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REVIEW

Functionalized Carbon Nanotubes: Towards the Delivery of Therapeutic Molecules

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In the last decade, carbon nanotubes (CNT) have attracted the attention of several scientists, due to their unique and intriguing structure and properties. This is because it is possible to modify them, through several procedures, with a wide variety of molecules. The present report is focused on the potential applications of functionalized CNT (*f*-CNT) as viable delivery devices. In particular, the recent integration of CNT into biological systems has suggested their potential employment in the delivery of therapeutic molecules, such as drugs, antigens and genes.

Keywords: Carbon Nanotubes, Peptides, Proteins, Drug Delivery, Vaccine, Gene Delivery, Toxicity, Supramolecular Complexes.

CONTENTS

1. INTRODUCTION

The word "nano" defines the scale that is used to describe systems applied in the field of nanosciences.[1] Systems having small dimensions hold a lot of potential for their use in everyday life. In fact, especially in the field of life science, there is an increasing demand for novel nanosystems that are able to deliver for example active molecules to specific sites of action, preventing from side effects. In fact, particles of such dimensions generally show different structural, mechanical and electronic properties if compared to their macroscopic counterparts. As a consequence, new interesting applications can be envisaged. Nanosystems can exhibit advantages over existing technologies not only in terms of reduced size, but also in novel performance. For example, this is the case of nanodevices applicable in biomedical applications, which require biocompatibility and specificity. To be able to achieve such properties, chemical derivatization of the nanomaterial is desirable to improve their solubility and processability. Presently there are several ways, including microemulsions,[2] cyclodextrins,[3] liposomes and micelles,[4] polymers and tubes that are of particular interest to the field of drug delivery.[5] Within the different nanoobjects, which are currently available,[6–8] carbon nanotubes hold a lot of promise for applications in material sciences and medicinal chemistry.

This paper is mainly dedicated to the methodologies of manipulating CNT, with the aim of better use for medicinal purposes. Initially, a brief introduction and description of the different strategies for the functionalization of CNT

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will permit to comprehend how they can be made biocompatible. Then, the most promising studies concerning viable applications of CNT as new tools for biomaterial devices and delivery systems, as well as their toxicity properties will be described.

2. CARBON NANOTUBES

In the last few years, carbon nanotubes have been shown to possess intriguing properties, much more interesting than those connected with their initial definition as simply "tubular fullerenes." Fullerenes, and its most abundant form C_{60}, represent the third allotropic structure of crystalline carbon with diamond and graphite. CNT can be visualized by cutting C_{60} along the centre and connecting the two end-caps with a cylinder of graphite of the same diameter.

There are two main types of CNT: (i) the single-walled carbon nanotubes (SWNT), which are made up of a single graphene layer wrapped into a cylindrical structure,

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Alberto Bianco received his Laurea degree in Chemistry in 1992 and his Ph.D. in 1995 from the University of Padova, under the supervision of Professor Claudio Toniolo, working on fullerene-based amino acids and peptides. As a visiting scientist, he worked at the University of Lausanne during 1992 (with Professor Manfred Mutter), at the University of Tübingen in 1996–1997 (with Professor Günther Jung, as an Alexander von Humboldt fellow) and at the University of Padova in 1997–1998 (with Professor Gianfranco Scorrano). He currently has a position as a Researcher at CNRS in Strasbourg. His research interests focus on the synthesis of pseudopeptides and their application in immunotherapy, solid-phase organic and combinatorial chemistry of heterocyclic molecules, HRMAS NMR spectroscopy, and functionalization and biological applications of fullerenes and carbon nanotubes.

and (ii) multi-walled carbon nanotubes (MWNT), with a central tubule of nanometric diameter, surrounded by several graphitic layers spaced by a distance of about 0.34 nm. Most commonly, the diameter range of CNT varies between 0.4 and 2 nm for SWNT and between 1.4 and 100 nm for MWNT,[9] while the length for both can be as long as several microns (Fig. 1). Typically, SWNT exist as bundles of 10–30 nm in diameter because of strong van der Waals interactions, resulting in ropes, which are tangled with one another like "spaghetti."

Although CNT were first identified in the late 50s,[10] only from 1991 they started to be isolated in reasonable amounts after being generated during deposition of graphite on the negative electrode, using the arc discharge process for the preparation of fullerenes.[11] Since then, a wide variety of methods, including catalytic chemical vapor deposition (CCVD),[12] laser ablation or pulsed laser vaporization (PLV)[13, 14] and HiPCO[15] processes, have been optimized. All these methods allow to produce CNT, containing undesired impurities, usually metal catalyst particles (mainly Fe and Ni), and amorphous carbon. The presence of these contaminants can be reduced using either oxidative acid treatments or by simply diminishing the proportion of the hydrocarbon and mixing hydrogen in the process of production.[16] However, the oxidative treatment has the disadvantage to reduce the size of the tubes and to increase the number of structural defects. The resulting defective structures comprise topological variations (ring sizes different from hexagons), rehybridization (from sp^2 to sp^3), incomplete bonding defects and doping with other atoms than carbon.[17] These modifications affect locally the mechanical properties of the system. In atomic simulation studies,[18] it has been demonstrated that nanotube stiffness generally decreases with topological defects, especially if they are placed close to each other, while it is not significantly influenced by the curvature or by the diameter of the CNT. Moreover, the rigidity of CNT is influenced by the functionalization of nanotubes, mainly due to the change in hybridization of the carbon atoms, which can be reversibly converted to sp^2 again. In fact, thermal treatments lead to elimination of the functional groups from the CNT surface.[19] The defunctionalization of *f*-CNT has been proposed as an alternative method of CNT purification. The external walls of CNT have been functionalized via 1,3 dipolar cycloaddition, leaving insoluble metal particles as impurities. Carbon impurities were then eliminated by careful precipitation of *f*-CNT. Finally, heating at 350 °C under an inert atmosphere, followed by annealing at 900 °C permitted the recovery of pure and defect-free CNT.[20]

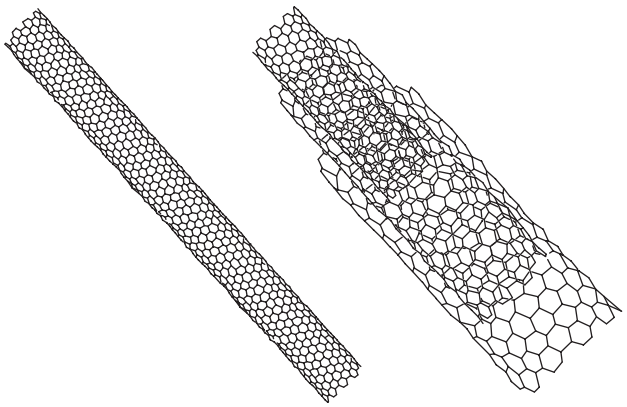

Fig. 1. Molecular structures of CNT fragments: (left) SWNT and (right) MWNT. The distance between the MWNT graphene layers is 0.34 nm.

3. PROPERTIES OF CNT

Purified CNT can exhibit metallic conductivity, chemical and thermal stability and extremely high tensile strength and elasticity. The electrical properties of CNT are due, to a large extent, to the peculiar structure of the graphene nature. While MWNT are always semiconducting, SWNT can be defined, using a vector **r** and a chiral angle θ (Fig. 2), through the Eq. 1,

$$\mathbf{r} = n\mathbf{a} + m\mathbf{b} \tag{1}$$

where **a** and **b** are vectors of the basic hexagon, and n and m are integers. If $(n-m)$ is a multiple of 3, the tubes exhibit metallic properties; otherwise they are semiconducting. In particular, depending on the roll-up vector **r**, three main structures can be visualized for the SWNT: the "armchair" (with $n = m$, and $\theta = 30°$), the "zigzag" (with n or $m = 0$, and $\theta = 0°$) and the chiral (with θ between 0° and 30°) states, which differ not only in their physical aspect, but also in showing different properties. The first one represents a metallic species, while the others can be semiconducting or metallic. In addition, SWNT show large thermoelectric power (TEP) with hole-like behavior at high temperatures.[21] Theoretical calculations on a metallic SWNT predict values significantly lower than the measured ones, which is justified by the self-assembly of nanotubes into crystalline ropes.

CNT have been thoroughly investigated for their potential use in materials science, particularly in relation to the development of new biosensors, and as substrates

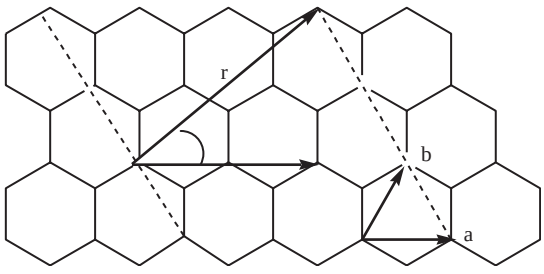

Fig. 2. Scheme to create the nanotubes cylinders from planar graphene sheets: a and b are primitive vectors of the hexagonal lattice, r is a roll-up vector.

for neuronal growth and tissue engineering.[22] Moreover, the possibility to be functionalized with different organic moieties, without being toxic to cells, suggested useful applications in biological devices, especially as delivery systems and scaffolds.[23]

4. SOLUBILIZATION

One of the limiting factors for the application of CNT in a biological context is their extremely low solubility both in organic and aqueous solvents. Several attempts have been made to overcome this problem, mainly focusing on different possibilities of sidewall functionalization. Although the dispersion of CNT in a specific medium is strongly dependent on: (a) the method of their production; (b) the amount of impurities and (c) the consequent purification steps; there are at least two main procedures to increase their solubility. One corresponds to the non covalent functionalization and the other to the covalent functionalization.

4.1. Non Covalent Functionalization

This methodology offers the advantage to preserve the aromatic structure of the nanotubes and thus their electronic characteristics. Strategies for non covalent modification include the use of: (a) organic solvents,[24] (b) organic polymers,[25, 26] (c) amphiphilic peptides,[27] (d) nucleic acids[28] and sugars,[3] and (e) detergents.[2, 29] Table I summarizes the different types of polymers and biopolymers used to bring CNT into solution.

In a recent report, it has been shown that it was possible to obtain sidewall functionalization of SWNT with proteins, maintaining high control and specificity.[30] In particular, 1-pyrenebutanoic acid, activated as succinimidyl ester, was irreversibly adsorbed on the hydrophobic surface of SWNT through π–π stacking interactions, leading to a system which was very reactive towards the amino groups of different proteins, including ferritin and streptavidin (Fig. 3). The same method was used to increase the solubility in water of CNT through the coupling of a pyrene derivative with ammonium groups.[31] In a different approach, SWNT were wrapped with conjugated polymers, such as poly(m-phenylenevinylene), able to induce an eightfold increase in electrical conductivity simply surrounding bundles of tubes, rather than wrapping around individual tubes.[32] Interestingly, CNT solubilization was also achieved using an amphipatic α-helical peptide, whose hydrophobic face interacted non-covalently with the surface of CNT, while the hydrophilic part resided in the aqueous phase through polar interactions. Such system did not only coat and solubilize carbon nanotubes, but also regulated the reassembly into supramolecular structures.[27] It was also possible to conjugate SWNT with surfactants, such as Tween 20 or Triton X100, which rendered the surface of the tubes highly hydrophilic and charge-neutral, thereby eliminating hydrophobic interactions and nonspecific electrostatic binding with proteins.[29] The co-adsorption of SWNT with Tween and poly(ethyleneglycol) allowed to achieve the formation of a complex able to selectively bind streptavidin while hampering adsorption of other proteins.[2] In addition, this procedure was used to analyze specific antigen–antibody interactions. In fact Tween 20 is a surfactant comprising a linear aliphatic chain and three polyethylene oxide (PEO) branches. It can be irreversibly adsorbed onto nanotubes. The three PEO-hydroxyl termini of Tween-coated nanotubes can subsequently be activated and coupled with a prominent autoantigen, which plays a crucial role in Systemic Lupus Erythematosus (SLE), an autoimmune disease that is characterized by complement deficiencies, modification of cytokine secretion, hyperglobulinemia and production of autoantibodies in particular against DNA. Recent studies have shown that such antigen, immobilized on PEO-CNT, retained its antigenicity, by binding to such antibodies specifically, confirming that it represents a useful target in the specific pathological condition.[33]

Overall, this kind of functionalization offers the possibility to associate CNT with several molecules and to produce nanotube biosensors for potential medical diagnostic

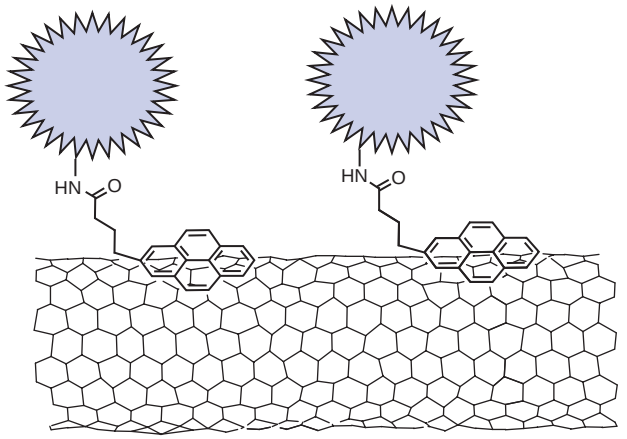

Fig. 3. Adsorption of 1-pyrenebutanoic acid onto SWNT as an anchor for protein immobilization.

Table I. Methods for CNT solubilization and dispersion based on non covalent functionalization.

Type of CNT	Type of solubilizing molecules	Reference	Solubility (mg/ml)
Non covalent functionalization			
SWNT	1-Pyrenebutanoic succinimidyl	30	—
SWNT	Poly(m-phenylenevinylene) ester	32	~0.5 (DMF or $CHCl_3$)
SWNT	α-Helical amphipatic peptide	27	0.7 (H_2O)
SWNT	Nucleic acids	28	—
SWNT	Surfactants: Tween 20	33	0.05 (H_2O)
	Triton X100	33	≤0.5 (H_2O)
	SDS	25	≤0.1 (H_2O)
	Surfactants + Polymer(PEG)	2, 29	—
SWNT	Starch	3	0.5 (H_2O)

and biological applications. However, the major problem of aggregation and precipitation after the release of the bioactive molecule from its complex with CNT, and the related undesirable effects, remain to be solved (*vide infra*).[34]

4.2. Covalent Functionalization

The chemical functionalization of CNT is based on two main approaches: (i) esterification or amidation of oxidized tubes[35] and (ii) side-wall covalent attachment of functional groups[36–41] (Table II). Concerning the first approach, the oxidation process is conducted under strong acid conditions. This treatment provokes the opening of CNT end-caps, generating carboxylic groups suitable for further derivatization.[35] In addition, carboxylic functions are created where defects of the nanotubes side walls are present. On the other hand, the direct sidewall functionalization of CNT with organic groups is possible by using reactive species such as nitrenes, carbenes and radicals.[36] To this end, alkyl azides, dipyridyl imidazolium salts and perfluoroalkyl radicals were employed, respectively, as efficient reagents.[37] The high solubility of the resulting adducts can be explained through mutual, electrostatic repulsion of the tubes.

The solvent-free functionalization technique can be considered as particularly innovative,[38] since it reduces the nanotube ability to bundle and even increases the exfoliation into individual tubes. By this procedure an aniline is treated with isoamylnitrite giving an arenediazonium salt, which then generates a reactive species that attacks the CNT. A mild heating is necessary to reduce the viscosity of the mixture, which is available to further polymer addition, offering the advantage of a scalable, cost-effective and environmentally benign derivatization.

Recently, the sidewall functionalization of SWNT and MWNT has been developed, through the 1,3-dipolar cycloaddition of azomethine ylides, a reaction successfully applied to fullerenes.[42] The easiest way for the preparation of such derivatives consists on the decarboxylation of immonium salts obtained by the reaction of an α-amino acid with an aldehyde.[43] In particular, a glycine modified at the N-terminus with a N-Boc-protected-triethylene glycol was proposed because of its high solubilizing power after removal of the Boc group. Both SWNT and MWNT with the free amino function have become extremely soluble in aqueous solvents and ready for further derivatization (Fig. 4).

Table II. Methods for CNT solubilization and dispersion based on covalent functionalization.

Type of CNT	Type of functionalization	Reference	Solubility (mg/ml)
Covalent funtionalization			
SWNT/ MWNT	Oxidation process HNO_3/H_2SO_4	35	1.77 (H_2O)
SWNT/ MWNT	Nitrenes	36, 37	1.2 (DMSO or TCE)
	Carbenes	36	—
	Radicals	36	—
SWNT	Solvent-free functionalization	38	—
	Fluorination/Amination	39	—
	Reduction of diazonium salts	40	—
	Electrophilic addition	41	—
SWNT/ MWNT	1,3-Dipolar cycloaddition	43	~20 (H_2O)

4.3. Modification with Natural or Bioactive Species

CNT represent an alternative to spherical particles, showing the interesting advantage of incorporating molecules (from small drugs to proteins) in their large inner volume. Moreover, their inner and outer surfaces have different characteristics, offering the possibility of loading the inner space with biologically active species and, at the same time, modifying the outer surface to make the nanotubes biocompatible. In addition, CNT could have open ends and functionalized surfaces, which would improve their interaction with molecular components abundant in living organisms, including carbohydrates, DNA and proteins. Starch, for example, is a natural carbohydrate that wraps itself helically around small molecules and can transport CNT into aqueous solutions.[3] The addition of glucosidases to the starched nanotubes results in their precipitation, offering a simple, fast and practical method of purification through a non covalent functionalization. Recently, another promising non-covalent functionalization method was proposed, which consists of the interaction between CNT and single-stranded DNA (ssDNA),[28] which allowed not only discrimination between metallic and semiconducting tubes, but also a diameter-based identification. In fact, the ssDNA-CNT hybrid carries an effective negative charge because of the phosphate groups on the DNA. Metallic tubes exert an electrostatic field that decreases the charge density while semiconducting tubes show less polarizability and a consequent increased linear charge density, strictly correlated to their diameter.

Another interesting aspect is that a wide variety of proteins can bind the CNT surface via nonspecific adsorption, especially if they can be inserted into the tubular space

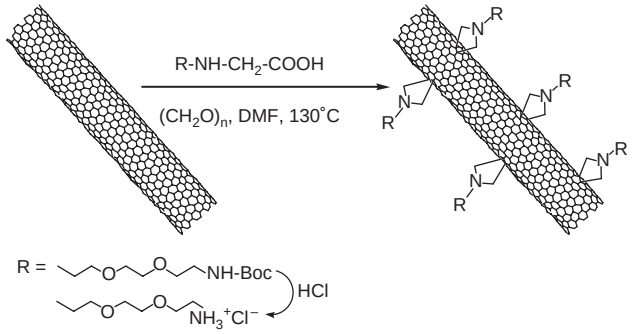

Fig. 4. Scheme of the 1,3-dipolar cycloaddition of azomethine ylides.

of open-ended MWNT.[44] The mechanism responsible for such binding seems to be more complicate than the well-known hydrophobic interaction. It was observed that the adsorption of proteins on CNT is insensitive to their isoelectric point, and strongly influenced by the amino affinity of nanotubes.[45] In the same context, peptide-nanotubes represent a useful model for protein-nanotube interactions, suggesting that aromatic, flexible and eventually amphiphilic peptides contribute to the observed affinity. However, it is worth considering that the secondary and even the tertiary structures of the proteins might strongly affect the nonspecific binding and the related properties. Lastly, new efforts to bridge nanomaterials and biological systems by preventing nonspecific interactions using biomimetic surface engineering have been made. Carbon nanotubes were modified with mucin mimics becoming water soluble, resistant to nonspecific binding of protein and linking specific molecules via receptor-ligand interactions.[46]

5. APPLICATIONS OF CNT AS VECTORS FOR DELIVERY OF THERAPEUTIC MOLECULES

5.1. Current Available Vectors for Delivery of Therapeutic Molecules

The applications of nanoparticles in biomedical sciences and biotechnology include their use as vehicles for biosensors, drug delivery, enzyme encapsulation and DNA transfection. Generally, almost half of the potential drug candidates, selected by high throughput screening, is rejected and never reach the step of final formulation, mainly due to their poor water solubility.[47] In fact, to achieve the target at a useful concentration, it is often desirable a compromise between hydrophobic and hydrophilic characteristics. In particular, drug delivery systems (DDS) are designed to improve the pharmacological profile of a drug, to alter the pharmacokinetics (PK) and biodistribution (BD) of a molecule, or to act as drug reservoir. Within the different DDS, liposome is one of the most efficient systems, which has entered the market. Liposomes were described as drug delivery vehicles in the 70s, on the basis of their ability to increase the efficacy of the drug with reduced toxic side effects. Major obstacles are still partly unsolved, such as a limited physical stability of the dispersions, a nonspecific clearance by the mononuclear phagocytic system (MPS) or a difficulty in upscaling production of some advanced liposome types.[48] More recent approaches relate to the use of polymeric micelles, formed by amphiphilic polymers dispersed in aqueous media, allowing the possibility of extended circulation time, favorable biodistribution and site-specific targeting of the incorporated drug.[49] However, the cytotoxicity of the polymers after internalization into cells is a crucial aspect that has raised many concerns. An alternative is represented by the so-called solid lipid nanoparticles (SLN), made-up of a solid lipid matrix, which contains surfactants as stabilizers. The advantages of this system comprise an excellent physical stability, a protection from degradation and a controlled drug release. Unluckily, they often demonstrate an insufficient loading capacity and drug expulsion after polymorphic transition during storage.[5]

Several attempts have been made to increase the site-specific actions of DDS by associating them with ligands targeted against cell surface molecules, a process called "active" or "ligand-mediated targeting." One of the most interesting systems proposed is the resulting additive or synergistic activity between a signaling antibody used as the targeting moiety and a cytotoxic drug associated with the DDS,[50] suggesting a potential use as synthetic vaccines.

On the one hand, there are still some limiting characteristics of the delivery devices that do not allow to obtain satisfactory results in terms of yields and efficacy. On the other hand, scientists are attracted by the intriguing, fascinating possibilities to induce a specific response against a precise defect. In order for both of these aspects to be realized, introduction of new delivery systems, which should have all the crucial characteristics, should be envisaged. The next generation DDS might include carbon nanotubes for different reasons: inducible water solubility, high stability of the dispersion, lack of intrinsic immunogenicity and an efficient loading capacity. On the whole, further and more detailed investigations are still required, but it seems that CNT might find the way to become promising DDS.

5.2. Functionalized CNT for Drug Delivery

On the basis of their polymeric and cationic nature, soluble functionalized carbon nanotubes seem to represent an innovative DDS, since they are able to penetrate into the cell without altering their morphology.[51, 52] It has been demonstrated that carbon nanotubes, functionalized with fluorescein (FITC) or a fluorescent bioactive peptide, cross the cell membrane. While the CNT with the FITC mainly distributed into the cytoplasm and slowly moved into the nucleus, the CNT with the fluorescent peptide rapidly penetrated into the nucleus. These CNT conjugates entered the cell with a passive mechanism. Indeed, the translocation capacity was not modified by decreasing the temperature or by using inhibitors of endocytosis-mediated process. It has been evidenced, using transmission electron microscopy, that functionalized CNT cross the cell membrane as nanoneedles. CNT dispose perpendicularly to the cell membrane and subsequently penetrate without perturbing or destroying the membrane itself.[53]

In a similar approach, carbon nanotubes were used to deliver streptavidin into the cell. The mechanism responsible for their internalization was disclosed. CNT-protein complex seems to associate non-specifically with

hydrophobic components of the cell surface and enters by endocytosis.[52]

In summary, these results open the way of using CNT for delivery of drugs, also on the basis of a very low cell toxicity in comparison with that shown by insoluble materials or by the residual catalysts involved in their production.

5.3. Functionalized CNT for Antigen Delivery

CNT, after covalent functionalization, can theoretically be considered ideal carrier systems for peptide antigens, showing a potentially high loading capacity for cargo molecules. In particular, a study describing the conjugation of a peptide to SWNT has been recently reported.[54] The peptide sequence belongs to VP1 protein from foot-and-mouth disease virus (FMDV). Aiming to evaluate the antigenicity and the immunogenicity properties and the influence of the number of peptides covalently linked to the SWNT, a mono- and a bis-peptide derivatized CNT were prepared (see Fig. 5 for their molecular structures). Using ELISA test and surface plasmon resonance, the specific anti-peptide antibody recognition was measured.[55] It has been demonstrated that the peptide is efficiently recognized when linked to the carbon nanotubes or free in solution. In addition, immunization of mice with these conjugates elicited higher antibody responses as compared to the peptide alone. On the other hand, no anti-carbon nanotube antibodies were detected, suggesting that CNT do not have intrinsic immunogenicity properties. However, only the mono-derivatized CNT conjugate induced high levels of virus neutralizing antibodies. In fact, the increase of the number of peptide units around the CNT surface, although enhanced the immunogenicity, did not improve the neutralizing capacity.[55] This was attributed to a reduced specificity of the antibodies generated using the bis-conjugate, likely due to a conformation adopted *in vivo* by the peptide onto the carbon nanotubes different from the native protein. This result underlines the critical role, which a carrier system may play in the presentation of the linked peptide to the immune system.

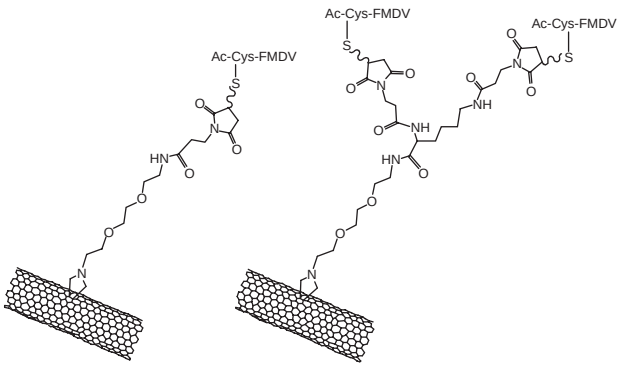

Fig. 5. Molecular structures of SWNT functionalized with a peptide derived from foot-and-mouth disease virus as a mono (left) and a bis-conjugate (right).

5.4. Functionalized CNT for Gene Delivery

One of the most promising approaches to correct a genetic defect or to cure a disease is gene therapy.[56] The main aim is to efficiently, specifically and safely introduce a DNA molecule into the cell nucleus. However, a major drawback associated to this methodology is the rapid degradation of the nucleic acids. To overcome this problem, one strategy is to use a vector system able to associate with plasmid DNA by self-assembly and assist its intracellular translocation. Some effective delivery systems for plasmid DNA include liposomes, cationic lipids and nanoparticles.[57] These systems offer several advantages, including an easy preparation in large quantities, flexibility towards the size of the DNA being transferred and reduced immunogenicity. The polymeric and cationic nature of functionalized carbon nanotubes seem to respond to the requirements for gene transfer. Complexation between carbon nanotubes and DNA may offer the possibility to achieve protection against DNA degradation before its release to specific target cell. The remarkable affinity between functionalized SWNT and the single-stranded DNA is presumably determined by hydrophobic interactions.[28] CNT were directly functionalized with DNA or PNA to form macromolecular wires following DNA hybridization.[58, 59] Concerning the application of CNT as gene vectors, preliminary studies have shown that complexes of a double-stranded, plasmid DNA expressing β-galactosidase, and amino functionalized nanotubes can achieve gene expression levels from 5 to 10 times higher than those observed when DNA is administered alone.[53] It has also been shown that the use of cationic f-SWNT and f-MWNT to condense DNA offers several advantages, such as an enhancement of cell membrane interactions due to electrostatic forces and an increased cellular uptake.[53] Recently, the translocation of the poly(rU) RNA using non-functionalized SWNT into breast cancer cells (MCF7) has also been reported.[60] As in the case of ssDNA, it was speculated that the hydrophobic bases of poly(rU) were able to bind the hydrophobic surface of CNT, through π-stacking with the carbon rings of SWNT, forming bundles with an average length of 400 nm. The translocation was confirmed by a radioisotope labeling assay, showing that SWNT-poly(rU)RNA hybrids penetrated into the cytoplasm and some of them were even detected in the nucleus, probably on the basis of a passive ratchet diffusion. These results are too preliminary to be compared with commercially available DNA and RNA transfection systems, but represent an important initial step towards the development of CNT technology in nucleic acid delivery for gene therapy and genetic vaccination.

5.5. Toxicity Studies

The laws of chemistry and physics work differently when particles get down to the nanoscale. Even substances that are normally harmless can potentially induce chemical

reactions and biological damage once at the nanoscale level. However, nanoscale substances are essential for the development of new materials for biomedical applications. The evaluation of their toxicity and biocompatibility is crucial to integrate them into biological substrates. It seems that the toxicity of carbon nanotubes is mainly related to the methods of their production. Manufacturing of nanotube materials relies on the use of transition metal catalysts, predominantly iron and nickel. The risks for health are likely associated with the presence of metal components even after their purification.[61] Indeed, transition metal complexes, as well as free iron and nickel, are known to catalyze free radical reactions that are dangerous in living systems.[61] There are only few preliminary toxicological investigations on the effects of nanostructures on cells or tissues. Adelman et al. in 1994 demonstrated that fullerenes are toxic on alveolar macrophages.[62] More recently, it has been shown that cytotoxicity of water-soluble fullerene derivatives is a function of the degree of surface modification.[63] C_{60} with an extensive surface functionalization is less toxic than its corresponding mono-adducts or the forms in which it is aggregated. If carbon nanotubes are considered an extension of the fullerene structure along one axis, it can be derived that CNT will be toxic as well. Lam and coworkers studied the pulmonary toxicity of some CNT derivatives showing that, regardless of the amount of metal, CNT products induced a dose-depending formation of epithelioid granulomas.[64] Such granulomas consisted of aggregates of macrophages laden with black nanotube particles. Other experimental studies in rats indicated that carbon black particles might produce a significant lung toxicity that potentially increases by decreasing particle size and by increasing surface area.[65] Biocompatibility of SWNT has been investigated using HEK293 human embryo kidney cells.[66] It has been found that nanotubes inhibit cell growth by inducing apoptosis and decreasing cell adhesion ability.[66] Stacked carbon nanofibers have also been implanted subcutaneously.[67] Histological and ultrastructural investigations showed macrophages around the materials, although the inflammatory response such as necrosis was not severe. The physicochemical properties of the different types of carbon nanotubes may influence their toxic activity. Indeed, the toxicological values should be considered carefully, since they could be determined, in large part, by the nature of the CNT, which are highly electrostatic and generally do not disperse but exist as ropes. In this context, the sidewall functionalization of carbon nanotubes, and the consequent water-solubility, increases enormously their biocompatibility. It has been demonstrated that non-functionalized SWNT-RNA polymer hybrids not only translocate inside the MCF7 breast cancer cells, but also they do not cause significant cell damage.[60] A concentration of 1 mg/ml of this complex had no effect on metabolism and cell viability. In addition, it has been reported that almost 90% of the fibroblast cells remained viable when incubated with soluble SWNT functionalized with fluorescein up to 5 μM concentration.[55] In addition, Wender and Dai indicated that cells survived after treatment with increasing doses of as-oxidized and cut tubes, and the extensive cell death observed after conjugation with streptavidin was attributed to the protein.[52]

Overall, the integration of novel materials such carbon nanotubes with biological systems requires a thorough evaluation of safety, as well as an understanding of their impact on the environment.[34] Even though the currently available information is somehow promising, more experiments are certainly highly necessary.

6. CONCLUSION

The unique characteristics of carbon nanotubes have given sufficient reason for excitement among materials scientists, physicists, chemists and biologists. Theoretical calculations have shown that their electronic properties can be tuned into the metal-semiconductor range, suggesting a potential use in material sciences and in biomedical research. One of the limiting factors is represented by their low water solubility, even if several studies demonstrated that is possible to overcome such problem through their functionalization. Furthermore, after conjugation with a specific molecule, they can be targeted to the desired site with a therapeutic effect. Though further investigations are still required, preliminary experiments showed promising results, especially in the potential application of carbon nanotubes as vectors in drug delivery, immune recognition and gene transfer.

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