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Carbon Nanotubes for Thermal Therapy CNTs: Effect of Nanotube Structure and Doping on Photothermal Properties, Anticancer Efficacy of CNT-Enhanced PTT and Systemic Delivery and Biocompatibility of CNTs for PTT

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Abstract: From manufacturing to health-related therapies, nanotechnology is causing a revolution in many different sectors. The remarkable mechanical, electrical, and physicochemical characteristics of carbon nanotubes (CNTs) have made them an attractive medicinal prospect in the field of nanomedicine. An increase in therapeutic efficacy or a decrease in toxicities of medicinally active substances is the primary motivation for creating nanocarrier drug delivery systems. Traditional methods involve the use of liposomes and other spherically shaped vesicle nanocarriers to accomplish this. As an alternative, carbon nanotubes (CNTs) are basically just carbon atoms arranged in a cylindrical shape. Carbon nanotubes (CNTs) are continuous graphene sheets wound into a cylinder with a high aspect ratio, a diameter of less than 1 nm, and a length of several micrometres. The cylinders can be open-ended or capped. Multiwalled carbon nanotubes (MWNTs) are composed of multiple graphene sheets, whereas single-walled nanotubes (SWNTs) are formed by CNTs manufactured from a single sheet of graphene. Biomedical uses of CNTs were not possible until methods to make them soluble and functionalized them with organic groups were developed. They can absorb or conjugate with many different medicinal compounds because of their large surface area. A new carrier system for both large and small medicinal compounds, carbon nanotubes (CNTs) have only lately been introduced. To control their biological or physical characteristics, CNTs can be surface-engineered with certain functional groups, a process known as functionalization. In addition to their versatility as therapeutic chemical carriers, carbon nanotubes (CNTs) have found usage in photothermal cell death because to their enormous surface area and the ease with which their physical dimensions and surfaces may be controlled.

Keywords: Carbon Nanotubes, Thermal Therapy, Doping, Photothermal Properties, Anticancer

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Introduction

Various forms of targeted regulation of tissue temperature for therapeutic objectives are collectively known as "thermal therapy." Carbon nanotubes (CNTs) have attracted a great deal of attention due to their many potential uses in fields such as atomic force microscopy, energy storage, field emission, and molecular electronics. The unusual electrical properties, exceptional strength, and extremely high specific heat and thermal conductivity are only a few of the remarkable properties exhibited by these nanoparticles. Iijima (1991) was the first to describe CNTs almost twenty years ago. At first, they were defined as pure carbon cylinders with lengths on the order of several microns and diameters anywhere between 30 and 50 nanometers for concentrically oriented multiwalled nanotubes (MWNT) [1-3] and 1.4 nanometers for single-walled nanotubes (SWNT). Recent developments in nanotube synthesis and processing have enabled the production of nanotubes with narrow length ranges spanning from several microns to ultrashort nanotubes with well-defined wall numbers. According to Hata et al. (2004), carbon nanotubes can be produced by a variety of methods. These include arc discharge, magnetic field synthesis, chemical vapour deposition using gaseous metal catalysts such iron, nickel, molybdenum, or cobalt, or water-assisted chemical vapour deposition. Compound nanotubes have very high aspect ratios, which measure the proportion of the longer to the shorter dimension. Nanoparticles with a wide surface area can amplify signals produced by peptides, proteins, nucleic acids, radionuclides [4-6], other nanoparticles, and medicines by conjugating them to their surfaces. It is not unexpected that CNTs have been investigated for potential uses to improve the treatment of human cancers because to these exploitable properties.

Research on carbon nanotubes (CNTs) and their potential biomedical uses has exploded in the last decade. One intriguing aspect is their capacity to transport anticancer drugs, such as nucleic acids, radionuclides, and chemotherapeutic agents. They have also demonstrated promise as imaging and diagnostic agents for cancer and as delivery vehicles for anticancer vaccines in animal studies. Graphene nanotubes (CNTs) are appealing for these uses because they can pass cell membranes with relative ease and because they can be functionalized with chemicals that selectively target cancer cells. Another feature of CNTs that renders them particularly well suited for anticancer therapy is their ability to act as high efficiency absorbers of near-infrared radiation (NIR) to promote the generation of therapeutic heat in tumours. CNTs are amenable to stimulation by a range of energy sources including NIR, microwave (MW), and radio-frequency (RF) radiation emitters directed at the site of the CNTs from outside the body. Electromagnetic radiation causes carbon nanotubes to produce vibrational energy, which in turn transfers a great deal of heat to the tumour site. The imaging capabilities of CNTs allow for more precise localization of heat delivery [7–11], and their ability to provide repeated rounds of heat therapy noninvasively bodes well for their usage in thermal treatment of cancer. While other nanomaterials do possess some of these characteristics, carbon nanotubes (CNTs) may provide the most advantageous set of features for creating a thermal therapy that does not involve invasive procedures. Following this, we will go into the ways in which different types of radiation and carbon nanomaterials differ in their heating capabilities. We will examine the present status of CNT-based anticancer photothermal therapy (PTT)[12,13] and think about what we know so far that could improve CNT designs for heat production and localization. The potential for bringing photothermal therapies based on CNTs to the clinic will be discussed lastly.

Investigating the Photothermal Characteristics of Carbon Nanotubes

Many fields rely on how light interacts with carbon nanotubes (CNTs), and PTT is one of them. Photothermal therapy (PTT) involves delivering a photothermal sensitizer to cancer cells, which are then excited by light at a specific wavelength—typically in the near-infrared (NIR) range of 700–1100 nm—causing the photosensitizer to enter an excited state and release vibrational energy—which is then converted into heat—thus killing the cancer cells [14–17]. Very little near-infrared (NIR) light is absorbed by biological systems due to the absence of chromophores that absorb in this spectrum. Notably, CNTs have an incredibly wide electromagnetic absorbance spectra, which includes the near-infrared (NIR) window, the radio frequency (RF) bands, and the microwave (MW) bands (Gannon et al. 2007). This provides more evidence that CNTs, when coupled with the right energy source, can cure deep lesions without invasively penetrating the tumour site. Raman scattering, fluorescence, and nonlinear optical analysis have all been used to study the photophysical factors that control how CNTs interact with electromagnetic radiation [18–23], but further study is required to fully comprehend these characteristics. It would appear that CNTs function as ballistic

conductors because of the low dimensionality and quantum confinement of electrons within the carbon lattice of the nanotube wall, which leads to quantized resistance and a lack of energy dissipation through electron movement. For this reason, the effectiveness of this material in photothermal applications is highly dependent on the nano-tube's specific structure, particularly its wall count. Next, we'll take a look at how the structure of nanotubes affects the effectiveness of CNTs' photothermal heat transmission.

Nanotube Structure and Its Impact on Photothermal Characteristics

Vibrational (phonon) modes generated by phonon-phonon and phonon-electron interactions are displayed by CNTs after they have been excited by electromagnetic energy. The thermal characteristics of carbon nanotubes are mostly determined by these phonon interactions. Each nanotube structure has its own unique heat capacity and ability to produce and transfer heat; in general, more phonon modes emerge with increasing nanotube diameter and unit cell size. One way of looking at phonons is as quanta of heat [24–29]. Therefore, in order to comprehend the photothermal heating behaviour of various nanotubes, it is fundamental to have a fundamental knowledge of their unique thermal conductivity characteristics. In this article, we will take a quick look at the thermal conductivity and magnetic resonance spectroscopy (NIR) of SWCNTs, MWCNTs, and similar nanostructures. The thermal conductivity of SWCNTs, measured in bulk samples at room temperature, is more than 200 W/(m K). The thermal conductivity of a single SWCNT measured along its axis at ambient temperature ranges from 2200 W/(m K) to about 3500 W/(m K), which is significantly lower, due to the disorganised orientation of the nanotubes in bulk samples. As the temperature increases, the phonon thermal conductivity drops, reaching a maximum at about 100 K. Umklapp processes, which include increased phonon-phonon and electron-phonon scattering interactions, are expected to cause thermal conductivity to decrease in a roughly linear form as temperatures rise. The SWNHs. One end of SWCNTs can be sealed to make them different. Accumulating into formations with average diameters ranging from 50 to 100 nm, these nanoparticles are known as single-wall nanohorns (SWNHs). Laser ablation of pure graphite is used to make SWNHs [30-33]. Metal catalysts are not used in this process. Changing the growth time and laser pulse duration during manufacture allow for diameter and size adjustments, respectively. When compared to SWCNTs and MWCNTs, nanohorns have received less attention in the research on their potential photo thermal applications. Nevertheless, it was discovered by Whitney et al. that the optical attenuation coefficient is directly proportional to the concentration of SWNHs. It is highly probable that SWNHs will be heated most effectively when exposed to NIR radiation with shorter wavelengths, as they also showed that the attenuation coefficient rose with shorter wavelengths. Photothermal uses of SWNH may be restricted to surface illness treatment due to the fact that tissue penetration is much improved at longer NIR wavelengths (Konig 2000). While SWCNTs and MWCNTs can produce enough heat for photothermal ablation of tumours, the few studies that have looked at SWNH for PTT have needed far longer NIR exposure durations and much higher SWNH concentrations. MWCNTs. Because of their high phonon mode density, MWCNTs are also excellent heat conductors, just like SWCNTs [34–37]. At ambient temperature, the measured conductivities of MWCNTs range from 3000 W/(m K) to 6600 W/(m K), suggesting that their thermal conductivity along their axis is higher than that of similar SWCNTs. On the other hand, several study teams have found a slightly lower thermal conductivity, ranging from 1500 to 3500 W/(m K) depending on the tube's diameter[38-41]. It is unclear whether these discrepancies result from different MWCNT preparations, different ways of measuring conductance, or some other factor. Using bulk MWCNT bundles, researchers discovered a significantly reduced thermal conductivity of just 20 W/(m K) at ambient temperature. This reduced value could be caused by CNT flaws or, alternatively, it could indicate that aggregation-induced resistive thermal junctions are inhibiting heating. The predicted rise in thermal conductivity with decreasing diameter of MWCNTs provides more evidence that interwall photon and electron interactions influence conductivity. In comparison to SWCNTs, nanoshells, and other plasmonic nanoparticles, MWCNTs have a wider absorption spectrum. According to Hanson (2005), the most efficient optical coupling of light and CNTs happens when the nanotube length is comparable to half that of the incident radiation's wavelength (Wang 2004; Hanson 2005), which is in line with the classic behaviour of dipole antennae and is caused by the larger number of electrons available for transport in MWCNTs compared to SWCNTs. This is also supported by a smaller electronic bandgap or metallic behaviour. Consequently, MWCNTs with lengths equal to or slightly longer than the wavelength of a 1064-nm laser easily become heated when exposed to light. But MWCNTs whose length was just 330 nm—a third of the laser's—did not produce any noticeable heat. Another sign of this antenna effect is that MWCNTs show polarisation effects [42–46]. This means that when the electric field of the incoming radiation is polarised

perpendicular to the dipole axis of the CNTs, the antenna response of the CNTs is inhibited (Wang 2004). Because of their larger mass per particle and abundance of electrons (carriers) accessible for photon interactions, MWCNTs seem to be significantly more efficient at heat creation than SWCNTs after exposure to electromagnetic radiation. Also, unlike MWCNTs, which display metallic behaviour in the as-produced tubes, the majority of SWCNTs function as semiconductors. Because of this, MWNCTs have a higher potential for electron excitation and subsequent heat release than the majority of SWCNTs. It has been found in experimental experiments that aqueous dispersions of MWCNTs absorb around three times more light per particle than similarly dispersed SWCNTs, according to comparisons of their near-infrared (NIR) absorbance at 1064 nm. It is worth noting that for the same dose of NIR, MWCNTs have been demonstrated to generate up to 20 times more heat than SWCNTs. We still don't know how this impact happens, but it's significantly larger than what the optical absorbance data would suggest.

One noteworthy aspect of MWCNTs could be their different heating rates compared to SWCNTs; this would allow for a lower dosage of MWCNTs to produce the same temperature increase after NIR exposure. As a result, compared to SWCNTs, MWCNTs may be able to produce comparable therapeutic benefits with doses that are less prone to cause systemic toxicity and off-target effects.

How Doping Affects the Photothermal Characteristics of Carbon Nanotubes

A way to change the photoelectronic characteristics of the tubes chemically instead than by changing specific geometry is to dope non-carbon atoms into CNTs. Antenna behaviour in nanotubes is caused by an increase in conductivity, which can be achieved via doping, which changes the electronic band structure. A wide variety of dopants can be introduced into CNT walls through various methods. These include substitutional doping, encapsulation in the interior space, coating on the surface, molecular absorption, and covalent sidewall functionalization. Electron donors like alkali metals or acceptors like halogens can be intercalated into CNT walls.

Adding additional atoms to carbon nanotubes (CNTs) can significantly change their photothermal characteristics, making them more effective in absorbing light and transferring heat. When carbon atoms are substituted in the lattice for non-carbon ones, a process called substitutional doping, changes the Fermi level of the valence band. The strength of this shift is proportional to the amount of doping. Near the Fermi level, the valence and conduction bands of pure CNTs seem to be symmetrical. To put it in perspective, nitrogen doping places an impurity 0.27 eV below the conduction band bottom while boron doping produces a level 0.16 eV above the top of the valence bands observed in undoped CNTs. A greater number of conduction channels can be introduced without substantial carrier dispersion when the Fermi level is lowered by boron dopants (Dai 2002). Since the electron-phonon interaction is weak, boron-doped nanotubes exhibit metallic behaviour [49–52]. The valence band of boron-doped MWNTs is filled with a strong acceptor-like peak around the Fermi level, in contrast to undoped CNTs that, even under idealised conditions, display a modest band gap (semiconducting or semi-metallic behaviour). Doping MWNTs seems to enhance their optical coupling to near-infrared radiation (NIR) by making more free carriers available, which in turn causes them to generate higher temperatures after exposure to NIR compared to undoped tubes of the same type, though there is only a small amount of experimental evidence supporting this claim. Fewer studies have investigated how photothermal characteristics are affected by filling CNTs with non-carbon atoms. Compared to iron-free MWCNTs, those with a high concentration of the iron-based catalyst ferrocene in their lumen seem to heat more efficiently [53-57]. After being exposed to the same dose of NIR, the former can reach temperatures up to 5-7°C higher. Meanwhile, some researchers have discovered that heating properties of MWCNTs are unaffected by increasing ferrocene concentrations in their lumen. The improved heating effect seen by Levi-Polyachenko happened when nanotube concentrations above 100 μg/mL were heated, while Ding et al. solely examined heating samples with 100 μg/mL of MWCNTs, even though the mechanism explaining this difference is still not fully understood.

RF and MW Heating of CNTs

In addition to being excellent NIR absorbers, CNTs can also generate heat when exposed to microwave or radio frequency radiation. While the wider RF spectrum overlaps and extends from 3 kHz to 300 GHz, the MW spectrum spans 300 MHz to 300 GHz. The polarisation that results from MW heating of CNTs is analogous to the antenna effect that is observed with NIR heating. The reduced heating observed in experiments involving CNTs in thick viscous media was ascribed to the inhibition of vibrations, which in turn reduced photon-photon and photon-electron

interactions. Localised heating is made possible when CNTs exposed to MWs undergo conduction and dipolar polarisation, which generate heat. Since MW irradiation converts electromagnetic energy into mechanical vibrations and, eventually, heat, the mechanisms of CNT heat generation utilising MW/RF are comparable to NIR. Free charges donated by residual metals in CNTs may potentially aid in the acceleration of MW coupling [58-61]. The thermal characteristics of MW and RF irradiation of CNTs will be covered concurrently due to the wavelength overlap. Combinations of SWCNTs with polycarbonate, according to the theoretical modelling of Dumitricia et al. (2004), should be able to absorb microwave radiation in the 6- to 20-GHz band. This study predicts that capped SWCNTs treated with around 100 fs pulses will stay intact while 8 percent of their valence electrons are moved to anti-bonding states. Without harming the cylindrical structure, irradiation would also cause the caps to open. On the other hand, it is anticipated that heating bulk (noncapped) SWCNTs will produce particle fragmentation and the promotion of 10% of valence electrons. The estimated maximum temperature for both NT kinds was 800 K, which later stabilised at 300 K. Using 700 W at 2.45 GHz, SWCNTs expanded to twice their initial volume after heating before contracting once more, according to a different study. After being heated, a large number of SWCNTs fused together to create junctions [62–65]. If this effect is to take place, the temperatures reached in the experiment have to have been extremely high, possibly exceeding 1500°C. The degree to which CNTs are pure is another variable that might affect their heating. When heated, unpurified SWCNTs (those with Fe catalyst impurities) reached 1850°C, but pure SWCNTs only achieved 650°C. The two varieties of SWCNTs were subjected to radiation at 2.45 GHz with a power of 1000 W MW and a diameter of 1.1 nm. This points to the potential significance of residual metals in CNT heating. A single single-walled carbon nanotube (SWCNT) with a diameter of 1 nm and varying lengths was shown by Reulet et al. to undergo electron heating via mechanical energy dissipation when subjected to radiofrequency irradiation between 100 MHz and 10 GHz. Heating did not alter the resonance spectra, which points to the RF field's Coulomb force and electrostatic forces acting on the tube as the sources of excitation and vibrations. The length of the NT was shown to affect the resonance frequencies. A temperature increase of 30–40°C (1.6 K/s rate) was seen when SWCNTs were exposed to an 800 W RF field at 13.56 MHz. One possible explanation for the unexpectedly high heating rate is the SWCNTs' tendency to spontaneously self-assemble into longer antennae. A total of 130,000 W/g of thermal power deposition was measured, with the NTs accounting for more than half of that amount (75,000 W/g). It would appear that the stimulation of electronic transitions or resonance is not the cause of RF heating of CNTs due to the long wavelengths. Cell death was found to be dose-dependent when hepatocellular and pancreatic cancer cell lines were heated in vitro with RF at concentrations of 250-500 μg/mL of SWCNTs. Almost all of the treated cells were destroyed. Treatment with medium alone (i.e., without SWCNTs) resulted in the death of around 25% of the cells, indicating the possible influence of nonspecific ion stimulation on heat generation. Treatment of rabbits with a VX2 hepatocellular carcinoma xenograft in vivo led to the tumor's total thermal necrosis [64-67]. There was a 2- to 5-mm zone of therapeutic damage to the surrounding liver, but no toxicity was seen. Unlike NIR laser-based treatments, SWNTs have the potential to noninvasively treat tumours anywhere in the body, according to this evidence. Unfortunately, due to the potential for substantial off-target heating, there has been less investigation into the effectiveness of this treatment modality in both in vitro and in vivo settings.

Methods for Tracking the Photothermal Heating of Carbon Nanotubes and Localising Their Distribution

In order to minimise collateral damage to healthy cells and tissues and precisely localise heat for thermal ablation, precise control over the temporal and geographical distribution of heat is crucial. When it comes to photothermal applications, the effectiveness of the CNT target in converting laser energy into heat is the most important factor. The total amount of laser energy that hits the target also plays a role. The maximal laser output and feasible nanomaterial concentration are the only constraints on heat generation.

A number of factors, including the surroundings of the tubes, their closeness to heat absorbers, the solvent, and the substrate into which the tubes are distributed, influence the heat dissipation process away from the CNTs. Because heat transfers away from the site of irradiated nanomaterials, it is highly improbable that a continuous heating of nanoscale sources could generate a substantial rise in surface temperature immediately surrounding a nanoparticle, nanowire, or nanotube, as will be elaborated upon below, unless the heating power is extraordinarily high. Using high-powered, rapidly-pulsed lasers (on the order of femtoseconds to nanoseconds), it is possible to localise the temperature of isolated nanoparticles after NIR heating, according to multiple investigations. Pulses longer than a

picosecond transfer energy from the excited electrons to atomic thermal motion, which could lead to uncontrolled structural changes in the material, while ultrashort laser pulses of about 100 fs are thought to instantly promote electrons in CNTs to anti-bonding states. Due to the lack of time for heat dispersion from the few micrometre heated area, materials containing gold nanoparticles can experience temperature spikes of 150-300°C when exposed to nanosecond NIR pulses. After CNT uptake, comparable approaches using nanosecond pulsed lasers were effective in treating cancer cells that had dispersed. As said, temperature rises over greater volumes tend to be relatively minor following such brief exposures, and unfortunately, picosecond or nanosecond pulsed lasers are not usually available in clinical situations. One potential alternative to macroscale temperature increases for cancer treatment is the application of somewhat longer near-infrared (NIR) pulses to irradiated carbon nanotubes (CNTs). One experiment used low-intensity near-infrared light (NIR) (800 nm; 50-200 mW/cm2) for 60 seconds on cancer cells that had taken up SWCNT bundles. Treatment resulted in a little increase in body temperature. Rather, water molecules trapped inside and between the bundles of SWCNTs boiled. As the water vaporised, tremendous pressures built up within the bundles, and they burst, killing cancer cells in their vicinity. Utilising bundles of SWCNTs is crucial to this approach because, unlike with well-dispersed samples, the "nanobomb" effect does not manifest. Using a millisecond pulsed laser (1064 nm; 200 mW/cm2) for 20 seconds, Kang et al. (2009) killed 85 percent of cancer cells that had taken up CNTs. This was in a separate investigation. Notably, there was hardly any discernible rise or fall in temperature, leading scientists to speculate that the cells were killed by a photoacoustic explosion caused by photon-electron interactions. This explosion would have physically ruptured the cells' membranes. Although other treatment tactics may have some benefit, as mentioned earlier, continuous NIR treatment seems to be the most effective way to treat large tumours because the heat it generates can diffuse all around the tumour (Biris et al. 2009). Anticancer treatment requires the extensive heating of the entire tumour volume, which is achieved by repeatedly heating a large number of nanoparticles distributed throughout the tumour under in vivo thermal ablation conditions. This causes a much larger global temperature rise compared to the localised rise near each particle. Long heating intervals (several seconds or more) cause heat to transfer away from the target location, which diminishes the therapy's efficacy and can even cause collateral harm to healthy tissue nearby. Minimising the total electromagnetic energy deposited into the tissue is important for reducing the spread of heat from the tumour target to the surrounding tissue. This will ensure that only the amount of heat necessary for therapy is supplied to the targeted area. This necessitates keeping track of the temperature changes caused by NIR irradiation of CNTs in real time, regardless of their location or duration. Infrared cameras and MRI-based approaches are two of various ways to track the temperature distribution in a tumour volume after CNT administration and NIR irradiation. Optimal control of CNT content and NIRirradiation parameters required to produce a target temperature has been made possible with the help of infrared cameras.

profiles in tumor-bearing animals and model tissues. Unfortunately, noninvasive imaging of temperature changes deep into tissue is not an option with NIR cameras. An MRI-based thermometry technique called proton resonance frequency (PRF) MR temperature mapping [68, 69] is a noninvasive way of temperature mapping that further permits the superimposition of temperature data over high-resolution anatomical pictures acquired at any depth. This method relies on the fact that a linear relationship exists between temperature and the PRF shift that results from the breaking of hydrogen bonds between water molecules in tissue as a function of increasing temperature. By connecting the treatment temperature to real thermal tissue damage, PRF MR temperature mapping gives clinicians control over treatment outcomes. Burke et al. (2009) showcased the feasibility of CNT enhanced photothermal cancer therapy in a kidney cancer mouse model, showing that it could be employed to both pinpoint the tumour target and determine the thermal dose delivered to the same tissue after near-infrared (NIR) exposure. Using PRF MRI thermometry, it was found that tumours injected with 100 μg of MWCNTs reached a maximal temperature of 76°C after a 30-second NIR exposure (1064 nm; 3 W/cm2). The identical NIR treatment resulted in a maximum temperature increase of 46°C when CNTs were not present. As a method for reducing the risk of collateral damage to nearby normal tissue and for monitoring whether ther-mal ablative temperatures are reached, the demonstration that CNTs are compatible with PRF MR temperature mapping is an important step towards future clinical applications [70, 71]. It would be ideal if CNTs employed in thermal therapy could also enhance MR contrast, as this would allow for more precise monitoring of nanomaterial distribution in the tumour and image-guided placement of the NIR source; nonetheless, there is room for improvement in this technique. Several investigations have demonstrated that CNTs can be detected by noninvasive imaging by including materials that increase MR contrast, such as iron or gadolinium (Gd). To get there, researchers

looked at iron-containing MWCNTs as a possible dual-modality agent for improving MR contrast and transferring photothermal energy. Important information for pretreatment planning and laser placement determination for MR image guided PTT of tumours in mice was provided by this study: an accurate depiction of the distribution of iron-containing MWCNTs inside the tumour as revealed by MR imaging. Even after attaining thermal ablative temperatures, the contrast and heating properties of these MWCNTs remained unchanged even after many rounds of NIR illumination. This would allow for the tracking of MWCNT dispersion over time, which might lead to fractionated or repeated laser treatments directed at the tumour as needed, all without the need for further injections. Attenuation of MR signals is a possible drawback of iron-containing MWCNTs. While this makes iron-containing MWCNTs great T2 contrast agents, it also has the ability to significantly weaken MR signals, which could mess with temperature mapping using PRF MR thermometry. In an ideal world, a CNT designed for the clinical use of nanoparticle-assisted photothermal cancer treatment would work in tandem with a noninvasive temperature mapping method to verify that the correct thermal dose was administered to the target region, and with an imaging modality like MRI to precisely pinpoint the lesion's boundaries and evaluate the nanoparticles' distribution inside the tumour [72–74]. To fully use the potential of nanoparticle enhanced PTT, it is essential to optimise such a material.

Efficiency of CNT-enhanced PTT in Combating Cancer

The results of CNTs' anticancer experiments, both in the lab and in living organisms, are quite promising. Although MWCNTs have since shown efficacy, Kam et al. (2005) reported the first paper detailing the use of SWCNTs for PTT of cancer cells in 2005. The main treatment technique entails exposing adherent cancer cells, cancer cells in suspension, or tumours grown in mice to CNTs followed by irradiation with an external NIR laser. Various variables will be addressed in depth below, but in general, this is how it works. Cell types such as cervical, renal, oral, breast, adenocarcinoma, ascitic, and lymphoma have all responded positively to this method, which has also been called nanotube-enhanced PTT, in preclinical studies. Table 17.3 summarises the results from a review by Iancu and Mocan (2011) that looked at the efficacy in in vivo cancer models in mice and human xenografts generated in mice. A photothermal ablation technique called laser-induced thermotherapy (LITT) directly heats a target tissue, like a tumour, above the thermal ablation temperature threshold of about 55°C. This technique forms the basis of the clinical model for the use of nanomaterials as heat transduction agents. Cell death follows subsequent denaturation of proteins, lysis of membranes, and coagulative necrosis. One of the main problems with LITT is that it can't reliably reach thermoablative temperatures all over the target lesion and can't limit treatment to only the tumour. Thus, for CNTs to have any therapeutic value, they need to significantly enhance heat deposition after NIR exposure. The possibility that CNTs could enhance LITT by increasing heat deposition after NIR exposure has been the subject of multiple investigations. The initial description of SWCNTs for PTT was provided by Kam et al. (2005). Little heat was created in the absence of SWCNTs after 60 seconds of exposure to 808-nm laser light. The absorbance was strong without SWCNTs, but it increased the solution temperature to over 55°C (i.e., into the known thermoablative range) when they were present. After a short incubation period, HeLa cells—a well-studied cervical carcinoma cell line—killed themselves at this higher temperature because they had internalised SWCNTs. For these and other nanomaterials to be used to treat non-superficial malignant lesions in vivo, it is essential that they convert tissue penetrating NIR wavelengths into heat efficiently. Following this preliminary investigation, several in vitro and in vivo studies examined the heating of cancer cells [77-79] using SWCNTs in response to NIR irradiation. Both the amount of heat produced and the therapy's effectiveness were found to improve as the concentration of SWCNTs and the amount of laser exposure (energy deposition) were increased, according to Huang et al. (2010). Similar research found that intra-tumoral injection of 1 mg/mL SWCNTs and irradiation with a low power (200 mW/cm2) near-infrared (NIR) laser for 10 minutes reduced tumour size and provided a modest survival benefit in a model of subcutaneous squamous cell carcinomas in mice. According to infrared thermometry, the treatment was successful when the tumor's temperature increased by a maximum of 18°C, marking the therapeutic ablation threshold. Unfortunately, long-term cancer remission was not achieved with this treatment. Necrosis in neighbouring healthy tissue, along with uneven tumour ablation and tumour recurrence, suggests substantial heat transfer from the tumour site to the non-tumor region. In contrast, research by Moon et al. (2009) showed that, with a higher intensity laser irradiation (3 W/cm2) for a shorter duration (3 min), the therapeutic efficacy and heat localization of SWCNT-mediated PTT may be increased significantly. There was no toxicity, adverse effects, or tumour recurrence after several months of follow-up after intratumoral injection of SWNCTs (120 μg/mL) into mice bearing subcutaneous xenografts of human mouth cancer

cells and NIR irradiation. The tumours were entirely eradicated. In contrast to the surrounding normal tissue, tumour sections showed strong staining for TUNEL, indicating cell death through apoptosis. Nevertheless, the target region was severely burned by the irradiation technique even when SWCNTs weren't present. In order to minimise damage to non-target areas, it is preferable to use as little energy as possible to induce thermal ablation. As mentioned earlier, MWCNTs have a far more efficient absorption of near-infrared radiation (NIR) than SWCNTs do due to their unique structure. This means that a much smaller amount of incident radiation or concentration is needed to produce the same temperature increase with MWCNTs compared to SWCNTs. Based on the literature review, it appears that the majority of studies use SWCNT concentrations higher than 100 μg/mL and total energy requirements ranging from 100 to several hundred Joules to accomplish thermal ablation in vitro. The use of MWCNTs allowed for the attainment of thermal ablative temperatures in vitro after exposing 10 μg/mL of MWCNTs to as little as 4 J, leading to the 99% mortality of treated cancer cells. There hasn't been a comprehensive effort to find the optimal combination of CNT type, concentration, and NIR needed to minimise the energy dose for therapeutic heat generation, even though Ghosh et al. (2009) performed extensive experiments to investigate the effect of nanotube concentration, laser power, and duration of laser exposure on the heating of SWCNTs and MWNCTs. A large range of treatment parameters is thus still present. As an illustration, the cells were successfully treated with MWCNTs at a concentration of 100 μg/mL after being incubated with them. The cells were then exposed to an NIR laser in the range of 780-1400 nm at 3.5 W/cm2 for 1.5-2 minutes, while the culture media was heated to a temperature between 50°C and 70°C. In their study, Fisher et al. exposed human prostate cancer cell lines PC-3 and renal adenocarcinoma cells (RENCA) to 100 μg/mL MWCNTs for 5 minutes followed by near-infrared light (15.3 W/cm2 at 1064 nm). The cells were killed off by the 42°C temperature increase that followed in the surrounding culture media. Using fluorescence and transmission electron microscopy, the internalisation of MWCNTs by cells was assessed in the later investigation. The uptake and translocation of MWCNTs to the nucleus by cells is in agreement with prior findings [71, 72]. The amount of MWNTs found in cellular vacuoles and nuclei increased when the incubation duration was increased. Further discussion of the impact of cell binding and internalisation of CNTs on treatment efficacy will be provided in Section 17.6. Research on the optimal concentration of MWCNTs per cell for efficient thermal treatment has been conducted. Monolayer cultures of human CRL1932 renal adenocarcinoma cells were exposed to varying doses of nitrogen-doped MWCNTs, with ratios of 1:1, 100:1, and 1000:1 matching to the estimations. There was a 23°C temperature increase and near-complete cell death in the culture media of cells exposed to MWCNTs at the 1000:1 ratio after 4 minutes of exposure to NIR (3 W/cm2 of 1064 nm NIR). Cells treated to MWCNTs at 1:1 or 100:1 ratios did not show any significant differences in cell mortality when heated compared to control cells heated without MWCNTs. For the purpose of in vitro cancer cell death, Burke et al. (2009) showed that cellular absorption of MWCNTs prior to NIR irradiation was unnecessary. According to Burke et al. (2009), murine RENCA cells were evenly distributed in phosphate buffered saline with 100 μg/mL of MWCNTs. After only 30 seconds of exposure to near-infrared light (3 W/cm2) at 1064 nm, 98% of cancer cells were eliminated, whereas after 45 seconds of treatment, all of the cells were killed. After exposing RENCA tumours implanted in the flanks of nude mice to NIR (3 W/cm2 of 1064 nm NIR for 30 s), in vivo studies showed that injecting increasing doses of MWCNTs (10, 50, or 100 μg) intratumorally reduced tumour growth and increased survival in a dose-dependent manner. This laser treatment caused very little surface blistering when MWCNTs weren't present. Eighty percent of the mice that received the 100 μg dose of MWCNTs in combination with NIR experienced a lasting remission throughout the six months of the research. Despite the persistence of MWCNTs at the injection site, the absence of toxicity was noted. On the other hand, no matter how many MWCNTs were given to mice without NIR, they all died after three months, and there was no discernible difference in tumour growth between the treated and untreated groups. A crucial finding of this work was that MWCNTs combined with laser irradiation can improve tumour in vivo treatment by causing more regulated thermal deposition and consequently more tumour harm. Beyond that. Heat shock proteins (HSPs) are endogenous cellular indicators of thermal stress that are induced by high temperatures, usually above 43°C. Untreated tumours showed very low levels of HSP expression, whereas tumours treated with NIR alone, without MWCNTs, showed very high levels of HSP expression. The expression of HSP27, HSP70, and HSP90 was shown to be maximal close to the skin, where the incident laser was located, and to gradually decrease as the depth increased. However, considerable HSP induction was not possible in tumours treated with NIR in conjunction with MWCNTs because the temperature elevation was high enough to cause coagulative necrosis throughout the tumour. On the other hand, HSPs were

detected in lower levels of tissue, close to the tumor-normal tissue interface. These findings show that the depth of thermal therapy can be increased by combining NIR irradiation with MWCNTs. Fisher et al. (2010) have found similar results in vitro, showing that MWNTs and NIR can significantly reduce cell viability without increasing HSP expression, which may suggest a necrotic rather than apoptotic cell death mechanism. There is no selective pressure to trigger the creation of treatment-resistant cancer cell clones when cells die by necrosis, which is a major benefit over many traditional therapies that depend on inducing pro-death signal transduction pathways. Although not all cell deaths caused by NIR and MWCNTs have been shown to involve necrotic, Kratz (2010) found that Hep G2 human hepatocellular carcinoma cells had a significant uptick in apoptosis after being exposed to both substances. Factors that may affect the mechanism(s) of cell death triggered by the combination of CNTs and NIR have not been thoroughly studied so far, and this is obviously an area that requires additional information. Beyond direct thermal ablation of cancer cells, CNT-generated thermal effects may have other useful applications. The permeability of tumour vasculature, for instance, can be enhanced by heat [73-77]. Together with chemotherapy or radiation, this can improve drug delivery to tumours and increase cytotoxicity in a synergistic fashion. Specifically, a murine ascites tumour model demonstrated improved cancer cell death and increased uptake of codelivered chemo-therapeutic drugs when MWCNTs were gently exposed to near-infrared light to quickly heat cancer cells below the thermal ablation threshold. Similarly, it was shown that chemical conjugation of SWNTs with platinum-based chemotherapeutics, when heated by photothermal means, had far better results than either therapy alone. Chemotherapeutic medicines may become more cancer selective with the use of these techniques, or the harmful side effects of these therapies could be lessened by lowering the effective dose.

Improving Anticancer PTT via Tumour Selective Nanotube Binding and Uptake

Cell membranes are no match for CNTs once they make contact with cells. The physicochemical characteristics of the tubes have a significant impact on the internalisation mechanism of CNTs, which is yet not fully understood. Their active transport by endocytosis or receptor-mediated endocytosis is a theory, and their passive entry into the cell occurs through diffusion across the lipid membrane. The potential for very localised heating effects is presented by the internalisation of CNTs by cells. For instance, Kam et al. showed that endocytosed SWCNTs can be excited briefly with near-infrared light (six pulses, each 10 s long, at 1.4 W/cm2) to cause endosomal rupture without causing any noticeable harmful effects. In addition, it was demonstrated that NIR and SWCNTs worked together to selectively release DNA and other noncovalent molecular payloads from nanotube carriers. Cells that had internalised SWNTs were shown to undergo substantial cell death when subjected to continuous NIR radiation for 2 minutes at a strength of 1.4 W/cm2, in stark contrast to the nontoxic effects seen after short, pulsed exposure to NIR. This finding provided a clue that near-infrared (NIR) irradiation of SWNTs might selectively activate or induce cell death in cancer cells while causing no harm to normal cells if the nanotubes could be internalised into cancer cells. One way to make therapy more targeted would be to actively direct CNTs towards tumours. Conjugation of peptides, proteins, or antibodies to the surface of CNTs can do this [78-81], showing an improvement in tumour targeting selectivity. Incorporating targeted ligands into CNTs changes their surface chemistry, which in turn changes their thermal and optical properties. Adding targeting moieties to SWCNTs does not compromise their optical characteristics, according to multiple investigations conducted under constrained experimental settings. Research comparing the photothermal behaviour of functionalized tubes to that of their nonfunctionalized predecessors under a variety of situations has been lacking up to this point. The utilisation of folic acid (FA) as a targeting ligand to guide CNTs to FA receptors (FR), which are overexpressed on numerous tumours, was an early strategy for selective targeting that was embraced by multiple groups. Researchers in one in vitro investigation targeted human cervical cancer cells lacking FR expression or HeLa cells expressing an excess of FR with SWCNTs (average length 150 nm) coupled to FA. Luminescent labelling confirmed that the targeted SWCNTs were internalised by the FR over-expressing cells, but normal cells with low FR expression failed to absorb the CNTs. Cells with minimal FR expression remained viable and demonstrated normal proliferative behaviour after being heated continuously for 2 minutes using an 808-nm laser at 1.4 W/cm2. In contrast, cells with high FR expression showed severe cell death. Similarly, Zhou (2009) and Kang et al. (2009) found the same thing when they used human hepatocellular carcinoma cells (HepG2) overexpressing FR to deliver FA conjugated SWCNTs. According to the second study, the impact of NIR in conjunction with FA-targeted SWCNTs on HepG2 cells overexpressing FR varied with the concentration of nanotubes. Cell death increased from 50% in cells treated with 2 μg/mL of CNTs to over 85% at 20 μg/mL and higher concentrations. At SWCNT

concentrations of 20 μg/mL and below, a comparable treatment of HepG2 cells with low FR expression resulted in a cell death rate of less than 10%. Nevertheless, the treatment's selectivity was significantly diminished at higher nanotube concentrations; HepG2 cells expressing low levels of FR showed substantial cell death (35-40%) after being treated with 50 μg/mL SWCNT dispersions, suggesting that the treatment's selectivity could be diminished by nonspecific binding and uptake of CNTs. In contrast to the homogenous elevation of FR observed in as many as 90% of ovarian and brain malignancies, the expression of folate receptors is more varied in solid tumours like breast cancers, with only approximately 50% exhibiting overexpression. Consequently, folate is effective against a small subset of malignancies when used as a targeted modality. Thanks to their adaptability, carbon nanotubes (CNTs) can show a wide variety of ligands, not only tiny compounds like folate, but also antibodies that bind to the Her2 receptor, which is associated with breast cancer. Research by Xiao et al. (2009) found that anti-Her2 conjugated SWCNTs bound to Her2-expressing SK-BR-3 human breast cancer cells, but did not internalise them. After being exposed to anti-Her2 conjugated SWCNT, Her2 negative MCF-7 breast cancer cells showed minimal binding. Following the removal of unbound CNTs and the addition of new medium to the cells, 97% of the SK-BR-3 cells were killed by treating them with an NIR laser at 5 W/cm2 for 2 minutes. Surprisingly, when nontargeted SWCNTs were coupled with NIR, very little cell death was seen; in contrast, the Her2 antibody on its own had no discernible impact. Similarly, under the same experimental circumstances, MCF-7 cells were not subjected to heat ablation because the anti-Her2 conjugated SWCNTs did not adhere to them. By further conjugating SWCNTs to an anti-Her2 antibody, Marches et al. (2011) further refined this therapy method. Here, after attaching to cancer cells expressing Her2, the anti-Her2 targeting moiety caused the SWCNTs to be internalised. Marches et al. found that cells with internalised CNTs were more susceptible to NIR-mediated thermal ablation than cells that bind to but do not internalise the CNTs based on their time-dependent tracking of the relative cell binding and internalisation of the anti-Her2 conjugated SWCNTs. Another interesting finding is that in a cell population that contains both Her2 expressing and non-expressing cells, the NIR-mediated cell damage was mainly limited to the Her2 expressing cells that bound and internalised the CNTs. This suggests that this type of therapy could be customised to target specific cells. Carbon nanotubes (CNTs) have a huge surface area to volume ratio, which allows them to display several targeting ligands on their surface. This is a major advantage over other nanoparticles. In order to increase the specificity and tropism of cancer-targeted CNTs, it is possible to modify CNTs such that they bind to several receptors that are overexpressed on cancer cells. Attached to SWCNTs, for instance, were antibodies that target Her2 and insulin-like growth factor 1 receptor (IGF1R), another receptor that is overexpressed in breast tumours. Both the MCF-7 cells (which express IGF1R but not Her2) and the BT474 cells (a human breast ductal carcinoma cell line that expresses but not IGF1R) were able to be targeted with specificity in cell culture investigations because the binding of each targeting ligand did not impact the other. Nearly every cell containing targeted SWCNTs died after NIR irradiation. Cells treated to nonspecifically targeted SWCNTs were less lethally affected by near-infrared (NIR) irradiation (less than 50% cell death). An order of magnitude lower than other techniques, the estimated power utilised to kill each cell was roughly 200 nW. This is a significant finding. It was recently shown that SWCNTs engineered to accumulate at an intracellular target can induce selective heat ablation of cancer, which is arguably the most remarkable example of this possibility. Coating with a phospolipid modified to incorporate polyethylene glycol (PEG) in the head group causes SWCNTs to aggregate intracellularly at the mitochondrial membrane, as shown by Zhou et al. (2010). Upon exposure to near-infrared light, these SWCNTs preferentially kill the mitochondria of interest, leading to depolarization of the mitochondria, release of cytochrome c, and activation of caspase 3. These modified SWCNTs were found to inhibit tumour growth and even cause total regression of tumours in a model of murine breast cancer when administered in vivo (Zhou et al. 2011). Not only can SWCNTs be used, but there are other strategies as well for targeting CNTs to cancer cells specifically for thermal ablation therapy. Several investigations have shown that MWCNTs can be treated in a manner analogous to that of SWCNTs, enabling the utilisation of MWCNTs' enhanced heat transmission capacity for targeted PTT. To combat the carbohydrate antigen overexpression in neuroblastomas, one study conjugated MWCNTs to a monoclonal antibody aimed GD2. Only neuroblastoma cells expressing GD2 were seen to bind and internalise these MWCNTs; in contrast, control rat neuroendocrine tumour cells lacking GD2 expression did not absorb these MWCNTs. The 808 nm laser was used to heat the cells, with a 10-minute ramp up to 6 W/cm2 and a 5-minute hold. As confirmed by calcein staining, this treatment resulted in necrosis in virtually all GD2+ cancer cells, but no such reaction was observed in normal cells. (Wang et al., 2009) albumin to target the albumin-binding Gp60

receptor expressed in hepatocellular carcinoma cells (Kratz, 2010), and only cells within the laser zone were killed. The boundary between the treatment zone and cells not illuminated by the laser was clearly defined by living cells. In contrast to normal hepatocytes, Gp60-expressing HepG2 cells exhibited specific internalisation. Receptor binding and caveolin-dependent endocytosis were found to be the mediators of uptake. The death of cells after near-infrared (NIR) irradiation (808 nm; 2 W/cm2) for 2 minutes was proportional to the concentration and duration of CNT exposure. The rate of cell death in cancer cells was five to six times higher than in normal liver cells. Both cancer and non-cancer cell lines were treated with nonfunctionalized MWCNTs, and there was no discernible difference in cell death.

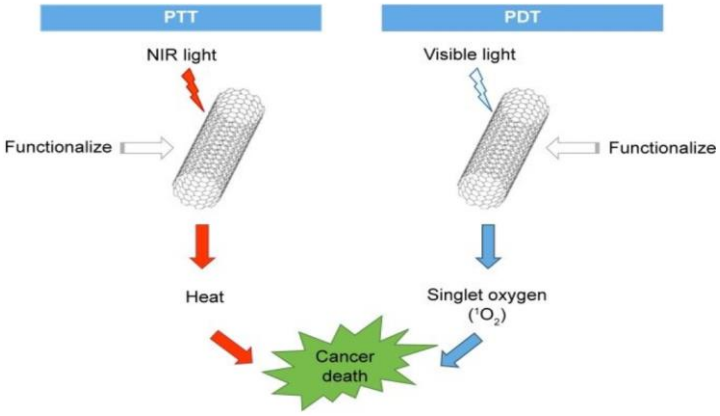

Figure 1. Processes of PTT and PDT using CNTs. Carbon nanotubes, near infrared, photodynamic therapy, and photothermal therapy are all acronyms.

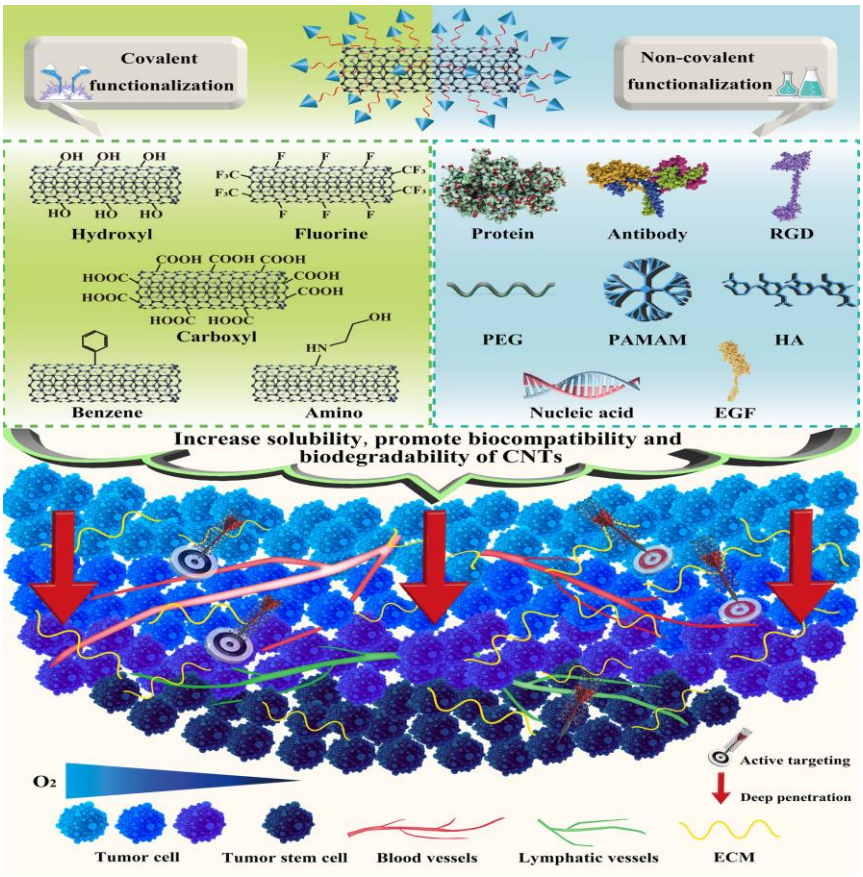

Figure 2. Various techniques of functionalizing CNTs are illustrated schematically. Covalent and non-covalent functionalization of CNTs with ligands, polymers, and chemical groups improves their biocompatibility, solubility, and biodegradability, making them more suitable for active medication targeting and deep tumour penetration.

Currently, glioblastomas and other malignancies that are extremely resistant to conventional treatments are undergoing trials of targeted thermal ablation therapy based on CNTs. It seems that the CD133 receptor is a cancer stem cell marker linked to malignancy, tumour recurrence, and poor survival in glioblastomas and other brain tumours. Theoretically, numerous tumour forms contain populations of cancer stem cells that are resistant to self-renewing therapies. The CD133+ subpopulations in glioblastomas are responsible for repopulating tumour cells after radiation therapy; they are also resistant to chemotherapy and radiotherapy. Potentially effective in halting the emergence of treatment resistance are treatment approaches that zero in on this specific subset.

More recently, MWCNTs chemically conjugated to a monoclonal antibody directed against CD133 were utilised to target these cells by Wang et al. (2011). They found that glioblastoma cells that expressed CD133 were the only ones that specifically internalise these targeted MWCNTs via endocytosis in cell culture tests. This is crucial to note: these were not immortalised cells, but rather newly obtained cells from patients. For the purpose of evaluating the selectivity of nanotube-enhanced PTT, a mixture of the two cell types was exposed to an 808-nm laser at 2 W/cm2 for 5 minutes after being incubated with 2.5 mg of MWCNTs for 6 hours. According to flow cytometry, CD133+ cells were destroyed but CD133- cells were left unharmed. These results showed that an in vivo xenograft model that was further replicated by injecting CD133 expressing glioma cells into mice after pretreatment with targeted MWCNTs. Following NIR illumination, the cells absorbed the MWCNTs, xenograft development was halted, and no metastases were found. Crucial evidence of CNTs' ability to treat glioblastomas and other incurable malignancies was presented here.

Breast cancer stem cells have been extensively studied because of their remarkable resistance to conventional radiation and chemotherapy. Burke et al. (2012) details how MWCNT-mediated thermal ablation can cure this group of cells that would normally be resistant to therapy. To start, breast cancer cells (both bulk and stem) were heated in a circulating water bath, simulating a clinical hyperthermic treatment setting, and the effects on cell viability were assessed. Breast cancer stem cells, as compared to bulk breast cancer cells, showed a markedly higher resistance to hyperthermia across the board when treated with this approach.

Alternatively, stem and bulk breast cancer cells responded similarly to MWCNT-mediated thermal therapy when both kinds were heated to the same end temperature using 50 μg/mL amidated MWCNTs and NIR laser radiation (1064 nm; 3 W/cm2). The scientists proved that this was because, unlike conventional hyperthermia, MWCNT-mediated heat treatment induced fast and robust necrotic cell death. Taken together, our results show that hyperthermia induced by nanotubes is functionally different from hyperthermia induced by other methods, and it could be a huge step forward in treating stem cell-driven malignancies that have not responded to conventional treatments.

How CNTs Are Delivered Systemically and Are They Biocompatible for PTT

It is critical that tumour cells selectively take up CNTs rather than normal cells for CNT enhanced PTT to avoid heating normal tissue. If tumor-targeted CNTs were administered systemically, this would be the ideal outcome. A lot of work has gone into developing passive and active targeting methods to selectively deliver nanoparticles to tumour locations [82, 83]. In passive targeting, the physicochemical and surface properties of the nanoparticle are correlated with the pathophysiology and anatomy of the target region in an effort to accomplish tumour delivery without utilising specific biological (ligand-receptor) interactions.

In addition to passive delivery, which is necessary for active delivery to reach the tumour site, nanoparticles can be modified to attach ligands with a higher binding affinity to complementary cellular receptors, allowing them to associate or interact with specific biological moieties. Enhanced permeability and retention effect have been used passively over the last 20 years as a means to selectively distribute nanoparticles to tumour locations. The theory behind the approach is that long-lasting nanoparticles can target tumours specifically by entering the tumour site via extravasation through the tumor's surrounding leaky vasculature. Coating nanoparticles with steric stabilisers like PEG increases their systemic circulation and decreases blood clearance. This is because the uptake of nanoparticles by macrophages and other components of the mononuclear phagocyte system is reduced. Following intravenous injection of CNTs into mice, multiple groups have shown that PEG coating significantly enhances tumour location.

This method of intravenously targeting tumours with nanotubes has recently demonstrated efficacy for nanotube-enhanced PTT. Mice with 4T1 murine breast tumours were injected with short (140 nm) PEG-coated SWCNTs at a

dose of 3.6 mg/kg via the tail vein. When the optical imaging system detected the SWCNTs' inherent near-infrared photoluminescence (1100-1400 μm wavelength) three days later, it confirmed that SWCNTs had accumulated at the tumour site.

After 5 minutes of heating at 0.6 W/cm2 with an 808 nm laser, the tumours were successfully abated. No harm was seen, and all treated animals survived the 6-month study without recurrence. This was a major milestone in the search for a systemically administered photothermal ablation agent that selectively targets tumours. Keep in mind that neither the nanotubes nor the cancer cells themselves were altered to exhibit a particular targeting ligand in this experiment [84–87]. If active targeting does provide an extra benefit in vivo, further research is required to confirm it. The process of transforming carbon nanotubes (CNTs) from an intriguing nanomaterial into a useful pharmaceutical product is yet in its infancy, but these studies show that CNTs have substantial potential for targeted hyperthermia of cancer. Their long-term clinical fate will be decided by their toxicity and, by extension, by our capacity to assess the possible risk-benefit balance of these materials.

Unfortunately, it is not an easy task to produce a reliable toxicity profile of CNTs. Surface functionalization changes adsorption properties, electrostatic interactions, hydrophobicity/hydrophilicity, and the stability of CNT dispersions; structural features like length and diameter also impact CNT toxicity. Biological and toxicological responses after CNT injection are influenced by both of these factors. Lastly, there has been a thorough evaluation of the toxicity of CNTs elsewhere; nonetheless, it is possible that residual catalysts like Co, Fe, Ni, and Mo, which are by-products of CNT production, are to blame. Because of this, we will simply present a handful of the most important studies. It should be mentioned that the majority of toxicity research have concentrated on the effects of ambient exposure after inhalation of unprocessed, "as produced" CNTs. For example, concerns have been raised regarding the possibility of very long (10–20 μm) nanotubes to induce an asbestos-like reaction, or for inhalation of nanotubes to cause dose-dependent granulomatous pneumonia, oxidative stress, and acute inflammatory and cytokine responses, with fibrosis and decrease in pulmonary function, and the possibility that CNTs may elicit an immune or allergic response (CNTs are inherently hydrophobic, and the toxicity of the pristine CNTs may be largely due to their hydrophobicity. CNTs used for biomedical applications must be modified in some way from their pristine, as-produced, condition in order to render them suitable for dispersion in aqueous environments. Typical modifications include acid oxidation of the CNT exterior to introduce carboxyl groups , “wrapping” CNTs in long-chain surfactants (reviewed by Nakashima and Fujigaya 2007), and as noted above, linking antibodies or other targeting moieties to the CNT surface both to aid in their dispersion and promote their accumulation in tumor tissue. By modifying them chemically, CNTs can be made less hazardous and cleared from the body more quickly. Nano-medicines made of CNTs will have to find a way to get past the body's defences, which could include injecting them intravenously, which could lead to different kinds of toxicity. The proof that CNTs are compatible with blood is particularly crucial since intravascularly administered particles have the propensity to generate undesirable thrombotic events. It was recently investigated how chemical functionalization of CNTs affected blood toxicity after intravenous injection in mice. After being injected intravenously into rats, researchers discovered that unfunctionalized MWCNTs were far more thrombogenic than chemically modified MWCNTs. Pure MWCNT caused pulmonary vascular obstruction and was immediately fatal when administered at a dosage of 250 μg. Covalently functionalized MWCNTs, on the other hand, had no discernible impact on coagulation in vivo; the only observable effect was a temporary reduction in platelets. In line with this, the vast majority of research that have tested injecting chemically functionalized CNTs into mice to enhance their aqueous dispersion have failed to find any chronic or long-term toxicity. It indicates that CNTs can be engineered to be biocompatible and appropriate for systemic distribution; nonetheless, larger and longer-term investigations are needed to completely understand CNT toxicity.

Views on the Possible Translational Use of CNT-Enhanced PTT

In the next iteration of photothermal agents, CNTs may play a crucial role. In comparison to existing therapeutic vectors, they provide a novel opportunity to tailor optical, thermal, and cancer-selective characteristics. In addition to providing localised heating quickly in response to near-infrared light (NIR), clinical applications of carbon nanotube (CNT) enhanced photothermal ablation could work with noninvasive imaging to pinpoint the exact location of the target lesion, evaluate how the CNTs were distributed inside the tumour, make sure the right amount of heat was applied, and monitor the treated area's reaction to therapy as time went on. By combining these properties, CNTs are

able to circumvent many of the problems with conventional thermotherapy, which might lead to more widespread usage of image-guided LITT in clinical settings and better therapeutic results for cancer patients after treatment.

In theory, CNTs might be safely administered to patients through non-invasive procedures. Such a procedure could involve directly injecting CNTs into a tumour or using intraoperative ultrasound to locate the tumor's major blood supply. Then, a mini-laser could be used to irradiate the tumour using the CNT dispersions. The procedure would be guided by a videoscopic or other real-time imaging modality. Reduced postoperative discomfort, faster recovery and less hospitalisation, fewer surgical or wound problems, and improved cosmetics are some of the potential advantages of this therapeutic approach, as pointed out by Iancu and Mocan (2011). Furthermore, nanoparticles may play a role in the next generation of heat therapy for both tissue ablation and the transport of chemotherapeutic drugs to cancer cells. The effectiveness of CNT therapy might be further improved with the development of efficient methods for localising CNTs to tumours after intravenous or arterial injection. To thoroughly examine the pharmacologic characteristics and possible toxicity of newly engineered targeted CNTs, additional research is necessary. It is essential to do thorough materials assessment prior to clinical development in order to determine these qualities, as they will be dependent on the specific particle being studied. Based on the promising outcomes shown with different types of CNTs thus far, it appears that their medicinal potential has only just been explored.

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