Oxygen adsorption by carbon nanotubes and its application in radiotherapy

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Abstract: The ability to deliver large molecules, for example nucleic acids, to cells using carbon nanotubes has been reviewed. Potential applications of functionalised nanotubes to deliver oxygen to cancer cells to enhance the effects of radiotherapy are considered.

1 Introduction

Since carbon nanotubes (CNTs) were discovered by Radushkevich and Lukyanovich in 1952 [1], they have evoked great interest in many scientists worldwide. Their special characteristics including the small dimensions, strength and the remarkable physical properties make them a very unique material with a whole range of promising applications in the areas of chemistry, physics, materials and biology.

Pristine CNTs are insoluble in all solvents; the development of efficient methodologies for the chemical modification of CNTs has stimulated the preparation of soluble CNTs that can be employed in several biological applications, among which drug delivery appears to be particularly promising [2–5].

Two functionalisation approaches are widely used for modification of CNTs. CNTs can be oxidised using strong acids, resulting in the reduction of their length while generating carboxylic groups, which increase their dispersibility in aqueous solutions [6]. Alternatively, addition reactions to the CNT external walls and tips make them soluble in water [7, 8]. Solubility under physiological conditions is a key prerequisite to make CNTs biocompatible. In addition, functionalised CNTs (f-CNTs) can be linked to a wide variety of active molecules, including peptides, proteins, nucleic acids and other therapeutic agents.

An efficient way to functionalise the external walls of CNTs is based on the 1,3-dipolar cycloaddition of azomethine ylides. CNTs undergo the addition reaction when heated in DMF in the presence of an α-amino acid and an aldehyde [9]. The scope of this reaction is very broad and produces f-CNTs that possess high solubility in a wide range of solvents. By carefully choosing the reactants, it is possible to modulate solubility in organic solvents or aqueous solutions [10].

In the present paper, we review CNTs as potential carriers for larger molecules and hydrogen. A resultant hypothesis is proposed that oxygen is adsorbed in CNTs, and this shows promise for application in radiobiological works.

2 DNA, RNA delivery by CNTs and uptake mechanisms

2.1 Uptake mechanisms

An important characteristic of f-CNTs is their high propensity to cross cell membranes [11, 12]. CNTs labelled with a fluorescent agent were easily internalised and could be tracked into the cytoplasm or the nucleus of fibroblasts using epifluorescence and confocal microscopy [11].

The mechanism of uptake of this type of f-CNTs appears to be passive and endocytosis-independent. Incubation with cells in the presence of endocytosis inhibitors did not influence the cell penetration ability of f-CNTs. Furthermore, f-CNTs showed similar behaviour when incubation with the cells was carried out at lower temperatures. Cellular uptake was confirmed by Dai and colleagues [12, 13], who, in later studies, used oxidised CNTs to covalently link fluorescein or biotin, allowing for a biotin–avidin complex formation with fluorescent streptavidin. Again the nanotubes were observed inside the cells. In this case, the protein–CNT conjugates were found in endosomes, suggesting an uptake pathway via endocytosis. The CNTs can also be visualised inside the cells using transmission electron microscopy (TEM) [14]. Functionalised watersoluble CNTs were incubated with HeLa cells. The cells were subsequently embedded into an epoxy resin that was sliced using a diamond microtome. Each slice was mounted on a TEM grid and observed under the microscope. Some tubes were also identified at the cell membrane during the process of translocation. The conformation of CNTs perpendicular to the plasma membrane during uptake suggested a mechanism similar to nanoneedles, which perforate and diffuse through the lipid bilayer of plasma membrane without inducing cell death. Dynamic simulation studies have shown that amphiphilic nanotubes can theoretically migrate through artificial lipid bilayers via a similar mechanism [15]. Nanopenetration was also recently suggested by Cai et al., who proposed an efficient in vitro delivery technique called nanotube spearing [16]. MCF-7 breast cancer cells were grown on a substrate and incubated with magnetic CNTs. A rotating magnetic field first drove the nanotubes to spear the cells. In a subsequent step, a static field pulled the tubes into the cells. On the basis
of SEM images, it seems that the tubes cross the cell membrane like tiny needles. Another efficient way to observe CNTs intracellularly was developed by Weismann and colleagues who used near-infrared fluorescence [17]. They showed that macrophage cells could ingest significant amounts of nanotubes without apparent toxic effects. The internalised tubes remained fluorescent and could be identified at wavelengths beyond 1100 nm. Therefore there is mounting evidence that f-CNTs are capable of efficient cellular uptake by a mechanism that has not yet been clearly identified. However, the nature of the functional group at the CNT surface seems to play a determinant role in the mechanism of interaction with cells.

As is well known, almost all materials are selectively absorbed into cells by active or passive transport. The two mechanisms described earlier [11, 12] represent these two forms of transport, respectively, which would inevitably lead us to be in confusion. Why is it that CNTs can be transported by both active and passive manners? We hypothesise that it is the nature of CNTs that makes them to be capable of entering cells in an active or passive manner, depending up on whether they are pure CNTs or f-CNTs.

### 2.2 Nucleic acids delivery by CNTs

Ammonium-functionalised CNTs were tested for their ability to form supramolecular complexes with nucleic acids via electrostatic interactions. Many cationic systems have been reported by both active and passive manners? We hypothesise that it is the nature of CNTs that makes them to be capable of entering cells in an active or passive manner, depending on whether they are pure CNTs or f-CNTs.

The area of hydrogen storage in CNTs remains active and controversial. Extraordinarily high and reversible hydrogen adsorption in single-walled nanotubes (SWNT) containing materials [26–29] and graphite nanofibers (GNFs) [30] has been reported and has attracted considerable interest in both academia and industry. Table 1 summarises the gravimetric hydrogen storage capacity reported by various groups [31].

However, many of these reports have not been independently verified. There is also a lack of understanding of the basic mechanism(s) of hydrogen storage in these materials.

Materials with high hydrogen storage capacities are desirable for energy storage applications. Metal hydrides and cryo-adsorption are the two commonly used means to store hydrogen, typically at high pressure and/or low temperature. In metal hydrides, hydrogen is reversibly stored in the interstitial sites of the host lattice. The electrical energy is produced by direct electrochemical conversion. Hydrogen can also be stored in the gas phase in the metal hydrides. The relatively low gravimetric energy density has limited the application of metal hydride batteries. Because of their cylindrical and hollow geometry, and nanometer-scale diameters, it has been predicted that the CNTs can store liquid and gas in the inner cores through a capillary effect [32]. A temperature-programmed desorption (TPD) study on SWNT estimates a gravimetric storage density of 5–10 wt% when H₂ exposures were carried out at 300 torr for 10 min at 277 K followed by 3 min at 133 K [26]. If all the hydrogen molecules are assumed to be inside the nanotubes, the reported density would imply a much higher packing density of H₂ inside the tubes than expected from the normal H₂–H₂ distance. Upon cutting the nanotubes by an oxidation process, the amount of absorbed H₂ molecules increased to 4–5 wt%. A separate study on higher purity materials reports ≈8 wt% of H₂ adsorption at 80 K, but using a much higher pressure of 100 atm.

### Table 1: Summary of reported gravimetric storage of H₂ in various carbon materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Max. wt% H₂</th>
<th>T, K</th>
<th>P, MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWNTs (low purity)</td>
<td>5–10</td>
<td>133</td>
<td>0.040</td>
</tr>
<tr>
<td>SWNTs (high purity)</td>
<td>≈4</td>
<td>300</td>
<td>0.040</td>
</tr>
<tr>
<td>GNFs (tabular)</td>
<td>11.26</td>
<td>298</td>
<td>11.35</td>
</tr>
<tr>
<td>GNFs (herringbone)</td>
<td>67.55</td>
<td>298</td>
<td>11.35</td>
</tr>
<tr>
<td>GNS (platelet)</td>
<td>53.68</td>
<td>298</td>
<td>11.35</td>
</tr>
<tr>
<td>Graphite</td>
<td>4.52</td>
<td>298</td>
<td>11.35</td>
</tr>
<tr>
<td>GNFs</td>
<td>0.4</td>
<td>298–773</td>
<td>0.101</td>
</tr>
<tr>
<td>Li–GNFs</td>
<td>20</td>
<td>473–673</td>
<td>0.101</td>
</tr>
<tr>
<td>Li–graphites</td>
<td>14</td>
<td>473–674</td>
<td>0.101</td>
</tr>
<tr>
<td>K–GNFs</td>
<td>14</td>
<td>&lt;313</td>
<td>0.101</td>
</tr>
<tr>
<td>K–graphite</td>
<td>5.0</td>
<td>&lt;313</td>
<td>0.101</td>
</tr>
<tr>
<td>SWNTs (high purity)</td>
<td>8.25</td>
<td>80</td>
<td>7.18</td>
</tr>
<tr>
<td>SWNTs (≥50% pure)</td>
<td>4.2</td>
<td>300</td>
<td>10.1</td>
</tr>
</tbody>
</table>
suggesting that nanotubes have the highest hydrogen storage capacity of any carbon material. It is believed that hydrogen is first adsorbed on the outer surface of the crystalline ropes. An even higher hydrogen uptake, up to 14–20 wt%, at 20–400°C under ambient pressure was reported [27] in alkali-metal intercalated CNTs. It is believed that in the intercalated systems, the alkali metal ions act as a catalytic centre for H₂ dissociative adsorption. An electrochemical absorption and desorption of hydrogen experiment performed on SWNT reported a capacity of 110 mAh/g at low discharge currents [29]. The experiment was done in a half-cell configuration in 6 M KOH electrolyte and using a nickel counter electrode. Experiments have also been performed on SWNTs synthesised by a hydrogen arc-discharge method [28]. Measurements performed on relatively large amount materials (50% purity, 500 mg) showed a hydrogen storage capacity of 4.2 wt% when the samples were exposed to 10 MPa hydrogen at room temperature. About 80% of the absorbed H₂ could be released at room temperature [28].

The potential of achieving/exceeding the benchmark of 6.5 wt% H₂ to system weight ratio set by the Department of Energy (DOE) has generated considerable research activities in universities, major automobile companies and national laboratories. At this point, it is still not clear whether carbon nanotubes will have real technological applications in the hydrogen storage applications area. The values reported in the literature will need to be verified on well-characterised materials under controlled conditions. What is also lacking is a detailed understanding of the storage mechanism and the effect of materials processing on hydrogen storage. Perhaps the ongoing neutron scattering and proton nuclear magnetic resonance measurements will shed some light in this direction. In addition to hydrogen, CNTs readily adsorb other gaseous species under ambient conditions which often leads to drastic changes in their electronic properties [34–36]. This environmental sensitivity is a double-edged sword. From the technological point of view, it can potentially be utilised for gas detection [36]. On the other hand, it makes very difficult to deduce the intrinsic properties of the nanotubes, as demonstrated by the recent transport [34] and nuclear magnetic resonance [35] measurements. Care must be taken to remove the adsorbed species that typically require annealing of the nanotubes at elevated temperatures under at least 10-6 torr dynamic vacuum.

4 Possibility of oxygen molecules adsorbed by CNTs

Great efforts have been made both in theoretical and experimental works for hydrogen storage in CNTs. Although there has been some debate on its hydrogen storage capability, undoubtedly CNTs are promising materials for hydrogen storage.

With the different physical structures and chemical characteristics, molecular oxygen absorbed by CNTs and its allotropes, such as graphite, have been focused on recently. Although a few experimental works were reported, most scientists discussed the theoretical model of molecular oxygen adsorption and desorption. Zhu et al. [37] have carried out a systematic study for adsorption and desorption of an oxygen molecule on CNTs using the density functional (DF) calculations (Fig. 1). Some key intermediates are identified in the adsorption process. An O₂ molecule adsorbs on the nanotube wall and diffuses into the edge. They also showed that several precursor states exist on the armchair edge, which may delay the onset of O₂ molecular adsorption, whereas an O₂ molecule dissociatively chemisorbs without an activation barrier on the zigzag edge. They evaluated the desorption barriers of a CO molecule from the CNT edges in a concerted desorption pathway, and proved that fullerene and α-carbon can be oxidatively etched away more easily than CNTs due to lower desorption barriers, and therefore CNTs can survive selectively during the oxidative etching process.

The adsorption of molecular oxygen on the clean graphite (0001) surface has been studied experimentally by Janiak et al. [38]. It was found that O₂ physisorbs on the clean graphite surface at low temperature (T < 47 K) with the low adsorption energy 0.1 eV, and the O–O stretch frequency almost identical to the value of gas phase, illustrating that the perturbation of the O₂ electronic structure by the graphite surface is minimal. More recently, Ulbricht et al. reported that the low-coverage binding energy of O₂ on the clean graphite (0001) surface is 0.12 eV using thermal desorption spectroscopy [39]. On the theoretical aspects, Lamoen and Person [40] have investigated the adsorption of O₂ on a clean graphite surface by means of GC-LDA method and found that the O₂–graphite interaction is purely repulsive. On the other hand, Sorescu et al. found a weak adsorbed O₂ species when O₂ molecule was constrained to lie parallel to the graphite surface by using the PW91-GGA method and predicted that O₂ molecule only weakly physisorbs on the graphite surface by low adsorption energy 0.89 kcal/mol [41]. All these studies both experimentally and theoretically consistently agree that O₂ molecule is weakly physisorbed on the clean graphite basal surface. The large distance of adsorbed O₂ from the surface and low adsorption energy (0.26 kcal/mol) indicate the fact that it belongs to an extremely weak adsorption in agreement with the weak nature of interaction of O₂ with the clean graphite basal surface and also comparable to the above results obtained from periodic slab models [38–42]. Good candidates for the adsorption of O₂ are possibly the graphite surface with some defect sites.

It is well known that, under most circumstances, the surface catalytic reactivity is closely related to the presence of extended and point defects, including low-coordinated sites (edge, step and kink), impurity atoms, adatoms and
surface vacancies, with respect to metal, metal oxides surface as well as the graphite surface [43–47]. Lee et al. investigated the reactivity of vacancies generated by low energy Ar+ ion bombardment towards an oxygen molecule by using scanning tunneling microscopy and density functional theory [48]. They found that the O2 molecules exothermally dissociate and adsorb at the dangling bonds at a vacancy. In fact, other previous studies regarding small molecules (such as H2, NO and SO2) pointed out that the unsaturated edge defect sites are capable of exhibiting active role in the catalysis process [40, 49, 50].

5 Promising application of CNTs conjugated with oxygen in radiobiology and radiotherapy

As is well known, solid tumors, which make up more than 90% of all cancers, typically have areas of very low oxygenation or hypoxia. This is because the cells grow faster than the blood supply can keep up with, especially as blood flow is sluggish with very tortuous vessels, and so cells go further away from blood vessels than the diffusion distance of oxygen (100–150 μm). These hypoxic cells are a problem. First, they are resistant to killing by both radiation (used in radiotherapy) and anticancer drugs (used in chemotherapy). These hypoxic cells also promote malignant progression and make the tumours more likely to metastasise, or spread throughout the body. This means that the more hypoxic the tumour, the harder it is to cure.

CNT–oxygen conjugations may throws some light on this area. Following reasons could support this

1. Soluble CNTs have the propensity to cross cell membranes and are biocompatible.
2. Molecular oxygen are possibly conjugated with CNTs and released with no energy barrier.
3. Bind energy of O–C is weak and easily destroyed by radiations.
4. When tumour cells (including molecular oxygen) are exposed to radiations, free radicals produced by radioanalyzation will kill them.

Although there is much positive information, which will encourage further research, some difficulties cannot be neglected. More work must be done to resolve the following problems.

First, how to realise oxygen adsorption by CNTs? As described earlier [31], high atmosphere pressure, low temperature or both are conditions for hydrogen adsorption. If these given conditions were absent, absorption rate would be low or nil. To create a gentle physiological environment of patients prepared to be treated with radiotherapy, it is important to get stable oxygen adsorption under common pressure and temperature. It is well known that metal elements in the CNTs could interact with hydrogen and consequently form metal hydrides at high temperature [31]. These metal hydrides are relatively stable when temperature resumes normality and can be easily desorpted. Whether oxygen has these characteristics should be further studied.

On the other hand, how to target oxygen-adsorbed CNTs to cancer volume is essential. Binding tumour-recognising materials onto CNTs can be considered. For example, prostate cancers are mostly androgen-dependent, which could be treated by regulating the gonadotrophin-releasing hormone (GnRH) level through the pituitary/gonadal axis. GnRH and its analogues could enter prostate cancer cells by recognizing the characteristic GnRH receptors existing in the cell membrane [51, 52]. By binding this specific peptide onto it, oxygen-adsorbed CNTs could easily enter prostate cancer cells.

6 Conclusion

CNTs have a wide range of applications in many areas, such as biology. There are large possibilities that CNTs absorb oxygen and deliver it into tumour cells. Thus CNT–oxygen conjugations express a significant landscape in radiobiology and radiotherapy in order to change the hypoxia status of tumour tissues and consequently enhance the efficiency of radiotherapy.

7 Acknowledgments

This work was jointly supported by the National Science Foundation of China (Grant No. 10475109, Grant No. 10335050) and the MOST 973 Program (Grant No. 2006CB705600, Grant No. 2003CCB00200).

8 References


