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Teaser The present review provides a novel platform to scientist for CNTs and their conjugation chemistry, interactions, conjugation, and their potential biological applications in drug delivery perspectives.

Interactions between carbon nanotubes and bioactives: a drug delivery perspective

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Applications of carbon nanotubes (CNTs) in the biomedical arena have gained increased attention over the past decade. Surface engineering of CNTs by covalent and noncovalent modifications enables site-specific drug delivery and targeting. CNTs are available as single-, double-, triple-, and multiwalled carbon nanotubes (SWCNTs, DWCNTs, TWCNTs, and MWCNTs, respectively) and have unique physicochemical properties, including a high surface area, high loading efficiency, good biocompatibility, low toxicity, ultra lightweight, rich surface chemistry, non-immunogenicity, and photoluminescence. In this review, we highlight current understanding of the different types of physical and chemical interaction that occur between therapeutics and CNTs, and the potential application of the latter in drug delivery and imaging. Such understanding will aid exploration of the utility of multifunctional CNTs as pharmaceutical nanocarriers, and potential safety and toxicity issues.

Multifunctional CNTs: a new contour in drug delivery and targeting

Carbon nanomaterials, including carbon nanohorns (CNHs), graphenes (GRs), carbon nanorods (CNRs), polyhydroxy fullerenes (PHF) and CNTs, represent safe and efficacious carrier systems for drug delivery and drug targeting because of their unique physicochemical properties. CNTs were first discovered by Roger Bacon in 1960, and were described fully by Sumio Iijima. CNTs are now the focus of many studies exploring their applications in drug delivery and drug targeting, as well as cosmetic products [1,2].

CNTs are ultra-light-weight, tubular, hollow monolithic structures, with a high surface:aspect ratio (length/diameter), rich functional surface chemistry and high drug-loading capacity. They are also biocompatible, nonimmunogenic, and photoluminescent, making them attractive nanocarriers for drug delivery and imaging. CNTs do not require any type of fluorescent labeling for detection because they can be detected directly because of their electron emission properties [3–6]. CNTs are available as SWCNTs, DWCNTs, TWCNTs and MWCNTs, with cylindrical graphitic layers [7–10].

Functionalization is a well-known approach for altering the surface of nanocarriers by attaching a variety of different bioactives. Functionalized CNTs (*f*-CNTs) have been used to

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deliver both lipophilic (paclitaxel and docetaxel) [11,12], and hydrophilic drugs (doxorubicin hydrochloride; DOX) [13–16]. CNTs readily cross different biological barriers, passing through the plasma membrane and entering the cytoplasm through a 'tiny nanoneedle' mechanism, which facilitates the transport and delivery of the cargo molecules or therapeutics into the target tissue [2,6,17].

Purification, dispersion, and oxidation of CNTs

The physicochemical properties of CNTs, such as surface topography, solubility, hybridization state, mechanical properties, thermal conductivity, and structural and metallic or carbonaceous impurities, need to be determined before they can be used in pharmaceutical and biomedical applications [2,10,18]. There are several factors (e.g., metal content, oxidation time, and oxidizing agents and temperature used) that affect the purification efficiency and yield of CNTs. The need for a mixture of strong acids (oxidizing agents) and corrosive solutions results in safety issues and, therefore, appropriate precautions, such as the use of acid-resistant gloves and adequate shielding, must be taken during their production. Strong acid treatment not only removes any metallic impurities, but also cuts the nanotubes into shorter pieces, generating oxygen-containing functional groups, such carboxylic (–COOH) and hydroxyl (–OH), around the sidewalls and tips of the tubes, where the curvature of the tubes results in a higher strain on nanotubes structure [18–20]. The effect of oxidation on the structural integrity of nanotubes was studied following acidic (nitric acid and a mixture of sulfuric acid and hydrogen peroxide) and basic (ammonium hydroxide/hydrogen peroxide) oxidation processes. The increase in the number of surface oxygens per chemical treatment (oxidation) followed the order: hydrochloric acid (HCl) < ammonium hydroxide (NH_4OH)/hydrogen peroxide (H_2O_2) < piranha (H_2SO_4:HNO_3) < refluxed nitric acid (HNO_3). Oxidation of CNTs with HNO_3 under extreme conditions increases the formation of defective CNTS because of shortening of the length of nanotube [18].

Recently, Chajara and co-workers developed a fast, microwave-assisted, organic solvent-free method for the efficient primary purification of nanotubes [21]. The method dissociates and disperses nonnanotube carbon in an organic solvent, resulting in CNTs of high purity in few minutes, and with low few defects [21]. Alternatively, strong acids have also used to oxidize CNTs for improving their dispersibility and purification [22]. The five methods used for the dispersion of hydrophobic nanotubes are: (i) dispersion, reaction; (ii) dissolution, dispersion, precipitation; (iii) dispersion, dispersion, precipitation; (iv) melt, powder, mixing; and (v) no fluid mixing (reviewed in [23]). Methods (iv) and (v) do not use any solvents [23].

The use of surfactants, such as sodium dodecyl sulfate (SDS) and sodium dodecyl benzene sulfonate (SDBS), as coating agents to improve the dispersibility of CNTs results in better long-term stability. As an alternative, chitosan (CHI; a natural cationic polysaccharide biopolymer obtained from the deacetylation of chitin) can also be grafted onto the nanotube surface because of its nontoxic, biocompatible and biodegradable properties [24,25]. CHI is an attractive way to encapsulate CNTs through hydrogen bonding; for example, it enhanced the stability and sustained release *in vitro* of DOX (degradation of chitosan and diffusion through chitosan shell) from DOX-loaded CHI–folic acid conjugated CNTs (CHI-FA-CNTs) as a result of their hydrophilic and cationic charges [24].

Horie and coworkers examined the cellular influences of chemical or biological reagents, such as pluronic F-127 and F-68, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC, the pulmonary surfactant preparation Surfactent®), bovine serum albumin (BSA), and Tween 80 as dispersants of CNTs in an experiment with the human lung carcinoma A-549 cell line [26]. The adsorbed dispersant on the surface of the nanotubes was shown to induce oxidative stress in the cells [26]. The poly-L-lysine (PLL)–MWCNTs aqueous solution after appropriate modification enhanced the aqueous dispersibility. After sonication, the black MWCNT–PLL aqueous solution was stable for up to 240 days at pH 5.0 (Fig. 1) [27]. The dispersion of MWCNT–SDBS (453 nm) was better compared with that of MWCNT–PLL (488 nm) and MWCNT–SDS (758 nm); however, tangle and aggregates were seen in water with unfunctionalized MWCNTs (899 nm). These results show clearly that SDS, SDBS, and PLL improve the aqueous dispersibility of MWCNTs, and that MWCNT–SDBS and MWCNT–PLL disperse better than MWCNT–SDS. Galactosylated CHI-grafted oxidized MWCNTs (*O*-CNTs-LCH-DOX) were synthesized for pH-dependent sustained release and hepatic tumor targeting of DOX. The particle sizes of *O*-CNTs, *O*-CNTs-LCH, and *O*-CNTs-LCH-DOX were 176.1 ± 2.4, 217.5 ± 3.2, and 286.3 ± 4.1, respectively, with a polydispersity index (PDI) of 0.39 ± 0.02, 0.35 ± 0.06, and 0.31 ± 0.01, respectively. A venous irritation study was performed on New Zealand white rabbits after intravenous injection of *O*-CNTs, *O*-CNTs-LCH, and *O*-CNTs-LCH-DOX in normal saline at a 5-mg/kg DOX dose for three consecutive days. The *O*-CNTs-LCH-DOX formulation showed good biocompatibility, low toxicity, higher cellular uptake, and higher antitumor activity, as well decreased vascular irritation (Fig. 1), compared with *O*-CNTs, *O*-CNTs-LCH, and free DOX in HepG2 cells [28].

The potential of biosurfactants to aid the effective dispersion of nanotubes needs to be explored further to render the CNTs safer. Only after obtaining a clear dispersion of CNTs using various chemical functionalization strategies, will we begin to understand the interactions of CNTs with therapeutics.

CNT interactions with theragnostics

CNTs can be considered as good adsorbents because of their ability to interact with guest molecules via different mechanisms on their surface. The adsorption of guest molecules into CNT bundles can occur inside the tubes (internal sites), in the interstitial triangular channels between the tubes, on the outer surface of the bundle (external sites), or in the grooves (major and minor) formed at the contacts between adjacent tubes. The influence of chemical modifications resulting from acid treatment, followed by triethylenetetraamine (TETA), has been studied at each stage of chemical treatment using different analytical tools (Fig. 2) [22]. However, unavoidable imperfections or vacancies, such as Stone–Wales defects, pentagons, heptagons, and dopants, have a crucial role in determining the adsorption properties of CNTs [29,30]. Apart from these imperfections, five interactions (i.e., hydrogen bonding, hydrophobic effects, covalent, electrostatic, and π–π stacking interactions) could have a role in the attachment of biomolecules [31]. In terms of the closed ends of CNTs, small molecules are easily