Thermal ablation the rapeutics based on CN_{x} multi-walled nanotubes

Suzy V Torti¹ Fiona Byrne² Orla Whelan² Nicole Levi² Burak Ucer² Michael Schmid² Frank M Torti⁴ Steven Akman³ Jiwen Liu² Pulickel M Ajayan⁵ Omkaram Nalamasu⁵ David L Carroll²

¹Department of Biochemistry, Wake Forest University Health Sciences, Winston-Salem, NC, USA; ²Center for Nanotechnology and Molecular Materials, Department of Physics, Wake Forest University, Winston-Salem, NC, USA; ³Cancer Biology; and the ⁴Comprehensive Cancer Center, Wake Forest University Health Sciences, Winston-Salem, NC, USA; ⁵Department of Materials Science and Engineering, Rensselaer Polytechnic Institute, Troy, NY, USA

Correspondence: David L Carroll Department of Physics, Wake Forest University, 100 Olin Physical Laboratory, 7507 Reynolda Station, Winston-Salem, NC 27109-7507, USA Tel +1 336 758 5508 Fax +1 336 758 6142 Email carroldl@wfu.edu **Abstract:** We demonstrate that nitrogen doped, multi-walled carbon nanotubes (CN_x -MWNT) result in photo-ablative destruction of kidney cancer cells when excited by near infrared (NIR) irradiation. Further, we show that effective heat transduction and cellular cytotoxicity depends on nanotube length: effective NIR coupling occurs at nanotube lengths that exceed half the wavelength of the stimulating radiation, as predicted in classical antenna theory. We also demonstrate that this radiation heats the nanotubes through induction processes, resulting in significant heat transfer to surrounding media and cell killing at extraordinarily small radiation doses. This cell death was attributed directly to photothermal effect generated within the culture, since neither the infrared irradiation itself nor the CN_x -MWNT were toxic to the cells.

Keywords: nitrogen doped, multi-walled carbon nanotubes, photothermal effect, photoablation

Introduction

Since the discovery of carbon nanotubes by Iijima in 1991 (Iijima 1991), the field of nanotechnology has expanded rapidly. An area of particular interest has been nanotubes application to medical technologies, which offers opportunities to improve the diagnosis, monitoring, and treatment of diseases such as cancer.

Thermal ablation therapies are routinely performed by application of lasers directly to the tumor target area. Limitations include thermal degradation of the non-tumorigenic surrounding tissues and lack of access to deeply embedded tumors without surgical invasion. Therefore, a preferred method is to use a sensitizing agent that couples to the incident radiation to increase localized heating and minimizes the intensity of the laser source and consequently undesired tissue damage. Photothermal therapies using chromophoric dyes like indocyanine green (ICG), which absorbs strongly in the nearinfrared, have been repeatedly studied for effectiveness of tumor destruction (Chen, Adams, Bartels et al 1995; Chen, Adams, Heaton et al 1995). However, diffusion of dyes can hinder repeated thermal ablations which may be necessary for complete eradication (Chen et al 1996).

Carbon nanotubes have physical, mechanical and electronic properties that can be exploited for medical applications. Carbon nanotubes are hollow structures that do not break easily upon bending and are currently the strongest known fibers (Schonenberger and Forro 2000). Multi-walled nanotubes (MWNT) have a strong optical absorbance in the near infrared (NIR) region of light (Brennan et al 2003). Optical coupling of light to nanotubes is predicted to be most efficient for nanotube lengths that are at least half the wavelength of the incident light as determined by classical antenna theory (Jackson 1999). However, unlike classical antennae, currents within the nanotube have extraordinarily long dephasing times. This means that the currents travel essentially without scatter (ballistically on/within the tube), resulting in a giant oscillator strength material, or a "super-antenna." Such antenna effects are well known in other materials and have been reported in nanotubes as well (Webster et al 2005).

Intrinsic and engineered defects (dopants) may be used to enhance the antenna properties of MWNT. Defects result in scattering of currents along the tube and augment heating of the tube. Defect density can be controlled by introducing substitutional dopants into the carbon lattice (Liu, Webster et al 2005). For example, we have previously shown that nitrogen can be introduced into the lattice at percentages up to ~5%, and that these result in pyridine-like rings within the lattice. The dopants add charge to the nanotube in a way that is analogous to N-doping of silicon. The extra carriers provided by the pyridines make the nanotubes significantly better conductors by filling in the small band gap of the MWNT. Since the overall "antenna efficiency" of the material also depends on the number of free carriers in the system, dopants may enhance optical coupling, as has been shown in nonlinear optical experiments on suspensions (Xu et al 2004). In addition, CN_x-MWNT have been found to have decreased toxicity as compared to undoped MWNT (Carrero-Sanchez et al 2006).

A number of approaches to the treatment of cancer involve the local application of heat, including radio frequency ablation, either delivered from external or directed intratumoral probes. However, recently, nanomaterials have also been explored as heat delivery vehicles for thermal ablation of tumors. These methods include gold nanoshells (Hirsch et al 2003) and single walled nanotubes (SWNT) (Kam et al 2005).

In this manuscript, we examine the use of a novel N-doped form of MWNT (CN_x -MWNT) in light activation and heat transfer, and test whether MWNT can be exploited to thermally ablate cancer cells. We demonstrate that these nanotubes are not inherently toxic to cultured cells, regardless of the specific form or length. We show that exposure to NIR light induces these particles to produce heat and kill cultured kidney cancer cells, and that killing occurs effectively when suspensed nanotubes are within their heating radial distance from the cells. Further, we define the length of the nanotube required for optimal heat delivery and cytotoxicity.

Materials and methods MWNT growth and characterization

 CN_x -MWNT were produced by carbon vapour deposition (CVD) as described previously (Liu, Czerw and Carroll 2005). As precursors the catalyst ferrocene (Fe(C₅H₅)₂) and the carbon-nitrogen source pyridine (C₅H₅N) were used together with hydrogen as carrier gas. X-ray photoelectron spectroscopy (XPS) was used to determine the overall nitrogen concentration in the CN_x-MWNT, which

was found to be 1% - 2%. In order to produce CN_{x} -MWNT of various lengths, the nanotubes were ultrasonicated in a mixture of concentrated sulfuric and nitric acid (3:1) for 7, 24 and 60 hours. After extensive washing and drying, the purity and length distribution of all samples were examined using a Philips 400 transmission electron microscope (TEM) operating at 80 keV. All samples showed almost no catalytic or carbonaceous particles on the MWNT surface; this is indicative of highly purified nanotube material. The length distribution of the samples sonicated for 7, 24 and 60 hours is shown in (Figure 1). Their average length was found to 1100 nm (7 hours), 700 nm (24 hours) and 300 nm (60 hours). Average widths were 15 nm, 15 nm and 13 nm, respectively. Since in the present experiments the ratio of nanotubes to cells was varied, all experiments were normalized to nanotubes per cell. In order to count the nanotubes in each of the samples, a simple TEM based method was devised based on placing a known volume of dispersed CN₂-MWNT on a TEM grid, evaporating the aqueous buffer, and counting the number of nanotubes on the grid. Before use, CN_x-MWNT were homogeneously suspended in Hepes Buffered Saline (HBS) by sonication in a Bronson 3510 sonicator for approximately one hour.

Cell culture and viability

CRL 1932 cancer cells were obtained from the American Type Culture Collection and cultured at 37 °C in a humidified chamber containing 5% CO₂ in Mc Coy's 5A media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were seeded at approximately 1.5×10^4 cells/well in 48-well tissue culture plates. 0.1 ml of (CN_x-MWNT) suspended in HBS were mixed with 0.9 ml CRL 1932 cancer cells in McCoy's 5 A modified media. The number of viable cells per ml of cell suspension was determined using a hemocytometer following staining with trypan blue. The dishes were incubated at 37 °C overnight.

The ratio of nanotubes to cells was varied in order to find the threshold for cell death. The concentration of nanotubes in the original suspension was determined by counting the number of nanotubes following deposition of a known volume of nanotube suspension onto a TEM grid as described above. Using this method and the conventional cell counting method described above, calculated ratios of nanotubes/cell of 1000:1, 100:1 and 1:1 were used. Where necessary, nanotubes suspensions were diluted in HBS. The concentrations for the 1000:1 samples were 0.083 mg/ml (1100 nm MWNT), 0.027 mg/ml (700 nm MWNT) and 0.005 mg/ml (300 nm



Figure I Sonication reduces mean length of CN_x -MWNT. CN_x -MWNT were sonicated in acid as described in Materials and Methods for 7 hours (a), 24 hours (b), and 60 hours (c). Average length was assessed by TEM.

MWNT). Furthermore, for each experiment a control sample of HBS without nanotubes was prepared. After incubation with or without nanotubes for 24 hours, the cells were irradiated with a NIR quasi-CW-YAG laser operating at a wavelength of 1064 nm and laser power of 3 W/cm². Each well was exposed to the laser for 4 minutes. Cell viability was assessed by trypan blue exclusion or in some cases by crystal violet staining. Temperature was measured using a thin thermocouple wire, attached to a Fluke thermometer, suspended in the growth media so that increases in the media

temperature could be determined as a function of lasing time and nanotube length.

Results and discussion Nanotube length and lack of inherent toxicity

Our initial objective was to determine whether CN_x -MWNT exhibit inherent toxicity in the absence of IR treatment. To test this, we first used CN_x -MWNT of a fixed average length of 1100 nm. These were produced by limited acid treatment, as shown in Figure 1A. Cells were mixed with CN_x -MWNT at a calculated ratio of 1000 nanotubes to one cell and effects on viability assessed over 48 hrs. As shown in Figure 2, CN_x -MWNT by themselves had no discernible effect on cell viability over this timeframe. Thus, CN_x -MWNT are not inherently cytotoxic, in accordance with the recent literature (Worle-Knirsch, Pulskamp, and Krug 2006).

Heat generated by CN₂-MWNT

We next assessed the degree to which irradiation of CN_x -MWNT with NIR could induce an increase in temperature. These experiments were performed with 700 nm MWNT. Nanotubes were diluted in media and irradiated for various time at different concentrations. As shown in Figure 3, an increase in temperature was attained when nanotubes were irradiated with NIR in aqueous media. The concentrations used were 1000×10^5 nanotubes per unit volume (marked 100%), 500×10^5 nanotubes per unit volume (marked 50%), 250×10^5 nanotubes per unit volume (marked 10%). We note that the heating of media drops as the concentration of nanotubes drops. We address this point below.

Cell viability following CN_x-MWNT induced photothermal effect

To test whether a decrease in viability could be induced by combined treatment with 1100 nm CN_x -MWNT and IR, cells were incubated with various concentrations of CN_x -MWNT, allowed to adhere overnight, and then exposed to $3W/cm^2$ IR



Figure 2 CN_x-MWNT do not inhibit cell growth. 1.5×10^4 cells/well CRL 1932 cancer cells were plated with 1.5×10^7 1100 nm nanotubes in 35 mm wells. Control samples contained no MWNT.Viability was measured using a crystal violet absorption assay at 595nm after 8, 12, 24, 36 and 48 hours of culture. Means and standard deviations of triplicate determinations are shown. -------- is 1000 nanotubes per cell.



Figure 3 Effect of nanotube concentration on heating. Concentrations of nanotubes used start at 1000×10^5 nanotubes/unit volume (marked as 100%). The concentration was then diluted to 500×10^5 , 250×10^5 , and 100×10^5 nanotubes/unit volume, (marked 50%, 25% and 10% respectively). Starting temperature was approximately 23 °C.

light for 4 minutes. Cells were incubated with CN_x-MWNT at nanotube:cell ratios of 1:1, 1:100, and 1:1000. Control cells were incubated with CN_x-MWNT, but were not treated with laser, or were treated with laser in the absence of nanotubes. Viability was assessed in all cultures. As shown in Figure 4A, in the absence of nanotubes, treatment with NIR had no effect on viability. Thus, cells have a high transparency to NIR light, and neither exposure to NIR nor exposure to nanotubes alone is sufficient to induce cell death. However when cells were incubated with CN_-MWNT and subsequently exposed to NIR, there was a dramatic dose-dependent decrease in viability, with over 90% cell death at the highest dose tested (Figure 4A). The greatest decrease in viability was associated with the greatest increase in temperature: the average number of viable cells in 1000x sample decreased by 96.25% after exposure to laser, where a maximum temperature of 57.7 \pm 1.5 °C was attained.

We next cultured cells with CN_x -MWNT at cell:nanotube ratios of 1:1, 1:100, and 1:1000 (designated as 1x, 100x and 1000x, respectively), irradiated for a fixed time of 4 minutes each, and tested whether temperature increases occurred under these conditions. As can be seen in Figure 4B, there was a slight increase in temperature in the control, 1x and 100x samples. The highest temperature change was found in the 1000x sample, which reached a maximum temperature of 57.7 ± 1.5 °C.

Control of CN_x-MWNT nanotube properties by length modification

In order to predict and control the properties of CN_x -MWNT nanotubes as heating agents, it was important to determine whether their behavior could be modeled by classic antenna



Figure 4 Effect of 1100 nm CN_z-MWNT. (a) 1.5×10^4 cells/well were mixed with 0.1 ml of 1100 nm CN_z-MWNT in HBS at various concentrations in 10 mm wells. Cells were allowed to attach overnight and exposed to YAG laser for 4 minutes. Untreated controls are also shown. Viability was assessed by trypan blue exclusion. Means and standard deviations of 6 replicate cultures are shown. Similar results were obtained in six independent experiments. (b) Change in temperature of CRL 1932 cancer cells plated with various concentrations of N-doped MWNT after exposure to YAG laser for 4 minutes. Initial temperature was approximately 23 °C. Maximum temperature change of 23.5 ± 0.3 °C in 1000x N-doped MWNT (and Laser), NL (no laser).

theory. One prediction of this theory is that optical coupling should occur at nanotube lengths that are at least half the wavelength of the incident light. In order to assess whether the behaviour of CN_x -MWNT obeyed this prediction, we prepared nanotubes of different lengths and compared their activities as thermal coupling and cytotoxic agents.

As shown in Figure 1B, by increasing the length of acid treatment from 7 hrs (used to produce 1100 nm tubes) to 24 hrs, nanotubes of 700 nm average length were produced. Acid treatment for 60 hrs further reduced average length of CN_x -MWNT nanotubes to 330 nm as measured by TEM (Figure 1C).

These nanotube preparations were incubated with cultured cancer cells, and their effects compared to those of 1100 nm CN_x -MWNT. As seen in Figure 5A, similar to the results obtained with 1100 nm CN_x -MWNT, cells incubated with 700 nm N-doped MWNT in the absence of laser exposure maintained viability. Laser treatment by itself (in the absence of nanotubes) was also non-toxic (Figure 5A). However the combination of nanotubes and laser treatment again induced a dose-dependent cell death and temperature increase in the culture: There was little increase in temperature or effect on viability in cultures treated with CN_x -MWNT at nanotube:

cell ratios of 1:1 or 100:1. However, the average number of viable cells in cells treated with nanotubes at a ratio of 1000:1 decreased by 91.94% after exposure to laser with a maximum temperature of 50.65 ± 5.7 °C (Figure 5B). Although the extent of cell death and maximal temperature increase were slightly less than those observed with 1100 nm nanotubes, these results indicate that CN_x-MWNT of 700 nm length are also efficient at thermal coupling.

Finally, the effect of CN_x-MWNT of 330 nm average length were assessed. Like the 1100 and 700 nm nanotube preparations, these exhibited no toxicity in the absence of laser (Figure 6A). However, in contrast to results obtained with 1100 nm and 700 nm nanotubes, no cell death was seen at any concentration of these nanotubes. Maximal increase in temperature was correspondingly decreased: the maximum temperature reached in 1000x sample was 41.78 ± 2.85 °C (Figure 6B). This temperature was only slightly above the normal incubation temperature of 37°, and was thus insufficient to cause cell death. Since 300 nm CN_-MWNT are less than of half the wavelength of light used to activate the nanotubes (1064 nm), these results are consistent with the prediction that these nanotubes should not exhibit effective coupling to the laser. A comparison of all three nanotube lengths and corresponding cell survival is shown in Figure 7.



Figure 5 Effect of 700 nm CN_x-MWNT (a) 1.5×10^4 cells/well were mixed with 0.1 ml of 700 nm CN_x-MWNT in HBS at various concentrations in 10 mm wells. Cells were allowed to attach overnight and exposed to YAG laser for 4 minutes. Untreated controls are also shown. Viability was assessed by trypan blue exclusion. Means and standard deviations of 6 replicate cultures are shown. Similar results were obtained in seven independent experiments. (b) Change in temperature of CRL 1932 cancer cells plated with various concentrations of N-doped MWNT after exposure to YAG laser for 4 minutes. Initial temperature was approximately 23 °C. Maximum temperature change of 16.35 \pm 2.5 °C in 1000x N-doped MWNT sample.



Figure 6 Effect of 330 nm CN_x-MWNT (**a**) 1.5×10^4 cells/well were mixed with 0.1 ml of 330 nm CN_x-MWNT in HBS at various concentrations in 10 mm wells. Cells were allowed to attach overnight and exposed to YAG laser for 4 minutes. Untreated controls are also shown. Viability was assessed by trypan blue exclusion. Means and standard deviations of 6 replicate cultures are shown. Similar results were obtained in two independent experiments. (**b**) Change in temperature of CRL 1932 cancer cells plated with various concentrations of N-doped MWNT after exposure to YAG laser for 4 minutes. Initial temperature was approximately 23 °C. Maximum temperature change of 7.2 ± 0.8 °C in 1000x N-doped MWNT samples.

Nanotube NIR coupling and heat transfer

The dependence of heating and cell death on nanotube length also suggests that CN_x -MWNT behave as anticipated by classic antenna theory, which predicts that for nanotubes to couple effectively, they must be at least as long as one half the wavelength of the incident radiation (Riggs et al 2000, 2001; Wang et al 2004; Hanson 2005). These wavelengths allow the antenna to become an electrical dipole from incident radiation and absorb light efficiently. Consistent with this prediction, 700 nm and 1100 nm nanotubes delivered substantial heat to the cellular media when exposed to 1064 nm light, whereas 300 nm nanotubes coupled poorly to the radiation field and consequently did not absorb enough light to raise the media temperature.

Thermodynamic calorimetrics can be used to estimate how far these nanotubes transfer the heat generated within them into the surrounding liquid. The calorimetric equation that must be satisfied on an instantaneous level is:

$$M_{\rm NT} C_{\rm NT} \Delta T_{\rm NT}^{\rm i} = M_{\rm water} C_{\rm water} \Delta T_{\rm water}^{\rm f} + M_{\rm NT} C_{\rm NT} \Delta T_{\rm NT}^{\rm f} \qquad (1)$$

where the *M*'s are the masses, the *C*'s are the heat capacities, and the ΔT 's are the changes in temperature (Kittel and Kroemer 1980). This states that the amount of heat (Q) lost from the hot nanotube can be accounted for in the final temperature of the water (cellular milieu) and the nanotube combined. Because the system is not closed, this is only a first approximation of the experimental system. Further, bundling of the nanotubes is not accounted for in these experiments. However, this can provide us with an expectation of the simple best case scenario. To estimate the zone of heat transfer, we can ignore the second term, because it is small compared to the total mass of the water, and rearrange equation (1) to obtain equation (2):

$$R_{ZONK} = \left\{ \frac{\frac{3}{4} \pi (M_{NT} C_{NT} 100 \,^{\circ}\text{C})}{\rho_{water} C_{water} 50 \,^{\circ}\text{C}} \right\}^{\frac{1}{3}}.$$
 (2)

 $R_{\rm ZONK}$ is the "zone of nanotube killing" (ZONK), the radius from the surface of the nanotube that defines a volume in which the temperature has reached a minimum of 50 °C. $\rho_{\rm water}$ is the density of water. If we choose 100 °C as an initial temperature of the nanotube, then the zone of cell killing around a single N-doped MWNT can be estimated as follows: $C_{\rm NT} \sim 3000$, $C_{\rm water} \sim 1000$ (Hepplestone et al in press), and the radius of a MWNT of about 10⁻⁸ m. This gives a ZONK radius of roughly 10^{-7} m. If we allow for nanotube temperatures to be 1000 °C, then the radius extends to approximately one micron. These calculations demonstrate that in the best case, a single nanotube can effectively heat volumes immediately adjacent to it.

The combined contributions of nanotube mass and number may explain why cell killing required high nanotube: cell ratios. In these studies, we have used a relatively low



Figure 7 Comparison plot of 300, 700 and 1100 nm nanotube lengths versus cell survival. The fewest number of surviving cells corresponds to laser absorption by 1100 nm long tubes. The greatest number of surviving cells corresponds to the shortest nanotube length of 300 nm.

radiation dose (720J) in order to stay within a range that would not damage dermal layers should this be applied as a therapeutic. At these radiation doses, the heat is relatively well localized to one or two ZONK radii for an individual nanotube. As the number of nanotubes is increased, for a constant irradiation power, we expect the heat they can transfer will increase as the cube root of their mass. However, the number of antenna used increases the heat transfer linearly. So, from these simple considerations, we expect that larger nanotube masses will enhance the total volume of killing, but not nearly as rapidly as increasing their numbers. This is seen in Figure 3 where the increase in maximum temperature is nearly linear with concentration for a given time. The solutions used in Figure 3 are well dispersed and therefore we expect clumping to have little effect.

Recently, nanomaterials have been applied to thermal ablation of tumors. These include gold nanoshells (Hirsch et al 2003), and single walled nanotubes SWNT (Kam et al 2005). All of these materials are in early developmental stages. CN_-MWNT have some theoretical and practical advantages as a nanomaterials. First, as discussed above, MWNT are excellent antenna for electromagnetic energy, and will absorb approximately three times the light as SWNT. This is important because within the transmission window of 700 nm to 1000 nm of human tissues, light is attenuated by skin and subcutaneous tissues. Since near IR irradiation may be applied through the skin to kill embedded cancers, the decreased light intensities required to heat with MWNT may reduce damage to dermal layers, particularly when the cancer lies at the deeper end of the NIR penetration range (2-4 cm). A second advantage of MWNT is that they have broad band width compared to the specific resonance absorptions of SWNT and nanoshells, allowing them to be activated by broader band widths of electromagnetic radiation. A third distinction is that MWNT do not require cell uptake, since we have shown that if they are within the R_{zonk} killing radius, they are sufficiently close to impart enough thermal energy for cell damage.

Our experiments demonstrate that N-doped MWNT's can act as highly efficient heat transduction molecules in the extracellular environment. Further, we have shown that heat transfer and subsequent cell killing can be achieved extracellularly with relatively low total radiation doses of 1064 nm light. We have suggested that both the mass of the N-doped MWNTs and their length play an important role in radiation transduction and heat transfer. Since previous studies have shown that such long nanotubes cannot be taken up by cells in general, the fact that such effective cell killing was achieved outside of the cell demonstrates that photo-thermal techniques involving "nano-antennae" are not limited to those materials that can be endocytosed, which allows for a wide variety of therapeutic possibilities for the delivery of nanotubes to tissues. Additionally, the radiation wavelengths and doses used in this study suggest that such approaches might be feasible for the treatment of cancer with externally applied electromagnetic radiation.

Conclusion

The overall goal of our experiments was to test whether CN_-MWNT could be used as effective heat transducers for photoablation of cultured cancer cells. We hypothesized that these nanotubes would be non-toxic per se, but could be activated to release heat by exposure to near-infrared radiation, and that this in turn would lead to cell death. We further hypothesized that effects of nanotubes would be dependent on their length. We observed that nanotube lengths of 1100 and 700 nm, but not 300 nm, coupled with the radiation and caused significant increases in temperature and cell death. The low power levels (3 watts) necessary to effectively kill cells suggests that the efficiency of NIR coupling to the long nanotubes is high. In summary, these results demonstrate that CN_x-MWNT are versatile and effective heat-delivery and cytotoxic agents. The ability to control their properties through length and other modifications may facilitate the ultimate clinical application of CN₂-MWNT in the treatment of cancer.

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References

- Brennan ME, Coleman JN, Drury A, et al. 2003. Nonlinear photoluminescence from van Hove singularities in multiwalled carbon nanotubes. *Optics Letters*, 28:266–8.
- Carrero-Sanchez JC, Elias AL, Mancilla R, et al. 2006. Biocompatibility and toxicological studies of carbon nanotubes doped with nitrogen. *Nanoletters*, 6:1609–16.
- Chen WR, Adams RL, Bartels KE, et al. 1995. Chromophore-enhanced in vivo tumor-cell destruction using an 808-Nm diode-laser. *Cancer Letters*, 94:125–31.
- Chen WR, Adams RL, Heaton S, et al. 1995. Chromophore-enhanced lasertumor tissue photothermal interaction using an 808-Nm diode-laser. *Cancer Letters*, 88:15–9.

- Chen WR, Adams RL, Higgins AK, et al. 1996. Photothermal effects on murine mammary tumors using indocyanine green and an 808 nm diode laser: an in vivo efficacy study. *Cancer Letters*, 98:169–73.
- Hanson GW. 2005. Fundamental transmitting properties of carbon nanotube antennas. *Ieee Transactions on Antennas and Propagation*, 53:3426–35.
- Hepplestone SP, Ciavarella AM, Janke C, et al. In press. Size and temperature dependence of the specific heat capacity of carbon nanotubes. *Surface Science*, Corrected Proof.
- Hirsch LR, Stafford RJ, Bankson JA, et al. 2003. Nanoshell-mediated nearinfrared thermal therapy of tumors under magnetic resonance guidance. *Proc Natl Acad Sci USA*, 100:13549–54.
- Iijima S. 1991. Helical microtubules of graphitic carbon. Nature, 354:56–8.
- Jackson JD. 1999. Classsical electrodynamics, 3rd edn. New York: John Wiley and Sons.
- Kam NWS, O'Connell M, Wisdom JA, et al. 2005. Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. *Proc Natl Acad Sci USA*, 102:11600–5.
- Kittel C, Kroemer H. 1980. Thermal physics, 2nd edn. New York: WH Freeman.
- Liu J, Czerw R, Carroll DL. 2005. Large-scale synthesis of highly aligned nitrogen doped carbon nanotubes by injection chemical vapor deposition methods. *Journal of Materials Research*, 20:538–43.

- Liu JW, Webster S, Carroll DL. 2005. Temperature and flow rate of NH3 effects on nitrogen content and doping environments of carbon nanotubes grown by injection CVD method. *Journal of Physical Chemistry B*, 109:15769–74.
- Riggs JE, Walker DB, Carroll DL, et al. 2000. Optical limiting properties of suspended and solubilized carbon nanotubes. *Journal of Physical Chemistry B*, 12:7071–5.
- Riggs JE, Walker DB, Carroll DL, et al. 2001. Carbon nanotubes as optical limiters. Kadish KM, Kamat PV, Guildi DM, eds. The Electrochemical Society Inc.
- Schonenberger C, Forro L. 2000. Multiwall carbon nanotubes. *Physics World*, 13:37–41.
- Webster S, Reyes-Reyes M, Pedron X, et al. 2005. Enhanced nonlinear transmittance by complementary nonlinear mechanisms: a reversesaturable absorbing dye blended with nonlinear-scattering carbon nanotubes. *Advanced Materials*, 17:1239.
- Worle-Knirsch JM, Pulskamp K, Krug HF. 2006. Oops they did it again! Carbon nanotubes hoax scientists in viability assays. *Nano Letters*, 6:1261–8.
- Wang Y, Kempa K, Kimbal B, et al. 2004. Recieving and transmitting lightlike radio waves: antenna effect in arrays of aligned carbon nanotubes. *Applied Physics Letters*, 85:2607.
- Xu JF, Xiao M, Czerw R, et al. 2004. Optical limiting and enhanced optical nonlinearity in boron-doped carbon nanotubes. *Chemical Physics Letters*, 389:247–50.