Expert Opinion



# Opportunitiesandc hallenges of carbon-based nanomaterials for cancer therapy

Alberto Bianco†, Kostas Kostarelos& Maurizio Prato
†*CNRS, Institut de Biologie Moléculaire et Cellulaire, UPR 9021 Immonulogie et Chimie Thérapeutiques, 67000 Strasbourg, France*

The possibility of incorporating carbon-based nanomaterials into living systems has opened the way for the investigation of their potential applications in the emerging field of nanomedicine. A wide variety of different nanomaterials based on allotropic forms of carbon, such as nanotubes, nanohorns and nanodiamonds, are currently being explored towards different biomedical applications. In this review, we discuss the recent advances in the development of these novel nanomaterials for cancer therapy. A comparison between the characteristics, the advantages, the drawbacks, the benefits and the risks associated with these novel biocompatible forms of carbon is presented here.

**Keywords:** cancert herapy, carbonn anohorns, carbonn anotubes, drugd elivery, nanodiamonds

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## 1. Introduction

Carbon nanotubes (CNTs), nanohorns (CNHs) and nanodiamonds (NDs) belong to the family of carbon allotropes. They differ mainly in their physicochemical and structural properties. Carbon nanotubes, discovered in the 1960s/70s [1,2] and described in 1991 by Iijima [3], are constituted of graphene sheets rolled up to form a cylinder capped at the extremities by a hemi-fullerene. Carbon nanotubes can be either composed of a single plane of graphene (single-walled carbon nanotubes [SWNT]) or by multiple concentric layers (multi-walled carbon nanotubes [MWNT]). Their diameters are in the nanometer scale, while their lengths can reach several microns. Closely related to nanotubes are the carbon nanohorns, observed for the first time in the late 1990s, which are constituted of SWNT aggregated in a globular arrangement of several hundred nanometers in diameter, similar to sea urchins or dahlias [4]. The tips of the horns are generally closed with a cone-shaped cap. Nanodiamonds are three-dimensional structures in which carbon atoms have $sp^3$ hybridisation, as in diamonds, but the dimensions remain in the nanometer range [5]. All three of these carbon forms have a wide variety of uses in materials science and also have great potential in biomedical applications, due to their particular features. The size of all these new types of nano-objects is in the 1 – 100 nm range in at least one dimension. They can be considered as novel and innovative tools in the development of alternative methodologies for the delivery of therapeutic molecules [6]. Indeed, there is a continuous demand for novel delivery systems that are capable of protecting, transporting and releasing active molecules (i.e., drugs, antigens, antibodies, nucleic acids) to specific sites of action [7-14]. This is of fundamental importance, particularly in cancer therapy. Although we consider CNT technology to be still in its infancy, some advantages are clearly emerging. The pros and cons of the methodology that employs CNTs are illustrated in **Table 1**.



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**Table 1. Properties and parameters that determine the advantages and disadvantages of carbon nanotubes in nanomedicine.**

| Pros | Cons |
|---|---|
| High stability *in vivo* because of their mechanical properties | Non-biodegradable |
| Large surface area available for multiple functionalisation | Large surface area for protein opsonisation |
| Capacity to easily pass biological barriers leading to novel biocompatible delivery systems | Insolubility of as-produced materials – functionalisation is required for rendering the material compatible in physiological conditions |
| Unique electrical and conducting properties for the development of new devices for diagnostics | Strong tendency to aggregate |
| Empty internal space for encapsulation and transport of therapeutic and imaging molecules | Limited data on tolerance by healthy tissues |
| Bulk production associated to low costs | Extremely high variety of carbon nanotube types – standardisation difficult |

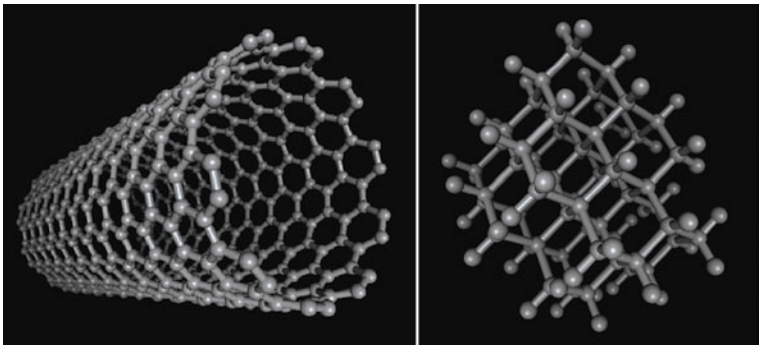

**Figure 1. Molecular structures of an opened single-walled carbon nanotube (left) and a nanodiamond (right).**

The aim of this article is to describe the potential of carbon nanotubes, nanohorns and nanodiamonds for the delivery of anticancer agents in the context of innovative cancer therapies. Each different carbon-based nanomaterial will be discussed separately to highlight its specific characteristics. The strategies to render them biocompatible, the functionalisation with the active drugs, the strategies for specific targeting and the options for imaging will be presented. Finally, we will compare the benefits and the risks of using each form of these novel drug delivery systems for clinical therapeutic treatments.

## 2. Carbon nanotubes

Among the materials that are currently being developed for cancer nanotechnology, carbon nanotubes can be considered a novel opportunity [15,16]. CNTs are capped cylinders of nanometric dimensions exclusively constituted of carbon atoms arranged in a hexagonal lattice (**Figure 1**, left).

CNTs can be either SWNT [17,18] or MWNT [3]. Most commonly, SWNT have a diameter from 0.4 to 3.0 nm and lengths that span from a few nanometres to a few microns, while MWNT are larger, with a diameter reaching 100 nm and a length ranging from 1 to several µm, or even longer (i.e., several millimetres) (**Figure 2**). Several methodologies for the production of both types of nanotubes have been reported in the literature [19,20]. They comprise arc-discharge [21], laser ablation [22], chemical vapor deposition (CVD) [23] and gas-phase catalytic process (HiPCO) [24].

CNTs are largely exploited in materials science for their mechanical, electronic, optical and magnetic properties [20,25]. In the field of biomedical applications, and in particular in the new discipline of nanomedicine, CNTs are attracting the interest of many research groups [26-30]. This is mainly owing to the established capacity of CNT to penetrate cells with remarkably reduced toxic effects [31-35]. Beside this important feature, one major concern with CNTs relates to the extreme difficulty of manipulating this material due to its insolubility in all types of solvents, particularly in aqueous solutions. This certainly limits the use of nanotubes in life sciences. However, several strategies are currently available to integrate nanotubes with physiological conditions. The two main approaches developed in recent years are based on the non-covalent and the covalent functionalisation [36]. Both approaches give rise to relatively soluble or dispersible conjugates, which consist of CNT modified with different

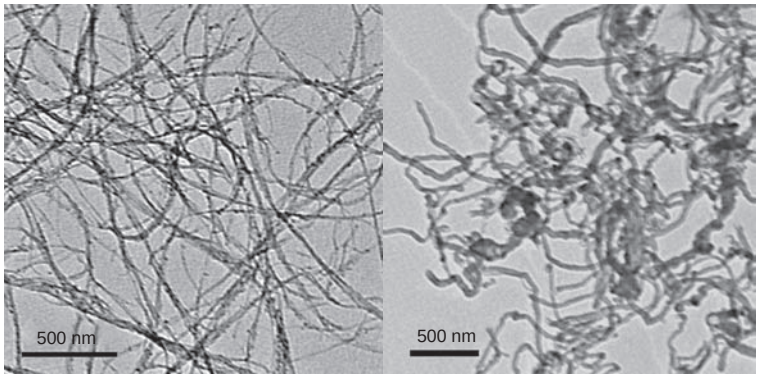


**Figure 2. Transmission electron microscopy (TEM) photographs of pristine SWNT (left) and MWNT (right).** For high resolution TEM images see, for example, [3] (MWNT) or [17] (SWNT). These images were taken on pristine samples purchased from Carbon Nanotechnology, Inc. (SWNT) and Nanostructured & Amorphous Materials, Inc. (MWNT).
SWNT: Single-walled carbon nanotubes; MWNT: Multi-walled carbon nanotubes.

types of biopolymers (i.e., peptides, proteins or nucleic acids). In terms of the differences between SWNT and MWNT, particularly in the field of biomedical applications, it is not still evident whether one system presents more advantages that the other. They are certainly both attractive because they have shown the capacity of cellular uptake. In addition, SWNT, for example, can be detected in a body because of its photoluminescence properties and has consequently been developed for diagnostic purposes [37]. On the other hand, MWNT has a wide internal diameter that can be exploited for the encapsulation of therapeutic molecules and a higher available external surface that offers increased possibilities of conjugation or interaction with active molecules than SWNT.

In the field of cancer therapy, the possibility of transporting anticancer drugs or radionuclides using carbon nanotubes is based on these two complementary approaches. One strategy is to form non-covalent complexes between the nanotubes and the drug or the radionuclide alone, or linked to a polymer, while the second method is based on the binding of the compounds to the tubes using a more stable covalent bond.

Another possibility of using CNTs in cancer therapy is to exploit their strong optical adsorption at low energy regimes. Indeed, CNTs have the intrinsic characteristic of adsorbing energy in the NIR and in a radiofrequency field [37]. Absorption of light induces a local increase in temperature with deleterious consequences for the malignant cells, tissues and organs, which have incorporated CNTs. The advantages and drawbacks related to these different CNT-based anticancer strategies will be discussed in the following paragraphs. **Table 2** summarises the different anticancer modalities and the characteristics of CNTs associated with each approach.

### 2.1 CNT thermal effect

Some examples have recently been reported showing the possibility of heating carbon nanotubes injected into cancer cells and thus provoking their death. Both *in vitro* and *in vivo* experiments using NIR radiation or radiofrequency irradiation were performed. In both such approaches the thermal effect of CNTs was combined with a non-covalent functionalisation approach, necessary to render the tubes biocompatible. CNTs were suspended in cell culture medium using phospholipid–polyethylene glycol chains containing a folate moiety for selective internalisation inside cancer cells that overexpress the folate receptors. Cell death was triggered by irradiating the cells with NIR light without damaging receptor-free cells [38]. Near-infrared phototherapy was also applied to destroy breast cancer cells using single-walled carbon nanotubes previously functionalised with two specific monoclonal antibodies [39]. In this case, antibodies against the membrane markers' insulin-like growth factor 1 receptor (IGF1R) and human endothelial receptor 2 (HER2) were separately conjugated to carbon nanotubes using a pyrene linker that adsorbed onto the SWNT backbone. Again, the thermal therapy was combined with non-covalent functionalisation. This approach is based on the capacity of the aromatic surface of CNTs to form strong $\pi$–$\pi$ interactions with the pyrene moiety. The stability of this type of complex has already been proved although studies on the *in vivo* stability and eventually the release of the attached therapeutic biomolecule are needed to validate a clinical use [40,41]. To prevent undesired interference with other proteins, CNTs have been also coated with polyethylene glycol to cover their free surface. The supramolecular complexes selectively bind the overexpressed receptors at the surface of two different types of cancer cells in comparison to the control hybrids functionalised with a non-specific antibody. Following excitation by infrared photons, the tumour cells treated with IGF1R and HER2 modified nanotubes died. Therefore this approach combined the thermal effects of CNTs with specific cell targeting using monoclonal antibody technology. Indeed, a multi-component strategy can lead to higher efficacy in the therapeutic action towards cancer. However, since the NIR light can only

**Table 2. Summary of the characteristics of carbon nanotubes associated with the different strategies developed for cancer treatment.**

| Anticancer modalities | *In vitro/in vivo* studies | Type of CNTs and functionalisation strategy | Targeted/non-targeted approach | Solubility* | Physico/chemical characterisation |
|---|---|---|---|---|---|
| ***CNT thermal effect*** | | | | | |
| NIR irradiation [38] | *In vitro* | SWNT – pristine Non-covalent | Targeted | 25 µg/ml | AFM, UV-Vis-NIR |
| NIR irradiation [39] | *In vitro* | SWNT – pristine Non-covalent | Targeted | 100 µg/ml | TEM, AFM |
| Radiofrequency [42] | *In vivo* | SWNT – pristine Non-covalent | Non-targeted Intratumoural injection | 500 µg/ml | ICP-MS, Raman |
| ***Anticancer delivery by non-covalent functionalised CNTs*** | | | | | |
| Doxorubicin delivery [51] | *In vitro* | SWNT – pristine and oxidised Non-covalent | Targeted | 50 µg/ml | AFM |
| Doxorubicin delivery [52] | *In vitro* | MWNT – pristine Non-covalent | Non-targeted | 40 µg/ml | TEM |
| siRNA delivery [53] | *In vitro/in vivo* | SWNT – oxidised Non-covalent | Non-targeted Intratumoural injection | 100 µg/ml | AFM, TEM, EDX |
| Radiolabelling [47] | *In vivo* | SWNT – pristine Non-covalent | Targeted | 50 µg/ml | AFM, Raman, PET |
| Platinum complex delivery [54] | *In vitro* | SWNT – pristine Non-covalent | Non-targeted | 400 nM | AAS |
| ***Anticancer delivery by covalent functionalised CNTs*** | | | | | |
| Methotrexate delivery [58,59] | *In vitro* | MWNT – pristine Covalent | Non-targeted | Not reported | TEM, NMR, UV-Vis |
| BNCT [60] | *In vivo* | SWNT – purified Covalent | Non-targeted | 24 µg/ml | TEM, SEM, FT-IR, NMR, UV-Vis, ICP-OES |
| Gonadotropin releasing hormone [61] | *In vitro* | MWNT – oxidised Covalent | Targeted | 50 µg/ml | SEM |
| Antibody (Rituximab) approach [48] | *In vivo* | SWNT – oxidised Covalent | Targeted | 50 mg/ml | AFM, ITLC-SG, HPLC |

*Solubility is reported in aqueous or buffer solutions.

AAS: Atomic adsorption spectroscopy; AFM: Atomic force microscopy; CNT: Carbon nanotubes; EDX: Energy dispersion x-ray spectrometry; FT-IR: Fourier transform infrared spectroscopy; HPLC: High-pressure liquid chromatography; ICP–MS: Inductively coupled plasma – mass spectrometry; ICP–OES: Inductively coupled plasma – optical emission spectroscopy; ITLC–SG: Instant thin layer chromatography – silica gel; NMR: Nuclear magnetic resonance; PET: Positron emission tomography; SEM: Scanning electron microscopy; TEM: Transmission electron microscopy; UV-Vis: Ultraviolet-visible spectroscopy; UV-Vis-NIR: Ultra violet-visible-near infrared spectroscopy.

penetrate a few centimetres of tissue, an alternative approach has recently been proposed. Radiofrequency waves were applied to kill malignant cells containing CNTs, since they can pass deeper into the body [42]. Indeed, the heat released in the radiofrequency field produces thermal cytotoxic effects in tumour cells that had previously uptaken nanotubes. A dispersion of SWNT coated with Kentera – a polymer based on polyphenylene ethynylene – was directly injected into the liver tumour of a rabbit. Application of a radio-frequency pulse destroyed the cancer cells, causing just a small amount of damage to the neighbouring healthy tissues. These novel antitumour technologies are very exciting, however they require a lot of development and precaution before they can be translated into clinically realistic cancer treatment modalities. In fact, all the above-mentioned studies are at a very early, proof-of-concept stage, as yet completely lacking systematic preclinical therapeutic data. Moreover, even in the cases where the cancer cells are reported to be dead, there is still a lack of statistically significant efficacy data (i.e., overall tumour elimination, tumour growth rate arrest, etc.) or any information on the fate of nanotubes following such procedures. The important issues of bio-distribution, accumulation and elimination of CNTs remains largely unknown and should be more thoroughly addressed before further work is recommended. The first studies in this direction started to appear recently [28,43-49]. Improved biocompatibility is one of the advantageous aspects that covalently functionalised carbon nanotubes offer, as has

recently been shown for both SWNT and MWNT [43,50], whereas carbon nanotubes that are non-covalently functionalised seem to present more hazards in terms of possible pharmacological side effects.

### 2.2 Anticancer agent delivery by non-covalent functionalised nanotubes

The strategies of coating carbon nanotubes with anticancer drugs can be diverse. The group of Dai [51] and our groups [52] have very recently shown that both SWNT and MWNT can be loaded with doxorubicin. SWNT were initially suspended with polyethylene glycol terminated by a lipid chain and subsequently adsorbed with doxorubicin, a molecule with an aromatic character which induces a π-stacking assembly [51]. According to the reported results, the release of the drug was controlled by the pH. However, the basic conditions used to bind doxorubicin to the nanotubes may not be compatible with the drug stability (as listed in the *British Pharmaceutical Codex 1973* (Pharmaceutical Press, 1973)). The release might partly be a consequence of drug degradation. In a similar approach, but instead using MWNT dispersed in water using the block copolymer Pluronic F127, we have demonstrated enhanced cell killing capacity of the adsorbed doxorubicin [52]. In this case, no release was observed by changing pH, although the non-covalent complexes showed a significant increase in drug activity using breast cancer cells *in vitro* compared to the drug alone. These two studies indicate that both SWNT and MWNT seem to offer available surface area for π–π interactions with the aromatic rings of doxorubicin, leading to an enhanced antitumoural effect. In view of these promising results, more mechanistic work is necessary to investigate whether the capability of CNTs to penetrate into cells is also exerted by the drug/nanotube complexes. In addition, other factors such as the timely and effective intracellular release of the drug molecule from the CNT complex that will determine the efficacy of drug action need to be studied.

Another promising tool to address cancer by inactivating tumour cells involves the use of RNA interference. Small interference RNA (siRNA) suffers from limited cell uptake and enzymatic instability. Cationic single-walled carbon nanotubes have been used to form stable complexes with siRNA able to silence the expression of telomerase reverse transcriptase (TERT), which is one of the attractive strategies for targeted cancer therapy [53]. In this work, the ability of carbon nanotubes to deliver TERT siRNA to knockdown the gene and inhibit cell proliferation and growth was demonstrated *in vitro* and following administration into the tumour in mice. This approach based on carbon nanotubes presents an interesting option for the delivery of siRNA therapeutics against cancer.

To improve the efficacy of a therapeutic modality, it is necessary to specifically direct it towards the injured (including ill, intoxicated, burdened or ill-fated) cells, tissues and organs. The possibility of tumour targeting has also been explored using non-covalent functionalised carbon nanotubes [47]. CNT have been wrapped in a lipid-polyethylene glycol conjugate modified with an integrin binding peptide (RGD) at the distal end of PEG and simultaneously with a radiolabelled (Cu-64) lipid-PEG for tracking purposes. The presence of such conjugates in the tumour was assessed using positron emission tomography and Raman spectroscopy after *in vivo* administration. Despite the interesting results, it remains to be demonstrated that it is possible to achieve tumour growth delay or preferably tumour elimination following such strategies by limiting the side effects. In addition, it is also necessary to verify that the system is safe for further translation into preclinical evaluation. In a similar approach, single-walled carbon nanotubes have been non-covalently coated by a lipid-polyethylene glycol chain bearing a platinum (IV) compound as a prodrug for the release of the cytotoxic anticancer cisplatin [54]. The use of platinum (II)-based drugs is limited by their deactivation once administered (i.e., platinum (II) complexes are sensitive to intracellular glutathione levels) [55]. To avoid this problem, it has been proposed to form complexes of platinum (IV) that can be reduced upon entering the cells, restoring the antitumoural activity of the metal. This concept was applied to carbon nanotubes, which play in this case the role of shuttle for the delivery of the drug. The efficacy of the system was established on testicular carcinoma cells. The cytotoxic effect of the conjugates was higher than the control cisplatin. The conjugates were found inside the cytoplasm and a substantial amount of platinum was detected in comparison to the molecule administered alone, which accounts for an improved cytotoxic activity. This approach is still at a very early stage of development. In addition, the platinum–CNT conjugates do not contain a specific targeting molecule for cancer cells.

### 2.3 Anticancer agent delivery by covalent functionalised nanotubes

The use of carbon nanotube covalent functionalisation to deliver anticancer agents has also been recently explored. Using this approach, control over a number of functional groups around the tubes is one of the key points. This issue can be solved, since the organic functionalisation of carbon nanotubes has become a powerful and controllable methodology [36]. Carbon nanotubes have been modified with methotrexate, a well-known and potent anticancer agent, used also to cure autoimmune diseases [56]. Methotrexate suffers from low bioavailability and toxic side effects [57]. Therefore, an increased bioavailability profile coupled with targeted delivery will be highly desirable. Preliminary results have shown that methotrexate conjugated to the nanotubes is as active as methotrexate alone in a cell culture assay where Jurkat cells were incubated up to 72 h [58,59]. Alternatively, carbon nanotubes can be

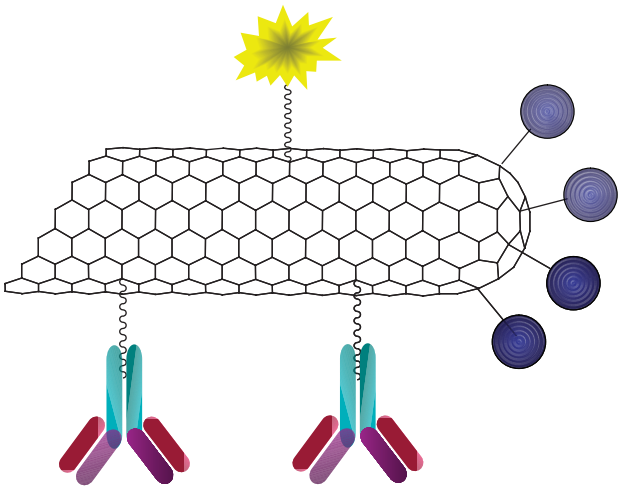

**Figure 3. A carbon nanotube functionalised with multiple moieties for cancer therapy (blue spheres), targeting (antibodies) and imaging (yellow star) presents multi-component capacities for biomedical applications.**

modified with a carborane cage for the development of boron neutron capture therapy (BNCT) [60]. BNCT is a binary radiation therapy modality that brings together two components that, when separated, have only minor effects on cells. The first component is a stable isotope of boron (boron-10) that can be concentrated in tumour cells by attaching it to tumour-seeking ligands. The second is a beam of low-energy neutrons. Boron-10 in or adjacent to the tumour cells disintegrates after capturing a neutron and the generated high energy, heavily charged particles destroy only the cells in close proximity to it, primarily cancer cells, leaving adjacent normal cells largely unaffected. The biodistribution on different tissues, following the intravenous administration, showed that the water soluble carborane–nanotubes were concentrated more in the tumour cells than in the other organs. These results were preliminary, although also promising for future applications of carbon nanotube boron-based agents for effective cancer therapies using boron neutron capture. Another interesting example of targeting and affecting cancer cells concerns the use of oxidised multi-walled carbon nanotubes covalently modified with gonadotrophin releasing hormone [61]. This hormone is overexpressed in the plasma membrane of several types of cancer cells. Its conjugation to carbon nanotubes allowed the generation of a hybrid capable not only of penetrating the malignant cells, but most remarkably to destroy them, which was not the case for the two entities administered alone. This novel toxic material, displaying both biocompatibility and bioadsorption, provides the basis for direct killing of prostate cancer cells, although this was demonstrated only *in vitro*. Cancer cells were also successfully targeted using antibody-functionalised carbon nanotubes [48,62]. Carbon nanotubes can be conceived as flexible, multi-presentation platforms which permit the simultaneous display of different moieties including targeting, imaging and therapeutic molecules (**Figure 3**). To develop radiotherapy devices based on carbon nanotubes, a specific monoclonal antibody was appended to water soluble carbon nanotubes together with a radionuclide. The construct was administered *in vivo* in a murine xenograft model of B-cell lymphoma showing a selective targeting of the tumour. In these proof-of-concept studies, the radioisotope was employed for biodistribution experiments, but it is assumed that, eventually, a radiotherapeutic nuclide can also be used.

## 3. Carbon nanohorns

Carbon nanohorns are alternative forms of carbon nanomaterials, closely related to nanotubes, which appear as spherical aggregates of single-walled carbon nanotubes with an average diameter of 100 nm (**Figure 4**) [4]. They are particularly promising because the methods used for their production are devoid of catalytic metal particles, which are present in most pristine nanotube preparations and are the cause of many safety concerns, as they may be responsible for some of the toxicity associated with carbon nanotubes before chemical treatment [63]. Due to their peculiar geometry, reminiscent of a sponge, carbon nanohorns can be exploited for their capacity to adsorb most types of molecules [64].

The advantage of such a property is that the horns can be used as reservoirs for controlled drug release, although there is also the risk that during functionalisation procedures the elimination of excess reagents is particularly difficult, requiring extensive washings to completely eliminate these unwanted molecules. It has to be stressed that the functionalisation of carbon nanohorns is mandatory to render this material biocompatible. Indeed, carbon nanohorns behave like carbon nanotubes and can be dispersed or solubilised into physiological or water solutions provided that they are modified at their surface with suitable functional groups. In addition to an extensive surface area, carbon nanohorns have a high number of interstices, which allow the adsorption of a large amount of guest molecules. Moreover, little holes can be generated at the tips of the tubes and can be exploited to insert different therapeutic agents into their empty space. Nanohorns have been loaded with different types of drugs including anticancer agents, like doxorubicin and cisplatin. Carbon nanohorns have been initially oxidised and subsequently complexed with polyethylene glycol (PEG) chains of different lengths, functionalised at one end with doxorubicin [65]. The preparation of the conjugates required the use of organic solvents like dimethylsulfoxide and dimethylformamide, which are not compatible with biological moieties (such as cell cultures), but can eventually be eliminated using chromatographic separation equilibrated in water. It has been demonstrated that carbon nanohorns adsorb PEG-doxorubicin via the doxorubicin moiety.

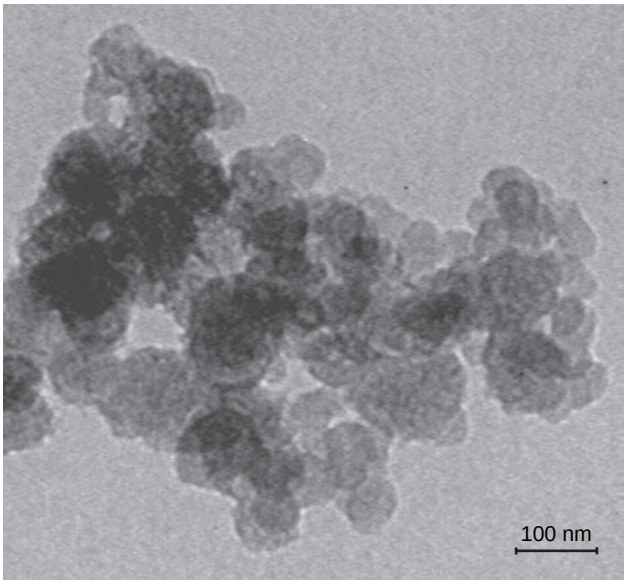


**Figure 4. Transmission electron microscopy photograph of pristine single-walled carbon nanotubes.** For high resolution transmission electron microscopy images see, for example, [64]. This image was taken on a pristine sample purchased from Nanocraft, Inc.

The complexes, which have a diameter of 160 nm, contain more than 250 mg of PEG–doxorubicin per gram of nanohorns. Preliminary *in vitro* tests have shown that the horns loaded with PEG-doxorubicin induce apoptosis of lung cancer cells to a certain extent, which was, however, lower than the control drug. It is probable that PEG–doxorubicin is retained on the surface of the nanohorns, thus reducing its therapeutic effect. However, it was not verified whether the nanohorn complexes were uptaken by the cells, which is necessary for effective drug action. The authors could not exclude the possibility that some amount of free doxorubicin remained in their preparation and was responsible for the apoptotic activity. In an alternative approach by the same group, cisplatin was trapped in the inner space of the horns [66-69]. Carbon nanohorns do not alter the structure of the anticancer agent, which was slowly released in aqueous solution [68]. Following the liberation of the drug, cell viability of human lung cancer cells was monitored for 48 h. The anticancer activity of the nanohorns containing cisplatin was almost comparable to the drug alone, while nanohorns used as controls presented no cytotoxic effects. Although carbon nanohorns can be easily dispersed in water, they have been shown to aggregate by their tendency to form clusters in the highly ionic and protein-rich cell culture media [66]. The presence of aggregates in the micrometer scale formed by both oxidised and cisplatin-containing nanohorns is a major concern with this approach that will need to be overcome in order to achieve *in vivo* applications. More specifically, we can imagine that these nanomaterials will be eliminated with extreme difficulty, leading to accumulation in tissues and organs upon *in vivo* administration. Very recently the same authors have devised an alternative methodology to maintain well-dispersed nanohorns in physiological conditions or cell culture media [67]. Cisplatin was encapsulated into the horns and subsequently coated with a PEG chain terminated with a synthetic peptide aptamer that specifically binds to the surface of the nanohorns. These complexes were able to exert a potent cytotoxic effect against cancer cells. This is probably the approach to follow to avoid the incapacitating aggregation phenomena previously described. However, the coverage of the nanohorns with different types of molecules might induce the risk of provoking other problems such as an undesired immune response. These studies using carbon nanohorns are very interesting, but more work is required to prove that carbon nanohorns are biocompatible drug carriers [70]. Although carbon nanohorns doped with magnetic nanoparticles have been administered into an animal model for MRI imaging purposes [71], the specific targeting of these carbon nanomaterials *in vivo* is something that requires further development.

## 4. Nanodiamonds

Diamonds are commonly known as stable and inert material (**Figure 1**, right) [5]. They are very difficult to manipulate and, being practically insoluble in any solvent, it is very difficult to imagine their use in nanomedicine. However, recent findings have shown that if the dimensions of diamonds are reduced to the level of nanometers or microns, they can be treated as constructs that can be eventually surface functionalised (**Figure 5**) [72-74]. This possibility increases their solubility and facilitates their manipulation remarkably. In view of this opportunity, nanodiamonds have been proposed as a versatile platform for diverse applications. They can be functionalised in a controllable manner for further interaction with therapeutic molecules. Huang *et al.* investigated the binding of proteins to nanodiamonds [75]. Nanodiamonds of 5 nm in diameter have been oxidised at the surface, generating carboxylic functions which have been exploited for the formation of a non-covalent complex based on electrostatic interactions with polylysine. Parts of the available amino functions of the cationic biopolymer were then covalently linked to cytochrome c via a heterobifunctional cross-linker. It was demonstrated that the immobilisation of the protein onto the nanodiamonds did not alter its stability or conformation. Alternatively, 2 – 8 nm diameter nanodiamonds highly functionalised with hydroxyl and carboxylic groups were used to adsorb doxorubicin, a drug extensively used in chemotherapy [76]. Non-covalent complexes were formed by the addition of NaCl, while reversible release of the drug was achieved by reducing the concentration of chloride ions (salt effect). The nanoparticles were able to enter into cells alone or complexed to doxorubicin. To prove the

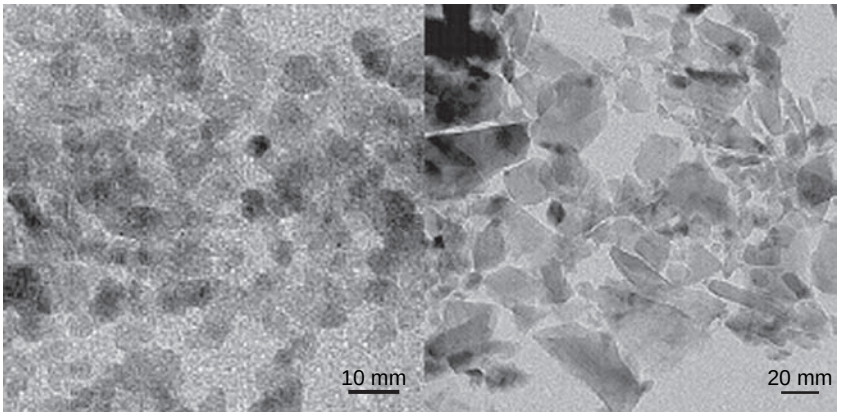


**Figure 5. Transmission electron microscopy photographs of non-functionalised nanodiamonds obtained by detonation (left) or high pressure/high temperature (right) (courtesy of Christelle Mansuy).** These images were taken on samples purchased from Gansu Lingyun Nano-Material Corp., Lanzhou, China and LM Van Moppes & Sons SA, Geneva, Switzerland, respectively. For high resolution scanning electron microscopy images see, for example, [5].

capacity of these nano-objects to pass the cell membrane, nanodiamonds were coated with a fluorescent polylysine derivative and localised inside the cytoplasm. The resulting nanodiamonds were also highly biocompatible, as demonstrated by the fact that cell viability was not reduced. The complexes with doxorubicin were uptaken and apoptosis was assessed as a consequence of the liberation of the drug from the complex. The effects of doxorubicin-induced cell death were tested in comparison to the drug alone. Nanodiamonds sequestered doxorubicin for a longer time, decreasing the efficacy compared to the drug alone, but were proposed as an alternative technology for a delayed and time-controlled drug release, prolonging efficacy during the treatment. However, such data is yet to be reported.

In addition, nanodiamonds can be doped with other atoms or can be modified by inducing defects and holes into their structure to render them fluorescent and therefore extremely useful as cellular biomarkers for imaging purposes. Indeed, we can imagine exploiting the fluorescence properties of the nanodiamonds functionalised at their surface with specific ligands to target cancer cells with an exceptionally high sensitivity in the detection, which is fundamental for early tumour diagnosis. The most common defect is the presence of a negatively charged nitrogen vacancy center in the nanodiamond structure. This defect center strongly adsorbs at 560 nm and emits fluorescence at 700 nm. Since the nitrogen atom is confined into an inert matrix, photobleaching is dramatically reduced if not completely eliminated, thus rendering such nanodiamonds very useful as markers for imaging [77]. These fluorescent nanoparticles are easily uptaken by the cells and display reduced cytotoxicity [78,79]. Bright nanodiamonds appear in the form of aggregates localised into the cytoplasm but not in the nucleus. Single particle tracking in live cells following the motion of the nanodiamonds into the cytoplasm allow analysis of its fate once internalised. Such applications may have great potential for *in vivo* studies using fluorescent nanodiamonds.

## 5. Conclusion

This review describes the potential applications of three different forms of carbon-based nanomaterials for cancer therapy. Carbon nanotubes, nanohorns and nanodiamonds can be functionalised with anticancer molecules following two main strategies. These novel nanomaterials can be either covalently or non-covalently modified to facilitate their manipulation and render them biocompatible. Such soluble/dispersible nano-objects in physiological conditions are then able to penetrate into the cells or they can be administered *in vivo* to deliver their cargo molecules, which eventually display anticancer activity.

## 6. Expert opinion

The development of novel delivery systems for the successful administration of anticancer therapeutics is currently one of the major challenges to improve the quality of human life. Among the new nanomaterials for application in cancer therapeutics, carbon nanotubes, nanohorns and nanodiamonds are receiving increasing attention and may play an important role in the future. These three different types of nanomaterials have different characteristics which are strictly related to their morphology and structure (**Table 3**). The biological properties described refer to the materials that have been functionalised with organic moieties to improve their biocompatibility.

**Table 3. Characteristics of functionalised carbon nanotubes, nanohorns and nanodiamonds.**

| | Nanotubes | Nanohorns | Nanodiamonds |
|---|---|---|---|
| Shape | Tubular/cylindrical | Spherical | Spherical/prismoidal |
| Dimensions | Diameter: 1 – 100 nm<br>Length: 0.01 – several microns/mm | Diameter: 80–100 nm | Diameter: 2–100 nm |
| Hybridisation | $sp^2$ | $sp^2$ | $sp^3$ |
| Non-covalent functionalisation | Yes | Yes | Yes |
| Covalent functionalisation | Yes | Yes | Yes |
| Biocompatibility | Yes | Yes | Yes |
| Biodegradability | None | None | None |
| Cell uptake | Good | Good | Good |
| Cytotoxicity | Very low* | Very low‡ | Very low‡ |
| *In vivo* organ accumulation | Yes | ND§ | ND§ |
| Rapid elimination | Yes | ND§ | ND§ |

*Assessed *in vitro* and *in vivo*.
‡Only few examples have been reported.
§Not demonstrated.

A specific discussion of the cytotoxic effects and pharmacokinetics of nanotubes, nanohorns and nanodiamonds is beyond the aim of this review. These topics have been carefully addressed in a series of interesting reviews recently [28,80-82]. From **Table 3** it is also evident that CNT, CNH and ND technologies for anticancer drug delivery require further investigation for validation and should carefully be addressed, mainly concerning the important aspects of the pharmacology and toxicology of these nanomaterials *in vivo*. Carbon nanotubes are one step ahead in terms of possible applications and assessment of some basic important issues concerning toxicity [80-82] and pharmacokinetics [28], however, *in vivo* efficacy studies against tumour models are clearly still lacking. Another important issue is the polydispersity of the starting material, which limits the reproducibility of the results and often affords inconsistent data. Indeed, the CNTs prepared by all currently known methods are mixtures of different tubes with a broad distribution in diameter and chirality and are often contaminated by impurities (mainly including amorphous carbon and catalyst particles). Various methods have been developed to purify CNTs, including oxidation of contaminants [83], chromatographic and centrifugation procedures [84-88]. Although these methods are quite efficient, they still need to be applied to a wide range of nanotube types to determine the extent of general applicability and scale-up. Nanohorns and nanodiamonds entered in the field of biomedical devices only very recently and many questions still remain concerning their real therapeutic uses. The structure of these materials is more similar to the traditional spherical nanoparticles, although a direct correlation between their properties and those of CNTs is not possible. The sizes of the different functionalised carbon hybrids described in this review might represent a limitation in terms of *in vivo* transport, however, proposed strategies that may lead to prolonged blood circulation half-lives and targeting ligands on the surface of such materials may overcome this drawback. The problem of specificity in cell targeting may be solved using epitope- and/or antibody-based cell membrane receptor recognition. Particularly interesting is the approach of multiple functionalisations, successfully applied to carbon nanotubes, which can amplify the efficiency of the delivery system and overcome the problem of heterogeneity of cell receptors. Another point of consideration should always be the comparison of such novel constructs with existing delivery technologies. At this stage it is almost impossible to directly compare nanotubes, nanohorns or nanodiamonds with other existing delivery technologies that are available and have been studied for decades. Finally, it is still very early to confidently determine whether carbon-based nanomaterials will become clinically viable tools to combat cancer. There is definitely room for them to complement existing technologies. Future investigations and the constant, systematic progress in the assessment of the biomedical potential of such nanomaterials will help us to determine the real opportunities from the unrealistic expectations.

## Declaration of interest

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## Affiliation


Alberto Bianco[†1], Kostas Kostarelos[2]& Maurizio Prato[3]
[†]Authorfor cor respondence
[1]CNRS,
Institut de Biologie Moléculaire et Cellulaire,
UPR 9021 Immonulogie et Chimie Thérapeutiques, 67000 Strasbourg, France
Tel: +33 388 417088; Fax: +33 388 610680;
E-mail: a.bianco@ibmc.u-strasbg.fr
[2]University of London,
Nanomedicine Laboratory,
Centre for Drug Delivery Research,
The School of Pharmacy,
London, UK
[3]Universitàd i Trieste,
Dipartimento di Scienze Farmaceutiche,
34127 Trieste,
Italy