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Carbon Nanotubes as Delivery System in Combating Diseases

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Abstract: ***Currently many chronic diseases e.g. chronic obstructive pulmonary disease, pulmonary fibrosis, cancer and metastases are treated in large part non-specifically for killing diseased cells to some extent, creating adverse systemic toxicity due to lack of proper drug selection and use. Carbon nanotubes (CNTs), especially single-walled, owing to their unique physicochemical properties such as high drug loading, cellular uptake, thermal ablation, and enhanced permeability and retention effect to differentiate normal cells from affected ones, have attracted remarkable interest in their application as nano-biotechnological delivery vehicle. In addition, their tubular form associated with axial symmetry, nanometer-sized diameters, ease of functionalizations and high aspect ratio, makes them capable for specific targeting to cells. Moreover, their appropriate surface modifications with the usages of ligands, make them unique to protect their toxic effect in the system. This review demonstrates their biomedical applications as delivery carrier against diseases.***

Keywords: Diseases; Carbon nanotubes; Properties; Specific targeting; Biomedical applications

1. Introduction

Life threatening diseases are toughest to be healed up, and most of the sufferers expire even when availed with modern developed medicinal technologies due to relapse of the original source. As the chemotherapeutic agents have their systemic toxic side effects owing to the lack of target specificity [1], considerable attempts are being directed to diminish the toxic side effect and to establish a drug delivery system that can target lead compounds to the selective diseased cells / tissues / organs. However, most of the researches is still in the preclinical stages and the results are not translated into differentiated progresses in clinics due to insufficiencies in the capability to administer therapeutics with appropriate selection and dosages, and least anticipated side effect. In addition, other disparities include the unable drug-access to diseased cells, mainly, due to the development of cellular drug resistance induced by the expressions of drug-efflux pump proteins and genes such as P-glycoprotein and other multidrug resistant proteins and genes. The conventional techniques are also associated with poor- bioavailability, insolubility, biocompatibility and systemic elimination of drugs that may cause systemic side effects. To overcome these obstacles, nanocarrier drug delivery systems have evolved a new direction towards the site specific targeting with more biological efficiencies. In this concern, carbon nanotubes (CNTs) have attracted attention as potent nanobiotechnological carrier for targeting their encapsulated components to specific cells.

CNTs are cylindrical molecules composed of carbon atoms, while graphene sheets roll into a seamless cylinder having open ended or capped like structure with a high aspect ratio, and their diameters become ~1 nm and the lengths of few µm. Single-walled carbon nanotubes (SWCNTs) are formed from a single graphene sheet while multi-walled carbon nanotubes (MWCNTs) from a few graphene sheets [2,3].

As CNTs normally exist to the fullerene carbon allotropes family having cylindrical shape due to their distinctive chemico-physical features [4,5], they not only can enable the anchorage of multiple drug components, but also can equip stealth molecules and targeting contents that may be eluded cleaning by the immune system. Thus, CNTs have higher drug loading capability compared to other nano-sized delivery systems owing to their higher surface areas and the ability of incorporation for extra therapeutic moieties either on their inner cavity or on the surface.

The tube- or fiber- like structure, especially of SWCNTs has increased their capability to acquire the extensive functionalization on the outer layer and loading of cargo for a variety of biomedical applications [6]. These shapes of the CNT have allowed them to enter the cell via various methods, such as passive diffusion across the lipid bilayer, endocytosis through their attachment to the cell-surface following engulfment by the cell membrane, or needle like penetration allowing direct cytoplasmic therapeutic payloads delivery [6-9]. They may be surface-modified for being more serum-stable and water-soluble to have less toxic at the cellular level [6,10]. Biological molecules and drugs may either attach to the nanoparticles-surface through functional groups or be encapsulated inside the CNTs by wrapping or filling binding-modes [11].

To improve their hydrophilicity, CNTs are functionalized with strong acid reactants to form a carboxylic group on their surface for increasing their dispersability in aqueous media. Alternatively, hydrophilic materials may be non-covalently or covalently anchored to the CNTs-surface [12,13] while polyethylene glycol (PEG) -coating may improve the biocompatibility, immunogenicity and hydrophilicity of CNTs [14,15]. This review demonstrates the consideration of SWCNTs as potent, efficient and therapeutic delivery system for targeting their components with thermal ablation to the specific sites of disease-development with insignificant systemic toxicity.

Synthesis, purification and functionalization of carbon nanotubes

CNTs are generally produced by three main procedures such as laser ablation / vaporization, electric arc discharge, and catalytic chemical vapour deposition.

CNTs are made by arc-vaporization of extremely pure two graphite cathode and anode electrodes placed end to end 1 mm distance in a reaction apartment filled with inert argon and helium gases at low pressure. CNTs are formed by the applications of high voltage current (50-100 amps) and 50-700 mbar pressure to generate elevated temperature emission between the two electrodes while carbon rods become vaporized and deposited on the cathode by forming rod shaped nanotubes [16,17]. The metal catalysts e.g. Pt, Ni, Co, Rh or Fe may be used for synthesizing SWCNTs [18]. In laser ablation, a solid graphite target impregnated with 1% Ni and Co is irradiated by laser graphite evaporation inside a 1200^{o}C furnace. A constant or throbbing laser is applied for vaporizing the graphite in an oven filled with inert gas for keeping the pressure at 500 torr [19,20] to synthesize highly pure, controlled size and chirality 70% yield [17]. In catalytic chemical vapour deposition, CNTs are synthesized in a quartz tube kept in an incinerator. Ethane, ethylene, methane, propylene, toluene, benzene, acetylene -hydrocarbons and electron beam i.e. resistive heating are utilized as raw substances and energy-source respectively while the molecules are decomposed as the reactive carbon species at 500-1000^{o}C [21]. After catalyst-deposition on substrate, catalyst-nucleation through chemical etching with ammonia or thermal annealing takes place. Source of carbon is then placed in a reaction apartment in gaseous stage, and allowed to transform into atomic state for diffusing towards shored metal catalyst on which CNTs- growth occurs [22,17].

After synthesis, CNTs are purified by surfactant aided sonication, acid refluxing, or air oxidation process for the elimination of fullerenes, transition metals and amorphous carbon contaminants used as catalysts for the synthesizing process [23-25].

Owing to the integral hydrophobic essence, affinity to agglomerate and water insolubility, CNTs are generally not suitable for targeted components delivery and therefore, needed surface modifications. The functionalizations of CNTs, not only increase their dispersabilities / solubilities, but also provide active functional regions to attach targeting ligands, drugs and other components to reduce their toxicity [26,27]. Depending on the nature of the biomolecules, CNTs are functionalized by forming covalent chemical bond and physio-adsorbing non-covalent linkages [28,29]. Ahead of covalent modification, carboxylic acid (-COOH) groups on CNTs-surfaces are allowed to become activated by utilizing reagents such as oxalyl chloride ($C_2O_2Cl_2$), thionyl chloride ($SOCl_2$) or N-hydroxysuccinimide (NHS) to get highly reactive intermediates to form strong covalent bondings between biomolecules and nanotubes [30]. Several oxygenated groups like carboxylic, hydroxyl, alcohol, phenolic and ketone are presented at 'end or defect' zones by oxidative treatments while oxidized groups are produced by the treatment of CNTs with the strong oxidative reagents -mixture (NH_4OH:H_2O_2::50:50 or H_2SO_4:HNO_3::3:1) not only to shorten the length of CNTs but also to form the oxidative groups on the surface of CNTs [31]. By altering the type, reaction conditions or acid-concentrations e.g. time duration, temperature and sonication, different functional groups may be integrated for increasing solubility / dispersion of CNTs [32]. Different functional groups may be anchored onto CNTs-surfaces by gas stage reactions while covalent side-wall modification generates sp^3 hybridized carbon zones. Different methods for side-wall modifications may be performed through esterification-amidation reaction, 1,3-dipole cyclo-addition reaction, grafting of polymers, Diels-Alder cyclo-addition reaction, and mechano-chemical functionalization [21,33]. Non-covalent functionalizations consist of hydrophobic, π-π and Van der Waals – interactions. In general, three types of molecules such as polymers, surfactants and biopolymers (nucleic acids and peptides) are engaged for CNTs-functionalizations [32]. Non-covalent functionalizations include Van der Waals interactions with biopolymers and lipids, π-π stacking with nucleic acids and aromatic organic compounds, and hydrophobic interactions with proteins and fluorophores. After surface modifications, CNTs become hydrophilic and suitable to link drugs or other components such as proteins, genes, enzymes, DNA for their targeted delivery to cells or organs.

Characterizations of carbon nanotubes

To determine various characteristic properties i.e. shape, size, solubility, purity, thermal conductivity and electromechanical features of CNTs, several instrumental analytical techniques are utilized. These include atomic force microscopy, scanning electron microscopy, transmission electron microscopy, infrared spectroscopy, thermo-gravimetric analysis, Raman spectroscopy and nuclear magnetic resonance.

Mechanism of action of carbon nanotubes

The toxic inductions of CNTs may be influenced by their physicochemical properties, i.e. shape, size, charge, agglomeration state, purity and morphology [34]. Owing to their nanosize and high surface area to volume ratio, they can penetrate cell membrane through a winding or spiralling motion, or may be endocytosed / phagocytosed via phagocytosis, macro-pinocytosis, clathrin-mediated endocytosis, caveolin / clathrin independent pathways or caveolin mediated pathways [35]. Their attachment or internalization to cells causes cell / organelles -membranes leaky / damage or induces oxidative stress generating reactive oxygen species (ROS) while ROS may lead to cellular apoptosis, necrosis, amino acids / nucleic acids / lipids -oxidations, DNA damage and enzymes-inactivation [36-38]. Higher level of oxidative stress may reduce the levels of systemic antioxidants such as glutathione (GSH), superoxide dismutase (SOD) and catalase. The presence of ROS and transition metal impurities may lead to inflammations and cytotoxicity resulting in cell death [39]. SWCNTs may activate MAPK, AP-1, NF-κβ and Akt signalling through the inductions of oxygen and hydroxyl radicals -generation and the elevations of the pro-inflammatory IL-8 and IL-1β cytokines to make the cells unviable [40-43]. In this concern, apoptosis of the cell may result from NADH-oxidase dependent mitochondrial dysfunction and release of pro-apoptotic factors associated with cytochrome c release and diminished cellular ATP content while DNA breakage with elevated levels of tumor suppressor proteins such as p21, p53 and bax and a relevant

G2/M cell cycle blockage to induce cell mortality by the activity of CNTs [44-46].

Carbon nanotubes as delivery vehicle

Some researchers have reported that carboxylated SWCNTs have capability to scavenge free radicals due to their presence of –COOH groups and are useful as antioxidant for the treatment of ageing and chronic ailments [47-49].

CNTs have now been exploited to apply biomedically in drug and gene deliveries, cancer and neurological degeneration therapies owing to their extraordinary, combinatorial electrical, mechanical and optical characteristic features [50]. Their functionalized moieties make them suitable for specific targeting interactions with cell surface receptors for internalization with low but maximum amount of components loading for disease treatment, minimizing systemic inflammation and toxicity [51].

The pore size of blood vessels in healthy tissues becomes 2-6 nm in contrast to tumor tissues (100-800 nm) [52]. The nanoparticles having sizes more than 6 nm, cannot pierce the healthy blood vessels but pass comfortably inside the tumor blood vessels and become accumulated at the tumor zones. The surface functionalizations of CNTs with ligands such as PEG, chitosan, sugar, protein, antibody, genes and other components make them suitable for specific targeting and controlled liberation in tumor environment at low pH due to enhanced EPR effect to overcome multidrug resistance and to kill cancer cells [51,53-60]. Additionally, utilizing a non-covalent perspective, peptide-functionalized SWCNTs stacked with tamoxifen exhibited efficient tumor targeting as well as high anti-carcinogenic effect [61]. Some investigators used SWCNTs as carrier for immunizing agent for delivering tumor antigens to induce immune responses against tumors [62,63].

Acetylcholine (Ach)-deprivation is associated with age-related diseases such as alzheimeris, perkinsonosis where neurons become unable to synthesize it. Researchers have shown that SWCNTs are capable to deliver Ach into the mice brain through transcytosis overcoming BBB and enzymatic degradation [64,65].

Other researchers showed that covalent functionalizations of SWCNTs with ester and amide bonds not only target diseased cells and organelles but also improve their half-lives and solubility with maximum amount components-delivery [66-68].

Ammonium surface functionalized SWCNTs have been exploited to deliver siRNA telomerase reverse transcriptase (TERT) into tumor cells to reduce their growth while siRNA-release silences the targeted TERT gene [69]. Ammonium functionalized DNA-SWCNTs complex multifunctionalized with phospholipid-PEG bearing tumor targeting folic acid, cationically modified CNTs coated with polyethylene-imine or pyridinium moieties with siRNA showed antitumor efficiency, enhanced silencing activity and reduced toxicity [70,71].

The hyperthermia therapy utilizing CNTs has suggested also an efficient strategy for the cancer treatment as these nanomaterials can generate heat significantly upon excitation with near-infrared region (NIR) light [72-74]. In this aspect, SWCNTs show high absorbance in the 700-1100 nm NIR while their photothermal consequence can induct the regional thermal tumor cells removal by massive heating of accumulated SWCNTs in the tumor site.

Moreover, CNTs themselves might have microbicidal activities via intra cellular oxidation of reduced glutathione leading to enhanced oxidative stress on the microbial cells and subsequent pathogen death [75].

Biodistribution, pharmacokinetics and elimination of carbon nanotubes

The biodistribution as well as pharmacokinetics of CNTs depend upon their chemico-physical features i.e. surface modifications, shape, aggregation, chemical composition and solubility. To assess their *in vivo* distribution, different radionuclide-based imaging techniques, mainly magnetic resonance imaging, positron emission tomography, single-photon emission computer tomography, photoacoustic imaging and optical imaging are used. Different surface coatings of CNTs can affect significantly their biocompatibility profile, blood circulation half-lives and agglomeration via Van der Waals forces. It has been reported that the half-life of blood circulation was increased to ~22.5 h after covalent SWCNTs -PEGylation whereas ~22 h after non-covalent PEGylation with PEG coated poly maleic anhydride-alt-1-octadecene when administered intravenously into mice [76,77], while pluoronic coating of CNTs showed ~1 h half-life of blood circulation [78].

Length of CNTs affects greatly *in vivo* pharmacokinetics owing to their alike one-dimensional shape while macrophages can engulf 220 nm -long MWCNTs more easily than 825 nm -long [79]. CNTs having length 100-300 nm are normally accumulated in the liver and spleen [80-82], 300nm-2µm undergone fast renal excretion due to their nanosize diameter (<5 nm) [83-85], and >2 µm undergone high accumulation in the lungs and significant retention in the spleen and liver [86], while long and needle-shaped CNTs influenced drastically their pharmacokinetic values [87].

It has been reported that SWCNTs can agglomerate inside the body by forming fiber-like constructions after intra-peritoneal entry, and form granuloma for the length >10µm while shorter agglomerates (<300nm) retain for upto 5 months without forming granuloma in the mouse-body [88]. Both MWCNTs and SWCNTs were also found to be excreted into the urine and the feces [89,90,82]. It has also been reported that CNTs may be broken down by neutrophilic myeloperoxidase into carbon dioxide and water significantly to be expelled out from the body monitored in mice [91, 23].

Interactions of carbon nanotubes with the immune system

It has been reported that CNTs may be identified by host immune system through the activation of the complement system consisted of over thirty five glycoproteins and proteins and the production of the inflammatory peptides C4a, C5a(c) and C3a [92,93] . In this concern, not only most functionalized and non-functionalized CNTs can induct the complement system, but also the other lectin pathway can also be induced by several CNTs to trigger the complement systems, while functionalization of CNTs can minimize the complement activation [94]. In addition, high concentration of MWCNTs can induce genes-over-expressions related to immune response and inflammation, while exposure of MWCNTs to HEK293 cells for 48 h caused a time-dependent remarkable IL-8 increment [45,95]. In this aspect, length and type of CNTs, metal catalyst impurities, functionalization, solubilising agent, aggregation and cell type may play a crucial role in the biological responses including oxidative stress, genotoxicity and membrane-injury to CNTs.

2. Conclusion and Future Perspectives

Carbon nanotubes are the novel class of nanomaterials for nanobiotechnological applications as they can move through cell membranes taking biomolecules, drugs, vaccines, genes and other components to the deep into the target diseased cells or organs overcoming all the biological barriers compared to unreachable ancient drug delivery methods. Though CNTs may be surface-modified with different biomolecules either non-covalently or covalently to reduce their cytotoxicity and enhance biocompatibility and to target to the specific site, the cytotoxicity has persisted as the restricted aspect for the uses of CNTs in the biological system. As the bioactivity of MWCNTs depends on their length, diameter and functionalization, and the toxicity of SWCNTs depends on their length and size associated with metal impurities, leading to oxidative damages [96-98], it is needed to emphasize more in the synthesis, design and proper functionalization to develop especially short length and nanosize SWCNTs with appropriate surface modification and / or coating with diseased cell oriented polymers / biopolymers / ligands to improve their site specificities, efficacies and controlled bioactive release to the target sites resulting insignificant side effects. In this concern, a short and long -term thorough *in vivo* study evaluating biodistribution, pharmacokinetics, biocompatibility, intracellular fate and possible cytotoxicity of intravenous and oral administered SWCNTs against normal and diseased animals are required to develop clinically-ready nanotube-based carrier with proper dose for biomedical therapy.

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