWNTs) reported in our previous studies.^{15,16}

Representative UV-Vis-NIR absorption spectra of stock dispersions of pristine and carboxylated BSA-SWNTs are shown in Fig. 1. The absorption peaks, which correspond to van Hove optical transitions in the electronic density of states for the metallic and semi-

conducting SWNT structures present in each dispersion, are superimposed on a nonresonant background, which includes contributions from the π -plasmon absorptions of SWNTs and amorphous carbon impurities not removed by centrifugation.²⁸ The key spectral feature for NIR-mediated heating experiments occurs at ~808 nm, the wavelength of the laser, where the pristine BSA-SWNT dispersions display a distinct absorption peak, undoubtedly a combination of several overlapping van Hove singularities, whereas the carboxylated BSA-SWNT dispersions do not.

Comparisons of the NIR-mediated heating of dispersions of pristine and carboxylated SWNTs involved preparing samples possessing equivalent optical densities at the wavelength of laser excitation in accordance with the experimental design of Huang *et al.*³ However, as shown in Fig. 1, the 808 nm optical densities of stock dispersions of pristine and carboxylated BSA-SWNTs are not equivalent even though both were prepared using the same mass of the respective SWNT starting material. Samples with equivalent optical densities at 808 nm were therefore prepared by appropriately diluting the respective stock BSA-SWNT dispersions with BSA solution. Representative absorption spectra of the resulting three matched sets (A, B and C) of pristine and carboxylated BSA-SWNT dispersions are shown in Fig. 2 and their respective 808 nm absorbances are reported in Table 1.

The amounts of carbonaceous species (i.e., SWNTs and amorphous carbon) in pristine and carboxylated BSA-SWNT dispersions were determined using SDS-PAGE with optical detection.²¹ In brief, when BSA-SWNT dispersions are subjected to SDS-PAGE, carbonaceous species accumulate in a sharp band at the interface between the loading well and the stacking gel, and intensities from the digitized images of these bands are proportional to the amounts of carbonaceous species loaded onto the gel. As shown in Table 1, the concentrations of carbonaceous species in pristine and carboxylated BSA-SWNT dispersions with matched absorbances (i.e., samples within a set) were statistically similar, and the relationship between the concentrations of carbonaceous species and 808 nm absorbances was linear ($R^2 = 0.99$) for both sets of pristine and carboxylated BSA-SWNT dispersions.

3.2. Temperature measurements of aqueous SWNT dispersions during NIR irradiation

The NIR-mediated heating of dispersions of BSA-coated SWNTs was evaluated by measuring the temperatures of the three matched sets of pristine and carboxylated BSA-SWNT dispersions relative to a BSA control solution that did not contain SWNTs. A calibrated liquid-immersion thermistor probe was used to measure solution temperatures, and a laser power density of 2.9 W/cm² was chosen because it was intermediate to the 1.4–5.0 W/cm² values used by others who measured the heating of purified SWNT dispersions exposed to 808 nm radiation,^{8,14,18} it does not significantly reduced the proliferation of mammalian cells that do not contain SWNTs,^{15,16} and it is ~25× less intense than the power density used to treat tumors xenografted on the backs of mice.¹⁴ Figure 3 shows the temperature versus time profiles for the three sets (A–C) of pristine and carboxylated BSA-SWNT dispersions described in Fig. 2 and Table 1. The temperature rise, defined as the difference between the mean temperatures of the BSA control solution and the respective BSA-SWNT dispersion after 2 min of exposure to 2.9 W/cm² of 808 nm radiation, was calculated for each sample and listed in Table 1. For example, an 18°C temperature rise was observed for both pristine and carboxylated SWNT dispersions containing ~8 µg/mL of carbonaceous species (set C), which is akin to the 14°C temperature rise reported by Xiao *et al.* where a 2-fold lower concentration of carboxylated SWNTs and a 1.7-fold higher power density of 808 nm radiation were used.¹⁸

Direct comparison of the NIR-mediated heating of dispersions of pristine and carboxylated BSA-coated SWNTs involved evaluating the relationship between the temperature rise and the concentration of carbonaceous species in each sample. As shown in Fig. 3 and Table 1, the temperature rises observed for pristine and carboxylated BSA-SWNT dispersions with matched 808 nm absorbances (i.e., samples within a set) were identical. Specifically, the observed temperature rise was proportional to the concentration of carbonaceous species for both the pristine BSA-SWNT dispersions ($R^2 = 0.94$) and the carboxylated BSA-SWNT dispersions ($R^2 = 0.94$), which is in agreement with the results of Moon *et al.* who also observed a linear relationship between the NIR-mediated temperature rise and the concentration of pristine SWNTs dispersed by polyethylene glycol.¹⁴

4. Discussion

The photothermal properties of a well-dispersed SWNT sample depend on a number of parameters such as SWNT diameters, lengths, chiralities and the extent of defect sites and surface functionalization, as well as, the SWNT concentration, impurity levels, the solvent and the coating material used to disperse the SWNTs.^{9,10,29} The pristine- and carboxylated-SWNT starting materials were chosen to be the same as those used in our previous works^{15,16}; they possessed similar distributions of lengths and chiralities and slightly different mean diameters (~1.0 nm and ~1.4 nm, respectively). Well-dispersed samples of each starting material were prepared using the same sonication/centrifugation purification protocol and water-soluble protein as surfactant, and the resulting dispersions possessed similar impurity levels. Specifically, all diluted sets of pristine and carboxylated BSA-SWNT dispersions contained sub-ppm metal levels, which is very important since higher levels of metals can contribute to the heating of a solution during exposure to NIR radiation.³⁰ While pristine and carboxylated BSA-SWNT dispersions both displayed an absorbance at 808 nm that can be attributed to the π -plasmon absorptions of SWNTs and amorphous carbon impurities,²⁸ only the pristine SWNTs displayed a significant resonant absorption band in this spectral region. Finally, pristine and carboxylated BSA-SWNT dispersions with matched 808 nm absorbances were shown to possess equivalent concentrations of carbonaceous species.

The key question of this research was whether disruptions of the sp^2 -hybridized carbon lattice in our carboxylated SWNT starting material would affect photothermal conversion efficiencies relative to our noncarboxylated, pristine SWNT material. In brief, since pristine SWNTs are carboxylated using a variety of oxidative methods, commercially-available carboxylated SWNTs are generally classified by their degree of surface functionalization: low (< 2 at.% carboxylic acid groups), moderate (3–6 at.%), or high (> 7 at.%). In our previous *in vitro* ablation studies,¹⁶ moderately-carboxylated SWNTs were chosen since the covalent attachment of large protein ligands did not require a high density of carboxylate groups on the SWNT surface. In addition, it was anticipated that moderately-carboxylated SWNTs would be more effective than highly-carboxylated SWNTs, and perhaps, as effective as pristine SWNTs, in absorbing NIR light and converting it into thermal energy. This is because, under mild oxidative reaction conditions, the most reactive sites on a SWNT surface (e.g., pre-existing sidewall defect sites and edge sites at the ends of opened tubes) will be the first sites to be carboxylated. In other words, if only pre-existing defect and edge sites of a pristine SWNT are carboxylated under mild reaction conditions, further perturbation of the phonon mean free path would be minimized. Conversely, as more aggressive reaction conditions are employed, and all pre-existing defect and edge sites have been reacted, further carboxylation reactions will be initiated at defect-free surface sites, which would likely disrupt the phonon mean free path further and decrease photothermal conversion efficiencies.

In our NIR-mediated heating experiments, pristine and carboxylated BSA-SWNT dispersions with matched 808 nm optical densities and equivalent concentrations of carbonaceous species were indistinguishable in their ability to absorb 808 nm radiation and heat an aqueous solution. This finding can be explained by the moderate degree of surface functionalization of the carboxylated SWNT starting material (~4 at.% carboxylic acid groups) relative to other highly-carboxylated SWNT materials. These results also indicate that the amount of carbonaceous species (i.e., SWNTs and amorphous carbon impurities) in purified SWNT dispersions is more important for converting absorbed 808 nm radiation into heat than whether or not the SWNTs were moderately carboxylated. In conclusion, the selection of moderately-carboxylated SWNTs as the material of choice for the targeted photothermal ablation of tumors has the following advantages: they have been shown to be as effective in targeting and killing tumor cells via NIR-mediated thermal ablation as pristine SWNTs,^{15,16} they can be used to covalently attach targeting ligands to ensure they remain associated with the NIR-absorbing SWNT material, and in contrast to NIR-absorbing nanoparticles comprising gold, carboxylated SWNTs have been shown recently to be biodegraded *in vivo*.³¹

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References

1. Nielsen OS, Horsman M, Overgaard J. Eur. J. Cancer. 2001; 37:1587. [PubMed: 11527682]
2. Hildebrandt B, Wust P, Ahlers P, Dieing A, Sreenivasa G, Kerner T, Felix R, Riess H. Crit. Rev. Oncol. Hematol. 2002; 43:33. [PubMed: 12098606]
3. Huang X, Jain PK, El-Sayed IH, El-Sayed MA. Photochem. Photobiol. 2006; 82:412. [PubMed: 16613493]
4. Hartman KB, Wilson LJ, Rosenblum MG. Mol. Diag. Ther. 2008; 12:1.
5. Weissleder R, Ntziachristos V. Nat. Med. 2003; 9:123. [PubMed: 12514725]
6. Hirsch LR, Stafford RJ, Bankson JA, Sershen SR, Rivera B, Price RE, Hazle JD, Halas NJ, West JL. Proc. Natl. Acad. Sci. USA. 2003; 100:13549. [PubMed: 14597719]
7. El-Sayed IH, Huang X, El-Sayed MA. Cancer Lett. 2006; 239:129. [PubMed: 16198049]
8. Kam NWS, O'Connell M, Wisdom JA, Dai H. Proc. Natl. Acad. Sci. USA. 2005; 102:11600. [PubMed: 16087878]
9. Jorio, A.; Dresselhaus, G.; Dresselhaus, MS. Carbon Nanotubes: Advanced Topics in the Synthesis, Structure, Properties and Applications. Berlin: Springer; 2008.
10. Dresselhaus MS, Eklund PC. Adv. Physics. 2000; 49:705.
11. Boldor D, Gerbo NM, Monroe WT, Palmer JH, Li Z, Biris AS. Chem. Mater. 2008; 20:4011.
12. Bassil A, Puech P, Tubery L, Bacsá W. Appl. Phys. Lett. 2006; 88 173113.
13. Shao N, Lu S, Wickstrom E, Panchapakesan B. Nanotechnol. 2007; 18 315101.
14. Moon HK, Lee SH, Choi HC. ACS Nano. 2009; 3:3707. [PubMed: 19877694]
15. Chakravarty P, Marches R, Zimmerman NS, Swafford AD-E, Bajaj P, Musselman IH, Pantano P, Draper RK, Vitetta ES. Proc. Natl. Acad. Sci. USA. 2008; 105:8697. [PubMed: 18559847]
16. Marches R, Chakravarty P, Musselman IH, Bajaj P, Azad RN, Pantano P, Draper RK, Vitetta ES. Int. J. Cancer. 2009; 125:2970. [PubMed: 19536775]
17. Marches R, Mikoryak C, Wang R, Pantano P, Draper RK, Vitetta ES. Nanotechnol. 2011; 22 095101.
18. Xiao Y, Gao X, Taratula O, Treado S, Urbas A, Holbrook RD, Cavicchi RE, Avedisian CT, Mitra S, Savla R, Wagner PD, Srivastava S, He H. BMC Cancer. 2009; 9:351. [PubMed: 19799784]

19. Lopez-Bezanilla A, Triozon F, Latil S, Blase X, Roche S. *Nano. Lett.* 2009; 9:940. [PubMed: 19191494]
20. Yehia HN, Draper RK, Mikoryak C, Walker EK, Bajaj P, Musselman IH, Daigrepont MC, Dieckmann GR, Pantano P. *J. Nanobiotechnol.* 2007; 5:8.
21. Wang R, Mikoryak C, Chen E, Li S, Pantano P, Draper RK. *Anal. Chem.* 2009; 81:2944. [PubMed: 19296592]
22. Wang R, Mikoryak C, Li S, Bushdiecker DK II, Musselman IH, Pantano P, Draper RK. *Mol. Pharma.* 2011; 8:1351.
23. Kam NWS, Dai H. *J. Am. Chem. Soc.* 2005; 127:6021. [PubMed: 15839702]
24. Edri E, Regev O. *Anal. Chem.* 2008; 80:4049. [PubMed: 18459735]
25. Karajanagi SS, Yang H, Asuri P, Sellitto E, Dordick JS, Kane RS. *Langmuir.* 2006; 22:1392. [PubMed: 16460050]
26. Matsuura K, Saito T, Okazaki T, Ohshima S, Yumura M, Iijima S. *Chem. Phys. Lett.* 2006; 429:497.
27. Elgrabli D, Abella-Gallart S, Aguerre-Chariol O, Robidel F, Rogerieux F, Boczkowski J, Lacroix G. *Nanotoxicol.* 2007; 1:265.
28. Pichler T, Knupfer M, Golden MS, Fink J, Rinzler A, Smalley RE. *Phys. Rev. Lett.* 1998; 80:4729.
29. Veca, LM.; Wang, W.; Lin, Y.; Meziani, MJ.; Tian, L.; Connel, JW.; Ghose, S.; Kong, CY.; Sun, Y-P. *Handbook of Carbon Nano Materials.* D'Souza, F.; Kadish, KM., editors. Vol. Vol. 2. Singapore: World Scientific Publishing; 2011. p. 755-786.
30. Vazquez E, Prato M. *ACS Nano.* 2009; 3:3819. [PubMed: 20025299]
31. Kagan VE, Konduru NV, Feng W, Allen BL, Conroy J, Volkov Y, Vlasova, Belikova NA, Yanamala N, Kapralov A, Tyurina YY, Shi J, Kisin ER, Murray AR, Franks J, Stolz D, Gou P, Klein-Seetharaman J, Fadeel B, Star A, Shvedova AA. *Nat. Nanotechnol.* 2010; 5:354. [PubMed: 20364135]

Fig. 1. Representative background-corrected UV-Vis-NIR absorption spectra of stock dispersions of pristine and carboxylated BSA-SWNTs, each prepared using 1.0 mg of the respective as-received SWNT powder.

Fig. 2. Background-corrected UV-Vis-NIR absorption spectra of three sets (A–C) of pristine and carboxylated BSA-SWNT dispersions. Each set of BSA-SWNT dispersions had different optical densities at 808 nm while each pair of pristine and carboxylated BSA-SWNT dispersions within a set possessed matched 808 nm optical densities.

Fig. 3. Temperature rise for pristine and carboxylated BSA-SWNT dispersions with matched 808 nm optical densities (sample sets A–C) during exposure to 2.9 W/cm² of 808 nm radiation. Solution temperatures were measured with a thermistor probe at 15-s intervals and the temperature rise was defined as the difference between the mean temperatures of the BSA control solution and the respective BSA-SWNT dispersion after 2 min of continuous exposure to NIR radiation. The data points represent mean values and the error bars represent standard deviations from triplicate measurements.

Table 1

Comparison of the NIR-mediated heating of pristine and carboxylated BSA-SWNT dispersions with matched 808 nm absorbances.

Sample sets	808 nm absorbance ^a (a.u.)	Carbonaceous species ^b (μg/mL)	Temperature rise ^c (°C)
A - Pristine SWNTs	0.44	15.6	32
A - SWNTs-CO ₂ H	0.44	16.5	32
B - Pristine SWNTs	0.32	11.7	28
B - SWNTs-CO ₂ H	0.33	12.4	28
C - Pristine SWNTs	0.21	7.8	18
C - SWNTs-CO ₂ H	0.22	8.3	18

Notes:

^aBackground-corrected absorbance peak heights at 808 nm were obtained from the spectra of the three sets (A–C) of pristine and carboxylated BSA-SWNT dispersions shown in Fig. 2. Note that the absorbances at 808 nm were different for each set but equivalent for each pair of pristine and carboxylated BSA-SWNT dispersions within a set.

^bMean concentrations of carbonaceous species were determined by SDS-PAGE with optical detection; the relative standard deviation for triplicate analyses was < 13%. The relationship between the concentrations of carbonaceous species and 808 nm absorbances was linear ($R^2 = 0.99$) for both pristine and carboxylated BSA-SWNT dispersions.

^cThe temperature rise after 2 min of exposure to 2.9 W/cm² of 808 nm radiation is reported as the difference between the mean temperatures of the BSA control solution and the respective BSA-SWNT dispersions shown in Fig. 3. The temperature rise was proportional to the concentration of carbonaceous species for both pristine BSA-SWNT dispersions ($R^2 = 0.94$) and carboxylated BSA-SWNT dispersions ($R^2 = 0.94$).