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CARBON NANOTUBES: A NOVAL CARRIER SYSTEM FOR DRUG DELIVERY & CANCER THERAPY

ABHINAV SHAHI

Department of Pharmacy, Pranveer Singh Institute of Technology, Bhauti, Kanpur, UP, India.

ABSTRACT

Carbon nanotubes (CNTs) have been introduced recently as a novel carrier system for both small and large therapeutic molecules. CNTs can be functionalized (i.e., surface engineered) with certain functional groups in order to manipulate their physical or biological properties. Carbon nanotubes (CNTs) are allotropes of carbon with a cylindrical nanostructure. CNTs made from a single graphene sheet results in a single-walled nanotubes (SWNT) while several graphene sheets make up multiwalled carbon nanotubes (MWNTs). This paper will discuss the therapeutic applications of CNTs with a major focus on their applications for the treatment of cancer.

KEY WORDS : **Cancer, Carbon, Cells, Nanocarrier, Nanotubes, Single-walled.**

ABHINAV SHAHI
Dept. of Pharmacy, Pranveer Singh Institute of Technology, Bhauti, Kanpur,UP, India.

***Corresponding author**

INTRODUCTION

The major aim of developing nanocarrier drug delivery systems is to reduce toxicity of therapeutically active materials or to enhance the therapeutic effect. This is conventionally achieved using spherically shaped vesicle nanocarriers such as liposomes (When phospholipids are dispersed in water they spontaneously form closed structure with internal aqueous compartments bounded by phospholipid bilayer membranes, these are called liposomes). Carbon nanotubes (CNTs) are allotropes of carbon with a cylindrical nanostructure. CNTs are graphene (one-atom-thick layer of graphite) sheets rolled into a seamless cylinder that can be open ended or capped, having a high aspect ratio with diameters as small as 1nm and a length of several micrometers. CNTs made from a single graphene sheet results in a single-walled nanotubes (SWNT) while several graphene sheets make up multiwalled carbon nanotubes (MWNTs) [1, 2, 39]

Diagram of single-walled carbon nanotube and multiwalled carbon nanotube

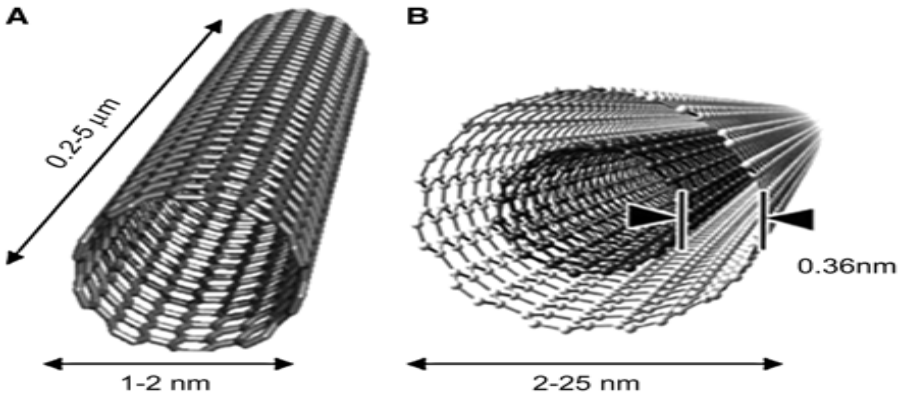

Figure 1

Conceptual diagram of single-walled carbon nanotube (SWNT) (A) and multiwalled carbon nanotube (MWNT) (B) delivery systems showing typical dimensions of length, width, and the separation distance between graphene layers in MWNTs.[34]

The way the graphene sheet is wrapped is represented by a pair of indices (*n*,*m*). The integers *n* and *m* denote the number of unit vectors along two directions in the honeycomb crystal lattice of graphene. If *m* = 0, the nanotubes are called zigzag nanotubes, and if *n* = *m*, the nanotubes are called armchair nanotubes. Otherwise, they are called chiral.
The diameter of an ideal nanotube can be calculated from its (n,m) indices as follows:[38]

$$d = \frac{a}{\pi}\sqrt{(n^2 + nm + m^2)}.$$

(where a = 0.246 nm.)

Nanotubes

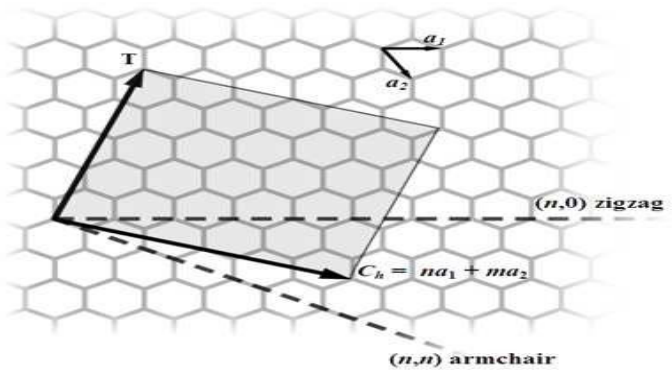

Figure 2

The (*n*,*m*) nanotube naming scheme can be thought of as a vector (C_h) in an infinite graphene sheet that describes how to "roll up" the graphene sheet to make the nanotube. T denotes the tube axis, and a_1 and a_2 are the unit vectors of graphene in real space.[35]

Since their discovery by Iijima in 1991, carbon nanotubes are seen by many as the breakthrough nanotechnology of the future. They are simple in design and yet have several intriguing and advantageous properties associated with them. There are several different types of carbon nanotubes such as single-walled, multi-walled, and nanotorus nanotubes. Due to their high surface area, they are capable of adsorbing or conjugating with a wide variety of therapeutic molecules.[1] Thus, CNTs can be surface engineered (i.e., functionalized) in order to enhance their dispersability in the aqueous phase or to provide the appropriate functional groups that can bind to the desired therapeutic material or the target tissue to elicit a therapeutic effect. CNTs might help the attached therapeutic molecule to penetrate through the target cell to treat diseases [3-6]

CELLULAR UPTAKE OF CARBON NANOTUBES

The cellular uptake of CNTs has been confirmed in various studies but the mechanism of CNT penetration into cells is still not well understood. Due to their needle-like shapes, CNTs might be able to perforate cellular membrane and pass into the cellular components without causing apparent cell damage [3, 4, 7–10.] An in vitro CNTs nanoinjector system has been developed by Chen and coworkers[6]. The nanoscale cell injection system (termed the nanoinjector) used carbon nanotubes to deliver cargo into cells. A single multiwalled carbon nanotube attached to an atomic force microscope (AFM) tip was functionalized with cargo via a disulfide-based linker. Penetration of cell membranes with this “nanoneedle” was controlled by the AFM. The following reductive cleavage of the disulfide bonds within the cell's interior resulted in the release of cargo inside the cells, after which the nanoneedle was retracted by AFM control.

AFM-controlled MWNT-based nanoinjector

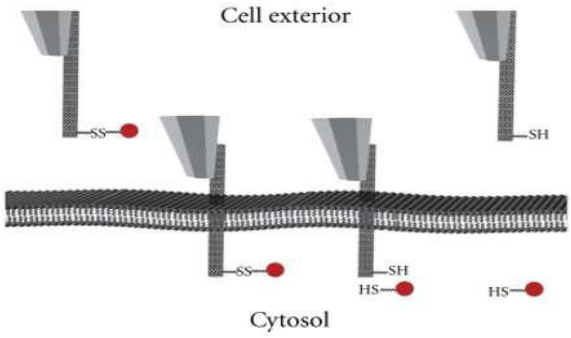

Figure 3

A schematic diagram showing that an AFM-controlled MWNT-based nanoinjector was able to penetrate into a cell and release the attached cargo compound after the breakage of the disulfide bond. This was followed by successful retraction of the nanoinjector with no apparent cell damage being produced[6].

The perpendicular positioning of the nanotubes to the cell membranes suggests that the uptake of CNTs was similar to that of nano needles which diffuse through cell membrane without causing cell death[5]

Perpendicular positioning of MWNTs

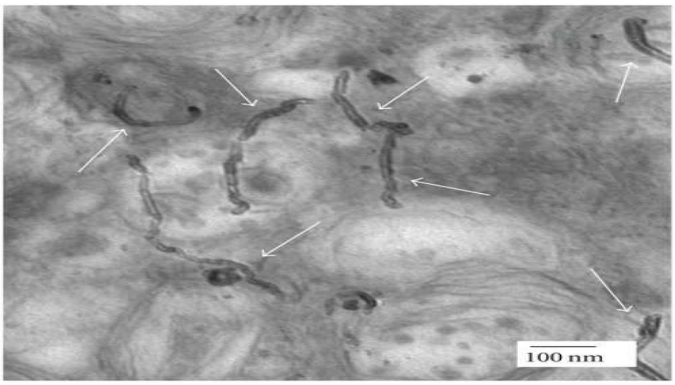

Figure 4

The perpendicular positioning of MWNTs (pointed at by the white arrows) during internalization into HeLa cells suggests that cellular uptake of CNTs by the cells was similar to that of nanoneedles[5].

CARBON NANOTUBES AS CARRIERS FOR DRUGS, GENES, AND PROTEINS

CNTs have been investigated as potential nanocarriers for the delivery of drugs, genes, and proteins. Most of the research on CNTs has focused on their potential for delivery of anticancer agents. This may be due to their unique needle-like shapes which enable them to be functionalized in order to adsorb or covalently link to a wide variety of therapeutic materials and internalize them into the target cell.

Approaches for CNT-based drug delivery

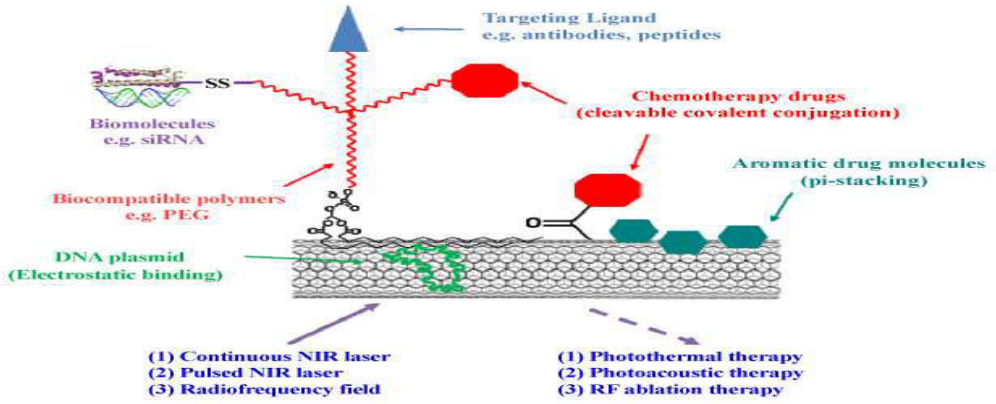

Figure 5

A schematic drawing showing various approaches for CNT-based drug delivery and cancer therapies.[36]

1. CARBON NANOTUBES AS CARRIERS OF ANTICANCER MOLECULES

It is well known that cancer cells over express folic acid (FA) receptors, and thus several research groups have designed nano carriers with engineered surfaces to which FA derivatives can be attached. Moreover, non spherical nano carriers (e.g., CNTs) have been reported to be retained in the lymph nodes for longer periods of time compared to spherical nanocarriers[11] (e.g., liposomes). Thus, CNTs might be used for targeting lymph node cancers as shown by various investigators[12,13,14]. In these studies, magnetic nanoparticles containing the anticancer cisplatin were entrapped into folic-acid-functionalized MWNTs. An external magnet was employed to drag the nanotubes to the lymph nodes where the drug was shown to be released over several days and the tumor to be selectively inhibited. Yang et al. have loaded the anticancer molecule gemcitabine into magnetic MWNTs and, using mice, they reported high activity against lymph node metastasis when the formulation was injected subcutaneously[15]. In another study, the poorly water-soluble anticancer camptothecin has been loaded into polyvinyl alcohol-functionalized MWNTs and reported to be potentially effective in the treatment of breast and skin cancers[16]. Dhar and coworkers[17] have developed the "longboat delivery system". In this a complex of cisplatin and FA derivative was attached to a functionalized SWNT via a number of amide bonds to comprise the "longboat" which has been reported to be taken up by cancer cells via endocytosis, followed by the release of the drug and its subsequent interaction with the nuclear DNA. Another anticancer, namely, carboplatin, after being incorporated into CNTs has been shown to inhibit the proliferation of urinary bladder cancer cells *in vitro*. In another study, anticancer effects have been shown to be dependent on the method used to entrap the drug in the CNTs, which highlighted the possible effects of preparation conditions on the therapeutic activity of therapeutic molecules associated with CNTs[18].

Paclitaxel is a poorly water-soluble anticancer molecule. In the commercialized paclitaxel product (Taxol), Cremophor EL is used to solubilise the drug. Unfortunately, Cremophor EL is toxic, which makes a possibility of finding a suitable alternative. Moreover, the circulation time of Taxol is very short. Coating the nanocarriers (e.g., liposomes) with hydrophilic polymers such as polyethylene glycol (PEG) has been established as a strategy to prolong the circulation of the nanocarrier-entrapped molecules in the blood by making the carrier highly evasive to uptake by the blood

macrophages[19,20]. PEGylation (process of covalent attachment of polyethylene glycol (PEG) polymer chains to another molecule, normally a drug or therapeutic protein) of paclitaxel increases the circulation time in the blood over Taxol[21]. Functionalized SWNTs were conjugated with paclitaxel through branched PEG chains via a cleavable ester bond. The resulting formulation was more effective in suppressing tumor growth *in vivo* than Taxol or paclitaxel-PEG conjugate in a 4T1 breast cancer animal model. The PEGylated nanotubes were able to prolong the circulation and greatly enhance cellular uptake of the drug by the cancer cells[22].

In vivo doxorubicin delivery with carbon nanotubes for cancer treatment

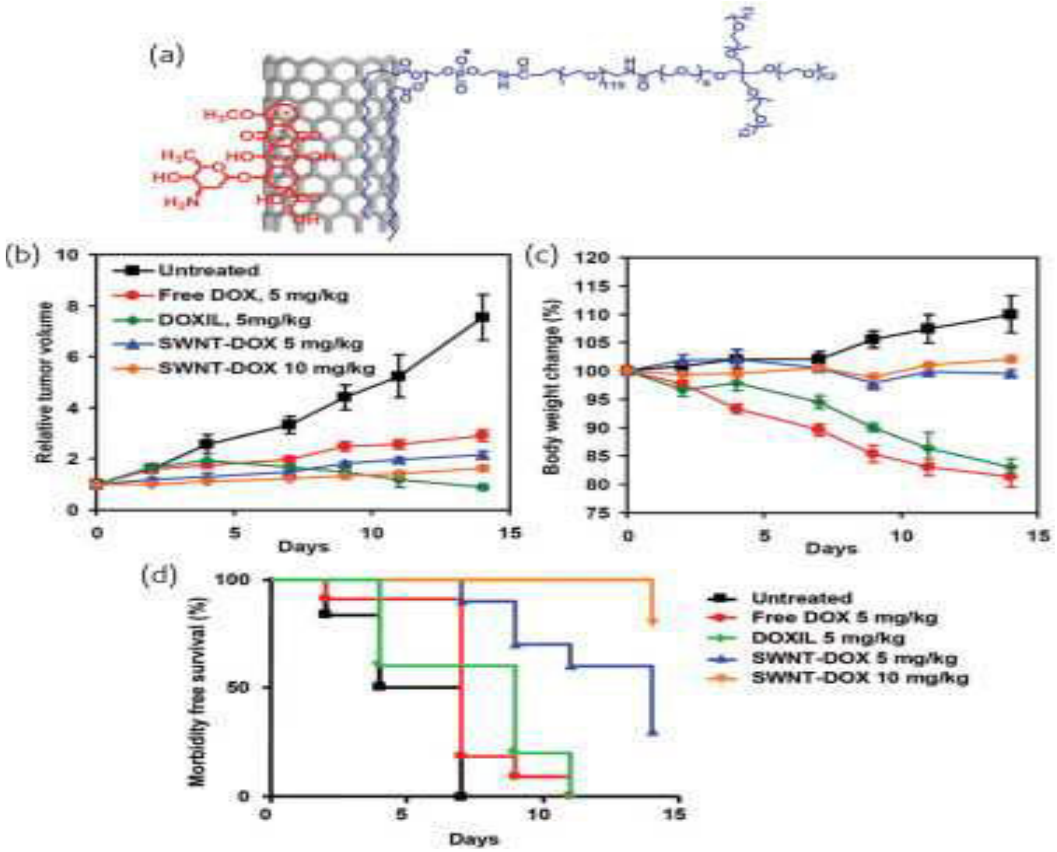

Figure 6

In vivo doxorubicin delivery with carbon nanotubes for cancer treatment. (a) A scheme showing supramolecular π-π stacking of DOX on PEGylated SWNTs. (b-d) Raji tumor bearing SCID mice were treated with different DOX formulations once per week on day 0 and day 7. (b) Tumor sizes of untreated (n = 7), 5 mg/kg free DOX treated (n = 10, 2 mice died in the second week), 5 mg/kg Doxil treated (n = 5), 5 mg/kg SWNT-DOX treated (n = 10) and 10 mg/kg SWNT-DOX treated (n = 10) mice were measured. (c) SWNT-DOX resulted in far less weight loss than DOX and DOXIL. Averaged tumor volumes and body weights were normalized to day 0. (d) Kaplan–Meier analysis of morbidity free animal survival post various treatments indicated P values: DOX 5 mg/kg versus SWNT-DOX 5 mg/kg or 10 mg/kg, $p < 0.001$; DOXIL 5 mg/kg versus SWNT-DOX 5 mg/kg, $p = 0.013$; DOXIL 5 mg/kg versus SWNT-DOX 10 mg/kg, $p < 0.001$). Error bars in (b,c) were based on the standard error of the mean.[37]

Multidrug resistance is a significant obstacle to successful anticancer drug therapy since the P-glycoprotein efflux transporter can interfere with the accumulation of anticancer drugs in the target cells, resulting in reduced effectiveness of therapy[23,24]. Recently, using

hepatoma cell lines, PEGylated MWNTs have been shown to accumulate in multidrug resistant cells as efficiently as in nonresistant cells, as observed by confocal microscopy[25].

2. CNTS AS CARRIERS OF IMMUNOACTIVE COMPOUNDS, PROTEINS, AND GENETIC MATERIALS

The ability of macromolecules (e.g., genes) to cross the biological barriers and be expressed within a target cell is particularly challenging, owing to their hydrophilicity and large molecular size. Gene therapy aims to use genetic material to treat diseased cells from repairing the cause of the disease. Since genetic materials are poorly able to cross the biological membranes, the use of viral or nonviral vectors to carry the gene and internalize it into the cell is necessary. Nonviral vectors are less efficient than viral vectors[26] and short lived[27]; however, they are far safer[28,29]. Pantarotto and coworkers have developed novel functionalized SWNT-DNA complexes and reported high DNA expression compared with naked DNA[4].

3. OTHER THERAPEUTIC APPLICATIONS OF CNTS

The use of CNTs has been expanding to include therapeutic applications other than cancer. Surface-engineered CNTs may be able to capture pathogenic bacteria in liquid medium[30–32]. Thus, CNTs themselves might have antimicrobial activity since microorganisms may be adsorbed onto the engineered surfaces of CNTs. Moreover, using E. coli as a model microorganism, it has been reported that the electronic properties of SWNTs may regulate their antibacterial activity. The antibacterial effect was attributed to carbon nanotube- induced oxidation of the intracellular antioxidant glutathione, resulting in increased oxidative stress on the bacterial cells and eventual death[33].

CONCLUSION

Carbon nanotubes (CNTs) are allotropes of carbon with a cylindrical nanostructure. CNTs are needle-like carriers of both small drug molecules as well as macromolecules such as genes and proteins. CNTs can be functionalized so that certain molecules are attached to their surfaces via covalent or noncovalent bonding. The needle-like shape of the CNTs enables them to perforate cellular membranes and transport the carried therapeutic molecules to the cellular components.

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