Carbon nanotubes in cancer therapy

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A careful examination of the carbon cathode used in the arc-discharge process for the production of fullerenes by Iijima[1], resulted in the historical discovery of carbon nanotubes (CNTs), ultra-thin carbon fibres with nanometre size diameter and micrometre size length. Iijima obtained only a multiwalled carbon nanotube (MWCNT) and that is indeed a milestone in the study of different forms of carbon. Subsequently, Iijima and Ichihashi[2] and Bethune *et al.*[3] reported the production of single-walled carbon nanotubes (SWCNTs). CNTs have been recognized as the quintessential nanomaterials and have acquired the status of one of the most active fields of nanoscience and nanotechnology. The MWCNT is composed of 2–30 concentric graphitic layers, the diameters of which range from 10 to 50 nm and length more than 10 μm. On the other hand, SWCNT is much thinner, with diameter ranging from 1.0 to 1.4 nm. CNTs exhibit unique electronic, mechanical and thermal properties. These properties of CNTs have led to their use in areas as diverse as sensors, actuators, field-emitting flat panel displays as well as energy and gas storage[4]. Application of CNTs in diagnosis and therapy of dreadful diseases is a field of current interest.

With more than 10 million new cases every year, cancer is one of the most devastating diseases[5]. Though the current treatments of cancer by surgery, radiation and chemotherapy are successful in several cases, these curative methods also kill healthy cells and cause toxicity to the patient. It would therefore be desirable to develop methods to directly target cancerous cells without affecting normal ones. Two recent papers[6,7] detail the potential use of SWCNTs to treat several types of cancers, with minimal or no toxic effects to normal cells.

In the field of healthcare, administration of drugs poses several problems like insolubility of drugs, inefficient distribution, lack of selectivity and side-effects. These problems are areas of current active research with the objective to improving efficiency, availability and toxicity profiles. Cell membranes also pose a problem in drug delivery by selectively allowing only certain structures to pass based on hydrophilicity : hydrophobicity ratios. It is interesting to note that among the currently available delivery systems, which include liposomes, emulsions, polymers and microparticles, CNTs have recently gained popularity as potential drug carriers, therapeutic agents and for applications in diagnosis[8].

As the sidewall of SWCNTs is highly hydrophobic, they are practically insoluble in water. Therefore, SWCNTs are functionalized by covalent or noncovalent routes that will help in disentangling the CNT bundles and make them soluble in water. Liu *et al.*[9] prepared a solution of SWCNTs wrapped in poly(ethylene glycol) (PEG) with a tumour-targetting cyclic arginine–glycine–aspartic acid peptide to the end of the PEG chains. This solution was injected into mice bearing tumours and it was observed that the targetted SWCNTs accumulated in tumours. Thus potential drug delivery applications have been achieved with efficient *in vivo* accumulation of SWCNTs in mice tumours[9]. The above finding has prompted studies to attach a cancer chemotherapy drug doxoyrubicin (DOX) molecule onto prefunctionalized nanotubes, possibly for *in vivo* cancer therapy[6]. SWCNTs are functionalized noncovalently by a surfactant (phospholipid (PL)–PEG, ~120 polyethylene oxide (PEO) units) or covalently by PEGylation (~200 PEO units) of COOH-groups on oxidized SWCNTs obtained by treatment with nitric acid. DOX was mixed with the prefunctionalized SWCNTs and kept overnight at a pH of 9. The solution was filtered to remove free, unbound DOX and characterized by spectral studies which indicate doxorubicin π-stacking (supramolecular assembly) onto unoccupied surface areas of PEG–SWCNTs forming a forest (PEG)–scrub(DOX) structures on SWCNTs (Figure 1). The DOX-loaded SWCNTs are stable in water and at pH 7.4 physiological buffers. DOX is a widely used chemotherapy drug and SWCNT without DOX loading did not show any toxic effects on malignant cells. Liu *et al.*[6] have demonstrated that DOX-loaded SWCNTs (PL–SWCNT–DOX) induced significant U87 cancer cell death and cell apoptosis similar to free DOX. The main advantage of using SWCNT as a drug carrier compared to free drug is

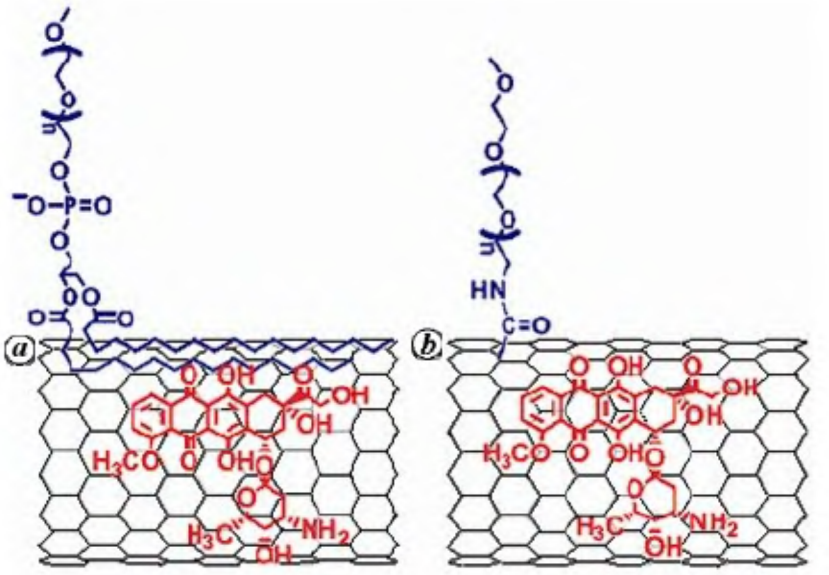

Figure 1. Supramolecular assembly of molecules on functionalized CNTs. DOX π-stacking onto a nanotube prefunctionalized noncovalently by PL–PEG (***a***) and covalently by PEGylation of a sidewall –COOH group (***b***). Reprinted with permission from Liu *et al.*[6]. Copyright (2007) from American Chemical Society.

the potential to target delivery for selective destruction of certain types of cells, reducing the toxicity to nontargetted cells. It has also been observed that with decreasing pH, the loading of DOX reduces on SWCNTs. This is due to increased hydrophilicity and higher solubility of DOX at lower pH caused by increased protonation of $-NH_2$ groups on DOX. This results in reducing the hydrophobic interaction between DOX and SWCNT, and DOX is released. The pH dependence of binding/release of DOX could be profitably exploited for drug delivery applications. As the micro-environments of extracellular tissues of tumours and intracellular lysosomes and endosomes are acidic, the situation will potentially facilitate active drug release from SWCNT delivery vehicles. This method provides a novel, easy-to-make formulation of the SWCNT–DOX complex with extremely high drug-loading efficiency, which is remarkably higher than that reported for conventional liposomes and dendrimer drug carriers[6].

Aqueous solution of functionalized SWCNTs on exposure to radiofrequency (RF) field experiences efficient heating and this property has been exploited by Gannon *et al.*[7] for a noninvasive and selective thermal destruction of human cancer cells with minimal or no toxic effects to normal cells. As 66% of the available SWCNTs are direct band-gap semiconductors, Gannon *et al.*[7] assumed that exposure to RF field would lead to significant heat release by SWCNTs. Both *in vitro* and *in vivo* tests were carried out with cancer cells. In the *in vitro* test, three human cancer cell lines were incubated with various concentrations of SWCNTs and then treated in the RF field. After 48 h, tumours were harvested to assess viability. The authors observed CNT concentration-dependent cellular cytotoxicity *in vitro* in all three cancer cell lines after 2 min of exposure to the RF field. At a concentration of 500 mg/l of SWCNT, cytotoxicity essentially was 100% in all three cell lines. In another set of experiments, hepatic VX2 tumours in rabbits were injected with SWCNTs or with control solutions and were treated in the RF field. Rabbits bearing tumours tolerated the RF treatment[7]. After 48 h, all SWCNT-treated tumours demonstrated complete necrosis. However, tumours treated only with SWCNTs or RF field were viable. These observations indicate the thermal destruction of cancer cells[7] in the presence of SWCNTs and exposure to RF field.

The two papers[6,7], one on supramolecular assembly of DOX on SWCNTs and the other employing SWCNTs and RF field, demonstrate that carbon nanotubes are capable of leading to new exciting directions and approaches in therapeutic oncology.

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Adaptation of *Listeria monocytogenes* to low temperature and high salt

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Listeria monocytogenes, a Gram-positive bacterium and causative organism of listeriosis, a serious food-borne infection, is widely distributed in nature. Its ability to grow at low temperatures and high salt environment, makes it a potential risk factor in storage of food materials and also an attractive model for studies on bacterial adaptation to low temperature and high osmolarity.

A couple of years ago, using SCOTS (Selective Capture of Transcribed Sequences), a novel procedure for differential cloning of c-DNA, selective expression of RNAs in *L. monocytogenes* caused by a downshift of environmental temperature from 37°C to 10°C, was studied. The RNAs, the steady-state level of which was elevated at low temperature, included those involved in synthesis of flagellar proteins, sigma factor Rpo N, histidine kinase (believed to be required for sensing environmental temperature), a transcriptional antiterminator, a repressor protein, some chaperone proteases, products related to the metabolism of some amino acids, enzymes required for some degradative metabolism and a novel fibronectin-binding protein. In addition to these, some genes unique to *L. monocytogenes* were also expressed at low temperature[1].

A recent study by Chassaing and Auvray[2] reveals the role of another protein in cold and salt tolerance of the bacterium. The investigators performed transposon mutagenesis of a strain of *L. monocytogenes* using the temperature-sensitive plasmid pTV32-OK containing the transposon Tn 917-*lac*. A total of 3720 random insertion mutants were screened for cold-sensitivity. A mutant that could grow at 37°C but not at 4°C in a microtiter plate was designated as cs 1. When cs 1 was checked for its ability to survive at 4°C over a period of 35 days with a culture of the wild-type strain maintained in the same condition as control, the mutant was found not only to survive but also to grow. However, its rate of growth was slower compared to