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A Review on Carbon Nanotubes: A Novel drug Carrier for Targeting to Cancer Cells

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Abstract
Carbon nanotubes (CNTs) have recently gained the interest in the area of novel drug delivery system. This novel carrier can effectively administer the drug for achieving safe and effective therapeutic regimen. Based on their structure CNTs have been classified in to single walled, double walled and multi walled carbon nanotubes. The surface walls of the CNTs can be functionalized with certain groups in order to alter their physiological and biological properties to enhance the solubility of many chemotherapeutic drugs for efficient tumor targeting as well as drug delivery. CNTs are having capable to penetrate in to cells and deliver the therapeutic molecules at targeted site, due to their larger surface area. The purity of the CNTs can be increase by different chemical and physical purification methods, since the raw form of CNTs having less solubility and more toxic effects to the bod organs. In this review we clearly described the methods for synthesis of CNTs, functionalization methods, purification methods for CNTs and their applications in drug delivery for different novel drug delivery systems.

INTRODUCTION

Carbon nanotubes (CNTs) are allotropes of carbon. A CNT is a one-atom thick sheet of graphite (called graphene) rolled up into a seamless cylinder with diameter of the order of a nanometer. Carbon nanotubes (CNTs) were firstly observed and described in 1952 by Radushkevich and Lukyanovich and later in 1976 the single or double walled carbon nanotubes were observed by [1]. In more recent history the discovery of CNTs is attributed to Iijima as the first scientist who described the multi walled carbon nanotubes (MWNTs). This results in a nanostructure where the length-to-diameter ratio go above 10,000. Such tubular carbon molecules have novel properties that make them potentially useful in a wide variety of applications in mechanical, structural, thermal, electronics, and optical, biomedical and other fields of science, engineering & medicine. They show astonishing strength and unique electrical properties, and are good conductors of heat. Their name is obtained from their size, since the width of a nanotube is on the order of a few nanometers (nearly 50,000 times smaller than the width of a human hair), while they can be up to numerous millimeters in length. There are two main types of carbon nanotubes that can have high structural perfection. Single walled nanotubes (SWNT), these consist of a single graphite sheet seamlessly wrapped into a cylindrical tube. Multi walled nanotubes (MWNT), these comprise an array of nanotubes one concentrically placed inside another like rings of a tree trunk.

Types

Based on their structure they can be classified into single walled (0.4-3nm), double walled (1-3nm) and multi walled (2-100nm) carbon nano tubes.

Single wall carbon nano tubes

Single wall carbon nano tubes (SWCNTs) [2] are discovered in 1993 and which is having diameter differs from 0.4 to 3.0 nm, with a tube length that may be many thousand times larger and up to orders of centimeters [3]. Single walled CNTs can readily penetrate into the cell and this property makes them a suitable carrier for drugs to be delivered in to the cells. SWCNTs are made up of single graphene layer wrapped into a hexagonal close packed cylindrical structure whose diameter differs from 0.4 to 3.0 nm and length ranges from 20 to 1000 nm and are held together by Vander Waals forces, which makes them effortlessly twistable and more pliable [4].

Double wall carbon nano tubes

Double-wall nanotubes (DWNT) are an important sub-segment of MWCNT. These materials having the diameter 1-3nm, same morphology and other properties of SWCNT, while significantly improving their resistance to chemicals. This property is especially essential when functionality is required to add new properties to the nanotube. Since DWNT are a synthetic mixture of both SWNT and MWNT, they have good electrical, thermal stability and flexibility when compared to both SWCNT and MWCNT.

Multiwall carbon nano tubes

MWCNTs consist of numerous coaxial cylinders, each made up with single graphene sheet surrounding a hollow core. The width of MWCNTs ranges from 2 to 100nm, while the inner diameter is in the range of 1–3 nm, and their length is 1 to several μm. Electric arc [5] and chemical vapor deposition (CVD) [6] are the main techniques for their production. Owing to the sp2 hybridization in MWCNTs, a delocalized electrons along the wall is generated which is responsible for the π-πinteractions between adjacent cylindrical layers in MWCNTs resulting in a less flexible and more structural defects.

Production of Carbon Nanotubes

There are various methods for production of carbon nanotubes such as arc discharge, laser ablation, flame synthesis, chemical vapor deposition, high pressure carbon monoxide (HiPco), pyrolysis, electrolysis etc. By depends upon the process of production they can be mainly classified into following groups.

1) Physical Processes
2) Chemical Processes
3) Miscellaneous Processes

Physical Processes

These are the processes, which make use of physical principles of carbon conversion into nanotubes. These include widely used process of carbon nanotubes production such as, arc discharge and laser ablation.

Arc Discharge

This is one of the oldest methods of carbon nanotube production. First utilized by [7] in 1991 at NEC's Fundamental Research Laboratory to produce new type of finite carbon structures consisting of needle-like tubes. The tubes were synthesized by using an arc discharge evaporation method likely to that used for the fullerene production. The carbon needles, varies from 4 to 30 nm in width and up to 1 mm in length, were developed on the negative end of the carbon electrode used for the direct current (DC) arc-discharge evaporation of carbon. Throughout the process Iijima used a pressurized chamber filled with a gas mixture of 40 Torr argon and 10 Torr methane .Two vertical thin electrodes were fitted in the center of the chamber. The lower electrode (cathode) contained a small piece of iron in a shallow dip made purposefully to grip iron. The arc was produced by running a DC current of 200A at 20 V between the electrodes. The three main components, namely argon, iron and methane, was critical for the production of SWNT. Carbon soot formed as result of arc-discharge settled and nanotubes grew on the iron catalysts contained in negative cathode. The nano-tubes had diameters of 1nm with a broad diameter varying in between 0.7 and 1.65 nm. In a similar process Bethune *et al*. used thin electrodes with bored holes as anodes, which were packed with a mixture of pure powdered metals (Fe, Ni or Co) (catalysts) and graphite. The vaporization of electrodes with a current of 95 -105 A in 100-500 Torr of Helium. SWNT were also synthesized by the variant of arc-technique by Journet *et al*. [8] as well. In his alternate, the arc was produced between two graphite electrodes in a reaction chamber under helium atmosphere (660 mbar). This method also gave large yield of carbon nanotubes. Ebbesen and Ajayan, [9] however, stated large-scale synthesis of MWNT by a variant of the standard arc discharge technique as well.

Laser Ablation Process

In the laser ablation process, as trike at graphite target in a high temperature reactor in the presence of inert gas such as helium which vaporizes a graphite target. The nanotubes formed on the cooler surfaces of the reactor, as the vaporized carbon condenses. A water-cooled surface is also involved in the most practical systems to collect the nanotubes. This method was first discovered by Small workers at Rice University in 1995 [10]. At the time of discovery they were identifying the effect of laser impingements on metals. They produced high yields (>70%) of Single walled CNTs by laser ablation of graphite rods containing small amounts of Ni and Co at 1200°C. In this method two-step laser ablation was used. Initial laser vaporization pulse was tailed by second pulse to vaporize target more fastly. The two step process reduces the amount of carbon deposited as soot. Tubes develop in this method on catalysts atoms and continued to grow until too many catalyst atoms aggregate at the end of the tube. The tubes formed by this method are in the form of mat of ropes 10 - 20 nm in diameter and up to 100 micron or more in length. By changing temperature, catalyst composition and other process parameters average diameter and length of carbon nanotube could be varied.

Chemical Processes

Chemical Vapor Deposition

In 1996 Chemical vapor deposition method for large scale production and synthesis of CNTs. This method is capable of monitoring growth directions on a substrate and synthesizing a large quantity of CNTs [11]. In this process a mixture of hydrocarbon gas (ethylene, methane or acetylene) and a process gas (ammonia, nitrogen, hydrogen) is made to react in a reaction chamber on heated metal substrate at temperature of around 700°C - 900°C, at atmospheric pressures. CNTs formed as a result of decomposition of hydrocarbon gas and deposit and grow on metal catalyst (substrate). The catalysts particle can stay at the bottom or top of growing CNT. The use of the catalyst and preparation of the most essential aspects in CVD, as this substrate will define the nature and type of CNTs formed. The usually substrate material is silicon, but glass and alumina are also used. The catalysts are metal nanoparticles, like Co, Fe and Ni, which can be deposited on substrates by means of electron beam evaporation, physical stammering or solution deposition. Porous silicon is model substrate for growing self-oriented nanotubes on large surfaces. The nanotube width depends on the catalyst particle size, hence, the catalyst deposition technique should be chosen carefully to yield desired results. A variant of CVD known as "plasma assisted CVD" is a process in which a plasma is generated in the process. By appropriately adjusting the geometry of reactor during plasma assisted CVD, it is possible to develop vertically developed CNTs. Without plasma CNTs produced are usually random groups just like bowl of spaghetti. However, under certain carefully con-trolled conditions even in the absence of plasma vertically aligned CNTs resembling that of first or carpet can be synthesized. Recently at University of California, Berkeley [12] researchers have also reported the production of double walled CNTs from CVD. Similar success has also been reported at University of California, San Diago [13].

High Pressure Carbon Monoxide Reaction (HiPco®)

This is a unique method developed at Rice University in1999 for the production of CNTs [14]. Unlike other methods in which the metal catalysts are deposited or embedded on the substrate before the deposition of the carbon initiates, in this method catalyst is introduced in gas phase. Both the catalyst and the hydrocarbon gas are fed into oven and then followed by catalytic reaction in the gas phase. This process is suitable for large-scale production, because the nanotubes are free from catalytic backings and the reaction can be operated continuously. Commonly CO gas is used as hydrocarbon gas which reacts with iron pentacarbonyl, Fe(CO)5 to form SWNT. This process is called HiPco process. SWNT have also been produced in a variant of HiPco process in which a mixture of benzene and

ferrocene, Fe(C5H5)2 reacts in a hydrogen gas flow to form SWNT [15]. In both methods, catalyst nanoparticles are formed through thermal decomposition of organometallic compounds, such as iron pentacarbonyl, ferrocene.

CoMo (cobalt and molybdenum) CAT® Process

Recently an effort has been made at university Oklahoma [16], to develop a process using Cobalt and Molybdenum catalysts and CO gases. In this method, SWNT are developed by CO disproportionation (decomposition into C and CO2) in the presence of CoMo Catalyst (specifically developed for the purpose) at 700°C - 950°C in flow of pure CO at a total pressure that typically ranges from 1 to10 atm. This process is able to grow a significant amount of SWNT (about 0.25 g SWNT/g catalyst) in couple of hours, keeping choosiness towards SWNT better than 80%. The secret of the process is in synergistic effect of Co and Mo. Catalyst is most active when both metals Co and Mo are present at a time on silica substrate with low Co and Mo ratio. The material produced by the HiPco process yields a much larger number of bands, which show a greater variety of diameters than the material synthesized by CoMoCAT Process. The distribution of diameters synthesized by the HiPco process reported in the literature is also significantly broader than that of the product obtained from the CoMoCAT process. This process brings solid prospects in it to be scaled up as large scale production process for the production of SWNT.

Miscellaneous Processes

Some miscellaneous and relatively less used methods of carbon nanotube production are given below.

HELIUM ARC DISCHARGE METHOD

Was reported in 2006 by scientists of NASA's Goddard Space Flight Center that they have developed a simple, safe, and very economical process of Single walled CNTs production. In this method scientists used a helium arc welding process to vaporize an amorphous carbon rod and then form nanotubes by depositing the vapor onto a water-cooled carbon cathode. This process yields bundles, or "ropes," of single-walled nanotubes at a rate of 2 grams per hour using a single setup. Further it was claimed, as method does not require any metal catalyst no metal particles need to be removed from the final product. Removing the presence of metallic impurities results in the SWCNTs exhibiting higher degradation temperatures (650°C rather than 500°C) and eliminates damage to the SWCNTs by the purification method. This method is under discussion for potential use as commercial scale process.

Electrolysis

In this method CNTs were produced by depositing alkali metals on a graphite cathode from a high temperature molten salt system. The deposited metallic atoms place into the gap between the graphitic sheets and diffuse towards the bulk of the graphite cathode, producing some mechanical stress inside graphite. This stress induces the ablation of separate graphitic sheets, which will change into CNTs due to interfacial forces, trying to recombining the broken carbon-carbon bonds. Though this method has been stated to yield good quality of carbon nanotubes. It is not scaleable to large scale production method to produce carbon nanotubes.

Flame Synthesis

This method is based on the synthesis of SWNT in a controlled flame atmosphere that produces the temperature, forms carbon atoms from the inexpensive hydrocarbon fuels and forms small aerosol metal catalyst islands. SWNT are grown on these metal islands in the same manner as in laser ablation and arc discharge. These metal catalyst islands can be prepared in three ways. The metal catalyst (cobalt) can either be coated on a mesh [17], on which metal islands resembling droplets were formed by physical vapor deposition. These small islands convert aerosol after exposure to a flame. The second way is to make aerosol small metal particles by burning a filter paper that is rinsed with a metal ion (e.g. iron nitrate) solution. The third way is the thermal evaporating technique in which metal powder (e.g. Fe or Ni) is inserted in a trough and heated. In a controlled way a fuel gas is partially burned to gain the right temperature of ~800°C and the carbon atoms for SWNT production. As optimization parameters the fuel gas composition, catalyst carrier surface and temperature can be controlled.

Purification of carbon nanotubes

CNTs usually contain a large amount of impurities such as amorphous carbon, metal particles and multi shell. There are different steps involved in purification of CNTs. Purification of CNTs is a method that separates carbon nanotubes from impurities included in the raw products, or from nanotubes having undesired numbers of walls. Purification has been an essential synthetic effort since the discovery of carbon nanotubes, and there are so many publications discussing different features of the purification method. Good review articles on the purification of CNTs are available in the recent literature [18]. The current industrial methods applied oxidation and acid-refluxing techniques that affect the structure of tubes. Purification problems are abundant because of insolubility of CNT and the limitation of liquid chromatography. CNT purification step (depending on the type of the purification) removes amorphous carbon from CNTs, increases surface area, decomposes functional groups blocking the entrance of the pores or induces additional functional groups. Most of these techniques are combined with each other to improve the purification and also to remove impurities at the same time. Those techniques are as follow.

Oxidation

Oxidation is a way to remove CNTs impurities. In this method CNTs and impurities are oxidized. The damage will occur to CNTs is less than the damage occur to the impurities. This technique is more preferable with respect to the impurities that are commonly metal catalysts which act as oxidizing catalysts. Altogether, the efficiency and yield of the procedure are highly depending on many factors, such as oxidation time, environment, metal content, oxidizing agent and temperature [19].

Acid treatment

Refluxing the sample in acid is effective in reducing the amount of metal particles and amorphous carbon. Different

types of used acids are hydrochloric acid (Hcl), nitric acid (HNO3) and Sulphuric acid (H2SO4), while Hcl is identified to be the good refluxing acid. Thus when a treatment in HNO3 had been used the acid had an effect on the metal catalyst only, and no effects were observed on the CNTs and the other carbon particles [19, 20]. If Hcl is used in treatment, the acid has also a little effect on the CNTs and other carbon particles [21, 22]. A review of literature demonstrates the effects that key variables like acid types and concentration & temperature have on the acid treatment [21, 22].

Ultra sonication

This technique is based on the separation of particles due to ultrasonic vibrations and also agglomerates of different nanoparticles will be more dispersed by this method. The separation of the particles are extremely dependable on the surfactant, solvent and reagents which are used [19, 20, 21, 22]. When an acid is used, the purity of the CNTs influenced on the sonication time. During the vibration of tubes in acid for a short time, only the metals are solvated, but in a more extended period, the CNTs are also chemically cut [21].

Micro-filtration

Micro-filtration is based on particle size. Generally CNTs and a small amount of carbon nanoparticles are trapped in a filter. The other nanoparticles (catalyst metal, fullerenes and carbon nanoparticles) are passing through the filter. A special form of filtration is cross flow filtration. Through a hole of fiber, the filtrate is pumped down at head pressure from a reservoir and the major fraction of the fast flowing solution is returned to the same reservoir in order to be cycled through the fiber again. A fast hydrodynamic flow down the fiber bore sweeps the membrane surface and prevents building up of a filter cake [19].

Surfactant aided sonication, filtration and annealing

After acid refluxing, the CNTs were purer but, tubes were entwined together, most of the impurities are trapping, such as catalyst particles and carbon particles, which were difficult to remove by using filtration. So surfactant-aided sonication was carried out. Sodium dodecyl benzene sulphonate (SDBS) aided sonication with ethanol (or methanol) as organic solvent were preferred because it took the longest time for settle down of CNTs, indicating an even suspension state was achieved. The sample was then filtered with an ultrafiltration unit and annealed at 1273 k in N2 for 4 h. Annealing process is effective in optimizing the CNT structures. It was verified the surfactant-aided sonication is effective to untangle CNTs, thus to free the particulate impurities embedded in the entanglement. Nanotubes can also be purified by using multi-step purification method [19].

Functionalization of carbon nanotubes

Despite of the advantages of CNTs in targeting various types of cancer cells, various constraints have been made on the biological and biomedical applications of CNTs due to their lack of solubility in aqueous medium as well as their toxicity caused due to hydrophobic surface. These toxic effects of CNTs can be overcome by Functionalization process [23]. CNTs are cytotoxic to the certain mammalian cells, without surface modification, for example, pure MWCNTs can injure to the plasma membrane of human macrophages. Therefore, approaches for surface functionalization including covalent and non-covalent functionalization are carried out on the synthesized CNTs. The process of functionalization is also helpful in conjugating the therapeutic molecule or the ligands to the CNTs either on the surface or on the ends of CNTs to render them active against cancer cells. In this situation, recently, a novel immunologically modified nanotube system was invented by Chen using glycated chitosan (GC), a potent immune adjuvant, as an effective surfactant for single-walled CNTs (SWCNTs). Upon laser irradiation of target tumor cells, administration of SWCNTGC resulted in highly effective tumor suppression in animal tumor models, and with complete tumor regression, long term survival in many cases.

Non-covalent functionalization of CNTs

The non-covalent dispersion of CNT in solution allows preservation of their aromatic structure and thus their electronic characteristics. The dispersion procedures normally involving ultra-sonication, centrifugation and filtration are quick and easy. Hydrophobic or k–k interactions are often evoked as likely responsible for non-covalent stabilization. Currently, three classes of molecules are mainly used for CNTs dispersion. Surfactants are used, since they are easily available and low cost. Polymers and biopolymers (nucleic acids and peptides) are also very efficient in the dispersion process.

Surfactants

A series of anionic, cationic and nonionic surfactants have been already proposed to disperse nanotubes. Sodium dodecyl sulfate (SDS) and Triton X-100 were used to obtain CNT suspensions up to 0.1 and 0.5 mg/mL, respectively [24]. However, the stability of this suspension was no longer than 1 week. A better result were attained by using sodium dodecyl benzene sulphonate (SDBS), which was able to provide stability over one month reaching 10 mg/mL concentration of the suspension. The combination of k–k interactions of aromatic moieties between CNT and SDBS and the long lipid chains of the SDBS increases the stability of the complex. Atomic force microscopy (AFM) and electronic transmission microscopy (TEM) studies of SDS/CNT dispersions showed that CNT are mainly present as individual tubes uniformly covered by the surfactant [25]. The types of amphiphilic molecules with long lipid chains are able to form a half-cylinder perpendicular or tilted around the tubes in a micelle-like arrangement [26]. Triton-X instead mainly interacts by k-stacking. Another approach for the adsorption/dispersion of CNT via k interaction resides on the use of 1-pyrenebutanoic acid activated as succinimidyl ester, which quickly reacts with the amino groups present in the proteins like ferritin or streptavidin. The solubility of CNT was between 0.1 and 0.7 mg/mL, which is slightly low but acceptable for biological use. Even though surfactants may be efficient in the solubilization of CNTs, they are known to permeabilize plasma membranes and have a toxicity profile of their own. Therefore, the implications restricting from use of surfactants interacting with biological systems can limit the

possible biomedical applications of such surfactant-stabilized CNT complexes.

Polymers

Polymers are widely used for example as molecular carriers for drug delivery [27]. In the solubilization of CNT they represent a good alternative to surfactants although they do not have a better dispersion efficiency [25]. The mechanism of dispersion is based in this case on wrapping of the polymer around the tubes [28]. Have used for example as poly (metaphenylenevinylene) to suspend SWNT in organic solvents. The polymer wraps around ropes of nanotubes. The driving force of the phenomenon is probable to be the steric repulsion of the polymer. When the polymer is attached to the surface of the nanotube, it offers adequate repulsive potential stabilizing the dispersion [29]. In the case of nonionic polymers, based on poly (oxyehtylene) copolymers, the effectiveness of the dispersion is instead due to their hydrophilic counterpart. For particularly high molecular weight polymers, the suspend ability is enhanced as the steric stabilization is increased by a wider coverage of the surface [25]. In a similar approach, CNT were dispersed by using cationic copolymers [30]. The nanotubes were covered by the hydrophobic backbone of the polymer while the positive tetraalkylammonium groups were exposed at the surface to display water solubility. This type of fluorescent polymers have also been employed to study the interaction with mammalian cells. Poly (vinylpyrrolidone) was conjugated with various fluorescent dyes. CNT were suspended in 1% SDS and mixed with the fluorescent polymers to form supramolecular complexes, which were identified to have potential applications as new molecular probes [31].

Biopolymers

The solubilization of CNT with biological components is certainly more appropriate towards integration of this new type of material with living systems. Self-assembly processes similar to k–k interactions typical of double-stranded DNA can be for example exploited to disperse the nanotubes. Nucleic acids are ideal candidates to form supramolecular complexes based on k-stacking between the aromatic bases and the CNT surface. Indeed, [32] have described an easy way to solubilize CNTs by simple sonication in the presence of a single-strand DNA. A molecular modeling study was done to explain the formation of the hybrids exerted by DNA wrapping and subsequent CNTs de-bundling. The DNA–nanotube complexes showed solubility in the range of mg/ml, and their good stability allowed the purification using ion-exchange chromatography. Amphiphilic peptides belong to other class of biopolymers that efficiently disperse CNT [33]. The presence of amino acids like phenylalanine, tyrosine tryptophan and histidine into the peptide sequence plays a key role on the solubilization process in water. These peptides might be selected from phage-display peptide libraries [34]. The design of highly specific peptides able to wrap around the nanotubes represent an interesting way to assure solubility and may even provide a useful tool for size separation. More recently, cyclic peptides were also proven to have similar capabilities [35].

Covalent modification of CNT surface

The covalent modification, namely the chemical modification of CNTs is an emerging area in materials science. Among the various approaches, the most common ones are:

i. Esterification and amidation of oxidized CNTs,

ii. Generation of functional groups on CNT sidewalls by cycloaddition reactions.

Oxidation of CNTs is a purification method for raw CNTs. Oxidation of CNTs can be carried out by reflexing raw CNTs in strong acidic media, e.g. HNO3/H2SO4. Under this state, the end caps of the CNTs are opened, and carboxylic groups are generated at these end caps and at some defect sites on nanotube sidewalls [36]. The carboxylic groups provide chance for further derivatization of the CNTs through esterification or amidation reactions. For example, some organic molecules with amine groups can be directly condensed with the carboxylic groups present on the surface of the CNTs [37]. Alternatively, the carboxyl moieties can be activated with thionyl chloride and subsequent react with amine groups [38]. These reactions are extensively applied for conjugation of water-soluble organic molecules, hydrophilic polymers, nucleic acid (DNA or RNA) or peptides to the oxidized CNTs, which result in multifunctional CNTs [38]. In most cases, the length of nanotubes is often shortened [36], but the electronic properties of such functionalized CNTs remain intact. Oxidation reaction only produces carboxyl groups on cap ends and defect sites on CNTs. To produce chemical bonds on sidewall and cap ends of CNTs, cycloaddition reactions are used [39]. Cycloaddition reaction is a very great methodology, in which the 1, 3-dipolar cycloaddition of azomethineylides can easily attach a large amount of pyrrolidone rings on sidewalls of nanotubes. Thus, the subsequent functionalized CNTs are highly soluble in water [40]. In addition, pyrrolidone ring can be substituted with many functional groups for different applications. In contrast to non-covalent surface modifications, which do not locally disrupt *sp*2 hybridization, or generate defects, the covalent surface modifications disrupt CNT *sp*2 conjugated structures and therefore, possibly will affect the electronic and optical performances.

DRUG DELIVERY BY CARBON NANOTUBES

Application of CNTs in Cancer Treatment

For decades, human immortal cancer cell lines have founded an accessible and easily usable set of biological models with which to investigate cancer biology and to explore the potential efficacy of anticancer drugs is of less tedious work. Presently, various ex-vivo studies, such as cellular uptake studies, fluorescent microscopy, cell line studies and flow cytometry, are carried out for this purpose. Various cancer cell lines were cultured with modified CNTs (functionalization on the surface and ends of the CNTs, and by conjugating CNTs with ligands) and evaluated for therapeutic efficacy, cell survival assays and cell apoptosis. Ex-vivo studies specifically used in the evaluation of CNTs for cancer chemotherapy.

Brain Cancer

Brain cancer is the leading cause of cancer-related death in the US in patients under the age of 35. Anaplastic astrocytoma's (Grade III) and glioblastoma (Grade IV) are most aggressive brain cancers with survival period of 24 and 9 months, respectively [41]. Children who survive their brain cancers (mainly medulloblastomas) often suffer substantial adverse effects related to the toxicities of therapy on the developing nervous system [42]. Currently available systemic chemotherapy is less effective due to presence of the blood-brain barrier (BBB) which restricts the penetration of most drugs into the brain. Recently, many of the CNT-based targeting approaches have been developed for the treatment of brain cancer and a brief account is presented below. Vittorio et al. investigated the biocompatibility of MWCNTs with cultured Human neuroblastoma cells SH-SY5Y. Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. ROS can damage cellular proteins, lipids, and DNA leads to fatal lesions in cells that contribute to carcinogenesis. In-vitro experiments revealed loss of cell viability was minimal with no intracellular ROS detected with prolonged cultures and continued propagation in the presence of 99%, 97% pure MWCNTs and acid treated 97% pure MWCNTs but no significant decrease in the proliferation of cells incubated for 3 days was observed with the cells cultured with 99%pureMWCNTs. After conducting WST-1 assay it was considered that, on increasing the concentration of MWCNTs from 5 μg/mL to 500 μg/mL, purity and surface oxidation of MWCNTs seem to down regulate the % cell viability and at 5 μg/mL 100% cells are viable. Therefore it was concluded that the concentration of 5–10 μg/mL seems ideal for gene and drug therapy against cancer [43].

Xing et al. synthesized phospholipid-bearing polyethylene glycol (PL-PEG) functionalized SWCNTs conjugated with protein A which was further coupled with the fluorescein-labeled integrin $\alpha v\beta 3$ monoclonal antibody to form SWCNT-integrin $\alpha v\beta 3$ monoclonal antibody (SWCNTPEG mAb). Confocal microscopy revealed that SWNT-PEGmAb showed a much higher fluorescence signal on integrin $\alpha v\beta 3$-positive U87MG cells and presented a high targeting efficiency with low cellular toxicity, while, for integrin $\alpha v\beta 3$-negative MCF-7 cells, no obvious fluorescence was observed which clearly reveals low targeting efficiency of the functionalized SWCNTs, indicating that the specific targeting of integrin $\alpha v\beta 3$-positive U87MG cells was caused by the specific recognition of integrin $\alpha v\beta 3$ on the cellular membrane by the $\alpha v\beta 3$ monoclonal antibody.

Oxidized MWCNTs cannot only be distributed in the brain but may accumulate in tumors after conjugating with specific ligands and also possess an ultrahigh surface area for efficient loading of anticancer drug. Ren et al. developed a dual targeting PEGylated MWCNTs and they were loaded with a targeting ligand angiopep2 (ANG) and doxorubicin, respectively, to target low density lipoprotein receptor-related protein receptor which is overexpressed on the blood brain barrier (BBB) and C6 glioma cells. In-vitro intracellular tracking and in-vivo fluorescence imaging demonstrated the ideal dual targeting of the developed system which was attained by the higher transcytosis capacity and parenchymal accumulation by the angiopep-2 and can be considered a material of choice to cross blood brain barrier as well as to specifically recognize their lipoprotein receptors present on the glioma cells for directing the site specific release of anticancer drug.C6 cytotoxicity, CD68 immunohisto chemical analysis and hematology analysis reveals better anti glioma effect with good biocompatibility and low toxicity of O-MWCNTs-PEGANG, when compared with that of free doxorubicin. The existence of cancer stem cells (CSCs) or stem-like cancer cells (SLCCs) is regarded as the cause of tumor formation and their recurrence. Though, the origin of such cells remains controversial with two competing hypotheses: CSCs are either transformed tissue adult stem cells or dedifferentiated from transformed progenitor cells [44]. The potential of CD133 monoclonal antibody (anti-CD133) conjugated SWCNTs for therapeutic targeting of CD133 CSCs. Glioblastoma (GBM)-CD133+ cells were selectively targeted and eradicated whereas GBM-CD133− cells are remained viable. Moreover, anti-CD133-SWCNTs pretreated GBM-CD133+ cells were irradiated with near infrared laser for 2 days and showed no sign of sustainability of CSCs for tumor growth after xenotransplantation in nude mice. From this report it is indicated that monoclonal antibody conjugated SWCNTs are capable of selectively targeting the CSCs as well as blocking their recurrence [45].

Blood Cancer

Leukemia is a cancer that begins in the bone marrow (the soft inner part of some bones), but in maximum cases, moves into the blood. Then it can spread to other parts of the body like organs and tissues. Acute lymphoblastic leukemia (ALL) is one of the main type of leukemia, is a slow growing blood cancer that starts in bone marrow cells called lymphocytes or white blood cells. If once these white blood cells are affected by leukemia, they do not go through their regular process of growing. The lymphocytes continue to replicate and build up and invade the blood fairly quickly. ALL is destructive type of leukemia; without treatment, maximum patients with acute leukemia would live only a few month. An enhanced targeted delivery of daunorubicin (Dau) to acute lymphoblastic leukemia was achieved, they developed a tertiary complex of Sgc8c aptamer, daunorubicin, and SWCNT termed as Dau-aptamer SWCNTs. Flow cytometric analysis exhibited that the tertiary complex was internalized effectively into human T cell leukemia cell (MOLT-4 cells) but not to U266 myeloma cells [46].

Breast Cancer

Breast cancer (BC) has become the most common malignancy and the leading cause of cancer-specific death in women, according to GLOBOCAN 2008 estimates [47]. Overexpression of human epidermal growth factor receptor-2 (HER2), also called as c-erbB-2 or HER2/neu, is approximately 20%–25% responsible for invasive BC. With an increasing understanding of the role of HER2 in tumor proliferation, and metastasis, angiogenesis novel special treatment strategies for this HER2-positive subtype

of BC have been confirmed and are increasingly used in clinical practice. One of the most important treatment approaches is to block the signal pathway of HER2/neu; this is defined as targeted therapy [48]. In a study, Pan et al. investigated the efficiency of MWCNTs to deliver gene to the tumor cell for cancer therapy. In this work, they invented MWCNTs modified with Polyamidoamine dendrimer which were further conjugated with FITC-labelled antisense c-myc oligonucleotides (asODN). Human breast cancer cell line MCF-7 cells and MDA-MB-435 cells were incubated with modified MWCNTs (asODN-dMNTs). Fluorescence developed by the FITC revealed the cellular uptake of asODN-dMNTs within 15min. These composites inhibit the cell growth in time and dose dependent means and down regulated the expression of c-myc gene (overexpression of this gene amplify the expression of HER2) and C-Myc protein [49]. A chemically functionalized SWCNT carrier has been developed for the effective delivery of siRNA and SiRNAMDM2complexes to the breast carcinoma B-Cap-37 cells. Results showed the high efficiency of F-SWCNT in carrying siRNAto the carcinoma cells and the newF-SWCNT-SiRNAMDM2 complexes caused 44.53% inhibition of proliferating B-Cap-37 carcinoma cells for 72 hours by down regulating the expression of c-myc gene [50]. The explosive nature of SWCNT which can act as a potent therapeutic nanobomb agents for killing breast cancer cells. In his work, he made water molecule adsorbed on the SWCNT, which upon exposure to laser light of 800 nm at light intensities of approximately 50–200MW/cm2 which is sufficient to transform optical energy to thermal energy and cause the evaporation of water molecules which built extreme pressure in SWCNT causing them to explode in the suspension of human BT-474 breast cancer cells in phosphate buffered saline solution and render the cells to death. The presence of bubbles around the dead cells revealed the boiling effect caused by SWCNT explosions [51]. A water soluble SWCNT-Paclitaxel (PTX) conjugate has been developed by conjugating PTX to functionalized polyethylene glycol SWCNTs via a cleavable ester bond. The SWCNT-PTX has been found to be highly efficient in suppressing tumor growth when compared with clinical taxol in a murine 4T1 breast cancer cells, which has been attributed to the prolonged blood circulation (due to PEGylation) and tenfold higher tumor PTX uptake by SWCNT delivery, probably through improved permeability and retention (EPR) effect [52].

Colon Cancer

Colorectal cancer is the leading cause of death amongst the men and women worldwide and afflicts more than 135,000 patients per year in America .This cancer has usually been viewed as a homogeneous entity rather than a complex heterogeneous disease developing through multiple genetic and epigenetic abnormalities, such as defective DNA mismatch repair (dMMR) and the CpG island methylator phenotype (CIMP). Abdolahad et al. utilize the vertical arrays of MWCNTs for entrapping the metastatic human colon adenocarcinoma SW-48 cells and HT-29 cancerous cells. Due to the dangerous deformability and softness of higher metastatic malignant cells, they showgreater fraction of entrapment by the vertically aligned MWCNTs as compared to the less deformable and rigid lower grades of metastatic cancerous cells. This novel application of MWCNTs distinguishes the healthy and highly deformable cancerous cells more precisely than SWCNTs and also showed better delivery of anticancer drugs to these cancer cells.[53] Triple functionalized SWCNTs were fabricated with an anticancer drug (Doxorubicin), a monoclonal antibody and a fluorescent marker (fluorescein) at the noncompetitive binding sites on the SWCNTs for targeting the cancer cells. Confocal laser microscopy shows the bovine serum albumin antibody specific receptor mediated uptake of SWCNTs by the human colon adenocarcinoma cell, wider cells with subsequent targeting of doxorubicin intracellularly to the nucleus [54].

Liver Cancer

Hepatocellular carcinoma (HCC) is a highly predominant malignancy, especially in Asia. Liver cirrhosis is the strongest predisposing factor for HCC, accounting for approximately 80% of patients with this disease. In the United States, Japan and Europe, hepatitis C virus (HCV) infection is the major etiology of liver cirrhosis and HCC. Hepatitis virus B (HBV) infection, however, is the foremost cause of HCC development in most Asian countries other than Japan. In addition to HBV and HCV infection, metabolic disorders and alcoholic cirrhosis can also act as risk factors for HCC. c-myc is among the most frequently overexpressed genes in human cancers. Overexpression of c-mycin hepatic cells leads to the development of hepatocellular carcinoma [55] However, an attempt has been made by Pan et al. to suppress the appearance of c-myc gene and C-Myc protein in the tumor bearing cell. Polyamidoamine dendrimer modified CNTs (dMWCNTs) were fabricated for the efficient delivery of antisense c-myc oligonucleotide (asODN) into liver cancer cell line HepG2 cells. AsODN-dMWCNTs composites were incubated with HepG2 cells and confirmed to enter into tumor cells within 15 min by laser confocal microscopy. These composites suppress the cell growth in time and dose dependent means and down regulated the expression of the c-myc gene and c-myc protein. These composites show maximal transfection efficiencies and inhibition effects on tumor cells when compared to CNT-NH2-asODN and dendrimer (asODN) alone [56]. Meng et al. constructed a highly effective targeted DDS based on chitosan and folic acid modified SWCNTs for controllable loading/release of anticancer agent doxorubicin (DOX).The obtained DDS not only effectively killed the hepatocellular carcinoma SMMC-7721 cell lines and depressed the growth of liver cancer but also displayed much less in vivo toxicity than free doxorubicin [57].

Lymph Node Metastasis

The presence of lymph node invasion is one of the strongest indicators for prognoses of distant metastasis and survival in most cancers. In the multistep process of cancer metastasis growth, invasion into a vascular or a lymphatic system has generally been believed to be a key step of tumor cell distribution. Once tumor cells obtain abilities of intravasation and survival in an unfavorable vascular environment, they circulate around the entire body parts to

form new tumors at the secondary site. Lymph node metastasis is a powerful predictor of recurrence and death in patients with cutaneous melanoma. Metastasis to regional lymph nodes grows during the course of the disease in approximately 30% of patients with cutaneous melanoma. Yang et al. compared the in vitro and in vivo potential therapeutic effect of gemcitabine (GEM) loaded magnetic MWCNTs (mMWCNTs) this carbon nano tubes act as magnetic-carbon particles (mACs). His finding reflects the high antitumor activity in human pancreatic cancer BxPC- 3 cells of both the systems when compared along with free drug. Owing to super paramagnetic behavior of mMWCNTs- GEM, their magnetic moments tend to align along the applied field leading to net magnetization which greatly affects the interaction of mMWCNTs-GEM with the cellular membrane and thus they were found to be superior than mACs-GEM in successful inhibition of lymph node metastasis after following subcutaneous administration under the impact of magnetic field [58].

Kidney Cancer

Renal cell carcinoma (RCC) is responsible for approximately 80% of primary renal cancers, and urothelial cell carcinoma (UCC) accounts for the majority of the remainder (20%). The most common histological subtype of RCC is the conventional or clear cell (ccRCC). The occurrence of ccRCC is due to the de functioning of the Von Hippel-Lindau (VHL) tumor suppressor gene (TSG), located on chromosome 3p. Loss of functioning of the VHL protein leads to stabilization of hypoxia-inducible factors and nuclear transcription factors that in turn can activate the transcription of many genes including those encoding vascular endothelial growth factor (VEGF) and platelet derived growth factor [59]. RCC is a highly aggressive tumor and also the most lethal of urologic malignancies with an estimated 88,400 new kidney cancer cases and 39,300 kidney cancer-related deaths from RCC in Europe [60]. The interaction between SWCNTs and human embryo kidney HEK-293 cells intended to explore SWCNT biocompatibility and safety. It was found that SWCNTs can prevent the proliferation of HEK-293 cells, prompt the cell apoptosis, and reduce cell adhesive ability in a time and dose dependent manner. SWCNTs induces variations in the cell cycle which could be attributed to the decrease in the number of cells in the S-phase due to up regulated expression of P16 which inhibits the cycline dependent kinase activity of CdK2, CdK4, and CdKr and therefore stops the cells from entering an S-phase and subsequently arresting the cell cycle in theG1 phase [61].

Cervical Cancer

Oncogenic human papillomavirus (HPV) has a causal role in nearly all cervical cancers and in many vulvar, penile, vaginal, and or pharyngeal cancers. HPV types 16 and 18 are mostly responsible for 70% of cervical cancers. In HPV-associated cancers, oncogenic antigens E6 and E7 were overexpressed on the tumor cells and thus, they represent a best target for developing antigen-specific immunotherapy for the control of cervical cancer [62]. Wu et al. developed a novel approach of utilizing natural biocompatible polymer chitosan for imaging the tumor cells. In this assay, SWCNTs were modified by using chitosan (CHIT) fluorescein isothyocyanate (FITC). This was further conjugated with folic acid (FA), as mostly cancers cells overexpress folic acid receptors, to construct the functional FITC-CHITSWCNT- FA conjugate. These new functionalized SWCNTs were found to be soluble and stable in phosphate buffer saline and can be readily transported inside the human cervical carcinoma HeLa cells. Combining the intrinsic properties of CNTs, flexibility of chitosan, and folic acid, FITC-CHITSWCNT-FA can be used as potential devices for targeting the drug into the tumor cells and also for imaging them [63]. Five types of CNTs suspensions were prepared by Zhang et al. by dispersing SWCNTs, acid-treated SWCNTs, MWCNTs, acid treated MWCNTs, and amylose wrapped SWCNTs, individually in water, and the effect of these scaffolds on human cervical carcinoma HeLa cells was investigated by WST-1 assay, acridine orange/ethidium bromide dual staining, and 1,1'-dioctadecyl-3,3,3',3'tetra methylindo carbocyanine perchlorate staining. The results indicated that both "dot like" and "dash like" focal adhesion kinases (FAKs) were mainly distributed at the periphery of the cells cultured on SWCNTs and on acid-treated SWCNTs and due to this they were found undergoing apoptosis with damaged cell membrane and condensed chromatin; however, cells cultured on MWCNTs, acid-treated MWCNTs, and amylose wrapped SWCNTs were found to be viable which is due to the distribution of "dot like" focal adhesion kinases (FAKs) in the whole cell body of the cells [64].

Prostate Cancer

Prostate cancer is a slow growing cancer and early propagation of cancer cells occurs before the disease become clinical. Cases of prostate cancer in USA estimates 238,590 in the year of 2013 out of which 29,720 cases of deaths due to prostate cancer have been reported in SEER stat facts sheet published by National Cancer Institute, USA. Prostate cancer antigen 3 (PCA3) has been validated as the principal molecule associated with prostate cancer (PCa). The PCA3 gene is located on the chromosome 9q21–22 and was molecularly characterized as the prostate cancer specific gene, extremely overexpressed in almost all prostate tumor specimens and PCa metastasis. Here we discuss a study using human prostate cancer cell line with respect to CNTs [65]. Li et al. developed a novel targeting SiRNA delivery system by using SWCNTs which was chemically functionalized with polyethylenimine and bound by DSPE-PEG 2000 maleimide for further conjugation with tumor targeting Asn-Gly-Arg (NGR) peptide. This novel system sufficiently crosses human prostate cancer cell PC-3 cell membrane in vitro and induces more severe apoptosis and suppression in the proliferating cells. The combine of near-infrared photo thermal therapy and RNAi significantly enhanced the antitumor activity without causing toxicity to other organs [63].

Carbon nanotubes for gene delivery

Gene therapy is an important treatment for cancer and other genetic diseases. Though, the effects of gene therapy are

limited by the efficiencies of transfection and system delivery. Since DNA and siRNA are macromolecules, they cannot pass through the cell membrane by themselves, carriers are required to take them inside of cells to take effects. Structurally, both DNA and siRNA have anionic phosphodiester backbone that can be complexed with cationic reagents, such as cationic polymers and lipids etc. For system delivery, the DNA or siRNA can be loaded into cationic nanoparticles made from cationic lipids or polymers [66]. The nanoparticles could protect them from nucleases degradation. Since CNTs are able to penetrate cells [67], they are investigated for gene delivery. Typically, three methods are used for loading nucleic acids to CNTs:

i. Electrostatic association with cationic molecule functionalized CNTs;

ii. Chemical conjugation of nucleic acids to functionalized CNTs via cleavable chemical bonds [68];

iii. DNA or siRNA are directly wrap to raw or oxidized CNTs.

Gene delivery by using cationic molecule functionalized CNTs via electrostatic interactions

As discussed early, cationic molecules, such as, polyethylene imine (PEI) and ammonium containing molecules can be covalently linked to chemically modified CNTs by oxidation or 1, 3-cycloadditions reactions. In one application, DNA was loaded into CNTs conjugated with ammonium-terminated oligo ethylene glycol(CNTs-OEG-NH3 +) for delivery. Using this transport vehicle, expression of test plasmid CMV-βgal was studied in-vitro. Result indicated that the transfection efficiency of CNTs carrier was 5-10 times higher than naked DNA; but, much lower than that of liposome [68]. It has been shown that charge ratio (ammonium groups on CNTs *vs* phosphate groups of the DNA backbone) is a determination factor for gene expression. In contrast to DNA delivery, the same CNTs carrier for delivery of cyclin A2 siRNA demonstrated pronounced silencing effect in-vitro. Surprisingly, *In-vivo* delivery of SOCS1 significantly inhibited SOCS1 expression and retarded the tumor growth in murine B16 tumor model. The studies with PEI functionalized CNTs also showed very positive results. PEI is an effective gene delivery reagent by its own, however, more amount of PEI is toxic to cells. The siRNA transport by PEI-grafted MWNTs showed improved gene expression to the equivalent amounts of PEI polymer alone but with reduced cytotoxicity [69].

Gene delivery by covalently conjugation to CNTs via cleavable chemical bonds

Alternatively, genes can be conjugated to amphiphilic polymers that are used for non-covalent CNT functionalization [70]. Incorporation of cleavable chemical bonds facilitates releasing of DNA or siRNA cargos from CNTs in a controlled manner [71]. Thiol modified DNA or siRNA were covalently conjugated to amino group of SWNT-PL-PEG- NH2via cleavable disulfide bond [72]. The genes were released by the cleavage of disulfide bonds by thiol digesting enzymes upon cellular internalization of CNT-PL-PEG-siRNA. The CNT-mediated siRNA delivery exhibited better gene transfection efficiency than liposome-based delivery system in hard-to-transfect human T cells and primary cells lines [71].

Gene delivery by wrapping directly on to the CNTs

Nucleic acids, DNA or siRNA, contains alternative amphiphilic motifs, which can be used to dissolve CNTs in water. The nucleic acids forms helical wrapping around the CNTs with the bases binding to the hydrophobic CNTs and the hydrophilic sugar-phosphate groups extending to the water phase. In this way, DNA or siRNA serves both CNT dispersing agent and the cargo. It has been revealed that the siRNA functionalized SWNTs readily enter cells and exerts its biological activity in cell culture. Studies with intratumoral injection of siRNA functionalized SWNTs showed significantly inhibition effect in-vivo [73].

CNTs for stem cell related therapies

There has been an increasing trend in attempts to design and develop different CNT based tools and devices for tissue engineering and stem cell therapy applications. In specific, CNT impregnated nanoscaffolds have shown multiple advantages over currently available scaffolds. This includes its good mechanical properties, similarity of structure with collagen fibrils and extracellular matrix and electrically conductive nature. These characteristics of the CNT based scaffolds and three dimensional nanocomposites have led to their diverse therapeutic applications in the field of myocardial therapy, muscle, neuronal regeneration and bone formation. These applications are generally based on one principle and that is to modulate the stem cell growth and differentiation in a more controlled and desirable manner.

CNTs for stem cell based heart therapy

Over the past two decades there has been significant advancement in stem cell therapy to repair and replace damaged tissues, such as heart muscle [74]. This is because of their ability to divide and differentiate into diverse specialized cell types. Recently, there has been growing body of evidence indicating that the extracellular matrix plays a critical role in stem cell viability, proliferation and differentiation [75]. Hence, designing a microenvironment prepared from polymeric scaffolds which imitate the physical characteristics of natural bio matrix has been the central strategy in tissue engineering. The development of nanomaterial's such as nanotubes provide opportunities to design such biocompatible scaffolds for hosting and directing stem cell differentiation [76]. Preliminary studies demonstrate that neonatal rat ventricular myocytes cultured on substrates of multiwall CNTs can interact with the nanofibres by forming tight contacts and show significantly improved mitotic and chemotactic effects [77]. Moreover, such mode of culture also altered the electrophysiological properties of cardiomyocytes, specifying that CNTs are capable to promote cardiomyocytes growth. Further research with a nanocomposite of PLGA: CNF show that cardiomyocytes density increases with greater amounts of CNF in PLGA [78]. The study also showed similar trends with neurons. The huge potential of this technology for myocardial therapy roots from the fact that this cardiac patch can not only promote myocardial cells, but also enhance the nerve cell growth that help the cardiac cells to

contract. In addition, it similarly supports endothelial cells that make the inner lining of the blood vessels supplying oxygen to the heart.

CNTs for stem cell based bone regeneration

In order to direct stem cell differentiation towards bone regeneration, there has been increasing attention by the researchers to explore topographical features of the cell culture substrate. Physical factors, for example rigidity of the extracellular environment, can effect stem cell growth and differentiation. This differentiation of human stem cells can be detected by altering the size of the nanotubes on which the cells are grown [79]. It has been reported that 70- to 100-nm diameter nanotubes can initiate rapid stem cell elongations, which increase cytoskeletal stress and selective differentiation into osteoblast-like cells, offering a favorable route for quicker and better recovery, for example, for patients who undergo to the orthopedic surgery. The group also exhibited that the differentiated stem cells express osteopontin and osteocalcin are the two essential osteogenetic protein markers. Moreover CNTs are promising materials for nanaoscaffold and implantation purposes due to the fact that CNTs are conductive, have tremendous mechanical properties and their nanostructured dimensions mimic the 3D structure of proteins found in extracellular matrices. Their dimensions are resembles closely with that of the triple helix of collagen fibrils which can promote for nucleation and growth of hydroxyapatite, the main inorganic component of bone. A recently developed nanocomposite scaffold of CNFs/CNTs has been shown to influence the cell behavior [80]. In-vitro study confirmed that, smaller dimension CNFs dispersed in polycarbonate urethane promoted osteoblast adhesion but did not promote the adhesion of chondrocytes, fibroblasts, and smooth muscle cells. But the mechanisms that attendant such cell functions are yet to be understood. Surface functionalizing the nanotube surface with bone morphogenetic protein-2 (BMP-2) further accelerates chondrogenic and osteogenic differentiation of MSCs [81,82]. This stimulation is a combined effect of the surface nanoscale geometry of the substrate nanostructures and their BMP-2 coating efficacy. In such kind of study, the system also exhibited higher cell proliferation rate, apart from enhanced differentiation [82,83]. Nanotubes can also be used for extended drug release has been proved by Hu et al, where drug loaded nanotubes, in combination with multilayers of gelatin and chitosan, have been revealed as a new way to use nanotubes as reservoir for storing drugs [83]. The system effectively promoted osteoblastic differentiation of MSCs. Further studies in this way can be beneficial in order to develop potential bone implants for improved bone osteo integration.

CNTs for stem cell based neuronal regeneration

The unique abilities of human embryonic stem cells (hESCs), such as their self-renewal and potency, hold abundant promise in the field of regenerative medicine and stem cell based therapy. The derivation of neuronal ancestries from hESCs holds promise to treat neurological pathologies of the central and peripheral nervous system such as multiple sclerosis, spinal cord injury, Parkinson's disease and glaucoma [84,85]. CNT based substrates have been shown to promote neuronal differentiation [86]. It has also been proposed that neurons grown on a CNT meshwork displayed better signal transmission, due to tight connection between the CNTs and neural membranes conducible to electrical shortcuts [87]. It was demonstrated that the MSCs and the neurosphere of cortex-derived neural stem cells (NSCs) can grow on the CNT array and both MSCs and NSCs interacted with the aligned CNTs. The results recommend that CNTs assist in the proliferation of MSCs and aid differentiation of cortex-derived NSCs [88]. However, due to the harsh external environment in the host body and lack of supportive substrates during transplantation, most of the transplanted cells lose its capability resulting in reduced therapeutic efficacy [89]. It has been reported that two dimensional thin film scaffolds, composed by biocompatible poly(acrylic acid) polymer grafted CNTs, can selectively differentiate human embryonic stem cells into neuron cells while maintaining the viability of transplanted cells. Even multiwalled carbon nanotube (MWNT) sheets showed to significantly enhance neural differentiation of hESCs grown on the CNT sheets. Axon outgrowth was also controlled by using nanoscale patterning of CNTs [90]. Recently, silk-CNT-based nanocomposite scaffolds are shown to protect and promote neuronal differentiation of hESCs [91]. Silks are natural polymers (protein) that have been widely used as biomaterials for many years. Fibroin, containing the major portion of the silk protein fiber, consists of 90% of amino acids including glycine, serine and alanine. Due to its good mechanical and flexible nature in thin film form, biocompatibility, and *in-vivo* bioresorbable properties, fibroin protein has been used as the building block for scaffolds. As per confirmed by scanning electron microscope (Figure5 A-C), similar results were found with the developed silk-CNT scaffold where cells grown on the silk substrate showed denser complex three-dimensional axonal bundle networks as well as better spatial density distribution of the networks compared to other scaffolds. Whole, the silk-CNT nanocomposite provided an efficient three-dimensional supporting matrix for stem cell-derived neuronal transplants, offering excellent opportunity for nerve repair treatments for patients with neurological disorder. In-vitro analysis indicated that β-III tubulin, representing the complete differentiated neurons and nestin, signifying the neuron precursors, were greatly expressed in hESCs grown on the silk-CNT substrate compared to the expression level of cells grown on the control poly-L-ornithine substrate (figure 5D). In addition, hESCs cultured on the silk-CNT scaffold exhibited higher maturity along with dense axonal projections.

CNTs for thermal destruction of tumors

Tissues are known to be highly transparent to 700- to 1,100-nm near-infrared (NIR) light, whereas, SWNTs display strong optical absorbance in this special spectral window. When continuously absorb energy in NIR region, SWNTs emit heat [92]. Continuous heating leads to killing of the cells. The SWNTs have been engineered with tumor recognition molecules for selective entering cancer cells. Upon NIR radiation, the cancer cells were killed by thermal

ablation [93,94,95,96]Previous studies have shown that folic acid decorated SWNTs more effectively killed folate receptor positive cancer cells[97]; monoclonal antibody (mAb) against human CD22 conjugated SWNTs only targeted CD22(+)CD25(-) Daudi cells; whereas, anti-CD25mAb coupled SWNTs only target CD22(-)CD25(+) activated peripheral blood mononuclear cells [98]. The thermal ablation effects can be combined with other therapies, for example Chemotherapy, by loading drugs on CNTs for synergic effect. Tumors, in general, contain a small population of tumor initiating stem-like cells, called as cancer stem cells. These cells are unmanageable by standard treatment modalities such as chemotherapy and radiotherapy and tend to persist after treatment [99]. Heat-based cancer treatments are increasingly becoming a potential alternative to approach this problem. Combining CNTs with hyperthermia based therapies can further enhance its efficacy by simultaneously eliminating both the stem cells and bulk cancer cells that constitute a tumor. In fact, CNTs offer numerous properties that make them promising candidates for such thermal therapy. This includes their capability for thermal conductance and strong absorbance of electromagnetic radiation. It produce significant amounts of heat upon excitation with near-infrared light which is transparent to biological systems including skins. Such type of photo thermal effect can be employed to induce thermal cell death in a noninvasive manner. Therefore, if CNTs can be localized to tumors, they can be stimulated by near-infrared radiation or radiofrequency energy to generate site-specific heat [100]. Preliminary in-vivo results show that a combination of multiwalled CNTs (MWNTs) and NIR can be useful for tumor regression and long-term survival in a mouse model [101]. Such CNT-mediated thermal therapy addresses the limitations of presently available medical strategies. This includes the slightly invasive site-specific heating which will greatly diminish the off-target toxicities, the uniform generation of temperature distribution throughout the tumor mass by the triggered CNTs, its compatibility with concurrent MRI temperature mapping techniques. It has also been recently stated that breast cancer stem cells, greatly resistant to conventional thermal treatments, can be effectively treated with CNT-based photo thermal therapies by promoting necrotic cell death [99]. Further studies in this direction shows that DNA-encased MWNTs are more efficient at converting NIR irradiation into heat compared to non-encased MWNTs and that this method can be effectively used in-vivo for the selective thermal ablation of cancer cells [102]. Glioblastoma multiform is the most common and aggressive malignant primary brain tumor involving glial cells and accounting for a large percentage of brain and intracranial tumor [103,104]. It is also known for its recurrence and overall resistance to therapy. CD133+ stem cells occurring among GBM cells are responsible for such huge recurrence risk [105]. Research has been focused on developing strategies to efficiently deliver CNTs to these target sites, harboring CD133+ cancer stem cells. In-vitro studies show that such targeted elimination of CD133 (+) cancer stem cells are possible by adding SWNTs functionalized with CD133 monoclonal antibody and then followed by irradiation with NIR laser light. In another study, embryonic stem cells, when administered with MWNTs, have shown to induce an enhanced immune boost and provide subsequent anticancer protection in mice with colon cancer by suppressing the proliferation and development of malignant colon tumors [106].

Vaccine delivery via CNTs

Peptide functionalized CNTs are capable of penetrating mammalian cell membrane and trans locating to the nucleus. CNTs can also bind to enzyme-linked immunosorbent assay plates, overcoming potential problems that may be encountered with the direct coating of peptide onto a solid support [107].

CNTs as protein carriers

CNTs can transport various types of proteins into the cells. The proteins must have a molecular weight less than 80 KDa, and covalently or non-covalently bound to nanotube sidewalls. Proteins bound to SWNTs transported inside the cell by endocytosis. Streptavidin, Fibrinogen, Protein-A, Bovine Serum Albumin, Erythroprotien and protein transported inside the cell by CNTs following the said mechanism [108].

Toxicity of carbon nanotubes

As has been mentioned in this paper, carbon nanotubes are a high-profile, nano-scale technology that is being considered in many technological fields. Increasingly however, concerns have been raised over potential toxicity issues with carbon nanotubes [109] and there is presently a lack of data and understanding about their impact on biological systems. Given the probable wide-spread use of CNTs in the future, it is imperative to understand their impacts on biological systems before they can be used in mainstream drug delivery. The most attractive properties of nano-materials for biomedical applications i.e. their small size, large surface area, high reactivity, and high aspect ratio, are also the main factors of potential cytotoxicity. It is thought that, although there may be several mechanisms of causing cell damage, the main way would be due to DNA damage [110].The study suggests that SWCNTs can induce adverse cellular responses through activation of molecular signaling associated with oxidative stresses (cancer inducing). Several groups have already observed that CNTs can exhibit behavior similar to that of asbestos fibres when conducting experiments on mice [111]. This concern becomes understandable when the structure of both compounds is compared. Structures of chrysotile asbestos (left) and MWCNT (right) are shown above. The main issue with asbestos (and the concern with CNTs) is that due to their nano-scale and light weight, they easily become airborne and are carried into the lungs. Asbestos is known to have caused scarring of the lungs (pulmonary fibrosis). This leads to a host of health problems and diseases due to the reduced surface area within the lung to transfer oxygen into the blood stream. CNTs resemblance to asbestos fibres, in terms of aspect ratio, bio-persistence and reactivity contribute to this concern. Whilst there is good reason to be concerned about the potential similarities to asbestos fibres, there is evidence to suggest that industrially produced MWCNTs in high doses do not result in cell death in lung epithelial (tissue) in the way that asbestos fibres do.

Additionally, long term exposure to pristine MWCNTs at low concentrations did not cause any major adverse effects [112].

Factors found to affect CNT toxicity

A list of factors that have been found to have an influence on the degree of toxicity of CNTs [113] follows below:

· Concentration / dose of CNTs.
· SWCNTs or MWCNTs
· Length of the tubes
· Catalyst residues left over during synthesis or functionalization
· Degree of aggregation
· Oxidisation
· Functionalisation.

Whilst many studies show conflicting results on some of these properties, two seem to yield the most concurrent results; concentration and functionalisation. Various studies have been conducted with regard to the effect of dose concentration on cell viability. The two parameters used to monitor this test are concentration of dose and incubation time. It has been shown using rat erythrocytes (red blood cells) that at MWCNT concentrations of 25 μg/mL no adverse effects to the cells were observed. At concentrations of 50 μg/mL however, erythrocyte haemolysis (breaking of the cell membrane) was increased. One likely explanation is that at these higher concentrations the MWCNTs agglomerate, which appears to accelerate the haemolysis process [114].

Several papers agree that high dose concentrations and prolonged incubation times both increase the induced toxicity and thus decrease cell viability. Research has shown cell viability decreases significantly in human bronchial epithelial cells [115]. The trend shows how DNA damage increases considerably with dose concentration of SWCNTs (non-functionalised SWCNT). The concentration and incubation time of a dose is an area of nanotechnology in cancer treatment which requires much further study, as it will be important to optimise these for the treatment and eradication of cancerous growths as well as to minimise the body's exposure to the drug (should it prove to have a degree of toxicity). The focus of a large body of research has been the degree to which functionalisation affects CNT toxicity. This is also likely to be one of the areas of research that receives most attention because active and passive targeting is directly related to the type and degree of functionalisation of the CNT. It has been demonstrated that increasing the degree of functionalisation of a SWCNT can dramatically decrease its cytotoxicity [116]. The executive director for the Centre for Biological and Environmental Nanotechnology (CBEN) has stated regarding this study 'it's the same answer: change the surfaces. This is an important demonstration that there are general trends in biological responses to nano-particles". Long side-chain functional groups on SWCNTs can lower toxicity and have been shown to increase the CNTs biocompatibility with cells. This property of CNTs for cancer treatment appears to be particularly promising, as the functionalisation of CNTs is essential for passive and active cancer treatment [117].

CONCLUSION

CNT represents a novel class of carriers for the delivery of drugs in a site specific and target oriented manner. CNTs possess excellent physical, chemical, and mechanical properties, which make them as a potent biological carrier to deliver anticancer drugs. Studies have clearly shown that functionalization of CNT and further derivatization with biodegradable polymers render them compatible with biological systems. Due to their unique chemistry, hexagonal arrangement of carbon atoms, various sites are available for both covalent and non-covalent functionalization with the therapeutically active molecule or protein macromolecules which envisaged the potential of CNT as nano carrier for the site specific delivery of therapeutic agent including peptides, proteins, nucleic acid, and other small drug molecules for targeting various cancer cells. With the prospect of gene therapy, cancer treatments, and innovative new answers for life-threatening diseases on the horizon, the science of nano medicine has become an ever-growing field that has an incredible ability to bypass barriers. The properties and characteristics of CNTs are still being researched heavily and scientists have barely begun to tap the potential of these structures. Single and multiple walled carbon nanotubes have already proven to serve as safer and more effective alternatives to previous drug delivery methods. They can pass through membranes, carrying therapeutic drugs, vaccines, and nucleic acids deep into the cell to targets previously unreachable. They also serve as ideal non-toxic vehicles which, in some cases, increase the solubility of the drug attached, resulting in greater efficacy and safety. Thus the CNT mediated drug delivery that specifically targets cells is a novel strategy that will have widespread applications in the near future.

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