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Carbon nanostructures as multi-functional drug delivery platforms

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Nanotechnology is providing exciting and new opportunities which are likely to revolutionize future clinical practice. The use of nanoparticles for biomedical applications is particularly exciting due to their huge potential for multi-modal approaches. This includes their use as drug delivery vectors, imaging contrast agents, hyperthermia systems and molecular targeting. Their ability to cross biological barriers, for example the blood brain barrier, makes them attractive for potential treatments in neurological disorders. There is also great hope that nanostructures will serve as platforms in future cancer therapies. Current cancer fighting strategies consist primarily of surgery, radiation therapy and chemotherapy. Each of these treatments is bound by a limit, known as the therapeutic window, which, if exceeded, causes undue harm to the patient. In the ongoing quest to improve our therapeutic arsenal, nanoparticles are emerging as exciting structures for a new generation of multi-modal therapeutics. Within this context, carbon nanostructures are amongst the leading contenders as building blocks to deliver multi-function drug delivery platforms. This review examines the various properties of carbon nanostructures that allow such multi-functionality. Recent advances on the development of novel approaches for functionalization, targeting and imaging *via* carbon nanostructures are discussed.

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1.0 Introduction

Nanotechnology is very diverse and holds much promise in many fields. At the same time there exists a significant debate on the future implications of nanotechnology. Within medicine, nanotechnology provides cause for much excitement. Nanotechnology could potentially offer diagnosis and therapy tailored to patients' genes. Moreover, it could be delivered with unprecedented precision. With these goals in mind, carbon

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nanostructures are being actively explored as supportive substrates as well as excipients for multi-functional drug delivery systems. Such systems are highly relevant in future strategies to combat and ultimately cure cancer. Current cancer treatments include surgery, radiation and chemotherapy. Although they can claim a degree of success, these approaches also kill healthy cells and cause toxicity to the patient. In addition, there are difficulties in the administration of drugs, such as insolubility of drugs, inefficient distribution, lack of selectivity and side-effects. In drug delivery, cell membranes also pose a problem by selectively allowing only certain structures to pass through depending on their hydrophilicity. Great efforts are being made to develop novel cancer strategies that directly target cancerous cells without affecting healthy ones. These strategies should offer improved efficiency, viability and toxicity profiles. Carbon nanostructures are amongst the leading contenders because their physicochemical properties offer both covalent and noncovalent functionalization with disparate functional groups. Moreover they can carry several moieties and in some cases provide enclosed payload options. They can also passively cross the membranes of many different types of cells.[1] They can enter a cell *via* energy-dependent endocytosis as well, and there is probably dependance on the size and shape of the nanostructure.

These features make them ideal building blocks for targeting, imaging and multi-therapy systems. The versatility of carbon nanostructures to combat numerous diseases as well as provide diagnostic ability is highlighted in Table 1 in which different carbon nanostructures functionalized in various manners are applied to a multitude of therapeutic approaches.

In this review we focus primarily on novel directions and approaches in therapeutic oncology built around carbon nanostructures. Many of the strategies presented are also applicable to a variety of other diseases. In the first section of this review, the types of carbon nanostructures currently under exploration are discussed in terms of their initial synthesis and purification through their functionalization and toxicity is presented. The second section addresses the studies being conducted on their potential for therapeutical application which includes hyperthermia, targeted drug delivery and gene therapy. The third section evaluates the state of the art in their development for diagnostics, *viz.* imaging. Finally, their future perspectives are briefly discussed.

2.0 Types of available platforms and their preparation

The variety of natural and artificial carbon allotropes is astounding and many of these carbon structural formations have exciting electronic, mechanical and physicochemical properties at the nanoscale. Indeed, their promising nano-based properties have excited scientists and engineers for a number of years, particularly in materials science and molecular electronics. More recently, we started realising how these wonderful structures can be exploited for their drug delivery and diagnostic potential. To this end, thus far, the following carbon-based nanostructures are being explored: carbon nanotubes, carbon encapsulates, graphene, fullerenes, carbon nanohorns and nano-diamonds. These structures are presented in Fig. 1. In this section, the synthesis, purification and functionalization for biomedical application of these nanostructures are addressed. In addition, brief comments on their toxicity are also presented.

2.1 Carbon nanotubes (CNTs)

The current excitement in carbon nanotubes (CNTs) was triggered by Sumio Iijima's Nature publication in 1991.[2] Their diameters range from sub-nanometers to several hundred nanometers. Their lengths can reach a few centimetres.[3] CNTs are artificial allotropes of carbon consisting of a single or multiple graphene layers rolled up concentrically and hence have a cylindrical structure. When the tubes are formed from a single graphene sheet then the structures are called single-walled carbon nanotubes (SWCNTs). In the case of multiple concentric sheets the structures are called multi-walled carbon nanotubes (MWCNTs). There are many routes to produce SWCNTs and MWCNTs. The most well-known are the flame synthesis,[4] arc discharge,[5–10] laser ablation[11–13] and chemical vapor deposition (CVD) systems. By far the most popular method to synthesize carbon nanotubes is the CVD route. In CVD usually a catalyst particle is employed to nucleate and grow the carbon nanotubes as well as to help decompose the carbon feedstock.[14–20] CNTs produced *via* CVD are often characterized by large diameter distributions. The as-produced material also contains impurities such as catalyst particles, amorphous carbon and encapsulated metal particles. Optimizing the synthesis parameters can minimize impurities, but rarely, if at all, fully prevent their formation. One example of this optimization for the high yield synthesis of SWCNTs was shown by Hata *et al.*[21] They demonstrated a water-assisted CVD route argued to yield a product consisting of 99.98% SWCNTs. The use of CNTs in biomedical applications requires that they should be of high purity with well defined properties. To achieve this, purification steps are needed. To this end, numerous methods have been elaborated. Carbonaceous impurities are usually removed by oxidation in fixed air or oxygen at $\sim$300 °C.[22–27] Other oxidizing methods apply oxidizing agents, *e.g.* nitric acid, *aqua regia* or potassium permanganate, using reflux or microwave digestion treatments.[22,28] The oxidation processes can open the ends of closed tubes, since the greater curvature of end caps makes them more reactive. In addition, the purification routes usually lead to surface functionalization. Impurities like metal catalysts or support materials are usually removed by acid treatments (*e.g.* hydrochloric, nitric, sulphuric acid or acid mixtures). Essentially the acid or acids applied dissolve unwanted material which can then be easily washed away.[22,23,28–31] Ultrasound treatments are sometimes employed in order to disperse the CNTs, to cut them (shorten) and accelerate dissolution.[28] Once purified, the nanotubes are ready for further functionalization, for example, to make them biocompatible. In addition, functionalization of carbon nanostructures in general allows them to be easily dispersed in aqueous solutions.

Table 1 Therapeutic approaches of functionalized carbon nanostructures discussed within the review

Functionalized carbon nanostructure	Incorporated molecule	Current usage	Advantage due to conjugation	References
CNTs	Folic acid antagonist methotrexate (MTX)	Anticancer drug	Reduce cytotoxicity and retain drug function	40
CNTs	Doxorubicin (DOX)	Anticancer drug	Reduce cytotoxicity and retain drug function	36,41
CNTs	10 Hydroxycamptothecin (HCPT)	Anticancer drug	Reduce cytotoxicity and retain drug function	42
CNTs	Paditaxel (PTX)	Anticancer drug	Reduce cytotoxicity and retain drug function	43
CNTs/CNHs	Cisplatin	Anticancer drug	Reduce cytotoxicity and retain drug function	44,48,49,187,195
CNTs	Phospholipid–polyethelene glycol (PL–PEG)	Dispersion in physiological environment	Increase biocompatibility of nanostructures	85
CNTs	Biotin	Molecular targeting for cancer cells	Increase specificity of nanostructures	45
CNTs	Cyclic RGD peptide	Molecular targeting for cancer cells	Increase specificity nanostructures	46,276
CNTs	Carboplatin	Anticancer drug	Controlled release and reduce toxicity	47
CNTs	Metal or metal oxides	Magnetic fluid hyperthermia, diagnosis, gene therapy	Biocompatibility, enhancement in image contrast, force the endocytosis for increasing transfection in gene therapy	40,50
CNTs	–COOH, –OH	Facilitate dispersion in physiological environment	Increase biocompatibility and functionalization sites on nanostructures	33
CNTs	Folic acid	Molecular targeting for cancer cells	Specific targeting in breast cancer cells	135
CNTs	RNAi	Gene therapy	Increase delay of tumor growth in comparison with liposomes	227,228
CNTs	Monoclonal antibodies	Molecular targeting for cancer cells	Efficient platform for specific targeting molecules	247
CNTs	Amphiphilic Gd^{3+} chelates	Contrast agent	Increase negative contrast in MRI	272
CNTs	Radionuclides	Radioprobes in cancer diagnosis	Increase diagnosis efficiency in nuclear medicine, *e.g.* SPECT	279
CNTs	Dapsone (DAP)	Antimicrobial and anti-inflammatory effect	Reduction of oxidative stress of CNTs retaining drug properties	190
CNTs	Ketoprofen	Anti-inflammatory effect	Controlled release of drug	191
CNTs	Amphotericin B (AmB)	Antifungal drug	Reduced toxic effect of drug	192
CNTs	Carvedilol (CAR)	Treatment of hyperthermia	Increase solubility of drug	193
NGO	Irinotecan	Anticancer drug	Reduce cytotoxicity and retain drug function	144
NGO	Gefitnib	Anticancer drug	Reduce cytotoxicity and retain drug function	145
NGO	Polyethyleneimine (PEI)	Platform for functionalization	Increase transfection of DNA efficiency	230
NGO	Monoclonal antibody TRC105	Molecular targeting for cancer cells	Increase specificity	291
NGO	^{64}Cu isotope	PET radiolabel	Increase diagnosis efficiency in nuclear medicine, *e.g.* PET	291
NGO	Polyvinyl alcohol (PVA)	Cartilage, tendons and menisci repair	Increase in gel/film mechanical properties	209,211
NGO	Poly methyl methacrylate (PMMA)	Cartilage, tendons and menisci repair	Increase in gel/film mechanical properties	209,210
NGO	Chitosan	Enhance bone formation	Increase in gel/film mechanical properties and bone formation	212
NGO	Pluronic	Dispersion in physiological environment	Steric stabilization and platform of different molecules	205
NGO	Angiopep-2	Molecular targeting for cancer cells	Specific targeting molecule	187
Fullerenes	Radionuclides	Radioprobes in cancer diagnosis	Increase diagnosis efficiency in nuclear medicine, *e.g.* SPECT	126

Table 1 (*Contd.*)

Functionalized carbon nanostructure	Incorporated molecule	Current usage	Advantage due to conjugation	References
Fullerenes	–OH	Free radical scavenging	Increase efficiency of scavenging	107,121,122
Fullerenes	Carboxyfullerenes	Free radical scavenging and treatment of Parkinson's disease	Increase efficiency of scavenging	123,124
Fullerenes	Metallofullerenes	Contrast agents	Improve contrast in MRI and X-ray diagnostic methods	108,109,126–129
Fullerenes	Fluoride anions	Osteoporosis	Increase solubility and absorption of drug	218,219
NDs	Polyethyleneimine (PEI)	Platform for functionalization	Increase transfection of DNA efficiency	217,235
NDs	Bovine serum albumin (BSA)	Dispersion in physiological environment	Stabilization in physiological media	289
NDs	Insulin	Regulation of glucose levels in blood	Controlled delivery into cells	200
CNHs	Glucocorticoid dexamethasone (DEX)	Anti-inflammatory drug	Controlled release into cells	198
CNHs	Polyamidoamine (PAMAN)	Platform for functionalization	Anchor for siRNA in gene therapy	231
SWCNHs	Prednisolone (PSL)	Anti-inflammatory drug	Increased anti-inflammatory effect	197
CEMNs	Metal or metal oxides	Magnetic fluid hyperthermia, diagnosis, gene therapy	Biocompatibility, enhancement in image contrast, force the endocytosis for increasing transfection in gene therapy	89,261,262
Fe/Co NPs coated with carbon, NGO, CNTs	Polyethelene glycol (PEG)	Dispersion in physiological environment	Increase biocompatibility of nanostructures	85,138,250
Co NPs coated with carbon, fullerenes	$-NH_2$	Facilitate dispersion in physiological environment	Increase biocompatibility and functionalization sites on nanostructures	92,93

The relatively large surface area and hollow core found with carbon nanotubes allow them to be functionalized in two basic ways. These versatile functionalization possibilities are presented in Fig. 2. In the first route, the exceptional ability of sp^2 carbon to form bonds with different materials is exploited *e.g.* molecules are attached to the outer surfaces of the tubes and this type of functionalization is referred to as exohedral functionalization. The second route, endohedral functionalization, takes advantage of the tubes hollow interior. Within the context of biomedical applications the demand for functionalized CNTs is to develop them into viable platforms for specific cell or tissue targeting, tracking or imaging and therapeutics. Various strategies to exohedrally functionalize CNTs have been designed to make them biocompatible.[32,33] Two basic approaches are adopted:

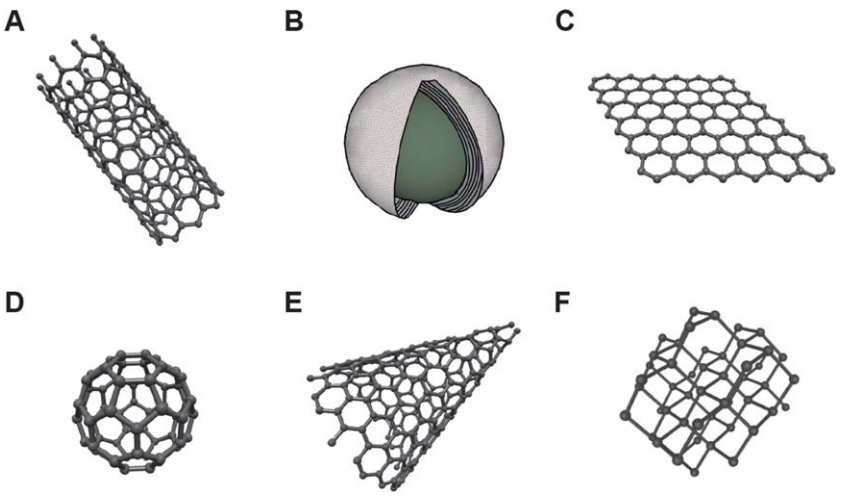

Fig. 1 Illustrations of the various carbon based structures described in this review. (A) Single-walled carbon nanotube. (B) Encapsulate (graphite layers encapsulate a particle). (C) Graphene. (D) Fullerene (C_{60}). (E) Single-walled carbon nanohorn. (F) Nano-diamond.

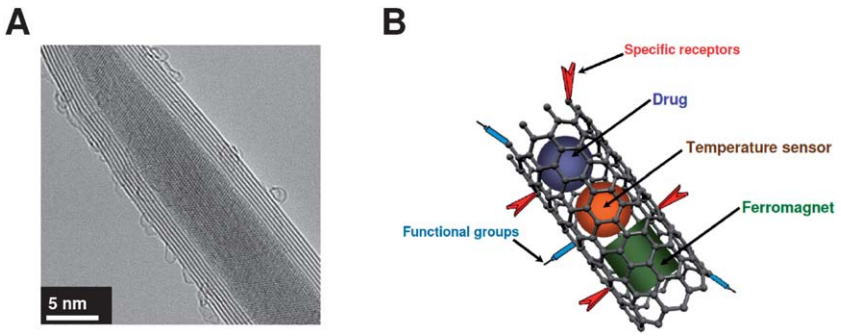

Fig. 2 (A) Transmission electron micrograph of an Fe filled MWCNT. (*Sample courtesy S. Hampel.*) (B) Schematic highlighting the versatile functionalization possibilities of carbon nanotubes.

non-covalent and covalent functionalization. The non-covalent functionalization routes involve the preparation of a stable dispersion by coating the CNT surfaces with molecules (*e.g.* copolymers, lipids, surfactants, and DNA).[34–38] For example, the use of synthetic tocopheryl polyethylene glycol succinate (TPGS) molecules was shown to disperse MWCNTs effectively at mass ratios (TPGS : MWCNTs) 1 : 4 or greater.[39] The covalent functionalization of the tube surface is typically realized through cycloaddition reactions which attach ammonium groups or *via* strong acid treatment to generate carboxylic acid groups.[33] Non-covalent and covalent functionalization offer well dispersed, flexible carbon nanotube platforms suitable for further derivatization *e.g.* loading with anti-cancer drugs. The loading of various anti-cancer drugs has been demonstrated to date. An example is folic acid antagonist methotrexate (MTX) which is toxic and has limited cell uptake. MTX can be covalently connected *via* different linkers (like cleavable linkers) to carbon nanotubes.[40] The cytotoxic activity is strongly dependent on the type of linker used for the functionalization. Other popular anti-cancer drugs loaded through surface functionalization are doxorubicin (DOX),[36,41] 10 hydroxycamptothecin (HCPT),[42] paclitaxel (PTX)[43] and cisplatin.[44] Prior to loading, the CNTs are dispersed in aqueous solution, for example, tri-block copolymer Pluronic F127,[36] or through covalent functionalization with polyethylene glycol (PEG), or non-covalently with a phospholipid (PL)–PEG surfactant.[41] The release of these drugs strongly depends on the diameter of the CNTs. This is due to the more efficient π-stacking of aromatic molecules on bigger nanotubes (flatter). Hence, for larger tubes drug release is less effective. Additionally, molecular targeting agents for cancer cells like *e.g.* biotin,[45] cyclic RGD (Arg-Gly-Asp) peptide[46] can be successfully connected on the tube surfaces. This helps the functionalized CNT reach cancerous cells more accurately. As mentioned previously, carbon nanotubes can also be functionalized endohedrally. Endohedral filling is usually accomplished before exhohedral functionalization. The placement of therapeutic molecules within tubes can be advantageous. For example, enclosure within the core can protect molecules sensitive to photo-degradation. One of the simplest ways to fill CNTs is to use capillarity, which is a process in which liquid spontaneously enters a narrow space and is due to inter-molecular attractive forces between the liquid and surrounding surface of the solid (the nanotube). Using this phenomenon *via* wet chemistry, various anticancer therapeutics can be loaded in the tube interior. Carboplatin[47] or cisplatin[48,49] can be loaded by mixing with dispersed CNTs and stirring over a period of time. Thereafter the mixture is filtered and the excess of therapeutic material is rinsed off. The encapsulation of ferromagnetic materials (*e.g. iron or iron-oxide*) inside CNTs is important for hyperthermia treatments.[40,50] Filling the tubes with ferromagnetic metals can be achieved through a variety of routes. The filling can be obtained simultaneously during the synthesis of the carbon nanotubes.[40,51–59] They can be filled *via* wet chemistry procedures (*e.g.* Fig. 2A)[60] or by hot vapor filling using organometallic compounds such as ferrocene, a cyclopentadienliron dicarbonyl dimer.[61] It is generally accepted that core fillings *e.g.* iron and anticancer drugs do not interact, however this has not been proven conclusively.

With regard to their toxicity there is still no clear evidence of the toxic influence of carbon nanotubes in biomedical applications. Studies on non-functionalized carbon nanotubes dispersed in different solutions with different concentrations[62–65] have been conducted. The results are somewhat unclear, in that some suggest serious health risks associated with carbon nanotube exposure. Most investigations are based on the CNT entry through the pulmonary route. However, the relevance of these studies is limited when considering biomedical applications because the dosing and administration parameters used are not applicable in this context.[32] Recently Kagan *et al.*[66] showed a novel enzymatic biodegradation route of SWCNTs relevant to respiratory exposures through the neutrophil enzymatic system. The study pointed out that the doses of nanotubes used in the toxicity tests might be too high, overwhelming the degradation capacity of this enzymatic system. Some studies indicate CNT length dependencies. Long and stiff carbon nanotubes (>10 μm) can accumulate in tissues and trigger cancer *e.g.* mesothelioma.[67,68] Studies on lipopolymer or surfactant-coated CNTs show that they tend to accumulate in the liver and cannot be metabolized there.[69–71] However, the use of short and functionalized carbon nanotubes (<1 μm) suggests an improved toxicological profile.[72] CNTs with small dimensions which are short and well dispersed in the body do not accumulate in tissue and are easily removed through urinary excretion.[73–76]

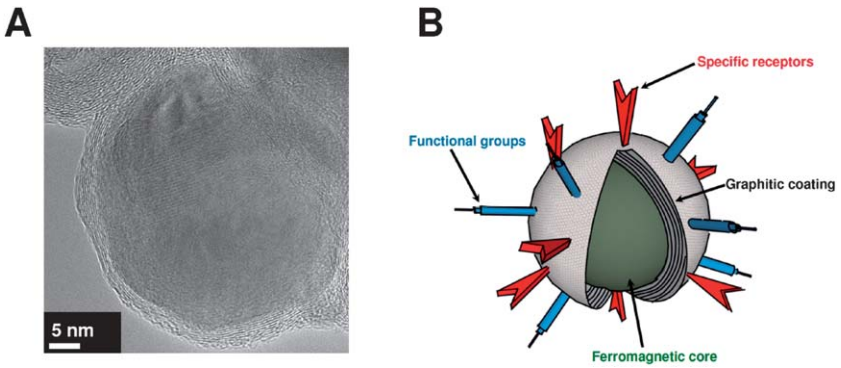

Fig. 3 (A) Transmission electron micrograph of an Fe nanoparticle encapsulated with graphite. (*Sample courtesy M. Bystrzejewski*.) (B) Illustration of a functionalized encapsulate.

2.2 Carbon-encapsulated magnetic nanoparticles (CEMNs)

Carbon-encapsulated magnetic nanoparticles (CEMNs) are core–shell structures, mostly spherical in shape with diameters ranging from a few nanometers up to tens of nanometers (see Fig. 3A). Their core consists of a nanosized metal or metal oxide particles (*e.g.* iron, cobalt, nickel or iron–cobalt alloy, iron oxides) and their shell is comprised of several graphitic layers.[77–85] CEMNs are usually produced by arc discharge,[78] thermal plasma synthesis[77,86] or *via* the thermal decomposition of compounds containing the required metal.[81,87] The cores of the carbon-encapsulated magnetic nanoparticles are sensitive to magnetic fields, thus apart from their potential as drug carriers[88] they also hold promise as contrast agents in magnetic resonance imaging (MRI)[89] and anti-cancer hyperthermia treatments.[81] The graphitic coating layers range from 1 up to 40 layers and play several important roles: in protecting the inner particle from oxidization, in isolating the magnetic nanoparticles from each other and in providing biocompatibility as well as affording the surface functionalization with antibodies, proteins, medical drugs, *etc.* for extended bio-applications. Usually the as-produced material contains unwanted species (impurities) such as amorphous carbon, uncoated metal or metal oxide particles, graphitic nanoparticles, unfilled graphitic capsules, carbon nanotubes, and carbon fibres.[77,78,81,87] Carbonaceous impurities can be easily removed through post-synthesis treatments. Examples include oxidation in air at ~300 °C, or exposure to oxidizing agents like hydrogen peroxide. Unwanted metal and metal oxides species are commonly removed through acid treatments (*e.g.* hydrochloric, nitric or sulphuric acid).[77,78] In addition, centrifugation steps can be used in order to separate (by mass) unfilled graphitic capsules from the heavier filled particles. After the purification procedures the carbon encapsulated magnetic nanoparticles are then prepared for biocompatibility through surface functionalization. This step is often accomplished by first oxidizing their surface in hot acid solutions (*e.g.* nitric acid or sulphuric acid, or their mixtures).[88,90] The oxidation process forms carboxylic groups (–COOH) on the surface of the particles. These groups enable further functionalization by conjugation with amino-containing molecules *via* diazonium chemistry,[91] amidation[92] or diimide-activated amidation.[93]

The resultant surface functionalized encapsulated nanoparticles are intermediates that can be yet further functionalized (Fig. 3B). For example, amine reactive intermediates can react with the carboxylic groups of biomolecules, *e.g.* proteins, and form stable amide bonds. These can be further functionalized for biocompatibility. This is often accomplished with poly(ethylene glycol) – PEG.[85] PEG-functionalized carbon-encapsulated magnetic nanoparticles are stable against aggregation under physiologically relevant conditions, are undetectable to the immune system and can be addressed to specific regions of the body. Moreover, *in vitro* and *in vivo* toxicity assays show no obvious cytotoxicity from these nanostructures and no obvious negative health problems for the tested organisms over a monitoring period *ca.* 6 months.[85] In addition, the nanoparticles remain stable in blood circulation for over 20 minutes, which, in this sense, makes them superior than standard MRI contrast agents.

2.3 Fullerenes

Zero-dimensional (0D) fullerenes were discovered in 1985 by Robert Curl, Harold Kroto and Richard Smalley at Rice University and Sussex University. Fullerenes are named after Richard Buckminster Fuller, a famous architect known for his geodesic domes. Fullerenes can exist with different numbers of carbon atoms in their structure, giving them different shapes and sizes. The most famous fullerene form is the C_{60} molecule (buckyball) which contains 60 carbon atoms. Carbon atoms in this object are linked together, forming 20 hexagonal and 12 pentagonal rings, similar to a traditional football. Other fullerene forms are C_{70}, C_{72}, C_{76}, C_{84} and C_{100}. Gram quantities of fullerenes were synthesized in 1990 for the first time by two physicists, W. Krätschmer and D. R. Huffman, by creating an arc between two graphite electrodes in a quenching atmosphere of helium.[93] Other methods to produce fullerenes are: the vaporization of graphite by heating in an inert gas,[94] combustion of unsaturated hydrocarbons in flames,[95] focusing sunlight on a graphite carbon target,[96] laser ablation of graphite (the technique in which fullerenes were first discovered)[97] and the laser ablation of polymers.[98] A substantial fraction of the as-produced black soot-like material consists of fullerenes. Mixed within the product are graphitic and amorphous impurities.

Therefore the fullerenes need to initially be separated from unwanted species by sublimation *via* heating in an inert gas or in a vacuum or by salvation, usually using toluene due to good solubility in this medium (2.8 mg ml^{-1}).[99] The resultant fullerene material contains $C_{60} \sim 80\%$, $C_{70} \sim 20\%$ and 1% of larger fullerenes. The various fullerene types are then fractioned using flash-chromatography. At this final stage the toluene is removed using a rotary evaporator.[100,101] Due to the unique carbon cage structure of fullerenes they can be easily functionalized with a wide range of molecules (exohedral functionalization) and endohedrally by metal ions.[102–109] This makes them promising for biomedical applications in therapeutics and diagnostics. However, fullerenes are hydrophobic, which restricts their direct use in bio-applications.[110] Hence, in order to make them viable as candidates for bio-applications they need to be suitably prepared. This is usually accomplished *via* surface functionalization. This includes chemical functionalization with *e.g.* amino acid, carboxylic acid, polyhydroxyl groups, amphiphilic polymers,[111–114] and therapeutic agents *e.g.* chemotherapeutics,[115] or encapsulation of fullerenes in special carriers such as cyclodextrins[116] or calixarenes,[117] polivinylpyrrolidone,[118] micelles and lyposomes.[119] Chemotherapeutic agents for cancer therapies can "decorate" the surface of fullerenes together with specific antibodies for targeted delivery; *viz.* the antibody guides the fullerene directly to the cancer cell by tracking its chemical signature.[120] Due to the unique structure of fullerenes which contain 30 conjugated double bonds, fullerenes can easily interact with other molecules and also react with free radicals and not be consumed in the process. An interesting derivative for free radical scavenging purposes is fullerenol ($C_{60}(OH)_{24}$). Fullerenol is usually prepared by adding NaOH solution and TBAH (tetrabutylamonium hydroxide) to a C_{60} toluene solution, and thereafter the functionalized fullerenes are separated from the organic and water phases.[107] Their free radical scavenging potential has been demonstrated both *in vitro*[121] and *in vivo*.[122] Another free radical scavenger is carboxyfullerene ($C_{60}C(COOH)_2)_2$. The preparation of this structure is usually performed by mixing C_{60} with diethyl bromomalonate under basic conditions.[107] Carboxyfullerenes can protect quiescence in human peripheral blood mononuclear cells against programmed cell death (apoptosis)[123] or can be used for the treatment of Parkinson's disease.[124]

Another class of fullerene derivatives are the endohedral metallofullerenes, which are fullerenes with metal ions trapped inside their cage (see Fig. 4). The metal atoms in these structures take off-center positions in the fullerene cages and electrons transfer from the metal atom to the cage. The preparation of doped metal fullerenes is achieved *via* laser evaporation or in an arc reactor. They hold potential for medical diagnostics inside living organisms as contrast agents for magnetic resonance imaging (MRI). The most common endohedral fullerenes used for biomedical applications are: gadolinium (Gd^{3+}),[108,109,125] scandium (Sc),[108] holmium (Ho^{3+}),[126] thulium (Tm^{2+}),[127] gallium (Ga^{3+})[128] and technetium (Tc^{2+}).[129] The basic concept of these fullerene derivatives as MRI agents is to isolate unstable metal atoms inside their carbon cages in order to protect against interaction with the outside environment, *viz.* to prevent any toxic influence. Before inserting endohedral fullerenes to living organisms they must be functionalized exohedrally in order to allow them to cross cell membranes. Tests have shown that fullerenes and their derivatives have no observable influence on *in vitro* cytotoxicity.[102,130] The distribution and metabolism of fullerene derivatives in living organisms is extensively investigated today. They can accumulate in specific places of the bodies, *e.g.* liver or bones (Ho^{3+} metallofullerenes), enabling detailed MRI imaging of specific areas. Various studies have shown that metallo-fullerenes applied to living organisms display ultra low levels of radioactivity. Moreover, endohedral fullerenes stay in the body after the application for approximately one hour, which is sufficient to image the circulatory system.

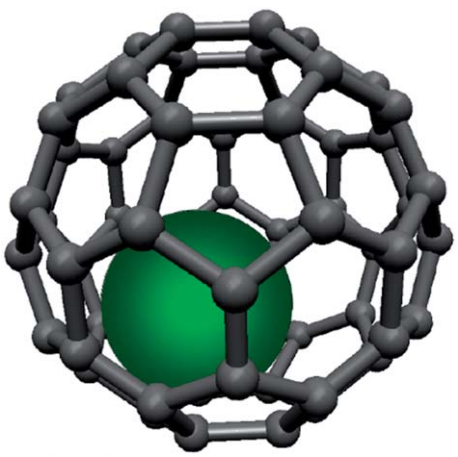

Fig. 4 Example of an endohedral fullerene.

2.4 Graphene

Two-dimensional (2D) material graphene was first isolated in 2004 by Geim *et al.*[131] Graphene is essentially an isolated atomic plane of graphite. It has exciting physical properties such as high mechanical stiffness[132,133] and excellent electronic transport properties.[131,134] Recently studies have begun to exploit the potential of nanosized pristine graphene and in its oxidized form (NGO) for biomedical applications. This includes their potential as drug delivery systems (DDS)[135,138] and for cellular delivery of genes and peptides or proteins.[139] Future biomedical applications of the graphene family may also include implantable sensors, tissue scaffolds or coating prosthetics and implanted devices.[140] The most used form of graphene in the biomedical field is the nanographene oxide. The most frequently adopted approach to prepare this material is by the Hummers method; initially the graphite is oxidized using a $KMnO_4$–H_2SO_4 mixture. Nanosizing the GO is usually accomplished by ultrasonication. Ultracentrifugation is then employed to separate the nano-sized sheets (<10 nm) from larger ones.[135–139] Nanoscale graphene oxide (NGO) prepared in this form is easily soluble in water due to the presence of functional groups *e.g.* hydroxyl groups (–OH), epoxide (R–CH–O–CH_2) and ester groups (R1–COO–R2), ketone groups (C=O),

and carboxylic acid groups (–COOH) on its surface. In order to prepare the structures for biofunctionalization the epoxy and ester groups need to be converted to hydroxyl groups and subsequently to carboxylic acid moieties (–COOH). This is achieved by adding chloroacetic acid under strong basic conditions to the NGO–water solution.[137,141] The NGO, now rich with carboxylic acid groups, can be subsequently functionalized with a biocompatible polymer like polyethylene glycol (PEG) – pegylation.[136,137] PEG is a particularly attractive polymer for conjugation with specific moieties, as it provides good water solubility, high mobility in solution, is not toxic and does not lead to immunogenicity. Moreover, it can readily be cleared from the body and also offers altered distribution in the body. These properties are all important aspects for bio-applications. The introduction of sulfonic acid groups is another functionalization route for stable dispersion in physiological solutions.[135,142] At this stage the NGO can be loaded with anticancer drugs *via* non-covalent bonding for targeted drug delivery. Because anticancer drugs are usually water insoluble, NGO is an attractive platform to overcome this drawback. Doxorubicin (DOX) or/and irinotecan, or gefitinib are commonly used as model anticancer drugs for application with NGO.[135–139] Doxorubicin is commonly used to treat some leukemias, Hodgkin's lymphoma, as well as cancers of the bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma, multiple myeloma, and others.[143] Irinotecan is a semisynthetic analogue of the natural alkaloid camptothecin. Its main use is in colon cancer, particularly in combination with other chemotherapy agents.[144] Gefitinib is currently only employed for the treatment of locally advanced or metastatic non-small cell lung cancer (NSCLC) in patients who have previously received chemotherapy.[145] In addition, to improve targeting, additional molecules like folic acid (FA) (to target cells with folate receptors),[135] or antibodies, *e.g.* rituxan (a B-cell specific antibody to selectively recognize and bind to B-cell lymphoma cells)[137] can be covalently conjugated.

Important results from cytotoxicity tests performed on pristine NGO and on functionalized NGO (*e.g.* NGO–PEG) have been conducted. They show that prior to drug loading they are practically non-toxic, even at very high concentrations (more than 100 mg L^{-1}).[137]

2.5 Single-walled carbon nanohorns (SWCNHs)

Single-walled carbon nanohorns (SWCNHs) were originally fabricated by Iijima *et al.* in 1999.[146] They are horn shaped nanostructures (average cone angle 120°) composed of single graphene sheets with lower diameters of around 2 nm. They usually form aggregates with diameters *ca.* 80 nm with a "dahlia-like" petal shape as can be seen in Fig. 5. The synthesis of single-walled carbon nanohorns is usually through CO_2 laser evaporization of graphite target at room temperature in a buffer gas (Ar, He, and N_2) at pressures ranging from 200 to 1000 mbar.[147,148] The as-produced material consists predominantly of SWCNHs. A small quantity of amorphous carbon is also present. The properties of SWCNHs are similar to carbon nanotubes, thus they are easily functionalized (*e.g.* fluorination, amidation),[149–152] solubilised and dispersed in water or physiological solutions following similar techniques used with CNTs.

2.6 Nanodiamond (NDs)

Carbon nanodiamonds (NDs) were first discovered in 1963 by K. V. Volkov during diamond synthesis studies through shock compression in a blast chamber.[153] NDs are nanosized particles with diameters smaller than 10 nm. They can be synthesized with a good yield and quality *via* the detonation of certain

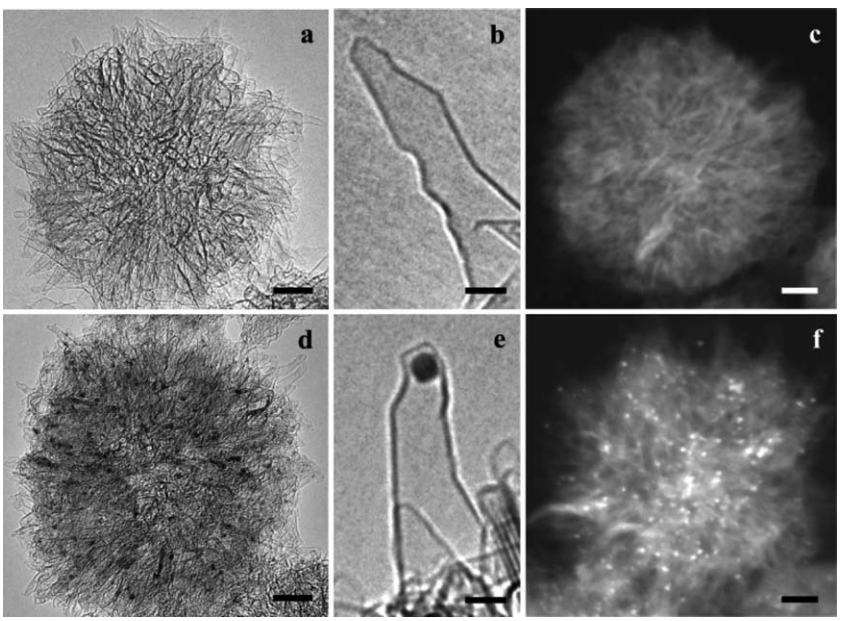

Fig. 5 (a and b) HRTEM images of oxidized SWNH (SWNHox) (scale bars of 10 and 2 nm, respectively). (c) *Z*-Contrast image of SWNHox aggregate (10 nm). (d and e) HRTEM images of cisplatin@SWNHox (10 and 2 nm) in which black spots are cisplatin clusters. (f) *Z*-Contrast image of cisplatin@SWNHox in which bright spots are cisplatin clusters (10 nm). (*Reproduced with permission from ref. 195.*)

explosives in a closed chamber.[154–157] Other synthesis methods like chemical vapor deposition (Fig. 6)[158,159] or shock compression of graphite[160] provide particles with large grain size or polycrystalline diamond films. The as-produced material, whilst mostly consisting of NDs, often also contains unwanted amorphous, graphitic and metallic species. Metal particles are commonly removed by acid treatment *e.g.* H_2SO_4, HNO_3 or mixtures.[161,162] To remove carbonaceous impurities different oxidative treatments can be applied, *e.g.* in solutions: KOH/KNO_3, Na_2O_2, CrO_3/H_2SO_4, and HNO_3/H_2O_2 under pressure or oxidation in air at *ca.* 400 °C.[163–165] A promising route is based on an autoclave method using HNO_3/H_2O_2, where at a high temperature, *ca.* 280 °C, unwanted carbon species are dissolved and transformed into a gaseous form without any other particles forming during the process.[161] In addition, any metal impurities present are easily dissolved and washed away. Nanodiamonds are characterized by low solubility in most solvents.[146] Hence, for bio-applications they must undergo surface functionalization.[166–169] Their surface can be functionalized with hydroxyls, carboxylics, ketones or amine groups which prepare them for further use in dispersions in aqueous or physiological solutions.[170] In order to improve solubility and biocompatibility of NDs they can be additionally covalently functionalized *via* diazitozation with diazonium salts,[169] fluorination,[168] chlorination, and silynation.[169] Non-covalent functionalization with organic and biological molecules like luciferase[171] and lysozyme[172] enables such functionalized NDs to serve as biomarkers. Small amounts of anticancer drugs like doxorubicin or paclitaxel (~25% of NDs mass) can be loaded on their surface when functionalized with $-NH_2$ groups. This makes them relevant for future drug delivery.[173] Nanodiamond particles can be successfully used as magnetic resonance imaging markers after covalently functionalizing them with –COOH groups on their surfaces with an amine functionalized gadolinium(III) complex.[174] Moreover, when prepared in a fluorescent-magnetic form they can be used as cellular fluorescence markers.[175] To make them fluorescent ND powder is first mixed with ferrocene and silicon powder and then treated in a microwave arcing process. The resultant material consists of iron nanoparticles connected with NDs and graphene sheets and is, in essence, a new nanocomposite material.[176,177] Next, fluorescent moieties are covalently attached to the previously formed magnetic NDs forming fluorescent magnetic nanodiamonds (FMNDs) *via* the surface attachment of poly(acrylic acids) and fluorescein *o*-methacrylate. The fluorescent-magnetic NDs are water soluble and can be successfully used for fluorescence imaging of cells (*e.g.* HeLa cells).[175] NDs also exhibit a degree of natural fluorescence.[178] *In vitro* experiments with different cell lines using both functionalized and unfunctionalized NDs showed practically no toxicity.[173,179–181] Additionally, *in vivo* (mice) studies showed no allergic response upon contact with skin to be induced.[173]

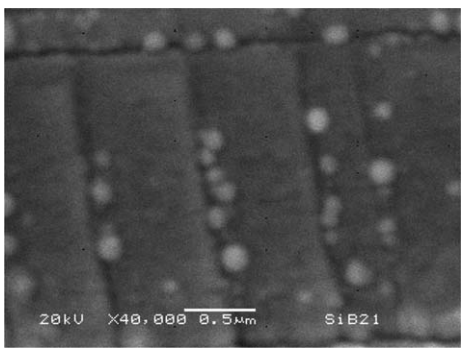

Fig. 6 SEM micrograph of nano-diamonds residing at terrace sites in Si formed with tequila as the feedstock. *Image courtesy of J. Morales and V. Castaño.*

3.0 Therapeutic applications

The demand for more efficient technologies to treat cancer which can substitute or work in combination with standard treatments is growing. The most promising results for cancer therapy stem from nanotechnology, where highly specific drug delivery systems and hyperthermia techniques or hybrid systems combining both are implemented. These platforms are briefly reviewed in this section.

3.1 Drug delivery

Humans have been experimenting with drug delivery ever since the first use of herbal plants as remedies. More recently, in the 1950s drug delivery through micro-encapsulated drug particles was developed. In the 1960s polymers began to be used to deliver drugs and our initial understanding of pharmacokinetic effects began.[182] There are many ways to deliver drugs in organisms. Examples include oral delivery, transdermal delivery, transepithelial delivery and intravenous delivery. These drug administration routes can be explored using nanotechnological approaches. A variety of drug nanocarriers are being extensively studied, namely, polymeric nanoparticles, liposomes, viral-based nanoparticles, magnetic nanoparticles and carbon nanostructures. All these nanosystems have been reported to have biomedical potential. Carbon nanostructures have the advantage of having great versatility in terms of loading (in and out) and easy functionalization. This review, however, focuses on the evolution and design of biocompatible carbon nanostructures as drug delivery systems in cancer nanotherapeutics.

3.1.1 CARBON NANOSTRUCTURES AS DRUG CARRIERS. CNTs show great potential as effective drug delivery systems for cancer therapy, as they can be grafted with cell-specific receptors and intracellular targeting molecules for the targeted delivery of therapeutic agents.[183] For example, delivery of anticancer drugs such as doxorubicin and platinum-based anticancer drugs has already been demonstrated.[184] Arlt *et al.*[185] compared the loading and releasing of carboplatin mediated by carbon nanotubes and carbon nanofibers in different tumor cell lines. They reported that both carbon nanostructures presented no significant toxic effects when unloaded, proving their intrinsic cytotoxicity even at high concentrations. They found the anticancer effect of carboplatin loaded nanotubes to be significantly better than for carboplatin loaded fibres. In

addition, the efficiency of the carboplatin loaded carbon nanotubes was more pronounced than that from free carboplatin in all the cell lines they tested. The functionalization of MWCNTs and SWCNTs with cisplatin, another antineoplastic agent commonly used to treat a wide variety of tumors, has also been successfully demonstrated.[49,186] The newly reported use of PEGylated MWCNTs conjugated with the molecule angiopep-2 was used as a specific-targeted complex system to deliver the anticancer drug DOX to brain glioma tumors.[187] The study successfully showed through *in vitro* and *in vivo* tests that the MWCNT–PEG–angiopep–DOX system was suitable to treat glioma tumors in mice. The conjugation of DOX with SWCNTs was also demonstrated to be more efficient in the treatment of cancer cells as compared to the drug alone.[188] CNTs have recently been reported to be good drug carrier platforms when functionalized with nanoliposomes.[189] This type of platform provides both an efficient cell uptake of CNTs and a high drug loading of liposomes which improves the treatment index of cancer. Tripisciano *et al.*[48] presented a study in which SWCNTs functionalized with cisplatin were incubated with prostate cancer (cell lines DU145 and PC3). They found that for certain concentrations they could reduce the number of living cells. However, in the case of the DU145 cell line, the SWCNT–cisplatin complex was not more effective than free cisplatin. This demonstrates that more detailed and systematic studies are required to better comprehend the processes involved.[188] CNTs have also been reported to deliver other types of drugs.[184] Dapsone (DAP), an antimicrobial and anti-inflammatory drug, has been modified onto MWCNTs and shown to trigger apoptosis only when incubated with cells for longer than 3 days and do not cause any oxidative stress. Thus, MWCNTs functionalized with DAP can be used for treating DAP-sensitive microorganisms and inflammatory diseases.[190] Ketoprofen, an anti-inflammatory drug, has also demonstrated to be effective when combined with MWCNTs.[191] In another study, MWCNTs were conjugated with the antifungal drug Amphtericin B (AmB) to reduce its toxic effects.[192] The work suggests that the complex AmB–MWCNTs are transported across mammalian cells without causing any cytotoxicity and AmB retains its high antifungal activity. CNTs have also been useful to improve the biocompatibility of carvedilol (CAR), which can be employed for the treatment of hypertension.[193] Moreover, CNTs may be used to treat Alzheimer's disease by carrying acetylcholine (Ach) into the brain.[194]

Another hollow carbon nanostructure reported to be functionalized with drugs is the SWCNH (see Fig. 5). Analogous to CNTs, the carbon nanohorns can also be loaded with the anticancer drug cisplatin. The successful release of cisplatin and suppression of cancer cell growth through *in vitro* studies have been demonstrated.[195] Apparently they exhibit a lower toxicity and a higher purity as compared to their tubular counterparts. Ajima *et al.*[196] showed, through *in vitro* studies, that the SWCNH–cisplatin complex is able to kill human lung cancer cells (NCI-H460). They also revealed the efficiency of the complex through *in vivo* investigations, and comparative studies with free cisplatin confirmed that SWCNH–cisplatin was better for suppressing the cell viability. Beyond cancer treatments, SWCNHs show promise in the fight against arthritis[197] when anchored with the anti-inflammatory drug prednilisone (PSL). Murakami *et al.*[198] described the possibility of using oxidized SWCNHs as drug carriers. They demonstrated that SWCNHs can be used to bind and release glucocorticoid dexamethasone (DEX), an anti-inflammatory drug. The complex DEX–SWCNH exhibited sustained release of DEX in mice cell lines ST2 and MC3T3-E1 cultures with no significant toxic effect.

Nanodiamonds are another carbon nanostructure with biomedical potential.[199] Shimkunas *et al.*[200] examined the adsorption and desorption of insulin on NDs as a platform for a protein-based drug. The study demonstrated that insulin's function is preserved after desorption from NDs. The adsorption of molecules on the surface of NDs also holds promise for cancer treatments as a vehicle for the delivery of chemotherapeutic agents. Huang *et al.*[201] successfully demonstrated that NDs functionalized with doxorubicin hydrochloride (ND–DOX), an apoptosis-inducing drug used in chemotherapy, were internalized by murine macrophages (RAW 264.7) and human colorectal carcinoma cells (HT-29). The ND–DOX complexes were capable of causing a significant decrease in cell viability. Lam *et al.*[202] used a different approach to deliver the drug doxorubicin using NDs. The chemotherapeutic drug conjugated with NDs (ND–DOX) was embedded within a parylene C polymer microfilm forming a patch-like structure as illustrated in Fig. 7. This structure assures a controlled release of the drug for over a period up to one month. Additionally, the patch can be placed directly where the drug should be released through the porous parylene film (see Fig. 7). The polymer assures biocompatibility and the controlled release of the drug, which is presumably driven by drug concentration gradients.

An important aspect in the design of carbon nanostructures as drug delivery systems is how to trigger desired functions once the nanostructure has reached its desired location, *viz.* the transport phase has been completed. The design stage also necessarily includes the choice of drug. Generally established anticancer drugs are used because their pharmacological and toxicological profiles are known. The simplest crystalline carbon nanostructure reported to be used as a drug carrier is nano-graphene. Graphene is a two-dimensional carbon sheet and holds great promise for nanoelectronics, sensors and nanocomposites.[203,204] However the use of graphene in bioscience has hardly begun. It has been shown that one can tailor graphene with insoluble anticancer drugs and load these graphitic nanostructures with DNA for gene therapy. An

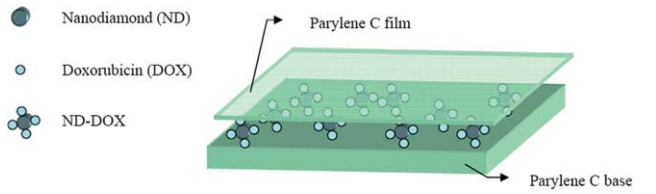

Fig. 7 Illustrative sketch of the anticancer patch proposed by Lam and colleagues. The drug complex ND–DOX is trapped between two layers of the polymer parylene C.

important step in the use of graphene in biomedicine is to make this molecule highly dispersible in a physiological solution. The insertion of functional groups onto the molecule through oxidation is the most common way to stabilize the molecules in electrolyte solutions. In addition to its electrostatic stability and dispersability in saline solutions, a second strategy employs triblock amphiphilic copolymer (pluronic F127) as a steric stabilizer of the NGO sheets.[205] These strategies aim to provide a means that enable other molecules to anchor and thus increase the effectiveness of drugs that are poorly soluble as well as to enhance the transfection of genetic material into cells.[137,141] Once the NGO is stabilized, further functionalization to further modify these nano-carriers can make them more effective. The most often used molecule to achieve this is PEG, however other polymers such as polyethylenimine (PEI)[206] have also been studied. Recently Wen *et al.*[207] showed the stabilization of NGO using PEG, which was also used as an intermediate link to the anticancer drug DOX. The work showed that the approach was successful for the delivery of the drug into HeLa cells and efficiently reduce viability. Sun *et al.*[138] covalently grafted PEG star polymers onto graphene oxide (PEG–GO). The PEG–GO exhibits photoluminescence from the visible to the NIR region in the electromagnetic spectrum and has the potential for cell imaging. In addition, the researchers loaded doxorubicin (an anticancer drug) onto the PEG–GO sheets *via* physisorption, as well as the antibody rituxan (anti-CD20) in order to selectively recognize and bind to B-cell lymphoma cells. Their work highlights the potential of functionalized graphene for high loading with anticancer drugs as well as selective targeting to specific cancer cells. Zhang *et al.*[136] used a different functionalization approach. They tailored graphene oxide with sulfonic acid to provide stability in biological environments and folic acid to specifically target the human breast cancer cell line MCF7. In addition, they loaded the functionalized graphene sheets with two different anticancer drugs, namely, doxorubicin and camptothecin. They demonstrated that the use of the combined drugs led to much higher cytotoxicity as compared to single drug delivery. The toxicity of GO has also been studied in bacteria, which could result in the future development of antimicrobial products. GO was shown to possess a strong antibacterial effect in both *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) bacteria.[208]

A rising field where graphene can be used is the reinforcement of biocompatible films, hydrogels and other scaffold materials frequently used for tissue engineering. Examples of this usage are polyvinyl alcohol (PVA) and polymethyl methacrylate (PMMA), which can be used as filters to repair cartilage, tendons and menisci, but have low mechanical strength or elasticity.[209] The incorporation of GO to these polymers increases their tensile strength and elasticity modulus without affecting their potential for osteoblast attachment.[210,211] The same results were obtained using chitosan which enhances bone formation. When chitosan is combined with GO it presented a superior mechanical strength and retained its morphological features under physiological and extreme pH conditions. Moreover the GO–chitosan complex significantly improved cell adhesion, proliferation and phosphate deposition in a mouse preosteoblast cell line.[212]

Another important carbon nanostructure which exhibits promise for a variety of therapies is the fullerene.[213,214] Fullerenes are unique carbon cage structures and many applications have been developed since their discovery in 1985. As highlighted in the reviews on fullerenes in biomedicine by Thakral and Mehta[215] and Partha and Conyers,[216] the acute toxicity of fullerenes is quite low, which make fullerenes promising building blocks for biomedical applications such as antiHIV activity, DNA cleavage, free radical scavenging and antimicrobial activity. They can even be used in the fight against osteoporosis. Bone-seeking drugs are useful in the treatment of osteoporosis and other bone disorders.[217] It is well established that bisphosphonate compounds are bone-active. Fluoride anions are also drugs currently used for the treatment of osteoporosis, however these drugs are not absorbed orally and are fairly toxic. Attractive routes taking advantage of the preferential localization of fullerene derivatives in bones are being developed.[218] An example is the use of polyfluoro bisphosphonated fullerene derivatives as bimodal drugs for osteoporosis therapy.[219]

It is hypothesized that free radicals play a role in carcinogenesis.[220,221] Free radicals are ubiquitously generated in our body through normal physiological processes. Strategies to reduce the number of these molecules are being developed. Fullerenes are known to have the remarkable property for trapping free radicals, which can prevent oxidative damage and deterioration of biological entities,[222] such as the DNA of cells. Bobylev *et al.*[223] suggested that sodium fullerenolates are able to prevent aggregation of amyloid fibrils with low cytotoxicity which could be useful against Alzheimer's disease. Dugan and colleagues[224] reported that free radicals influence neurodegenerative diseases, such as Parkinson's disease and Alzheimer's dementia. Their study suggested that fullerenes could be taken as novel neuroprotective agents. This notion was based on their experiments which demonstrated that carboxyfullerenes are capable of eliminating both superoxide anions and H_2O_2.

3.2 Gene therapy

An efficient way to trigger the biological functions and signal the destruction of cancer cells is through gene therapy. This technique is exciting in that, apart from its potential to fight cancer, it could also be applied to a large number of other diseases. Early investigations in this vein using carbon nanostructures suggest they are promising non-viral vector platforms for transfection in gene therapy. The first demonstration of carbon nanostructures as gene delivery systems used functionalized SWCNTs and MWCNTs with a pyrrolidine ring bearing a free amine-terminated oligoethylene glycol moiety. The amine-functionalized nanotube served to condense plasmid DNA to form supramolecular complexes (CNT–DNA). The CNT–DNA complex did not cause any cytotoxic effect on activated and non-activated lymphocyte.[225] Cheung *et al.*[226] reviewed the use of carbon nanotubes to deliver genetic sequences into cells. They highlighted the rapid development of

therapies based on RNA interference (RNAi), due to their high affinity and specificity to the target site and its potential to silence the targeted genes. Zhang *et al.*[227] functionalized SWCNTs with –CONH–$(CH_2)_6$–$NH^+_3Cl^-$ groups carrying small interfering RNA (siRNA) to silence genes inside tumor cells. *In vitro* and in-mouse models showed that the SWCNT–CONH–$(CH_2)_6$–$NH^+_3Cl^-$ successfully delivered siRNA into the cells and reduced the growth of tumor cells. Podesta *et al.*[228] conducted a comparative study to determine the cytotoxicity of cationic liposomes and amino-functionalized MWCNTs to suppress tumor growth. Both types of nanostructures carried a siRNA sequence. Their experiments showed cytotoxicity and cell death from both structures. However, the MWCNT–NH_3^+:siRNA complexes were shown to be better than the liposome complexes because only MWCNT–NH_3^+:siRNA complexes were able to delay tumor growth and increase the survival rate of animal models. Recently the polymer PEI has been argued as a promising anchor to the transfection of DNA fragments and siRNA into cells.[229] The development of efficient novel gene delivery systems based on NGO has been demonstrated to be comparable or even better with regard to DNA transfection efficiency in comparison with the polymers alone or viral vectors.[230]

The use of carbon nanohorns can also be effective vehicles for gene delivery. Guerra and collegues[231] conjugated polyamidoamine (PAMAM) dendrimers to serve both as an anchor to siRNA and to avoid aggregation of the nanohorns. The study showed that the CNH-siRNA could be transfected into cancer cells and diminish the expression of the protein p42-MAPK, which is directly involved in cancer development.

An important aspect in the use of nonviral vectors in gene therapy is to create an efficient transfection method. Early methods made use of specific functionalization for endocytosis of carbon nanostructures.[232] Cai *et al.*[233] reported a highly efficient technique to deliver molecules into cells using CNTs. They coined the term *nanotube spearing* for the technique. The technique provides a means to manipulate ferromagnetic-filled CNTs. They used nickel-filled carbon nanotubes (Ni-CNTs) as carriers for DNA plasmids containing an enhanced green fluorescent protein (EGFP) sequence. They demonstrated the expression of almost 100% of the EGFP in the Bal17 cells line. The efficiency of the technique was also confirmed in non-dividing mice cells. The technique demonstrates the effectiveness of carbon nanotubes as a nonviral platform for DNA plasmid transfection. The work also highlights the potential of nanotube spearing as a route for intracellular transport of proteins or peptides and RNAi. Carbon nanostructures are ever increasingly being tested as a vector for gene therapy. However, many of the physicochemical interactions between the nanostructures and DNA must be further elucidated for the construction of novel gene-transfer vector systems.[234]

Zhang *et al.*[235] described the use of NDs as a platform for non-viral gene delivery. They demonstrated the feasibility of NDs for delivering plasmid DNA either by surface-functionalized NDs with amine groups (ND–NH_2) or by noncovalent immobilization of 800 Da polyethyleneimine (PEI800) onto NDs (ND-PEI800). In both approaches, the nano-particles were internalized by HeLa cells, however only the ND-PEI800 exhibited high transfection efficiency. They claimed that this difference in DNA release is due to the fact that the ND-PEI800 can dissociate from endosomes upon cellular uptake. As a future prospect they suggest the use of polymer functionalization as a base for incorporating cell-specific targeting molecules or chemotherapeutic agents for improved performance.

3.3 Hyperthermia

The effect of heat in treating cancer has been known for decades and many molecular mechanisms involved in the process are understood. Since the 1960s numerous hyperthermia experiments have been performed, but the use of magnetic nanoparticles for heating purposes has only recently (1993) been studied.[236] The term magnetic hyperthermia is based on elevating tissue temperatures artificially to temperatures of 40–41 °C (ref. 237) through the application of external alternating (or rotating) magnetic fields.[238] The technique is based on the ability of magnetic nanoparticles to absorb energy from an alternating (or rotating) magnetic field and transform this energy into heat by reversing its magnetization (alternating field) or in the case of a rotating magnetic field, the magnetic particle rotates within a fluid suspension.[239] The heating power of the particles is described by the specific absorption rate (SAR) which quantifies the energy converted into heat per unit time per unit mass [$W\ g^{-1}$].[81,238,240] Thus, alternating or rotating magnetic fields exerted on magnetic nano-particles can raise cell temperatures which can modify various structural and functional properties of proteins, which in turn can alter cellular growth and functions, inducing apoptosis.[238]

3.3.1 CARBON NANOTUBES. The magnetic heating of tissue is a promising approach for cancer thermotherapy. The majority of the research in this field is focused on the use of magnetic iron oxides Fe_3O_4 (magnetite)[241] and γ-Fe_2O_3 (maghemite)[242] which have been proven to be tolerated by the human body.[243] The use of metallic iron could provide enormous advantages over its oxide counterparts because iron offers a higher magnetization saturation.[244] However their direct biomedical application is not possible due to oxidation in physiological environments. An exciting solution is the use of carbon coatings, namely magnetic nanoparticles encapsulated with carbon or simply magnetic-filled carbon nanotubes. The carbon shells efficiently isolate the encapsulated metallic core from the biological environment while retaining its magnetic properties. This makes such structures attractive candidates as biocompatible magnetic nanoparticles.[245]

With respect to the use of carbon nanotubes as hyperthermia systems, two main approaches are usually adopted: one using single-walled carbon nanotubes and the other using multi-walled carbon nanotubes. Interestingly, both forms of carbon nanotubes can be filled with magnetic material. Although it has been demonstrated that single-walled carbon nanotubes can be filled with iron and maintain their ferromagnetic behaviour at room temperature[60] their viability for hyperthermia has yet to be demonstrated. However, SWCNTs do emit heat when exposed to near-infrared radiation (NIR).[246] As biological tissues are

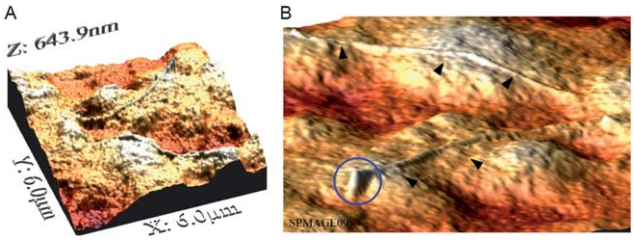

Fig. 8 (A) Shows a 3-dimensional representation of the topography of an AFM image of DU-145 cells incubated with Fe-MWCNTs. A magnified area of the 3-dimensional image is shown in (B) where the partial uptake of a tube can be better observed and is indicated by the arrows. The blue circle points out an image artifact.

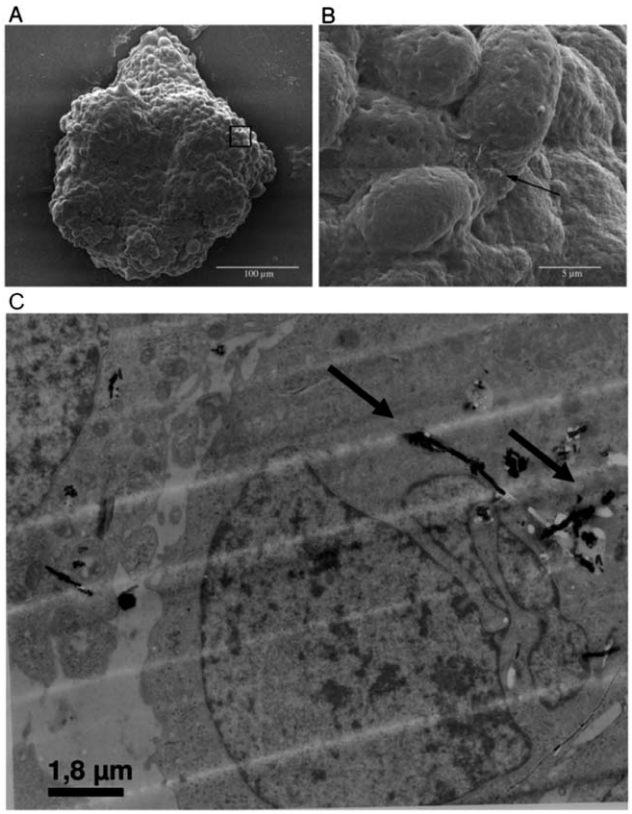

Fig. 9 (A) Cell aggregate incubated with MWCNTS. (B) Magnified region showing the MWCNTs (indicated by the arrow). (C) Transmission electron micrograph showing internalization of Fe-MWCNTs (indicated by arrows) by EJ-28 bladder tumor cells. (*Image courtesy A. Taylor.*)

relatively transparent to the NIR spectrum, the exposure of targeted SWCNTs to NIR light can cause the ablation of tumor cells. In a study by Chakravarty *et al.*[247] they demonstrated that the functionalization of SWCNTs was accomplished by tailoring biotinylated SWCNTs with moieties consisting of neutralite avidin (NA) coupled with different monoclonal antibodies (mAb–NA; RFB4–NA and RFT5–NA). The heat produced by the mAb–SWCNT complexes after exposure to NIR light exclusively ablated Burkitt's lymphoma cells *in vitro*. Unfortunately, the use of NIR is limited due to its low tissue penetration depth of a few centimeters,[248] which restricts their application to superficial tumors. Gannon *et al.*[37] also demonstrated the heating potential of SWCNTs. They applied a 13.56 megahertz radiofrequency (RF) field to SWCNTs functionalized with Kentera (Zyvex Corp, Richardson, Tex), a polymer based on polyphenylene ethylene. They studied the effect on two hepatocellular cancer cell lines (HepG2 and Hep3B) and a pancreatic adenocarcinoma cell line (Panc-1) (American TypeCulture Collection, Bethesda, MD) after 2 minutes of RF field exposure. At concentrations of 500 mg L^{-1} the cytotoxicity was 100% for all cell lines investigated.

Multi-walled carbon nanotubes usually have larger inner diameters as compared to SWCNTs and are more readily functionalized internally, including with magnetic material in relevant amounts suitable for hyperthermia. Various magnetic materials have been explored as fillants in the core of MWCNTs.[249,250] Fillants explored to date include iron,[251] cobalt,[252] and nickel.[253] Of these, iron is the most promising due to its high magnetic coercivity[254,255] and does not present toxic effects.[256] Krupskaya *et al.*[244] conducted AC inductive heating experiments using iron-filled MWCNTs (Fe-MWCNTs). The results showed a substantial temperature increase in liquid dispersions containing Fe-MWCNTs. Subsequent studies by Taylor *et al.*[257] showed no relevant cytotoxic effects from the Fe-MWCNTs prepared through the same synthesis route. In a study by Mendes,[258] DU-145 cells were incubated with Fe-SWCNTs. Various post-incubation microscopy studies, while not conclusive, suggest that the structures are taken up by cells (*e.g.* Fig. 8).

Studies with cell aggregates also point to the successful uptake of CNTs. The use of cell aggregates or spheroids (Fig. 9 panels A and B) is another important model for cancer research cells since they more closely resemble the morphology of tumors.[259] Studies in which CNTs are incubated with cell aggregates show the nanotubes can be taken up by cells (see Fig. 9).

These various studies collectively validate the potential of Fe-MWCNTs as hyperthermia agents. Analogous to SWCNTs, MWCNTs also have a strong optical absorbance in the NIR region of the electromagnetic spectrum. In addition, Torti *et al.*[260] found N-doped MWCNTs (CN_x-MWCNTs) to be less toxic than pristine MWCNTs. They showed that human renal carcinoma cells (786-O) incubated with CN_x-MWCNTs showed no discernible effect on cell viability. However, when the cells were exposed to NIR for 4 minutes after incubation with CN_x-MWCNTs there was a dramatic decrease in cell viability with over 90% cell death, whereas neither the MWCNTs nor the NIR light alone were capable of causing cell death. They also tested the length dependence of the MWCNTs. From the 3 different lengths used (1100 nm, 700 nm and 300 nm) only the two longer nanotube samples were able to cause a significant temperature increase and cell death.

3.3.2 CARBON ENCAPSULATES. Carbon-coated magnetic spherical nanoparticles are an alternative nanostructure suitable for magnetic hyperthermia. The production of encapsulates with various magnetic materials such as iron, cobalt and nickel has been demonstrated. High-frequency heat induced studies using such encapsulates have also been performed.[81,261,262] Heating rates of 7 and 11 °C min^{-1} have been shown for cobalt and nickel encapsulates, respectively. Iron

encapsulated particles showed no heating effect under similar conditions. This was attributed to the presence of paramagnetic γ-Fe particles dominating the sample which do not contribute to the heating process.[81] Like carbon nanotubes, CEMNS can be functionalized with selected biomolecules facilitating tailored targeted therapy. Moreover, they are smaller than nanotubes and a number of studies suggest such nanoparticles may have superior biocompatibility due to their morphology.[263,264] These aspects make carbon encapsulates highly promising for combined targeted therapy and hyperthermia systems for tumor destruction.

3.3.3 CARBON NANOHORNS. CNHs can be used as a thermal agent for laser-based treatment. These molecules act as a heating system when excited by an NIR light which can in turn be used for the destruction of cancer cells.[265] A comparative study showed that a more rapid and substantial viability decline was observed over time in samples exposed to SWNHs with laser treatment compared with samples experiencing laser heating or SWNH treatment alone. Zhang and colleagues[266] also reported the potential of functionalized single-wall carbon nanohorns for both photodynamic therapy (PDT) and photohyperthermia (PHT) cancer phototherapy. The technique takes advantage of the ability of SWCNHs to absorb NIR radiation. In addition, they loaded the SWCNHs with the photosensitizer, zinc phthalocyanine (ZnPc), through holes opened on the nanohorns (SWCNHox). The edges of these holes allow the formation of carboxyl groups which enabled the attachment of bovine serum albumin (BSA) to enhance their biocompatibility and dispersion (ZnPc–SWCNTHox–BSA). They injected the ZnPc–SWCNTHox–BSA into tumors formed by a 5RP7 cell line which was then subcutaneously transplanted into nude mice. Irradiation with a 670 nm laser was found to strongly supress tumor growth. The study showed that ZnPc or SWCNH–BSA applied individually were not as effective as the combined complex ZnPc–SWCNTHox–BSA.

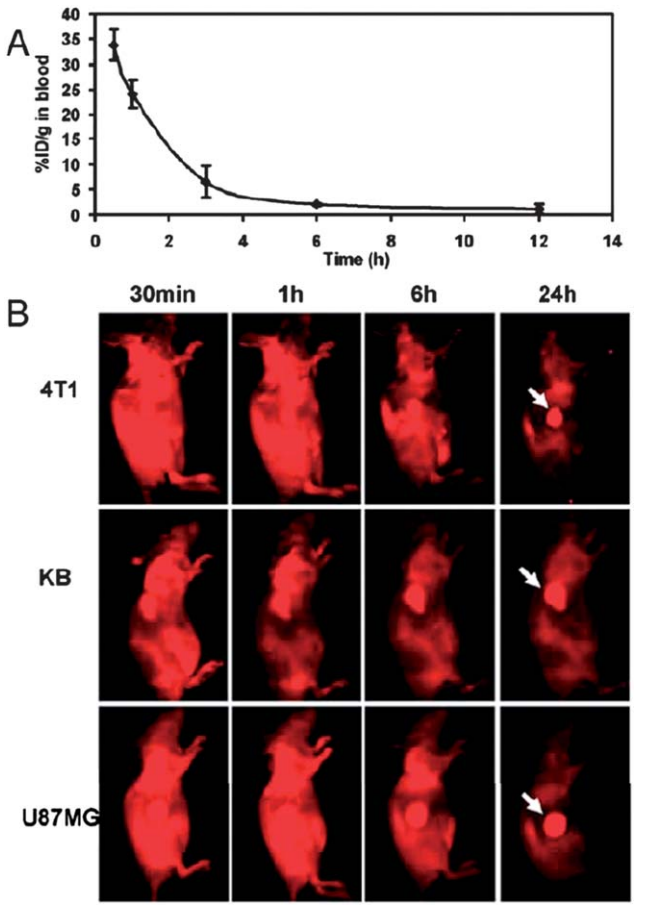

Fig. 10 *In vivo* behaviors of NGS–PEG–Cy7. (A) The blood circulation curve of NGS–PEG–Cy7 determined by measuring Cy7 fluorescence in the blood at different time points post injection. The unit was a percentage of injected dose per gram tissue (% ID/g). Error bars were based on triplicated samples. (B) Spectrally unmixed *in vivo* fluorescence images of 4T1 tumor bearing Balb/c mice, KB, and U87MG tumor bearing nude mice at different time points post injection of NGS–PEG–Cy7. Mouse autofluorescence was removed by spectral unmixing in the above images. High tumor uptake of NGS–PEG–Cy7 was observed for all of the three tumor models. Hairs on Balb/c mice were removed before fluorescence imaging. (*Reproduced with permission from ref. 267.*)

3.3.4 GRAPHENE. Nanographene sheets (NGS) strongly absorb in the NIR and hence also have potential in photothermal therapy. Yang *et al.*[267] used a 6-arm branched PEG conjugated to graphene oxide sheets *via* amide formation (NGS–PEG). The NGS–PEG was further labelled with the fluorescent dye Cy7 for *in vivo* tracking. Balb/c mice bearing 4T1 murine breast cancer tumors, nude mice bearing KB human epidermoid carcinoma tumors, and U87MG human glioblastoma tumors were injected intravenously with the NGS–PEG–Cy7 with a dose of 20 mg kg^{-1} and then imaged. The successful targeting of the tumor bearing mice is shown in Fig. 10. In addition, NGS–PEG was injected in Balb/c mice bearing a 4T1 tumor model. The same dose was used as in the imaging process and the mice were exposed to an 808 nm laser 24 hours after injection. The irradiation procedure was able to completely destroy the tumor, and no tumor regrowth was observed.

The use of PEGylated NGO also facilitates the loading of cancer drugs such as DOX, which can be used as a chemo-photothermal cancer therapy. Zhang and colleagues[268] demonstrated that the complex NGO–PEG photothermal treatment in combination with DOX resulted in the complete destruction of tumors without recurrence, which neither DOX nor NGO–PEG alone could accomplish. The photothermal properties of NGO was exploited for the treatment of Alzheimer disease by Li *et al.*[269] In this work amyloid aggregations were locally and remotely dissociated using thioflavin-S linked to graphene oxide (ThS–GO), which can selectively attach to the Aβ aggregates and form the complex GO–ThS–Aβ. By applying a low power NRI laser irradiation, local heat was generated and effectively dissociated the amyloid deposits both in buffer and in mice cerebrospinal fluid. In addition, the disaggregation of Aβ fibrils can be monitored by the fluorescence change of the Aβ staining dye, ThS.

4.0 Imaging applications

The use of magnetic nanoparticles coated with carbon as a therapy is innovative and promising for future treatments of various types of cancer. The aim of such therapies is to improve

the quality of life, increase life expectancy, reduce side effects and ultimately provide an effective cure. The early and precise detection of the disease is also very important. In this section, the promise of carbon-coated nanoparticles to contribute effectively to this goal is presented.

4.1 Carbon nanotubes

Amongst the family of carbon nanostructures, carbon nanotubes are hailed as the most promising for both diagnostic and drug delivery applications. Within the realm of diagnostics they can be used as contrast agents in magnetic resonance imaging, NIR fluorescence, Raman spectroscopy, photoacoustic tomography and even radionuclide-based imaging.[270,271] We begin with magnetic resonance imaging (MRI) which is one of the most powerful and noninvasive techniques available to physicians. The development of new contrast agents is required for improved resolution and hence diagnostic accuracy. Richard *et al.*[272] first showed the efficiency of noncovalent functionalization of amphiphilic Gd^{3+} chelates on the outer carbon shell of MWCNTs. These functionalized nanotubes exhibit great characteristics as a positive or negative contrast agent in MRIs. SWCNTs are also effective as MRI contrast agents. Ananta *et al.*[273] produced three types of SWCNTs (HiPCO SWCNTs, purified SWCNTs and ultra-short SWCNTs) and tested them as T2-weighted contrast agents. The results suggested that purified and ultra-short SWNTs could serve as a high performance T2-weighted MRI contrast agent. The ultra-short SWCNTs showed superior relaxation and, of the three forms tested, they are considered to be the most promising for future magnetic cell labeling and trafficking studies. The tracking of nanoparticles in cells is another important issue to be considered, in that it provides important feedback on the behavior of the nanoparticles. Carbon nanostructures can be tailored with fluorophores and be imaged in cells.[274] Kam *et al.*[275] used HiPCO SWCNTs adsorbed with a fluorescent Cy3-labeled single-stranded DNA. Using confocal microscopy they traced the internalization of Cy3-DNA-SWCNTs into HeLa cells at 37 °C, but not at 4 °C. This suggests an energy-dependent endocytosis mechanism for uptake. Furthermore, they demonstrated that when Cy3-DNA-SWCNTs incubated with HeLa cells are exposed to NIR light the Cy3-DNA strands are unwrapped and released, and can eventually freely diffuse across the nuclear membrane. Zavaleta *et al.*[276] established a different approach to target tumors. They used Raman spectroscopy to noninvasively localize pegylated SWCNTs functionalized with RGD peptide within a U87MG glioblastoma tumor model over several days. Using this technique they were able to study quantitatively the accumulation of RGD-SWCNTs in the tumor area with great accuracy. Their work established Raman spectroscopy as an ultrasensitive and noninvasive detection method for pre-clinical imaging (see Fig. 11) applications.

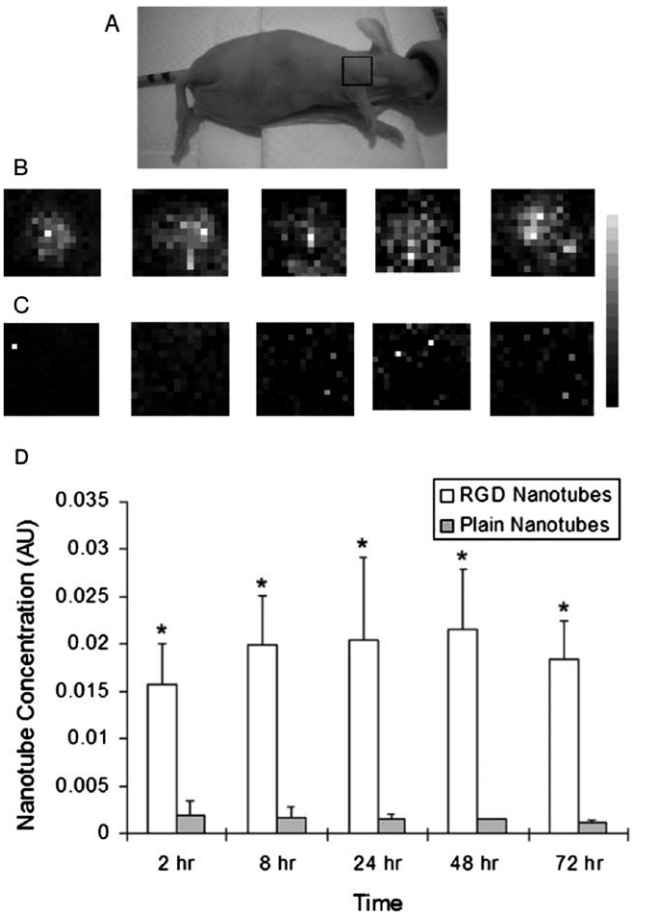

Fig. 11 Raster-scan images of the tumor area (750 μm steps) using Raman spectroscopy in conjunction with SWNTs. The grayscale bar to the right depicts the Raman intensity where white represents the maximum intensity and black represents no intensity. (A) Digital photograph of a tumor bearing mouse depicting the tumor area scanned with Raman spectroscopy (black box). (B) Panel of tumor maps from a mouse receiving RGD nanotubes at various time points post-injection starting from left to right with 2, 8, 24, 48, and 72 h. (C) Panel of tumor maps from a mouse receiving plain nanotubes at various time points post injection starting from left to right with 2, 8, 24, 48, and 72 h. Notice how the panel of tumor maps in panel b from the mouse that received RGD nanotubes shows a continued accumulation of nanotubes in the tumor area over 72 h, as opposed to panel c which shows no defined accumulation of nanotubes in the tumor area of a mouse that received plain nanotubes. (D) Bar graphic showing quantitatively the accumulation of RGD nanotubes and plain nontargeted nanotubes within the tumor over three days post-injection. (*Reproduced with permission from ref. 276.*)

Complementarily to the Raman spectroscopic technique to image RGD-SWCNTs, Zerda *et al.*[277] have shown that the same carbon nanotube complex can be used as a contrast agent for photoacoustic imaging of tumors. Direct intravenous injection of RGD-SWCNTs increased the photoacoustic signal by eight times. The study included high-resolution 3D photoacoustic images of tumors with substantial penetration depth.

Currently radionuclides are extensively used in the clinical routine for cancer diagnosis. Within this field there is a demand for improved and novel techniques, for a more selective release of the radiotracer with improved efficacy and safety.[278] Again, inspiration to achieve these goals is being sought in nanotechnology. Single-photon emission computed tomography (SPECT) and positron emission tomography are frequently used to image tumors through radionuclides. Hong *et al.*[279] presented a distinctive *in vivo* study in which the covalent functionalizations of radionuclide-filled single-walled carbon nanotubes were used as radioprobes. In this study, NaI^{125} was

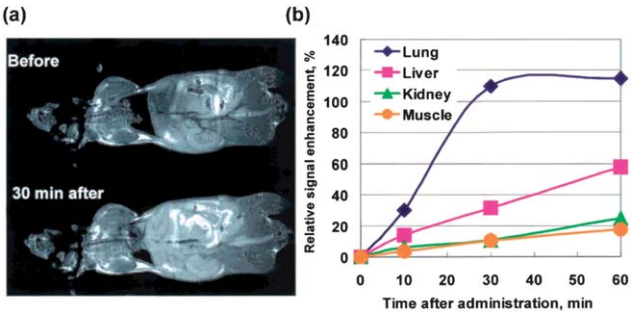

Fig. 12 (a) T1-weighted MRI of CDF1 mice before and 30 min after i.v. administration of Gd@$C_{82}(OH)_{40}$ *via* tail vein as the dose of 5 μmol Gd kg^{-1} [which was 1/20 of a typical clinical dose of Gd-DTPA (100 μmol Gd kg^{-1})] ($n = 3$), and (b) its time dependent signal intensity change in various organs. MRI conditions: 4.7 T Unity INOVA (Varian), at TR/TE 300 ms/11 ms. (*Reproduced with permission from ref. 287.*)

sealed inside SWCNTs with the outer surface covalently modified with carbohydrates. They demonstrated that the biodistribution of free NaI^{125} was completely different than that from the SWCNTs encapsulated radionuclide. While free NaI^{125} accumulated in the thyroid, stomach and urine, the radionuclide functionalized SWCNTs showed accumulation predominately in the lung with no detectable signal detected in the thyroid, stomach or bladder. This not only highlights the imaging power of these structures *in vivo*, but also evidences the effective and complete entrapment of radionuclides within SWCNTs.

4.2 Carbon encapsulates

Magnetic nanoparticles coated with carbon can also be used for diagnostics. Researchers have been conducting investigations on iron oxide nanoparticles for biomedical applications with great interest.[89,280] However, the use of iron nanoparticles is likely to produce a better signal in magnetic sensors or respond more readily to an applied magnetic field than superparamagnetic iron oxide nanoparticles (SPIONs) of the same size.[78] Other ferromagnetic materials such as nickel and cobalt have also been coated with carbon.[281,282] Leconte *et al.*[283] described iron nanoparticles coated with carbon and functionalized with amine groups as a promising contrast agent for magnetic resonance imaging. The dispersion of amine-functionalized nanoparticles was shown to be very stable in aqueous solutions under a wide range of pH. The magnetic properties were retained and displayed similar colloidal properties to commercially available contrast agents based on dextran coating. Seo *et al.*[85] described the synthesis of FeCo crystals coated with single-layered graphitic layers (FeCo@C). The outer surface of these carbon-encapsulated FeCo crystals was non-covalently functionalized with phospholipid–polyethylene glycol (PL–PEG) molecules. Their dispersion in PBS solutions was stable for over six months. To test the biological efficacy, the PL–PEG-functionalized FeCo@C nanocrystals were incubated with mesenchymal stem cells. The work showed spontaneous endocytosis of the nanoparticles and a high performance magnetic resonance contrast enhancement at lower doses than existing materials. Furthermore, toxicity assays found no obvious cytotoxicity to mesenchymal stem cells or other apparent negative health problems in rabbits injected with PL–PEG-functionalized FeCo@C.

4.3 Fullerenes

As mentioned before, fullerenes have promising therapeutic applications in biomedicine. Another promising application of fullerenes is cancer diagnosis. Fullerenes with an appropriate metal atom trapped inside their core, so-called metallofullerenes, can be used as contrast agents in magnetic resonance imaging.[284] The first reported use of fullerenes as carriers for diagnostic or therapeutic agents was conducted by Watson *et al.*[285] Mody and colleagues[286] proposed the use of perfluorinated metallofullerene ($C_{60}F_{60}$) as a contrast agent for

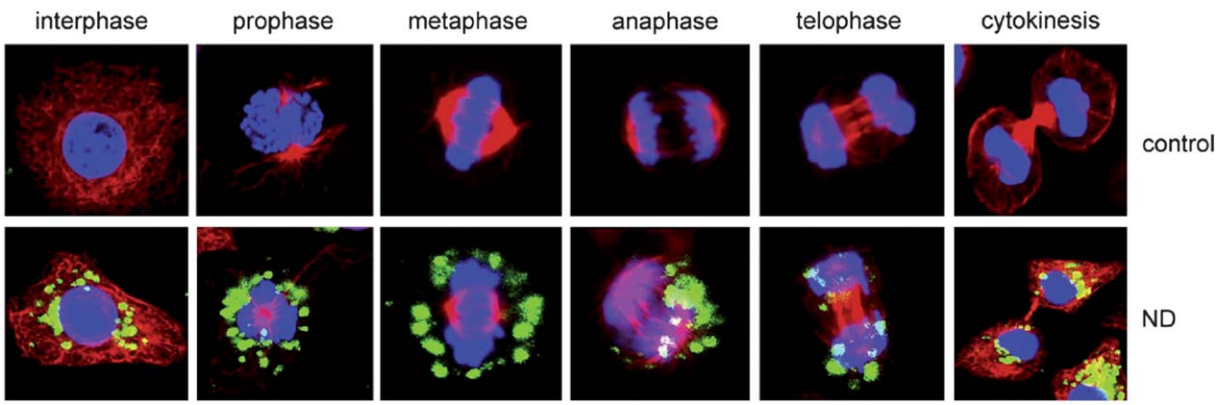

Fig. 13 The detection and distribution of ND particles in mitosis. A549 cells were incubated with or without 100 mg mL^{-1} ND particles for 48 h, and then replaced with fresh medium and recultured for 24 h. At the end of incubation, the cells were subjected to nuclear and microtubule staining and observed by laser scanning confocal microscopy. The microtubule was stained with anti-b-tubulin Cy3, presented in red. The nuclei were stained with Hoechst 33258, presented in blue. The green fluorescence from ND particles was excited with a wavelength of 488 nm and the emission was collected in the range of 510–530 nm. ND particles were located in the interphase and mitotic phases (prophase, metaphase, and telophase). During cytokinesis, these ND particles were separated into two daughter cells. (*Reproduced with permission from ref. 290.*)

MRI. Most of the efforts in this field use gadolinium (Gd III). Mikawa *et al.*[287] synthesized water-soluble gadolinium endohedral metallofullerenes ($Gd@C_{82}(OH)_{40}$) and demonstrated that these paramagnetic complexes have the ability to significantly enhance the contrast of magnetic resonance images. The biodistribution of $Gd@C_{82}(OH)_{40}$ in CDF1 mice was investigated and was found to accumulate more in the lungs as can be seen in Fig. 12.

The use of fullerenes as contrast agents is not only restricted to magnetic resonance imaging. These carbon nanostructures have also been reported to be precursors for X-ray contrast agents.[288] Another potential application of metallofullerenes is in the field of nuclear medicine, where they may be used as a more stable alternative for transporting radiometals. Cagle *et al.*[126] reported biodistribution studies of water-soluble C_{82} fullerenes entrapping a holmium (^{166}Ho) in mice. They determined that these nanosystems are not acutely toxic and can be used as a radiotracer compound with a blood clearance period of about one hour, and they are likely unmetabolized in the liver.

4.4 Nanodiamonds

The use of NDs as probes for tracking purposes has also been reported. The cell imaging is usually performed using fluorescent dyes in combination with a confocal microscope and stimulated emission depletion (STEAD) microscopy which yields higher resolution than a conventional microscope. A recent work in the field reported the possibility to image individual albumin-conjugated NDs uptaken by HeLa cells. Through the STEAD technique it was possible to image individual particles within clusters uptaken by the cells.[289] Liu *et al.*[290] studied the location and distribution of carboxylated NDs in cell division and differentiation as a promising non-toxic nanostructure for tracking cancer and stem cells. They investigated the endocytotic mechanism of 100 nm ND particles and observed that ND clusters that internalized did not interfere with normal cellular functions, including cell division and differentiation. The NDs were tracked using their natural fluorescence[178] upon laser excitation through scanning confocal microscopy. Furthermore, the uptake of ND particles was observed in all cell cycle phases, including interphases and mitotic phases, and they did not disturb spindle formation and chromosome segregation (see Fig. 13).

Chang *et al.*[175] reported that Fe nanoparticles coated with carbon can be used to functionalize NDs (Fe@CNP–ND). This provides them with magnetic properties useful as contrast agents and probes for biodistribution studies in magnetic resonance imaging. Manus *et al.*[174] described the conjugation of NDs with gadolinium (Gd(III)–NDs) for magnetic resonance imaging contrast enhancement. Their studies showed that the Gd(III)–ND complexes were able to increase the relaxivity of free Gd(III) nearly 10-fold. These encouraging results should stimulate further *in vivo* research of gadolinium conjugated with NDs.

4.5. Graphene

Graphene has also been shown to be a promising bioimaging platform.[229] The use of NGO as a platform that can be specifically directed to track tumor angiogenesis was recently demonstrated.[291] In this work a monoclonal antibody (TRC105) that binds to a vascular marker for tumor angiogenesis was incorporated onto NGO. In order to evaluate the biodistribution, pharmacokinetics and tumor targeting efficacy of functionalized NGO in tumor-bearing mice, serial PET imaging was performed. For this the NGO–TRC105 complex was labeled with the isotope ^{64}Cu. The study showed that this complex can be specifically directed to the tumor neovasculare sites *in vivo* through targeting the CD105 marker.

5.0 Summary

The studies to date highlight the versatility of carbon nanostructures as platforms suitable for multiple targeting, therapeutic and diagnostic options. These options can be installed in combination so that a single drug can detect, select, deliver cargo and trigger explicit responses. This is achievable due to the rich functionalization potential of carbon nanostructures. The ideal nanosystem must inherently contain specific targeting functional groups, trigger an explicit biological response and be detectable. These are all possible using carbon-coated nanoparticles, making them exciting nanovectors for the targeted delivery of drugs and imaging contrast agents. Carbon nanostructures hold promise in other biomedical applications also, such as neurological tissue stimulation,[292] tissue regeneration scaffolds[293,294] and as biosensors.[295] Nonetheless, the excitement that carbon nanostructures instill must also be matched with an appropriate degree of caution. Proper safety considerations for patients and health-care workers need to be developed. In addition, fast and safe regulatory approval protocols still need to be established. Many studies relevant to safety and approval protocols with both *in vivo* and *ex vivo* assessments are already in progress. Programs to inform and alleviate public fears associated with nanomaterials are also important. Continued investment and investigations in this field are essential so that an accurate understanding of their benefit against any risk they may pose can be obtained. The investigations reviewed here suggest the future is bright for carbon nanostructures to become a clinical reality.

List of abbreviations

AmB	Amphotericin B
BSA	Bovine serum albumin
CAR	Carvedilol
CEMNs	Carbon-encapsulated magnetic nanoparticles
CNTs	Carbon nanotubes
CN_x-MWCNTs	N-doped multi-walled carbon nanotubes
CVD	Chemical vapor deposition
DAP	Dapsone
DDS	Drug delivery system
DEX	Dexamethasone
DNA	Deoxyribonucleic acid
DOX	Doxorubicin
EGFP	Enhanced green fluorescent protein
FA	Folic acid

Fe-MWCNTs	Iron-filled carbon nanotubes
FMNDs	Fluorescent magnetic nanodiamonds
GO	Graphene oxide
HCPT	Hydroxycamptothecin
HiPCO	High pressure carbon oxide conversion
HRTEM	High resolution transmission electron microscopy
MRI	Magnetic resonance imaging
MTX	Methotrexate
MWCNTs	Multi-walled cabon nanotubes
NA	Neutralite avidin
NDs	Nanodiamonds
NGO	Nano-sized graphene oxide
Ni-CNTs	Nickel filled carbon nanotubes
NIR	Near-infrared radiation
NSCLC	Non-small cell lung cancer
PDT	Photodynamic therapy
PEG	Polyethylene glycol
PET	Photon emission tomography
PHT	Photohyperthermia
PL	Phospholipid
PMMA	Poly methyl methacrylate
PTX	Paclitaxel
PVA	Poly vynil alcohol
RGD	Arginine-glycine-aspartic acid
STEAD	Stimulated emission depletion microscopy.

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