WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 2, 906-926. Research Article ISSN 2277– 7105

CARBON NANOTUBE: A PROMISING DRUG CARRIER IN CANCER THERAPY

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Article Received on
21 Nov 2014,

Revised on 16 Dec 2014,
Accepted on 10 Jan 2015

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ABSTRACT

Carbon nanotubes (CNTs) are allotropes of carbon, made of graphite and constructed in cylindrical tubes with nanometer in diameter and several millimeters in length. Their impressive structural, mechanical, and electronic properties are due to their small size and mass, their strong mechanical potency, and their high electrical and thermal conductivity. CNTs have been successfully applied in pharmacy and medicine due to their high surface area that is capable of adsorbing or conjugating with a wide variety of therapeutic and diagnostic agents (drugs, genes, vaccines, antibodies, biosensors, etc.). They have been first proven to be an excellent vehicle for drug delivery directly into cells without metabolism by the body. Then other applications of CNTs have been extensively performed not only for drug and gene therapies but also for tissue regeneration, biosensor diagnosis, enantiomer separation of chiral drugs, extraction and analysis of drugs and pollutants. Moreover, CNTs have been recently revealed as a promising antioxidant. This minireview focuses the applications of CNTs in all fields of pharmacy and medicine from therapeutics to analysis and diagnosis as cited above. It also examines the pharmacokinetics, metabolism and toxicity of different forms of CNTs and discusses the perspectives, the advantages and the obstacles of this promising bionanotechnology in the future.

KEYWORDS: Carbon nanotube, Enantiomers, Bionanotecnology.

1. INTRODUCTION[1-3]

Carbon nanotubes (CNTs), discovered by Japanese scientist Iijima in 1991, are now considered to be a top class subject in academic researches as well as in various industrial areas. These nanomaterials are allotropes of carbon, made of graphite, and have been constructed in cylindrical tubes with nanometer scale in diameter and several millimeters in length. Their impressive structural, mechanical, and electronic properties are due to their small size and mass, their incredible mechanical strength, and their high electrical and thermal conductivity. Carbon nanotubes have been first used as additives to various structural materials for electronics, optics, plastics, and other materials of nanotechnology fields. Since the beginning of the 21st century, they have been introduced in pharmacy and medicine for drug delivery system in therapeutics. Thanks to their high surface area, excellent chemical stability, and rich electronic polyaromatic structure, CNTs are able to adsorb or conjugate with a wide variety of therapeutic molecules (drugs, proteins, antibodies, DNA, enzymes, etc.). They have been proven to be an excellent vehicle for drug delivery by penetrating into the cells directly and keeping the drug intact without metabolism during transport in the body. Many studies have demonstrated that when bonded to CNTs, these molecules are delivered more effectively and safely into cells than by traditional methods. This fantastic discovery has opened a new way for drug preparations that is completely different with traditional techniques used in pharmaceutical industry before and radically changed anterior concepts of pharmacology. It has been first applied to bind antineoplastic and antibiotic drugs to carbon nanotubes for cancer and infection treatments, respectively. Then, other linkages of biomolecules (genes, proteins, DNA, antibodies, vaccines, biosensors, cells, etc.) to CNTs havee been also assayed for gene therapy, immunotherapy, tissue regeneration and diagnosis of different ailments. Therefore, in a very short time, CNTs have become the focus of attention by scientists in a wide variety of disciplines. They may be promising antioxidants for health protective effect and ailment prevention in the future . However, it is notified that all these medicinal findings are in an experimental stage and are still not applied in men. Besides these main applications of CNTs, they have been shown as a powerful tool for enantiomer separation of chiral drugs and chemicals in pharmaceutical industry as well as in laboratory and for extraction of drugs and pollutants in different media by solid phase extraction before analysis. Our group has recently contributed to the development of different novel functionalized CNT techniques for drug delivery as well as for drug analysis and also to the study on the interactions between CNTs and albumin or drugs.

In this paper, an overview of different applications of CNTs is focused in the field of pharmacy and medicine. It briefly describes the structure of CNTs and their different types. It examines some main methodologies using CNTs as vehicle for drug and biomolecule delivery in the treatment and diagnosis of different dreadful diseases. It enumerates some recent researches of CNTs as antioxidants and also some novel analytical techniques using CNTs as tools for enantioseparation of chiral drugs and for solid phase extraction of drugs and pollutants in different media. Pharmacokinetics, metabolism, and toxicity of CNTs are also examined. The perspectives of this promising bionanotechnology in the future medicine are briefly commented, in the conclusion.

2. Carbon Nanotube's Properties and Types[4-6]

CNTs have several unique chemical, size, optical, electrical and structural properties that make them attractive as drug delivery and biosensing platforms for the treatment of various diseases[5] and the noninvasive monitoring of blood levels and other chemical properties of the human body, respectively.

2.1 Properties

2.1.1 Electrical and Structural

Carbon nanotubes can be metallic or semiconducting depending on their structure. This is due to the symmetry and unique electronic structure of graphene. For a given (n,m) nanotube, if n = m, the nanotube is metallic; if n − m is a multiple of 3, then the nanotube is semiconducting with a very small band gap, otherwise the nanotube is a moderate semiconductor. Thus all armchair (n=m) nanotubes are metallic, and nanotubes (5,0), (6,4), (9,1), etc. are semiconducting. Thus, some nanotubes have conductivities higher than that of copper, while others behave more like silicon.

2.1.2 Dimensional

Due to their nanoscale dimensions, electron transport in carbon nanotubes will take place through quantum effects and will only propagate along the axis of the tube. These electrical and structural properties best serve CNTs as far as biosensing is concerned because current changes in the CNTs can signify specific biological entities they are designed to detect. The fact that CNTs are small (nm scale) allows them to deliver smaller doses of drugs to specific disease cells in the body thus reducing side effects and harm to healthy cells unlike conventional drugs, whilst improving disease cell targeting efficiency.

2.1.3 Chemical

CNTs have been observed to have enhanced solubility when functionalized with lipids which would make their movement through the human body easier and would also reduce the risk of blockage of vital body organ pathways. As far as optical properties are concerned CNTs have been shown to exhibit strong optical absorbance in certain spectral windows such as NIR (near-infrared) light and when functionalized with tumor cell specific binding entities have allowed the selective destruction of disease (e.g. cancer) cells with NIR in drug delivery applications.

2.2 Types

2.2.1 Single walled

Most single-walled nanotubes (SWNT) have a diameter of close to 1 nanometer, with a tube length that can be many millions of times longer. The structure of a SWNT can be conceptualized by wrapping a one-atom-thick layer of graphite called graphene into a seamless cylinder. 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 honeycombcrystal 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.

$$d = \frac{a}{\pi}\sqrt{(n^2 + nm + m^2)} = 78.3\sqrt{((n+m)^2 - nm)}\text{pm},$$

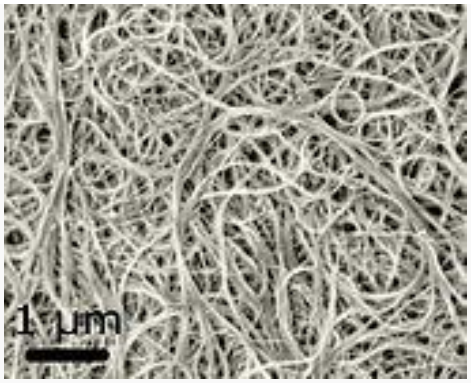
where a = 0.246 nm.

SWNTs are an important variety of carbon nanotube because most of their properties change significantly with the (n,m) values, and this dependence is non-monotonic (see Kataura plot). In particular, their band gap can vary from zero to about 2 eV and their electrical conductivity can show metallic or semiconducting behavior. Single-walled nanotubes are likely candidates for miniaturizing electronics. The most basic building block of these systems is the electric wire, and SWNTs with diameters of an order of a nanometer can be excellent conductors.[3,4] One useful application of SWNTs is in the development of the first intermolecular field-effect transistors (FET). The first intermolecular logic gate using SWCNT FETs was made in 2001. A logic gate requires both a p-FET and an n-FET. Because SWNTs are p-FETs when exposed to oxygen and n-FETs otherwise, it is possible to protect

half of an SWNT from oxygen exposure, while exposing the other half to oxygen. This results in a single SWNT that acts as a NOT logic gate with both p and n-type FETs within the same molecule.

Single-walled nanotubes are dropping precipitously in price, from around $1500 per gram as of 2000 to retail prices of around $50 per gram of as-produced 40–60% by weight SWNTs as of March 2010.

2.2.2 Multi-walled



Multi-walled nanotubes (MWNT) consist of multiple rolled layers (concentric tubes) of graphene. There are two models that can be used to describe the structures of multi-walled nanotubes. In the Ru Rotating model of multi-walled nanotubessian Doll model, sheets of graphite are arranged in concentric cylinders, e.g., a (0,8) single-walled nanotube (SWNT) within a larger (0,17) single-walled nanotube. In the Parchment model, a single sheet of graphite is rolled in around itself, resembling a scroll of parchment or a rolled newspaper. The interlayer distance in multi-walled nanotubes is close to the distance between graphene layers in graphite, approximately 3.4 Å. The Russian Doll structure is observed more commonly. Its individual shells can be described as SWNTs, which can be metallic or semiconducting. Because of statistical probability and restrictions on the relative diameters of the individual tubes, one of the shells, and thus the whole MWNT, is usually a zero-gap metal.

2.2.3 Double walled

Double-walled carbon nanotubes (DWNT) form a special class of nanotubes because their morphology and properties are similar to those of SWNT but their resistance to chemicals is significantly improved. This is especially important when functionalization is required (this means grafting of chemical functions at the surface of the nanotubes) to add new properties to

the CNT. In the case of SWNT, covalent functionalization will break some C=C double bonds, leaving "holes" in the structure on the nanotube and, thus, modifying both its mechanical and electrical properties. In the case of DWNT, only the outer wall is modified. DWNT synthesis on the gram-scale was first proposed in 2003 by the CCVD technique, from the selective reduction of oxide solutions in methane and hydrogen.

The telescopic motion ability of inner shells and their unique mechanical properties will permit the use of multi-walled nanotubes as main movable arms in coming nanomechanical devices. Retraction force that occurs to telescopic motion caused by the Lennard-Jones interaction between shells and its value is about 1.5 nN.

2.2.3 **Torus**

In theory, a nanotorus is a carbon nanotube bent into a torus (doughnut shape). Nanotori are predicted to have many unique properties, such as magnetic moments 1000 times larger than previously expected for certain specific radii. Properties such as magnetic moment, thermal stability, etc. vary widely depending on radius of the torus and radius of the tube.

2.2.4 **Nanobud**

Carbon nanobuds are a newly created material combining two previously discovered allotropes of carbon: carbon nanotubes and fullerenes. In this new material, fullerene-like "buds" are covalently bonded to the outer sidewalls of the underlying carbon nanotube. This hybrid material has useful properties of both fullerenes and carbon nanotubes. In particular, they have been found to be exceptionally good field emitters. In composite materials, the attached fullerene molecules may function as molecular anchors preventing slipping of the nanotubes, thus improving the composite's mechanical properties.

2.2.4 Graphenated carbon nanotubes (g-CNTs)

Graphenated CNTs are a relatively new hybrid that combines graphitic foliates grown along the sidewalls of multiwalled or bamboo style CNTs. Yu et al. reported on "chemically bonded graphene leaves" growing along the sidewalls of CNTs. Stoner et al. described these structures as "graphenated CNTs" and reported in their use for enhanced supercapacitor performance. Hsu et al. further reported on similar structures formed on carbon fiber paper, also for use in supercapacitor applications. The foliate density can vary as a function of deposition conditions (e.g. temperature and time) with their structure ranging from few layers of graphene (< 10) to thicker, more graphite-like.

The fundamental advantage of an integrated graphene-CNT structure is the high surface area three-dimensional framework of the CNTs coupled with the high edge density of graphene. Graphene edges provide significantly higher charge density and reactivity than the basal plane, but they are difficult to arrange in a three-dimensional, high volume-density geometry. CNTs are readily aligned in a high density geometry (i.e., a vertically aligned forest) but lack high charge density surfaces—the sidewalls of the CNTs are similar to the basal plane of graphene and exhibit low charge density except where edge defects exist. Depositing a high density of graphene foliates along the length of aligned CNTs can significantly increase the total charge capacity per unit of nominal area as compared to other carbon nanostructures.

2.2.5 Nitrogen Doped Carbon Nanotubes

Nitrogen doped carbon nanotubes (N-CNT's), can be produced through 5 main methods, Chemical Vapor Deposition, high-temperature and high-pressure reactions, gas-solid reaction of amorphous carbon with NH_3 at high temperature, solid reaction, and solvothermal synthesis.

N-CNTs can also be prepared by a CVD method of pyrolysizing melamine under Ar at elevated temperatures of 800^{o}C - 980^{o}C. However synthesis via CVD and melamine results in the formation of bamboo structured CNTs. XPS spectra of grown N-CNT's reveals nitrogen in five main components, pyridinic nitrogen, pyrrolic nitrogen, quaternary nitrogen, and nitrogen oxides. Furthermore synthesis temperature affects the type of nitrogen configuration. Nitrogen doping plays a pivotal role in Lithium storage. N-doping provides defects in the walls of CNT's allowing for Li ions to diffuse into interwall space. It also increases capacity by providing more favorable bind of N-doped sites. N-CNT's are also much more reactive

to metal oxide nanoparticle deposition which can further enhance storage capacity, especially in anode materials for Li-ion batteries. However Boron doped nanotubes have been shown to make batteries with triple capacity.

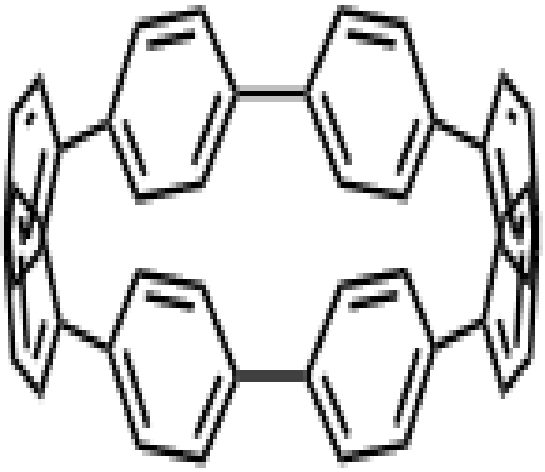
2.2.5 **Peapod**

A Carbon peapod is a novel hybrid carbon material which traps fullerene inside a carbon nanotube. It can possess interesting magnetic properties with heating and irradiating. It can also be applied as an oscillator during theoretical investigations and predictions.

2.2.6 **Cup-stacked carbon nanotubes**

Cup-stacked carbon nanotubes (CSCNTs) differ from other quasi-1D carbon structures, which normally behave as quasi-metallic conductors of electrons. CSCNTs exhibit semiconducting behaviors due to the stacking microstructure of graphene layers.

2.2.7 **Extreme carbon nanotubes**



The observation of the longest carbon nanotubes (18.5 cm long) was reported in 2009. These nanotubes were grown on Si substrates using an improved chemical vapor deposition (CVD) method and represent electrically uniform arrays of single-walled carbon nanotubes.

The shortest carbon nanotube is the organic compound cycloparaphenylene, which was synthesized in early 2009.

The thinnest carbon nanotube is armchair (2,2) CNT with a diameter of 3 Å. This nanotube was grown inside a multi-walled carbon nanotube. Assigning of carbon nanotube type was

done by combination of high-resolution transmission electron microscopy (HRTEM), Raman spectroscopy and density functional theory (DFT) calculations.

The thinnest freestanding single-walled carbon nanotube is about 4.3 Å in diameter. Researchers suggested that it can be either (5,1) or (4,2) SWCNT, but exact type of carbon nanotube remains questionable. (3,3), (4,3) and (5,1) carbon nanotubes (all about 4 Å in diameter) were unambiguously identified using aberration-corrected high-resolution transmission electron microscopy inside double-walled CNTs.

The highest density of CNTs was achieved in 2013, grown on a conductive titanium-coated copper surface that was coated with co-catalysts cobalt and molybdenum at lower than typical temperatures of 450 °C. The tubes averaged a height of 0.38 μm and a mass density of 1.6 g cm−3. The material showed ohmic conductivity (lowest resistance ~22 kΩ).

3. Method of Preparation[7-8]

powder of carbon nanotubes

Techniques have been developed to produce nanotubes in sizable quantities, including arc discharge, laser ablation, high-pressure carbon monoxide disproportionation, and chemical vapor deposition (cvd). most of these processes take place in vacuum or with process gases. cvd growth of cnts can occur in vacuum or at atmospheric pressure. large quantities of nanotubes can be synthesized by these methods; advances in catalysis and continuous growth are making cnts more commercially viable.

3.1 Arc discharge

Nanotubes were observed in the carbon soot of graphite electrodes during an arc discharge, by using a current of 100 amps, that was intended to produce fullerenes. During this process, the carbon contained in the negative electrode sublimates because of the high-discharge temperatures. Because nanotubes were initially discovered using this technique, it has been the most widely used method of nanotube synthesis.

The yield for this method is up to 30% by weight and it produces both single- and multi-walled nanotubes with lengths of up to 50 micrometers with few structural defects.

3.2 Laser ablation

In laser ablation, a pulsed laser vaporizes a graphite target in a high-temperature reactor while an inert gas is bled into the chamber. Nanotubes develop on the cooler surfaces of the reactor as the vaporized carbon condenses. A water-cooled surface may be included in the system to collect the nanotubes.

The laser ablation method yields around 70% and produces primarily single-walled carbon nanotubes with a controllable diameter determined by the reaction temperature. However, it is more expensive than either arc discharge or chemical vapor deposition.

3.3 Plasma torch

Single-walled carbon nanotubes can also be synthesized by a thermal plasma method. In this method, the aim is to reproduce the conditions prevailing in the arc discharge and laser ablation approaches, but a carbon-containing gas is used instead of graphite vapors to supply the carbon necessary for the production of SWNT. Doing so, the growth of SWNT is more efficient (decomposing a carbon containing gas can be 10 times less energy consuming than graphite vaporization). It is also continuous and occurs at low cost. To produce a continuous process, a gas mixture composed of argon, ethylene and ferrocene is introduced into a microwave plasma torch, where it is atomized by the atmospheric pressure plasma, which has the form of an intense 'flame'. The fumes created by the flame are found to contain SWNT, metallic and carbon nanoparticles and amorphous carbon.

Another way to produce single-walled carbon nanotubes with a plasma torch, is to use the induction thermal plasma method. The method is similar to arc-discharge in that both use ionized gas to reach the high temperature necessary to vaporize carbon-containing substances

and the metal catalysts necessary for the ensuing nanotube growth. The thermal plasma is induced by high frequency oscillating currents in a coil, and is maintained in flowing inert gas. Typically, a feedstock of carbon black and metal catalyst particles is fed into the plasma, and then cooled down to form single-walled carbon nanotubes. Different single-wall carbon nanotube diameter distributions can be synthesized. The induction thermal plasma method can produce up to 2 grams of nanotube material per minute, which is higher than the arc-discharge or the laser ablation methods.

3.4 Chemical Vapor Deposition (CVD)

Nanotubes being grown by plasma enhanced chemical vapor deposition the catalytic vapour phase deposition of carbon. During CVD, a substrate is prepared with a layer of metal catalyst particles, most commonly nickel, cobalt, iron, or a combination. The metal nanoparticles can also be produced by other ways, including reduction of oxides or oxides solid solutions. The diameters of the nanotubes that are to be grown are related to the size of the metal particles. This can be controlled by patterned (or masked) deposition of the metal, annealing, or by plasma etching of a metal layer. The substrate is heated to approximately 700°C. To initiate the growth of nanotubes, two gases are bled into the reactor: a process gas (such as ammonia, nitrogen or hydrogen) and a carbon-containing gas (such as acetylene, ethylene, ethanol or methane). Nanotubes grow at the sites of the metal catalyst; the carbon-containing gas is broken apart at the surface of the catalyst particle, and the carbon is transported to the edges of the particle, where it forms the nanotubes. This mechanism is still being studied. The catalyst particles can stay at the tips of the growing nanotube during growth, or remain at the nanotube base, depending on the adhesion between the catalyst

particle and the substrate. Thermal catalytic decomposition of hydrocarbon has become an active area of research and can be a promising route for the bulk production of CNTs. Fluidised bed reactor is the most widely used reactor for CNT preparation. Scale-up of the reactor is the major challenge.

CVD is a common method for the commercial production of carbon nanotubes. For this purpose, the metal nanoparticles are mixed with a catalyst support such as MgO or Al_2O_3 to increase the surface area for higher yield of the catalytic reaction of the carbon feedstock with the metal particles. One issue in this synthesis route is the removal of the catalyst support via an acid treatment, which sometimes could destroy the original structure of the carbon nanotubes. However, alternative catalyst supports that are soluble in water have proven effective for nanotube growth.

If a plasma is generated by the application of a strong electric field during growth (plasma-enhanced chemical vapor deposition), then the nanotube growth will follow the direction of the electric field. By adjusting the geometry of the reactor it is possible to synthesize vertically aligned carbon nanotubes (i.e., perpendicular to the substrate), a morphology that has been of interest to researchers interested in the electron emission from nanotubes. Without the plasma, the resulting nanotubes are often randomly oriented. Under certain reaction conditions, even in the absence of a plasma, closely spaced nanotubes will maintain a vertical growth direction resulting in a dense array of tubes resembling a carpet or forest.

3.5 Super-growth CVD

In this process, the activity and lifetime of the catalyst are enhanced by addition of water into the CVD reactor. Dense millimeter-tall nanotube "forests", aligned normal to the substrate, were produced.

The synthesis efficiency is about 100 times higher than for the laser ablation method. The time required to make SWNT forests of the height of 2.5 mm by this method was 10 minutes in 2004. Those SWNT forests can be easily separated from the catalyst, yielding clean SWNT material (purity >99.98%) without further purification. For comparison, the as-grown HiPco CNTs contain about 5–35% of metal impurities; it is therefore purified through dispersion and centrifugation that damages the nanotubes. Super-growth avoids this problem. Patterned highly organized single-walled nanotube structures were successfully fabricated using the super-growth technique.

The mass density of super-growth CNTs is about 0.037 g/cm^3. It is much lower than that of conventional CNT powders (~1.34 g/cm^3), probably because the latter contain metals andamorphous carbon.

The super-growth method is basically a variation of CVD. Therefore, it is possible to grow material containing SWNT, DWNTs and MWNTs, and to alter their ratios by tuning the growth conditions. Their ratios change by the thinness of the catalyst. Many MWNTs are included so that the diameter of the tube is wide.

The vertically aligned nanotube forests originate from a "zipping effect" when they are immersed in a solvent and dried. The zipping effect is caused by the surface tension of the solvent and the van der Waals forces between the carbon nanotubes. It aligns the nanotubes into a dense material, which can be formed in various shapes, such as sheets and bars, by applying weak compression during the process. Densification increases the Vickers hardness by about 70 times and density is 0.55 g/cm^3. The packed carbon nanotubes are more than 1 mm long and have a carbon purity of 99.9% or higher; they also retain the desirable alignment properties of the nanotubes forest.

3.6 Natural, incidental, and controlled flame environments

Fullerenes and carbon nanotubes are not necessarily products of high-tech laboratories; they are commonly formed in such mundane places as ordinary flames, produced by burning methane, ethylene, and benzene, and they have been found in soot from both indoor and outdoor air. However, these naturally occurring varieties can be highly irregular in size and quality because the environment in which they are produced is often highly uncontrolled. Thus, although they can be used in some applications, they can lack in the high degree of uniformity necessary to satisfy the many needs of both research and industry. Recent efforts have focused on producing more uniform carbon nanotubes in controlled flame environments. Such methods have promise for large-scale, low-cost nanotube synthesis based on theoretical models, though they must compete with rapidly developing large scale CVD production.

3.7 Removal of catalysts

Nanoscale metal catalysts are important ingredients for fixed- and fluidized-bed CVD synthesis of CNTs. They allow increasing the growth efficiency of CNTs and may give control over their structure and chirality. During synthesis, catalysts can convert

carbon precursors into tubular carbon structures but can also form encapsulating carbon overcoats. Together with metal oxide supports they may therefore attach to or become incorporated into the CNT product. The presence of metal impurities can be problematic for many applications. Especially catalyst metals likenickel, cobalt or yttrium may be of toxicological concern. While un-encapsulated catalyst metals may be readily removable by acid washing, encapsulated ones require oxidative treatment for opening their carbon shell. The effective removal of catalysts, especially of encapsulated ones, while preserving the CNT structure is a challenge and has been addressed in many studies. A new approach to break carbonaceaous catalyst encapsulations is based on rapid thermal annealing.

4. Application of carbon nanotube[9-13]

4.1. Carbon Nanotubes Used for Cancer Therapy

4.1.1. By Drug Delivery

CNTs can be used as drug carriers to treat tumors. The efficacy of anticancer drugs used alone is restrained not only by their systemic toxicity and narrow therapeutic window but also by drug resistance and limited cellular penetration. Because CNTs can easily across the cytoplasmic membrane and nuclear membrane, anticancer drug transported by this vehicle will be liberated in situ with intact concentration and consequently, its action in the tumor cell will be higher than that administered alone by traditional therapy. Thus, the development of efficient delivery systems with the ability to enhance cellular uptake of existing potent drugs is needed. The high aspect ratio of CNTs offers great advantages over the existing delivery vectors, because the high surface area provides multiple attachment sites for drugs.

Many anticancer drugs have been conjugated with functionalized CNTs and successfully tested in vitro and in vivo such as epirubicin, doxorubicin, cisplatin, methotrexate, quercetin, and paclitaxel.

For avoiding the harmful effect of anticancer drug on healthy organs and cells, our group has linked epirubicin with a magnetic CNTs complex obtained by fixing a layer of magnetite (Fe_3O_4) nanoparticles on the surface of the nanotubes with necklace-like type and on the tips of shortened MWCNTs. Other authors have used the epirubicin magnetic CNTs complex for lymphatic tumor targeting. Such a system can be guided by an externally placed magnet to target regional lymphatic nodes.

For the same previous reason, chemotherapeutic agents can be bound to a complex formed by CNT and antibody against antigen overexpressed on the cancerous cell surface. By the

attraction of antigen-antibody, the CNTs can be taken up by the tumor cell only before the anticancer drug is cleaved off CNTs; thus, targeting delivery is realized. A major obstacle to effective anticancer therapy is the multidrug resistance caused by enhanced efflux of anticancer drugs by the overexpressed p-glycoprotein, resulting in poor anticancer effect . Li and coworkers have shown that SWCNTs can be functionalized with p-glycoprotein antibodies and loaded with the anticancer agent doxorubicin. Compared with free doxorubicin, this formulation demonstrated higher cytotoxicity by 2.4-fold against K562R leukemia cells.

The in vivo administration of SWCNT paclitaxel conjugate in a murine breast cancer model has been observed with higher efficacy in suppressing tumor growth and less toxic effects to normal organs. The higher therapeutic efficacy and lower side effects could be attributed to prolonged blood circulation, higher tumor uptake, and slower release of drug from SWCNTs.

4.1.2. By Antitumor Immunotherapy

Some studies have demonstrated that CNTs used as carriers can be effectively applied in antitumor immunotherapy. This therapeutic consists of stimulating the patient's immune system to attack the malignant tumor cells. This stimulation can be achieved by the administration of a cancer vaccine or a therapeutic antibody as drug. Some authors have validated the use of CNTs as vaccine delivery tools. Yang's group observed that the conjugate of MWCNTs and tumor lysate protein (tumor cell vaccine) can considerably and specifically enhance the efficacy of antitumor immunotherapy in a mouse model bearing the H22 liver tumor. In vitro, the conjugate of CNTs and tumor immunogens can act as natural antigen presenting cells (such as mature dendritic cells) by bringing tumor antigens to immune effector T cells; this action is due to the high avidity of antigen on the surface and the negative charge. The complement system activation effects of CNTs and also their adjuvant effects may play a role in the stimulation of antitumor immunotherapy; however, the mechanism remains unknown.

4.1.3. By Local Antitumor Hyperthermia Therapy

The hyperthermia therapy using CNTs has been recently suggested as an efficient strategy for the cancer treatments. SWCNTs exhibit strong absorbance in the near-infrared region (NIR; 700–1100 nm). These nano-materials are considered as potent candidates for hyperthermia therapy since they generate significant amounts of heat upon excitation with NIR light. The photothermal effect can induce the local thermal ablation of tumor cells by excessive heating

of SWCNTs shackled in tumor cells such as pancreatic cancer. Some progress in the technique has been achieved in recent years, and it has shown feasibility in clinical application.

4.2. Carbon Nanotubes for Infection Therapy

Because of the resistance of infectious agents against numerous antiviral, antibacterial drugs or due to certain vaccine inefficacy in the body, CNTs have been assayed to resolve these problems. Functionalized CNTs have been demonstrated to be able to act as carriers for antimicrobial agents such as the antifungal amphotericin B. CNTs can attach covalently to amphotericin B and transport it into mammalian cells. This conjugate has reduced the antifungal toxicity about 40% as compared to the free drug. Our group has successfully combined an antimicrobial agent Pazufloxacin mesilate with amino-MWCNT with high adsorption and will be applied to experimental assays for infection treatment.

Functionalized CNTs can also act as vaccine delivery procedures. The linkage of a bacterial or viral antigen with CNTs permits of keeping intact antigen conformation, thereby, inducing antibody response with the right specificity. The fixation of functionalized CNTs with B and T cell peptide epitopes can generate a multivalent system able to induce a strong immune response, thereby becoming a good candidate for vaccine delivery . Thus, functionalized CNTs can act a good carrier system for the delivery of candidate vaccine antigens. Besides, CNTs themselves might have antimicrobial activity since bacteria may be adsorbed onto the surfaces of CNTs, such as the case of E. coli. 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 cell death.

4.3. Carbon Nanotubes for Gene Therapy by DNA Delivery

Gene therapy is an approach to correct a defective gene which is the cause of some chronic or hereditary diseases by introducing DNA molecule into the cell nucleus. Some delivery systems for DNA transfer include liposomes, cationic lipids and nanoparticles such as CNTs recently discovered.

When bound to SWCNTs, DNA probes are protected from enzymatic cleavage and interference from nucleic acid binding proteins, consequently, DNA-SWCNT complex exhibits superior biostability and increases self-delivery capability of DNA in comparison to DNA used alone. Indeed, stable complexes between plasmid DNA and cationic CNTs have

demonstrated the enhancement of gene therapeutic capacity compared with naked DNA. CNTs conjugated with DNA were found to release DNA before it was destroyed by cells defense system, boosting transfection significantly.

The use of CNTs as gene therapy vectors has shown that these engineered structures can effectively transport the genes inside mammalian cells and keep them intact because the CNT-gene complex has conserved the ability to express proteins. Pantarotto and coworkers have developed novel functionalized SWCNT-DNA complexes and reported high DNA expression compared with naked DNA.

4.4. Carbon Nanotubes for Tissue Regeneration and Artificial Implants

The knowledge advances of cell and organ transplantation and of CNT chemistry in recent years have contributed to the sustained development of CNT-based tissue engineering and regenerative medicine. Carbon nanotubes may be the best tissue engineering candidate among numerous other materials such as natural and synthetic polymers for tissue scaffolds since this nanomaterial is biocompatible, resistant to biodegradation, and can be functionalized with biomolecules for enhancing the organ regeneration. In this field, CNTs can be used as additives to reinforce the mechanical strength of tissue scaffolding and conductivity by incorporating with the host's body.

Indeed, MacDonald et al., have successfully combined a carboxylated SWCNTs with a polymer or collagen (poly-l-lactide or poly-D,L-lactide-co-glycolide) to form a composite nanomaterial used as scaffold in tissue regeneration. Other tissue engineering applications of CNTs concerning cell tracking and labeling, sensing cellular behavior, and enhancing tissue matrices are also studied recently. For example, it has been reported that CNTs can effectively enhance bone tissue regenerations in mice and neurogenic cell differentiation by embryonic stem cells in vitro.

4.5. Carbon Nanotubes for Neurodegenerative Diseases and Alzheimer Syndrome

As a promising biomedical material, CNTs have been used in neurosciences. Because of their tiny dimensions and accessible external modifications, CNTs are able to cross the blood-brain barrier by various targeting mechanisms for acting as effective delivery carriers for the target brain. Yang et al.have observed that SWCNTs were successfully used to deliver acetylcholine in mice brains affected by Alzheimer's disease with high safety range. Many other functionalized SWCNTs or MWSCNTs have been used successfully as suitable delivery

systems for treating neurodegenerative diseases or brain tumors . Overall, the results of these studies have indicated that conjugates of CNTs with therapeutic molecules have better effects on neuronal growth than drugs used alone.

4.6. Carbon Nanotubes as Antioxidants

The theory of oxygen-free radicals has been known about fifty years ago. However, only within the last two decades, has there been an explosive discovery of their roles in the development of diseases, and also of the health protective effects of antioxidants. Nevertheless, the potential role of CNTs as free-radical scavengers is still an emerging area of research. Some scientists have recently reported that CNTs and in particular carboxylated SWCNTs are antioxidants in nature and may have useful biomedical applications for prevention of chronic ailments, aging, and food preservation. Francisco-Marquez et al. observed that the presence of –COOH groups would increase the free radical scavenging activity of SWCNTs and that carboxylated SWCNTs are at least as good as, or even better, free radical scavengers than their nonfunctionalized partners. Their antioxidant property has been used in anti-aging cosmetics and sunscreen creams to protect skin against free radicals formed by the body or by UV sunlight. More investigations of different CNT forms in the future are needed to develop their precious effect of free radical scavenger for biomedical and environmental applications, since free radicals are well known to be very damaging species.

4.7. Carbon Nanotubes as Biosensor Vehicles for Diagnostic and Detection

A biosensor is an analytical device, used for the detection of an analyte that combines a biological component with a physicochemical detector. The use of CNTs in biosensing nanotechnology is recent and represents a most exciting application area for therapeutic monitoring and in vitro and in vivo diagnostics. For example, many searchers have coupled CNTs with glucose-oxidase biosensors for blood sugar control in diabetic patient with higher accuracy and simpler manipulation than biosensors used alone. Other CNT-enzyme biosensors such as CNT-based dehydrogenase biosensors or peroxidase and catalase biosensors have also been developed for different therapeutic monitoring and diagnostics.

For electrical detection of DNA, the assay sensitivity was higher with alkaline phosphatase (ALP) enzyme linked to CNTs than with ALP alone. The sensitivity of the assay using SWCNT-DNA sensor obtained by integration of SWCNTs with single-strand DNAs (ssDNA) was considerably higher than traditional fluorescent and hybridization assays. This CNT-biosensor-linked assay can be modified for antigen detection by using specific

antibody-antigen recognition. Thus, it could provide a fast and simple solution for molecular diagnosis in pathologies where molecular markers exist, such as DNA or protein.

Moreover, CNTs have been assayed to detect some organophosphoric pesticides by using acetylcholine esterase immobilized on CNT surface with electrochemical detection. Owing to their length scale and unique structure, the use of CNTs as biosensor vehicle is highly recommended to develop sensitive techniques for diagnostics and analyses from the laboratory to the clinic.

4.8. Carbon Nanotubes for Enantioseparation of Chiral Drugs and Biochemical

In pharmaceutical industries, 56% of the drugs currently in use are chiral products and 88% of the last ones are marketed as racemates consisting of an equimolar mixture of two enantiomers. Recently, US Food and Drug Administration (FDA) recommended the assessments of each enantiomer activity for racemic drugs in body and promoted the development of new chiral drugs as single enantiomers. Therefore, a wide range of new technologies for chiral separation has been developed, among them carbon nanotechnology. Silva et al. have recently used a microcolumn packed with SWCNT as chiral selector for separation of carvedilol enantiomers, a -blocker, with fluorescent detection. Yu et al. have developed a chiral stationary phase of MWCNT cross-linked with hydroxypropyl--cyclodextrin for enantioseparation of racemic clenbuterol, a bronchodilator, with-high-resolution factor. It is also notified that CNTs are chiral forms due to the helical winding of the graphitic rings around the tube axis. However, they might not be effective enantio-specific adsorbents. In contrast, chiral selector modified CNTs have been successfully assayed to separate enantiomers from many racemic drugs.

4.9. Carbon Nanotubes for Solid Phase Extraction of Drugs and Biochemicals

Due to their strong interaction with other molecules, particularly with those containing benzene rings, CNTs surfaces possess excellent adsorption ability. Non-functionalized or functionalized CNTs have been investigated as Solid phase Extraction (SPE) adsorbents used alone or in conjugation with classical SPE sorbents (C18 silica, XAD-2 copolymer) for the analytical extraction of drugs, pesticides or natural compounds in different media such as biological fluids, drug preparations, environment, plants, animal organs, and so forth. In several comparative studies CNTs exhibit similar or higher adsorption capacity than silica-based sorbents or macroporous resins. Many applications of CNTs in SPE can be found in different recent review articles dealing with general aspect. For examples, many drugs such

as benzodiazepines, sulfonamides, non-steroidal anti-inflammatory (NSAI), barbiturates, antidepressants, propranolol, cinchonine and quinine, and so forth, have been extracted by SPE using either SWCNTs or MWCNTs as adsorbents in different matrices cited above, then analyzed by different physicochemical techniques. Recently, our group has developed a novel molecularly imprinted magnetic solid phase extraction materials fixed on magnetic carbon nanotubes as support for the extraction and determination of an antibiotic, gatifloxacin (GTFX), in serum samples coupled with HPLC. The results of this novel adsorbent phase showed excellent specific recognition toward GTFX. Moreover, it was easily separated from the suspension by an external magnet, giving a best selective extraction of drug from biological fluids.

Many other applications of CNTs in SPE have been performed for the analysis of diverse pesticides (carbofuran, iprobenfos, parathion-methyl, etc.), phenolic preservatives, and natural compounds (piperine from pepper). Moreover, CNTs can be utilized for the extraction of inorganic ions and organometallic compounds as well as for the preparation of stationary phases of GC or LC columns.

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