5

Carbon nanotubes: A new biotechnological tool on the diagnosis and treatment of cancer

Benjamín Pineda[1], Norma Y. Hernández-Pedro[1], Roxana Magaña Maldonado[1], Verónica Pérez-De la Cruz[2], Julio Sotelo[1]

[1]Neuroimmunology and Neuro-Oncology Unit, Instituto Nacional de Neurología y Neurocirugía.
[2]Neurochemistry Unit, Instituto Nacional de Neurología y Neurocirugía.

Outline:

Introduction

Cancer is one of the leading causes of death worldwide. Treatment of cancer requires a careful selection of one or more intervention, such as surgery, radiotherapy, and chemotherapy. However, these standard treatments do not improve the prognosis and quality of life of patients. Nanotechnology allows highly personalized and safer medicines with the potential of improve cancer diagnosis and therapy. A wide variety of nanomaterials are under investigation, including polymeric/non-polymeric nanoparticles, dendrimers, quantum dots, carbon nanotubes, lipid- and micelle-based nanoparticles. These nanomaterials reduce toxicity associated with cancer therapy, their ability to carry and controlled deliver site-specific cytotoxic drugs as paclitaxel, docetaxel, cisplatin and multivalent-ligand targeting.

Carbon nanotubes (CNTs) have received considerable interest for diagnosis and treatment of cancer due to their minimum toxicity and biocompatibility, although CNTs are safety, they present some cytotoxic effects [1]. They represent an important group of nanomaterials with attractive geometrical, electronic and chemical properties. There are two main kinds of carbon nanotubes (CNT), single-walled nanotubes (SWNTs) consisting of a single graphite sheet seamlessly wrapped into a cylindrical tube and multiwalled nanotubes (MWNTs) comprise an array of such nanotubes that are concentrically nested like rings of a tree trunk. CNTs have been studied for intracellular delivery of proteins, peptides, drugs and fluorescence contrast agents to MRI. They are often functionalized with cationic molecules or polymers in order to interact electrostatically with negatively charged siRNAs or plasmid DNAs and also for vaccine development. The CNTs may become attached to the surfaces of biological membranes by adsorption or electro-static effects, causing damage to cells by generating reactive oxygen species, resulting in lipid peroxidation, protein denaturation, DNA damage, and ultimately cell death.

Recently, CNTs has been coupled to diverse quantum dots (QDs) and they have been used for localization of cancer cells due to their nano size and ability to penetrate individual cancer cells and high-resolution imaging derived from their narrow emission bands compared with organic dyes. The conjugation of QDs to CNTs offers the opportunity for simultaneous diagnosis and treatment of cancer. Initially they allow localization of the cancer cells by imaging with QDs, and subsequent cell killing, via drug release or thermal treatment, due to their ability to deliver drugs to a site of action or convert optical energy into thermal energy. Likewise, CNTs release substantial vibrational energy after exposure to near-infrared radiation; this produces heating localized within a tissue, which could be used as potentially phototherapy in the treatment of cancer.

In the same way, CNTs provide a versatile, biodegradable, and non-immunogenic delivery alternative to viral vectors for molecular therapy or immunotherapy and direct delivery of antigens to antigen presenting cells (APCs). Once of CNTs are delivered, they were connected to tumor proteins by formation of a covalent bond with polypeptides or formation of complexes between CNTs and tumor proteins. The CNT-tumor protein complex promotes phagocytosis of dendritic cells in the tumor tissue, the enhancing of immunogenicity is through to augment the ability of lymphocytes to attack and destroy the tumor.

CNTs have been diversely modified to improve or increase their effect under cancer cells. The preparation and attractive performance of carbon-nanotube modified glassy-carbon (CNT/GC) electrodes for improved detection of purines, nucleic acids, and DNA hybridization are described. The surface-confined MWCNT facilitates the adsorptive accumulation of the guanine nucleobase and greatly enhances its oxidation signal.

Even though CNTs have emerged as important in the treatment of cancer, their cytotoxicity has limited their use. *In vitro* studies have shown that CNT have many toxic effects, including decreased cell viability, induction of apoptosis, disruption of the cell cycle, generation of oxidative stress and inflammatory responses. CNT can damage the respiratory system of mice by entering the alveolar space, causing a chronic inflammatory reaction characterized by intermittent granulomatous lung tissue and finally pulmonary fibrosis, with significantly greater toxicity than ordinary carbon black. CNT distribute throughout the body via the circulatory and lymphatic systems in mice; therefore, their toxicity is not limited to the site of administration. It is possible that CNT have toxic effects in several organ systems. Moreover, it has been confirmed that CNT pass through the blood-brain barrier into the central nervous system in mice, and neuronal apoptosis due to peroxide-induced inflammation and oxidative stress in stimulated neurons and glial cells has been observed.
This chapter is focus on the application of carbon nanotubes in diagnosis and therapy that are under preclinical and clinical trials and the new possibilities to use them on the diagnostics and prognosis of cancer patients. We also discuss the possible challenges that have to be resolved before the establishment of used nanomedicine in cancer.

Carbon nanotubes characteristics

Nanotubes have the simplest chemical composition and atomic bonding configuration but exhibit perhaps the most extreme diversity and richness among nanomaterials in structures and structure-property relations. Carbon nanotubes (CNT) consist of graphene sheets rolled up into a cylindrical shape with a high aspect ratio and a diameter in the nano-scale range. CNT are classified by their structure into two main types: single-walled carbon nano-tubes (SWNT) and multiwalled carbon nanotubes (MWNT) [2].
The SWNTs are characterized by strong covalent bonding, a unique one dimensional structure, and nanometer size of 0.4–2 nm; which impart unusual properties to the nanotubes including exceptionally high tensile strength, high resilience, electronic properties ranging from metallic to semiconducting, high current carrying capacity, and high thermal conductivity [2]. MWNT comprise multiple layers of concentric cylinders with the space from 2–100 nm (Meziani and Sun 2003). Nonetheless, MWNTs exhibit advantages over SWNTs, such as ease of mass production, low product cost per unit, and enhanced thermal and chemical stability. In general, the electrical and mechanical properties of SWNTs can change when functionalized, due to the structural defects occurred by C=C bond breakages during chemical processes. However, intrinsic properties of carbon nanotubes can be preserved by the surface modification of MWNTs, where the outer wall of MWNTs is exposed to chemical modifiers.
CNT can be differentiated in two zones: the tips and the sidewalls. The tips are reminiscent of the structure of a fullerene hemisphere and are relatively reactive [3]. The sidewalls can be approximately considered as curved graphite, the degree of curvature, of course, depending on the diameter of the tube [2]. The length of MWCNTs varies greatly depending on their application. The concentric nanotube layers are held together by secondary Van der Waals forces. The walls of each layer of MWCNTs lie parallel to their central axis [4] CNTs are available in diverse structural designs and various electron arrangements; due to these structural differences, SWCNT and MWCNT tend to exhibit different physical properties.

Functionalization

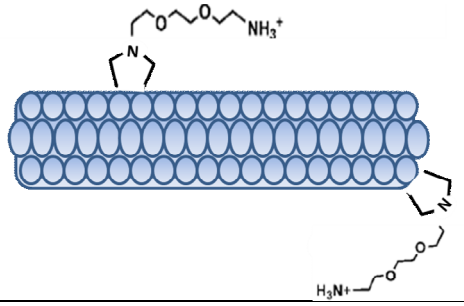
Functionalized CNT are highly promising as novel delivery systems especially based on their ability to cross biological barriers independently of the cell type. Generally, the functionalization require organic solvent or water-solubilization, enhancement of functionality, dispersion and compatibility or lowering the toxicity of CNT also, the process involves functional groups to carry simultaneously several moieties for targeting, imaging, and therapy.

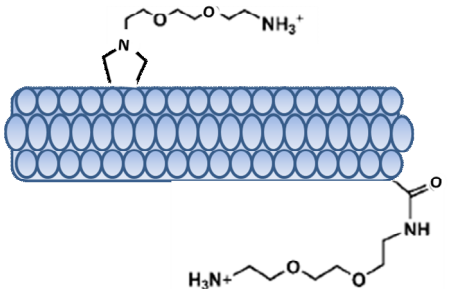
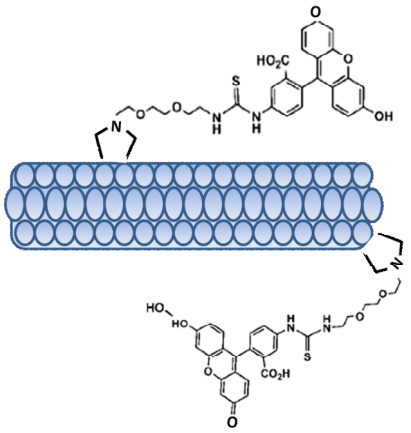
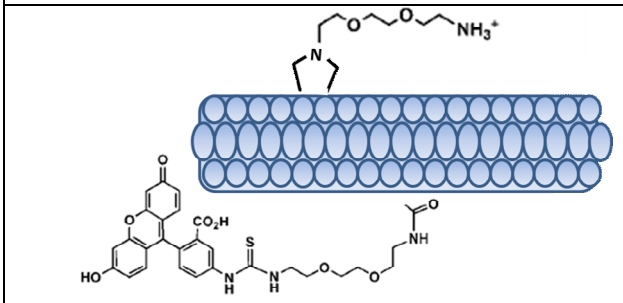
The attachment may be achieved via covalent or non-covalent bonding. Non-covalent functionalization has the advantage that it results in the preservation of the electronic structure of the nanotube atomic and it not cause noticeable toxicity when animals were treated [5, 6]. Poly ethylene glycol (PEG) is the most adopted species for functionalization, which increases the dispersity in aqueous solution and biocompatibility of CNTs. Adding PEG also allows for modification of the CNT with different functional groups such as terminal amine (PEG-NH2) and carboxyl (PEGCOOH) groups that offer further new functionalization sites for biomolecules [7, 8].

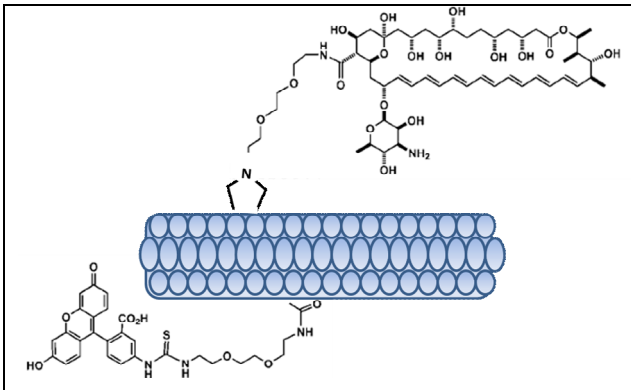
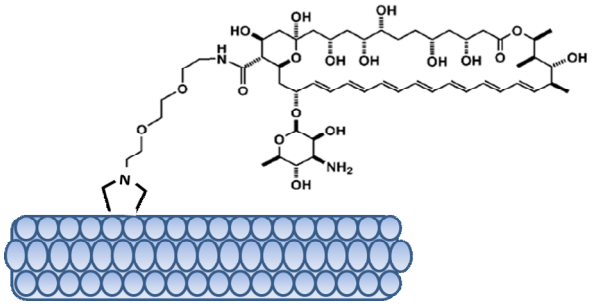
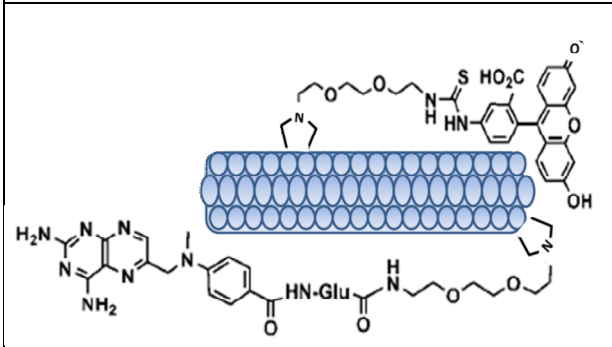
Functionalized SWNTs are attracting increasing attention as new vectors for the delivery of therapeutic molecules. Oxidized SWNTs can be functionalized at their carboxylic groups with proteins [9] peptide [10], nucleic acid [11], oligonucleotide [12], sugar moieties [13] and poly oxide derivatives [14].

Some studies show that ammonium-functionalized CNTs (f-CNTs) are able to associate with plasmid DNA through electrostatic interactions. Upon interaction with mammalian cells, these f-CNTs penetrate the cell membranes and are taken up into the cells. The nanotubes exhibit low cytotoxicity and f-CNT-associated plasmid DNA is delivered to cells efficiently; gene expression levels up to 10 times higher than those achieved with DNA alone were observed [10]. Although some of them are under *in vivo* and *in vitro* investigations, it could be a standard nano-treatment.

Carbon nanotubes were covalently modified by using a method based on the 1,3-dipolar cycloaddition of azomethine ylides. Both single-walled and multi-walled carbon nanotubes (SWNTs and MWNTs) were functionalized with a pyrrolidine ring bearing a free amino-terminal oligoethylene glycol moiety attached to the nitrogen atom. This modifications allow that the CNT are capable of traversing the plasma membrane and promoting the cellular uptake of small molecules and macromolecules (e.g. nucleic acids and peptides) [15].

Compounds	Bioassays
	Cell internalization [68-70] Intracelular trafficking([68-70] Cell viability [68] Plasmid DNA delivery [70, 71]

	Precursor for the praparation of CNT (4) and (5)
	Cell internalization [15, 69, 72] Intracellullar trafficking [15, 69, 72] Cell viability [15, 69, 72]
	Cell internalization [69].

	Cell internalization [69, 73] Cell viability [73]
	Antibiotic delivery [73]
	Cell internalization [74] Cell viability [74] Anticancer delivery [74]

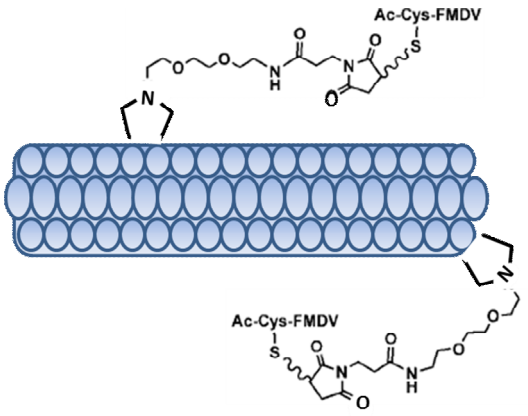
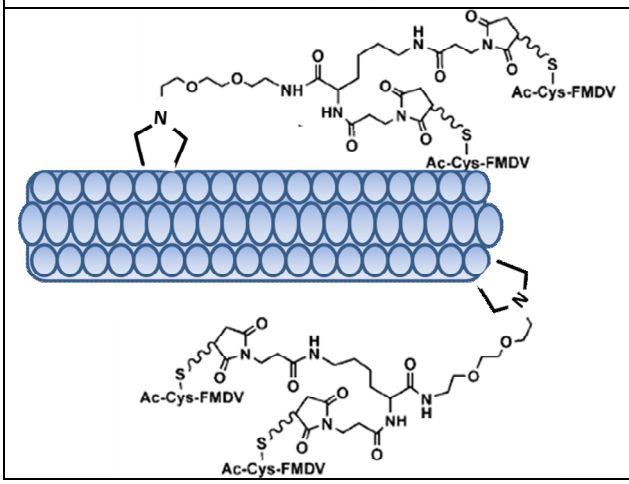
	Immunogenic activity [75, 76]
	Immunogenic activity[76]

FIGURE 5.1
Molecular structures of the carbon nanotubes conjugated with different therapeutic agents. The different components that can be used during the synthesis and the diverse biological assays according to their structural properties are show. The table was taken and modified by [67].

Other modification to functionalized CNT has been developed, such as novel *in situ* atom transfer radical polymerization (ATRP) to functionalize multiwalled MWNT. The living ATRP approach displays at least three merits: (1) both styrene and acrylate/acrylamide monomers can be directly used as the raw materials; (2) the initiating sites in the reaction system remain constant after the graft polymerization and purification of the products, so it is convenient to further perform block copolymerization and chain extension; and (3) it presents an access to prepare the polymers with a functional group in each repeating unit such as poly-(hydroxyethyl methacrylate)[16]

Carbon nanotubes on diagnosis

In the imaging field, the development of nanoparticles as contrast agents has enabled detailed cellular and molecular imaging, monitoring drug delivery specifically to tumoral areas to be carried out, and providing data for efficient surgical removal of solid tumors [17]. CNTs as emerging drug and imaging carrier systems show significant versatility. One of the extraordinary characteristics of CNTs as Magnetic Resonance Imaging (MRI) contrasting agent is the extremely large proton relaxivities when loaded with gadolinium ion (Gdn3+) clusters. MRI is a widely accepted modality for providing anatomical information and high spatial-resolution anatomic images primarily based on contrast derived from the tissue-relaxation parameters T(1)- and T(2)∗-weighted sequences[18]. Sitharaman et al. developed the first CNT-based contrast agent. They demonstrated that Gd@Ultra-short single-walled carbon nanotubes (gadonanotubes) drastically increase MRI efficacy compared to the traditional CAs [19]. However, the most challenging part of using CNTs in biological system is lack of solubility and hence its toxicity. Even though oxidation of CNTs improve their dispersibility, but it`s still not enough to call them as a suitable carriers. To overcome these disadvantages the gadonanotubes have been modified on their surface; addition of polyethylene glycol to this complex could improve its solubility, stability and more over MRI contrasting ability [20].

Clinically their main application is for imaging cancer cells,[21] they can be used for localization of cancer cells due to their nano size and ability to penetrate individual cancer cells and high-resolution imaging derived from their narrow emission bands compared with organic dyes.

Immunosensors

Immunosensors are a novel amplification strategy for SWNT and applications to the detection of a cancer biomarker in real biomedical samples. SWNT immunosensors can be adapted easily for the detection of other relevant biomarkers and have the potential for fabrication into arrays to facilitate multiplexed detection with very high sensitivity and selectivity [22].
Xin Yu et col. in 2006 reported the combination of electrochemical immunosensors using SWNT forest platforms with multi-label secondary antibody-nanotube bioconjugates for highly sensitive detection of a cancer biomarker in serum and tissue lysates. They used bioconjugates featuring horseradish peroxidase (HRP) labels and secondary antibodies (Ab2) linked to CNT at high HRP/Ab2 ratio. SWNT immunosensors were capable of sensitive quantitative measurement and it has an excellent correlation results obtained in two different ways for prostate specific antigen (PSA) in human serum samples with standard ELISA results[22].

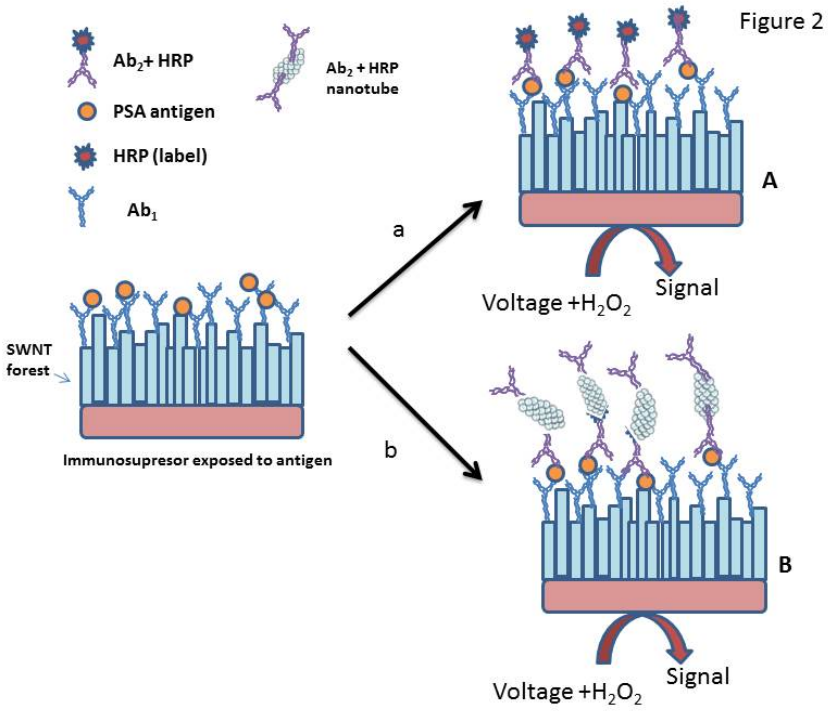


FIGURE 5.2
The components of Immunosensors. The SWNT forest serves as the immunosensor platform that it has been equilibrated with an antigen along with the biomaterials used for fabrication (HRP is the enzyme label). Primary antibody on the SWNT sensor binds antigen in the sample, which in turn, binds a peroxidase-labeled antibody (Scheme 1A), shows the imunosensor after treating with a conventional HRP-Ab2 providing one label per binding event, while section B shows the immunosensor after treating with HRP-CNT-Ab2 to obtain amplification by providing numerous enzyme labels per binding event. The final detection involves immersing the immunosensor after secondary antibody attachment into a buffer containing mediator in an electrochemical cell, applying voltage, and injecting a small amount of hydrogen peroxide. Amperometric signals are developed by adding small amounts of hydrogen peroxide to a solution bathing the sensor to activate the peroxidase electrochemical cycle, and measuring the current for catalytic peroxide reduction while the sensor is under a constant voltage. Taken and modified by [22].

Carbon nanotubes acopled to quantum dots

CNTs offer the potential for drug delivery and thermal treatment of tumors whilst QDs offer the potential for tumor imaging. QDs are semiconductor nanocrystals constituted by inorganic nanomaterials in range from 1–10 nm. They contain elements found in groups II–IV (eg, CdSe, CdTe, CdS, and ZnSe) or III–V (eg, InP and InAs) of the periodic table [23]. They have fluorescent properties which offer superior features to conventional organic dyes including high quantum yield,[24] broad absorption, narrow emission spectra, photostability of coated QDs against photobleaching and tolerance to changes in the pH of biological electrolytes [25].
QDs consist of an inorganic core, an inorganic shell and aqueous organic coating. The size of the inorganic core determines the wavelength (color) of light emitted following excitation. An inorganic core consisting of group III–V elements is preferable for clinical work in comparison to group II–IV

elements. This is mainly due to the higher stability and lower toxicity of the group III–V elements, the stability of these is known to be due to the presence of covalent rather than ionic bonding [26], but one of the main disadvantages of group III–V is its low quantum yield in comparison to group II–VI [27].
These nanomaterial properties offer the opportunity for QDs to be engineered allowing particle size, shape, and chemical composition to be used simultaneously in diagnosis and treatment of cancer. Two properties that are often manipulated are the size and composition of QDs; this will determine whether the QD is chemically excited in ultraviolet (UV) or NIR light. For example, nanocrystals of 2-nm size, comprising CdSe, emit light in the range 495–515 nm, whereas larger CdSe nanocrystals of 5-nm size emit light in the range 605–630 nm. The inorganic shell is responsible for increasing the photostability and luminescent properties of the QDs and the aqueous organic coating is used for conjugation of biomolecules to the QD surface [28] The photo stability of the inorganic shell has allowed QDs to be used as probes for imaging cells and tissues over long time periods.

Figure 3

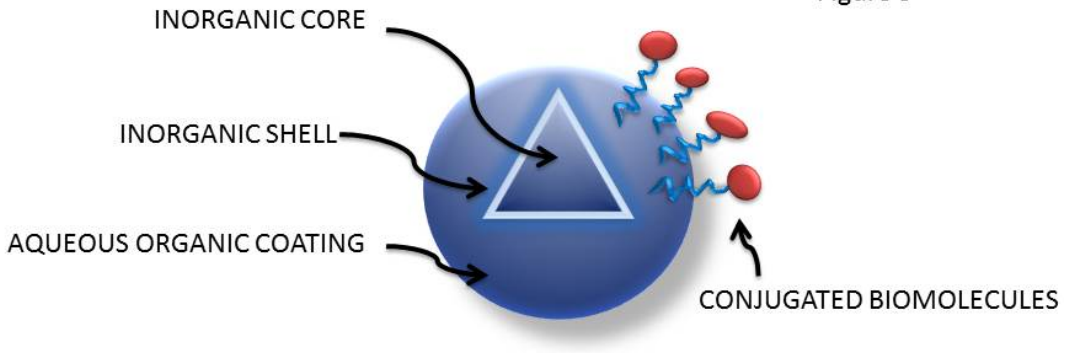


FIGURE 5.3.
Schematic diagram of quantum dots (QD) structure, QD consist in an inorganic core, and inorganic shell and aqueous organic coating.

Bimolecular coatings such as the attachment of antibodies enable the delivery of QDs to a specific organ or another site of action. The choice of antibody is important as antibody size may increase the overall size of the quantum dot to between 5–30 nm [29]

QDs have been shown to accumulate at disease sites and appear as bright and easily detected stains after illumination, which allows the location of diseased tissue to be identified [26, 30].
CNTs can destroy cancer cells via thermal ablation and functions as a tool for drug-delivery platforms [31]. Labeling CNTs with fluorescence materials such as QDs enables researchers to track the movement of CNTs [32].
The QDs may be linked to the CNT surface by either direct attachment or an intermediate molecule such as a polymer that has previously been conjugated to either the CNT or the QD. In covalent bonding, a linker between the functional group of the CNTs and QDs is needed [33]. Pre-functionalization of the surface of the CNTs with a polymer-wrapping technique may prove helpful. In this approach, the QDs form bonds with a polymer that coats the CNT side wall. Such a method may also prevent the CNT side wall from invasive damage and defects and also ensures stability of the QDs on the CNT.

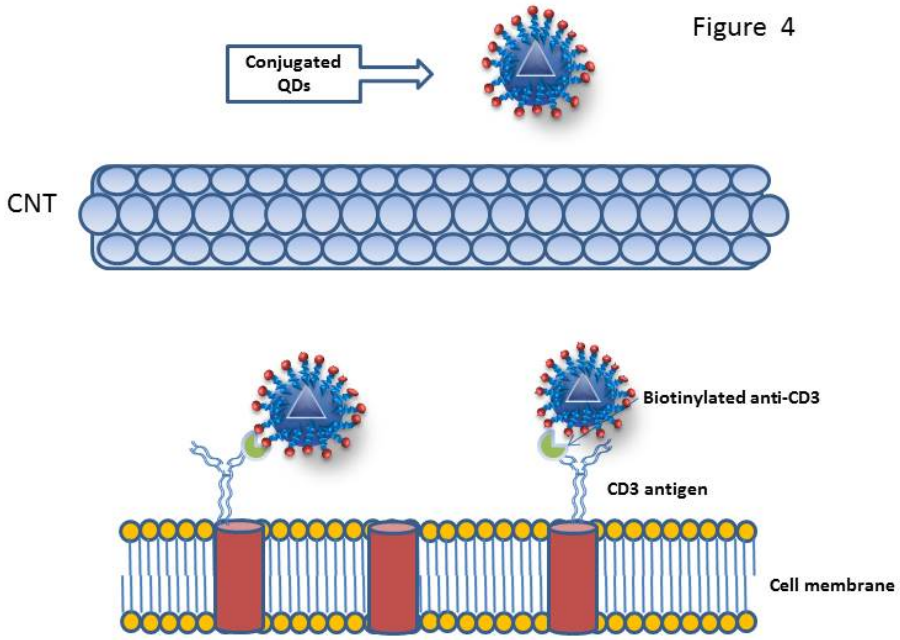


FIGURE 5.4
CNTs coupled with QDs. Bimolecular coatings such as the attachment of antibodies enable the delivery of QDs to a specific organ or another site of action. The choice of antibody is important as antibody size may increase the overall size of the quantum dot to between 5–30 nm. The image shows an example of the interaction between CD3 receptors on the Jurkat T leukemia cell membranes and a CNT-QD nanoassembly. A biotinylated anti-CD3 monoclonal antibody was used to link CD3 to the nanoassembly Taken and modified by[32].

The QD-CNT complex has applications in biomedical sciences, they been used in the optoelectronic and biosensor fields. These complexes due to the electrochemical luminescent properties have one of the major applications on intracellular fluorescent imaging. Furthermore, QD-CNT complexes can also function as biosensors and biological nanoprobes as well as tools for drug delivery into cells [34]. Combining QDs to CNTs may enable the CNT to be located to particular cell types and has been shown not to be a barrier to penetration into inaccessible tumor sites. By attaching different QDs to CNTs containing different drugs, the delivery of drugs to cancer cells could be monitored, which allows the efficacy of treatments to be evaluated [32, 35].

Drugs released

To improve the target of the drug-loaded CNTs to the site of action, the surface of these materials need to be modified, preferably with an antibody or a peptide.
Studies about biodistribution of the lipid-polymer polyethylene glycol (PL-PEG) functionalized CNT showed that CNT is safe because it can be excreted via the biliary and renal pathways after intravenous injection and it does not cause noticeable toxicity in the treated animals [39]. More importantly, a high tumor accumulation of PL-PEG functionalized SWNT could be achieved by conjugation of targeting ligands to SWNT. In recent years, SWNT have been encapsulating such as drug-loaded CNT into artificial cells for targeted delivery [40]. The polymeric membrane of artificial cells could prevent drug degradation from the harsh gastrointestinal environment in the route of oral delivery, and the surface

of artificial cells could be engineered for the targeted delivery. Also it has been incorporated pro-angiogenic genes functionalized CNT into stents for efficient gene therapy [41].
On the other hand, since the number of folic acid receptors on the surface of the cancer cells increases, the presence of folic acid on CNT allows the tubes to be used to target cancer cells and enhance drug delivery [35]. CNTs loaded with the anticancer drugs are injected into the circulation so that the antibody on the surface of the CNTs would direct these materials to the site of action. The drugs contained within the CNT are delivered to the cells depending on stimulation factors such as a change in the pH or by an enzyme produced by the tumor that may cleave the drug molecule and release them from the nanotube [32].
Wu et al. research about to delivery an anticancer drug, 10- hydroxyl camptothecin (HCPT), by covalent attachment on the outer surface of the MWCNT. Carbon nanotubes coated with HCPT and amino group were functionalized by carboxylic group. This enhances the cell uptake of MWCNTs-HCPT and increased blood circulation with high drug accumulation to the tumor [42]. Liu et al. had conjugated paclitaxel (PTV) to branched polyethylene glycol chain on SWNTs. SWNTs-PTV conjugate exhibited higher drug accumulation, higher bioavailability, and little toxicity. Murine 4T1 breast cancer model shows suppression in tumor growth, enhanced permeation and retention. SWNTs-PTV delivery is the promising treatment for cancer therapy in the future, with higher efficacy and minimum cytotoxic effect [43].
Carbon nanotubes are capable of penetrating the cell membrane and are widely considered as potential carriers for gene or drug delivery. Since the C-C and C=C bonds in carbon nanotubes are nonpolar, functionalization is required for carbon nanotubes to interact with genes or drugs as well as to improve their biocompatibility. Huang y col. (2013) produced polyethylenimine (PEI)-functionalized single-wall (PEI-NH-SWNTs) and multiwall carbon nanotubes (PEI-NH-MWNTs) to be used as nonviral gene delivery reagents. This complex has modified such as carriers for nonviral gene delivery, as opposed to viral transfection which applies viral vectors to achieve high transfection efficiency, carbon nanotubes are often functionalized with cationic molecules or polymers in order to interact electrostatically with negatively charged siRNAs or plasmid. The chemically modified with amino groups were capable of delivering plasmid DNAs into A549, HeLa, and CHO cell lines and induced cell deaths in a dose-dependent manner but were less cytotoxic compared to pure polyethylenimine (PEI)-functionalized single-wall[44].
Recently, it has been developed conjugates bounded to small interfering RNA (SiRNA) was targeting towards breast cancer. The SWNTs facilitate the coupling of siRNA specifically targeting Murine Double Minute Clone 2 (MDM2) to form MDM2 siRNA–f-SWNTs complexes and the efficiency of siRNA carried by f-SWNTs. The results showed an increase in the uptake of SWNTs-SiRNA into the breast carcinoma Bcap-37. The siRNA-MDM2 released silence MDM2 gene, which inhibited the functions of p53, and resulted in inhibiting cell proliferation and promoting apoptosis. This novel strategy of chemical functionalization is effective carrier system and is a very advanced or significant therapy for breast cancer in the future [45].

Thermal treatment

Hyperthermia is a therapeutic procedure used to raise the temperature of a region of the body that was affected by cancer. It is administered together with other cancer treatment modalities. A synergistic interaction between heat and radiation dose as well as CNT treatments has been validated in preclinical studies. The ability of CNTs to convert near-infrared (NIR) light into heat provides an opportunity to create a new generation of immunoconjugates for cancer photo-therapy with high

performance and efficacy [46]. Hyperthermia also preferentially increases the permeability of tumor vasculature compared with normal vasculature, which can enhance the delivery of drugs into tumors. Therefore, the thermal effects generated by targeted CNTs may have important advantages.

The ability of CNTs to NIR light into heat provides an opportunity to create a new generation of immunoconjugates for cancer photo-therapy with high performance and efficacy. The use of NIR light in the 700- to 1,100-nm range for the induction of hyperthermia is particularly attractive because living tissues do not strongly absorb in this range [47]. Hence, an external NIR light source should effectively and safely penetrate normal tissue and ablate any cells to which the CNTs are attached. The generation of targeting moieties consisting of mAb-NAs attached to dispersed biotinylated CNTs. The use of biotinylated CNTs (B-CNTs) and mAb-NAs gives the flexibility to ''assemble'' the targeted CNTs by using any cell binding mAb. The one-step strategy of generating dispersed CNTs by using biotinylated polar lipids has the advantage of preventing subsequent chemical treatments that remove the polar lipids and/or destroy their optical properties. Previously studies have demonstrated that folic acid-coated CNTs could be targeted to folate receptor (FR)-positive cells and that NIR light killed the cells [48]. These CNTs were also evaluated for *in vivo* biodistribution [49], but control peptide-CNTs were not used to demonstrate specificity. Another approach for targeting CNTs to cells is to non-covalently attach monoclonal antibodies (mAbs) that can be used in photothermal therapy or imaging [50]. However, attachment of mAbs by direct adsorption on CNTs involves a potential loss of the targeting function of the mAbs [50].

CNTs are well-ordered, all carbon, hollow graphitic nanomaterials with a high aspect ratio, high surface area and ultralight weight, in addition they contain unique physical and chemical properties [51, 52] CNTs also absorb near-infrared (NIR) light, generating heat. These unique properties facilitate the use of CNTs in drug delivery and thermal treatment of cancer [53].

Antibodies conjugated to nanoparticles

Tumor-specific targeting using nanotechnology is a mainstay of increasing efficacy of antitumor drugs. One of the most significant advances in tumor targeted therapy is the surface modification of nanoparticles with monoclonal antibodies (mAbs) alone or in combination with antineoplastic drugs in cancer therapy [54]. Another important advantage of this technology is the possibility of masking the unfavorable physicochemical characteristics of the incorporated molecule. In particular, the treatment of brain tumors takes advantage of these characteristics due to efficient and specific brain delivery of the anticancer drugs [55].These different strategies can be exploited for a variety of biomedical applications such as cancer immunotherapy that manipulate the immune system for therapeutic benefits and minimize adverse effects[56].

Single-walled carbon nanotubes attached to antibodies or peptides represent another approach to targeting cancer cells. Previously studies demonstrated that neutravidin (NA)-conjugated MAbs attached to a biotinylated (B) polymer that non-covalently coated to CNTs can specifically target and kill cells *in vitro* [57]. Subsequently the MAbs were stably attached to the CNTs modifications added to mouse IgG1 anti-human CD22. The tested on human Burkitt's lymphoma cell line shown that the conjugates bound specifically to target cells and the binding remained specific even after the MAb-CNTs were incubated in mouse serum. Both results showed not significant differences in the selectivity

and killing efficiencies between non-covalently and covalently conjugates, using identical targeting MAbs, MAb-CNTs of similar dimensions[58].

Immune response

As nanovectors, CNTs have the advantage of providing a versatile, biodegradable, and nonimmunogenic delivery alternative to viral vectors for molecular therapy or immunotherapy as direct delivery of antigens to antigen presenting cells (APCs) or microglia in the central nervous system [59]. Kateb et al. evaluated the efficacy of multiwalled carbon nanotubes (MWCNTs) as potential nanovectors for delivery of macromolecules into microglia (MG) using the cell line BV2 (a microglia cell line) to determine the capacity to uptake MWCNTs by BV2 cells *in vitro*, demonstrating the ability of BV2 cells to more efficiently internalize MWCNTs as compared to glioma cells without any significant signs of cytotoxicity. They were able to visualize ingestion of MWCNTs into MG, cytotoxicity, and loading capacity of MWCNTs under normal culture conditions, suggesting that MWCNTs could be used as a novel, nontoxic, and biodegradable nanovehicles for targeted therapy in brain tumors. On the other hand, this group also analyzed the internalization of these nanotubes in an intracranial glioma model and characterized some changes in tumor cytokine production following intratumoral injection of MWCNTs in GL261 murine glioma model. Authors demonstrated that MWCNTs were preferentially detected in tumor macrophages (MPs), and to a lesser extent in MG. In addition to MG and MP, a small fraction of glioma cells, which are not typically capable of phagocytosis, also became positive for MWCNTs; FACS and quantitative RT-PCR were performed to analyze the inflammatory response and cytokine profile. A transient influx of MP was seen in both normal brain and GL261 gliomas in response to MWCNTs; whereas no significant change in cytokine expression was noted in normal group [60].They concluded that CNTs can potentially be used as a nanovector delivery system to modulate MP function in tumors.

Toxicity

Toxicity of CNTs has been evaluated in a variety of cell or animal models. The CNT attributes contribute the most to pulmonary toxicity according to metallic impurities, aggregate size and both CNT length and diameter. Some studies have evaluated toxicity induced by CNT. Subcutaneous injection of the nanotubes induced paw edema; also elicited hyperalgesic response, seen by the increase of animal paw withdrawal. The oxidized multiwalled carbon nanotubes elicit inflammatory and hyperalgesic effects associated to severe tissue damage in rats [61]. In other hand, CNT are capable to induce inflammatory fibrosis in the peritoneal cavity, as the same manner to long asbestos fibers. Besides the accumulation of CNT induce macrophages attempt to phagocytosis which can result in frustrated phagocytosis and stimulated recruitment of inflammatory cells and mesothelial cell damage, leading to chronic inflammation and granuloma development [62, 63]

Studies have shown that CNT have many toxic effects, including decreased cell viability, induction of apoptosis, disruption of the cell cycle, generation of oxidative stress, inflammatory responses, also PEG-SWCNTs may cause occasional teratogenic effects in mice beyond a threshold dose[64]. Although CNT has shown a promissory field in the area or drug delivery, and imaging, some aspect need

improved to be possible applied clinically.

Conclusions

Studies on the biological composition, administrations and adverse events of new nanomaterials suited for biomedical applications, are important for therapeutic drug delivery and the development of innovative and better treatments [65]. Furthermore, the engineering of the particle backbone structure, size, shape of the nanoparticle surface and the core itself provides yet another dimension of physical control that can be directed toward an increased strength, increased chemical specificity or heat resistance. Most polymeric nanoparticles are biodegradable and biocompatible, and have been adopted as a preferred method for drug delivery. Since nanoparticles come into direct contact with cellular membranes, their surface properties may determine the mechanism of internalization and intracellular localization [66]. They also exhibit a good potential for surface modification via chemical transformations, provide excellent pharmacokinetic control, and are suitable for the entrapment and delivery of a wide range of therapeutic agents.

The use of nanoparticles could be a good option in diagnosis and treatment of gliomas. Studies suggest that a variety of NP's can be engineered to become part of the next generation of agents delivery and specific treatment on gliomas. The use of a biocompatible system of NP's conjugates should highly reduce the toxicity and side effects of systemic drugs administration, and therefore improve the quality of life in cancer patients. However, several studies conducted largely in mice; have shown undesired side effects such as inflammatory response including substantial lung neutrophil influx and mortality at high doses. In addition, NP's may feasibly represent a useful imaging tool to diagnosis and follow-up; also, it to be used to assess/monitor efficacy of anti-angiogenic or other anti-tumour treatments, and thus improving the clinical management of brain tumours. Nevertheless, additional research is required in multifunctional NP's based drug delivery systems to overcome the problems and understand how nanoparticles interact with biological systems and the environment for effective therapy.

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