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Artificial Cells, Nanomedicine, and Biotechnology

An International Journal

ISSN: 2169-1401 (Print) 2169-141X (Online) Journal homepage: https://www.tandfonline.com/loi/ianb20

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To cite this article: Mehrdad Samadishadlou, Masoud Farshbaf, Nasim Annabi, Taras Kavetskyy, Rovshan Khalilov, Siamak Saghfi, Abolfazl Akbarzadeh & Sepideh Mousavi (2018) Magnetic carbon nanotubes: preparation, physical properties, and applications in biomedicine, Artificial Cells, Nanomedicine, and Biotechnology, 46:7, 1314-1330, DOI: 10.1080/21691401.2017.1389746

To link to this article: https://doi.org/10.1080/21691401.2017.1389746

Published online: 18 Oct 2017.

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ARTIFICIAL CELLS, NANOMEDICINE, AND BIOTECHNOLOGY
2018, VOL. 46, NO. 7, 1314–1330
https://doi.org/10.1080/21691401.2017.1389746

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Magnetic carbon nanotubes: preparation, physical properties, and applications in biomedicine

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ABSTRACT

Magnetic carbon nanotubes (MCNTs) have been widely studied for their potential applications in medicine, diagnosis, cell biology, analytical chemistry, and environmental technology. Introduction of MCNTs paved the way for the emergence of new approaches in nanobiotechnology and biomedicine as a result of their multifarious properties embedded within either the carbon nanotubes (CNTs) or magnetic parts. Numerous preparation techniques exists for functionalizing CNTs with magnetic nanoparticles, and these versatile strategies lay the ground for the generation of novel and versatile systems which are applicable to many industries and biological areas. Here, we review and discuss the recent papers dealing with MCNTs and their application in biomedical and industrial fields.

ARTICLE HISTORY
Received 21 June 2017
Revised 3 October 2017
Accepted 4 October 2017

KEYWORDS
Magnetic carbon nanotubes; bioapplications; nanotechnology; nanobiotechnology; nanoparticles; MRI; drug delivery

Introduction

Nanoscience plays a major role in advanced science. By the means of nanotechnology, researchers are able to shape their ideas from the very first level, i.e. molecular level. Due to their unique properties and exceptional size, nanoparticles (NPs) offer great benefits, and magnetic nanoparticles (MNPs), in particular, attract attention for applications in such various areas as biomedicine, biotechnology, engineering, material science, and environmental science [1–3]. Nowadays, nanostructures are known to be applicable in a variety of fields, including drug carriers, labeling and imaging agents, implant reinforcing and coating structures, diagnostic sensors, and biomimetic scaffolds used for tissue and organ regenerations [4]. Ultrahigh surface area, high mechanical strength associated with ultralight weight, outstanding chemical and thermal stability, and rich electronic polyaromatic structure are the characteristics which set the stage for carbon nanotubes (CNTs) to be considered as promising therapy-enhancing nanostructures [5–8]. Therefore, CNT's surface can be modified with various methods, which makes them capable of loading miscellaneous substances for different usages, and also avoids unwanted cytotoxicity and immune reactions [9]. Magnetic delivery, provided by the means of an external magnetic field, has attracted a great deal of attention in biomedical applications, due to its ability to target a variety of therapeutic and diagnostic agents at specified tissues [4,10]. This targeting behaviour results in aggregation of NPs in desired sites, which in turn, enhances the magnetic resonance imaging (MRI) contrast, hyperthermia effects, and drug release. The most significant advantage of magnetic fields is their non-invasive nature, which makes them harmless to the tissue [11]. Desired tissues in body can be targeted employing an external magnet or a situated magnet inside the body [4,12]. CNTs have such a surprising active surface area that enables them to be functionalized and decorated by various types of biological and chemical substances. This privilege becomes significant by taking central hollow of CNTs into account, which can prevent loaded NPs from having interaction with biological solutions [13]. Another noticeable advantage of CNTs over MNPs is their needle like shape, which enables them to penetrate cell walls more easily, and also facilitates their flow within blood, which stems from reduced drag force [14]. CNTs, however, do not contain any magnetic properties, which restricts their potential for biomedical applications as a targeted drug delivery agent. In order to overcome this hurdle, a plethora of efforts have

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been made to blend CNTs and MNPs. Typical methods of producing magnetic carbon nanotubes (MCNTs) are the attachment of MNPs to the surface and loading them into CNTs central hollow [15,16]. By so doing, the beneficial characteristics of both CNTs and MNPs lead to high functionalizing capability and targetable carriers. The first part in this review provides a summary of physical and magnetic properties of MCNTs. In the following, we concerned with their preparation methods and we also discussed about their bio-application in different fields.

Structure and properties of CNTs

Carbon has the ability of constructing structures with entirely different properties through different banding manners. Carbon sp^2 hybridization forms a layered structure which has weak van der Waals out-of-plane and strong in-plane bounds. Multi-walled carbon nanotubes (MWCNTs) are constructed of coaxial cylinders with the ordered spacing between layers, around central hollow. Analysis of MWCNT images revealed that there are interlayer spaces varying between 0.34 and 0.39 nm [17]. The number of layers determines the inner and outer diameter of MWCNTs. Inner diameter varies from 0.4 nm and rises up to a few nanometers; and outer diameter diverges from 2 nm up to 20–30 nm. MWCNTs usually have closed by half-fullerene molecules, which have a dome-shaped structure, in both tips. Axial length of MWCNTs differs between 1 μm and a few centimeters. Single-walled carbon nanotube's (SWCNT) diameters, Similarly, differ from 0.4 to 2–3 nm, and their length is in the micrometer range [18].

MWCNT and SWCNT structures

SWCNTs have three diverse formations, which are known as armchair, chiral, and zigzag depending on their tube wrapping method (Figure 1(b)). A SWCNT's structure and also their electrical properties are characterized by structural indices (*n, m*) that define the chiral vector. Unit vectors number in the graphene's honeycomb crystal lattice alongside two directions is regulate by the n and m (which are integers). Generally, nanotubes with $m = 0$ are known as zigzag; nanotubes with $n = m$ are known as armchair nanotubes, and other ones are called chiral. Tube diameter *d*, also determines by the chiral vector $C = na_1 + ma_2$ (a_1 and a_2 are graphite's base cell vectors) [19,20], and this vector ascertain rolling direction of a graphene sheet (Figure 1(a)). Thus, carbon tube

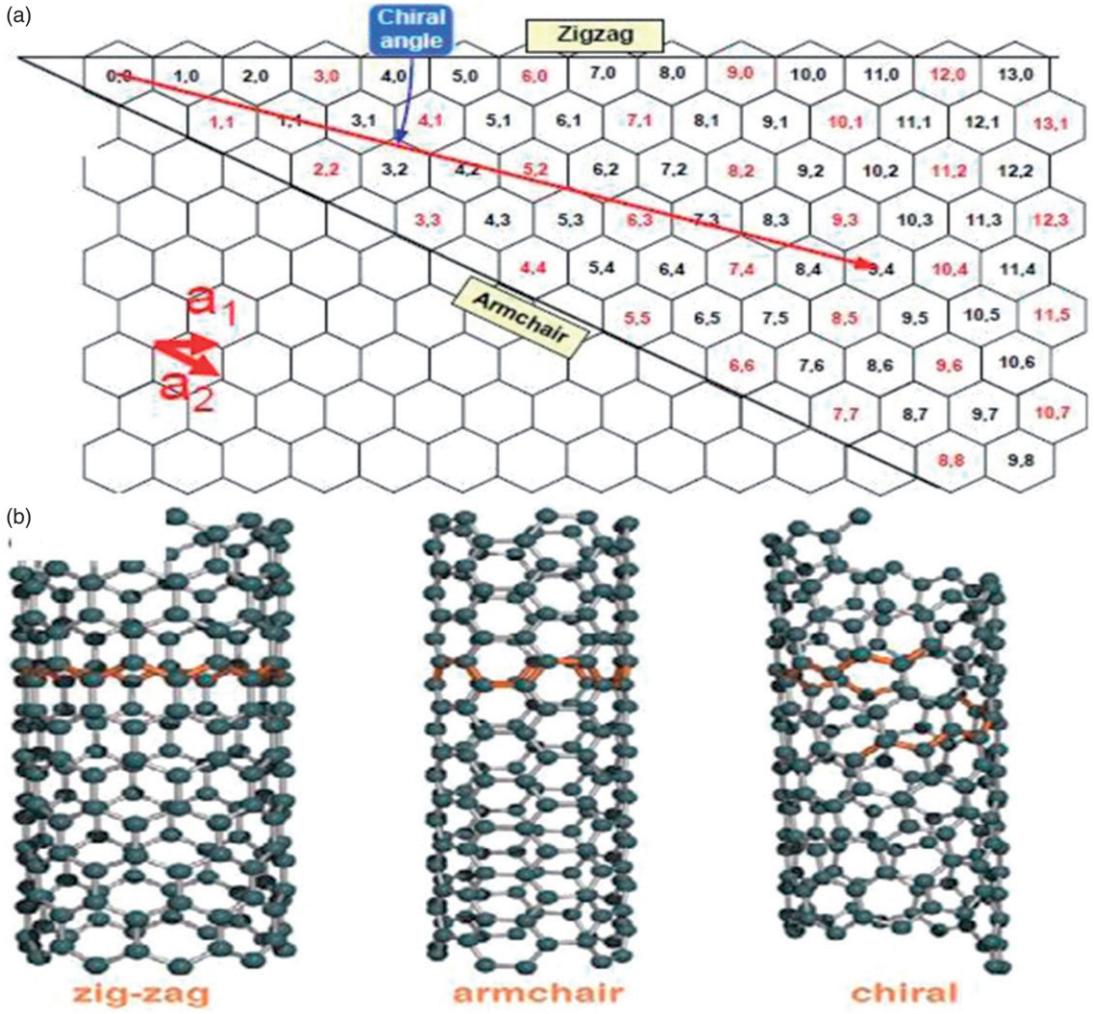

Figure 1. Different types of SWCNTs: (a) Illustration of chiral vector and angle and (b) schematic of three ideal SWCNT structures [21].

diameter can be calculated by:

$$d = \frac{a\sqrt{m^2 + mn + n^2}}{\pi},$$

where $a = 1.42 \times \sqrt{3}$ that is calculated from lattice constants of graphite sheet. When n - m is a multiple of 3, the nanotube is highly conductive and known as "metallic", and in other states the nanotube is a semi-metallic or semiconductor. The armchair form is always metallic, while other forms could be metallic or semiconductor.

A nanotube structure can be under effect of many parameters and vectors like [22]:

1. Chiral vector $= Ch = na_1 + na_2$ » (n, m)
2. Translational vector $= T = t_1a_1 + t_2a_2$ » (t_1, t_2)
3. Chiral vector's length $= L = a\sqrt{(n_2 + m_2 + n \times m)}$, where a is the lattice constant
4. Diameter $= dt = L/\pi$
5. Symmetry vector's rotation angle of the $= \psi = 2\pi/N$ (in radians)
6. Chiral angle $= cos\theta = (2n + m)/(2 \times \sqrt{(n_2 + m_2 + n \times m)})$
7. Hexagons number in the unit cell $= N = (2 \times (n_2 + m_2 + n \times m)/dR)$
8. Symmetry vector's Pitch $= \tau = ((m \times p - n \times q) \times T)/N$
9. Symmetry vector $= R = pa_1 + qa_2$ » (p, q).

MWCNTs have two structural models including Parchment and Russian Doll models. Russian Doll model refers to a CNT enclosing another nanotube inside. On the contrary, when a single graphene sheet is rolled around itself several times, it is known as the Parchment model. Generally, properties of MWCNTs and SWCNTs are similar. In a MWCNT, due to its multi-layer structure, the outer walls not only are able to protect the inner CNTs from chemical interactions with outside solutions, but also provide high tensile strength properties [23]. Due to sp^2 bonds between carbon atoms, CNTs have higher tensile strength than steel and Kevlar. It was reported that this bond contains more strength in comparison to the diamond's sp^3 bond. On the other hand, elasticity is another outstanding property of CNTs. A nanotube which exposed to various forces can withstand different deformations without damaging, and will refund its original construction. However, nanotube elasticity is limited, and by exposing to very powerful forces, it is possible to deform plastically. Furthermore, number of the structural defect such as rearrangement of the carbon bonds or atomic vacancies could weaken the nanotube's strength. Elastic modulus defines elasticity in all materials including SWCNTs and MWCNTs. The elasticity modulus of MWCNTs is determined with transmission electron microscopes (TEM) which exposed high strength bonds in CNTs and consequently, they tolerate high temperatures and show excellent thermal conductivity. They can endure up to 750 °C at normal atmospheric pressure and 2800 °C in vacuum [24].

Magnetic property

Materials can be categorized based on their reaction under external magnetic field. Therefore, because of neutral magnetic behaviour of MCNTs, it is possible to classify theme according to their MNPs behaviour. Magnetic moment orientation in a material is the key to identify different behaviour of magnetism. In this regard, basic types of magnetic behaviour are as follows: diamagnetism, ferromagnetism, paramagnetism, ferrimagnetisms, and antiferromagnetism. As a magnetic field applies, atomic domains that are produced by electrons orbital motion, weaken the applied magnetic field. Materials exhibit this form of weak response to an external magnetic field recognized as diamagnetism. In contrast, other forms of magnetism usually strengthen the applied magnetic field. Nowadays, it is clear that electrons configuration of diamagnetism behaviour takes place in materials with occupied electronic subshells, in which paired magnetic moments neutralize each other. Susceptibility of diamagnetic materials is negative ($\chi < 0$) and weakly beats back an applied magnetic field (e.g. quartz SiO_2) [25–28]. All other magnetic behaviours are assigned to materials which have relatively unpaired electrons in 3d or 4f layer of atomic shells. Paramagnetism is a magnetic behaviour of material which have uncoupled atomic magnetic moments; hence, there is no long-range order in moments of paramagnetic materials, and magnetic susceptibility is slightly positive ($\chi \approx 0$), e.g. pyrite [29,30]. Ferromagnetic materials have parallely ordered magnetic moments with equivalent enlargement and a crystalline structure that allows moments to couple directly, results in flux density severe intensify (e.g. Fe, Ni, and Co). Moreover, ferromagnetic material's aligned moments can result in an impulsive magnetization in a situation which there is no external magnetic field exists. Hard magnets are materials that keep their magnetization in the absence of an external magnetic field. Materials having antiparallel arranged atomic magnetic moments with equal magnitude exhibit anti-ferromagnetism behaviour (e.g. troilite FeS). In this type of behaviour, moments couple in an antiparallel manner, therefore, outcome is no magnetization [31]. Above the Néel temperature, thermal energy result is randomization of atomic moments, and consequently, loss of long-range moments order: which is known as paramagnetic behaviour. Ferrimagnetic including Fe_3O_4 and Fe_3S_4 are materials with atoms or ions which tend to be ordered, but in a non-parallel direction of moments in lack of applied magnetic field below the Néel temperature. Generally, antiparallel configuration of adjacent non-equivalent sublattices inside a magnetic domain results in net magnetization. Above the Néel temperature, the material shows paramagnetic behaviour (Figure 2) [32].

Preparation of MCNTs

During the last few years, much effort has been devoted to develop efficient synthetic routes in order to obtaining shape-controlled, highly stable, and well-defined magnetic carbon hybrid nanotubes. There are several common methods including filling process [34,35], template-based synthesis [36,37], chemical vapor deposition (CVD) [38,39], hydrothermal/solvothermal [40,41], pyrolysis procedure [42,43], sol–gel process [44,45], detonation induced reaction [46,47], and self-assembly [15,49] and some other techniques such as capillary action, condensed-phase electrolysis, arc discharge [48,49], electrospinning [50,51], sonochemical deposition [52],

microemulsion technique [53], electrochemical method [54,55], and pulsed laser irradiation [56]. In the following, a detailed discussion including a summary of most important preparation methods is presented. We also summarized their advantages and disadvantages in Table 1.

Filling process

The filling process is the simplest method in order to fabricate magnetic carbon composites in which the porous nature of CNTs plays a key role in this technique. Synthesis of MCNT is carried out with two major filling strategies: first, filling the pores of the CNT with ferrofluid; and second, filling with the precursor of the magnetic species and then reducing the precursor [57]. The very first attempt for loading commercial MNPs (paramagnetic iron oxide) into CNTs was done by Korneva et al. [34]. Their results showed that almost 100% of the nanotubes became magnetic and they can be simply manipulated with an external magnetic field. TEM images of obtained MCNTs are presented in Figure 3, and also the vibrating sample magnetometer (VSM) result illustrated in Figure 4(a). Bio et al. [58] have prepared MCNTs containing Fe and Ni NPs by successive feeding of nickelocene and ferrocene into the pores of CNTs and then reducing of the metal compounds to magnetic metals. The filling process has many advantages such as low cost in operation, and raw materials accessibility, but its major advantage is the convenience of the conducting procedure. Magnetic particles can be dispersed onto the surface of CNTs, instead of the pores which makes quality control very hard. In addition, loss of control on the mass ratio of carbon precursor and magnetic precursor may lead to the blockage of pore, which the property of the product is critically dependent on. The mentioned problem can in turn lead to bad reproducibility [59,60].

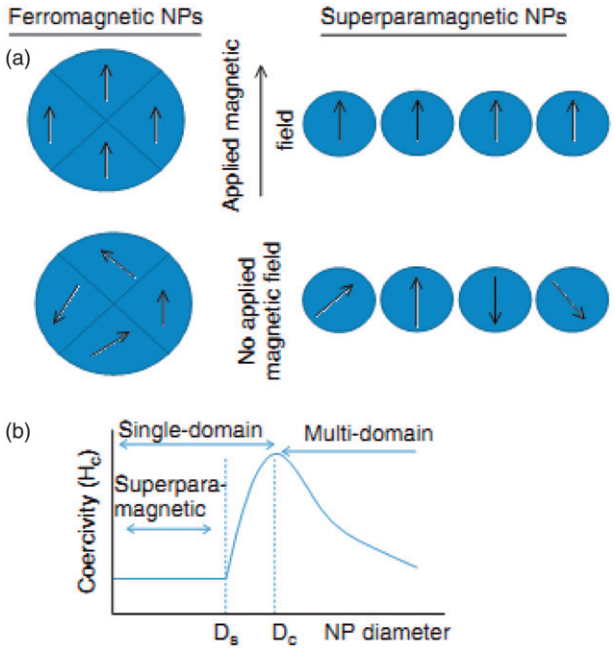

Figure 2. Magnetization behaviour of ferromagnetic and superparamagnetic NPs under an external magnetic field. (a) Domains of a superparamagnetic and ferromagnetic NPs align with the applied external magnetic field. In the absence of an external field, ferromagnetic NPs will keep a net magnetization, while superparamagnetic NPs will display no net magnetization since quick setback of the magnetic moment. (b) Relationship between NP size and the magnetic domain structures. Ds and Dc are the "superparamagnetism" and "critical" size thresholds [33].

Sol–gel process

In addition to synthesis of high-grade metal or metal oxides/silica nanocomposites, sol–gel process can be employed for synthesis of metal nanocrystals and oxides/carbon hybrid composites. Hydrolysis and condensation of precursor in solution are fundamentals of this process. The quality, shape, structure, size, and properties of the product could be fully governed regulating the parameters including solvent, temperature, concentration of the precursors, the pH, agitation, and so on [61]. MCNTs also can be prepared by the sol–gel process. In a research by Modugno et al. [62], Fe_2O_3-MWCNTs nanocomposites were synthesized using a modified sol–gel process. As the first step in this method, the MWCNTs surface was activated with carboxylic acid groups and subsequently, using sol–gel process in which the g-Fe_2O_3 NPs was attached to the MWCNTs surface, at the same time with their synthesis. The main advantages of sol–gel process are low temperature operation which prevents oxidation of precursors and low cost. However, the nature of this process increases the possibility of product contamination which is the major drawback of sol–gel process.

Chemical vapor deposition

CVD is a well-known synthesis method for solid materials with high quality and purity, in which a chemical process carries out in a chamber of reactive gas. Reaction chamber fills with gases containing the deposition materials and a coating deposits on a substrate [63]. CVD is one of the most applied techniques used for mass production of 1D nanomaterials,

Table 1. Advantages and disadvantages of MCNT production method.

Method	Advantages	Disadvantages
Filling process	Operations low cost, raw materials availability, procedure easement	Hardship of quality control, MCNT's central hollow blockage, weak reproducibility
Sol–gel process	Relative low temperature, low cost	Possibility of product contamination
CVD	Ultimate control on MCNT structure	Complex equipment, high cost, high energy consummation
Self-assembly	Full control over MCNT properties	Heterogeneity of produced MCNTs
Pyrolysis process	Mass production	Limited control over MCNTs, production of carbon black, certain need to inert atmosphere
Template-based synthesis	High quality of MCNTs	High-quality template, template elimination

such as CNTs and MCNTs [64]. In recent years, CVD has been developed by many research groups with the purpose of synthesizing MCNTs. Ni-filled MWCNTs were synthesized by Sengupta et al. [65] consuming a simple mixture of Ni (salen) and photoresist, using atmospheric pressure CVD with propane on Si at 850 °C. By their method, MCNTs have grown by a tip-growth mechanism and exhibited ferromagnetic behaviour. In another work, Tian et al. [66] prepared iron-filled CNTs using a floating catalyst CVD method in presence of ferrocene which acted as both catalyst precursor and iron source. The major advantage of this method is superior control over CNT structure and the main drawbacks of this method are using expensive equipment and it is high cost and energy consuming procedures.

Self-assembly method

Self-assembly is a term used to define processes in which a disordered system of pre-existing components spontaneously forms an organized structure. In addition to other well-known applications of self-assembly method, MCNTs preparation is also an important product of this method. One of the first attempts for using self-assembly method for MCNT preparation was carried out by Zhang et al. [67]. They used a two-step procedure: (i) first, potassium persulfate (KPS) was used as oxidant in one-pot modification in order to obtain hydrophilic CNTs; (ii) Second, they assembled the Fe_3O_4 on the surface of CNTs, using a hydrothermal process in the presence of hydrazine hydrate as the mineralizer. The XRD pattern of obtained MCNTs is presented in Figure 4(b). In a research by Zhou et al. [68], amino-magnetite NPs have successfully assembled onto the surface of poly(acrylic acid)-functionalized MWCNTs via covalent bonding. Such magnetic nanocomposites show good magnetorheological performance. Static electronic interaction is another method of connecting MNPs to CNTs, which is employed in various investigations [69,70]. The bottom-up nature of the self-assembly procedure permits ultimate control over MCNTs properties. Nevertheless, heterogeneity of product is the main disadvantage of this method.

Pyrolysis process

Pyrolysis is transformation of an organic compound to smaller and simpler compounds at elevated temperatures in the absence of oxygen (or any halogen). Pyrolysis is an irreversible process and in this process change in chemical composition and physical phase occurs in the same time. In a research by Shen et al. [71], SWCNTs coated by Fe_3O_4 were synthesized using thermal decomposition of 1-methyl-2-pyrrolidone solution of iron triacetylacetonate in the presence of poly(acrylic acid)-functionalized SWCNTs. Such magnetic carbon nanocomposites exhibit excellent magnetic and mechanical properties. In another study, Shan et al. [72] prepared

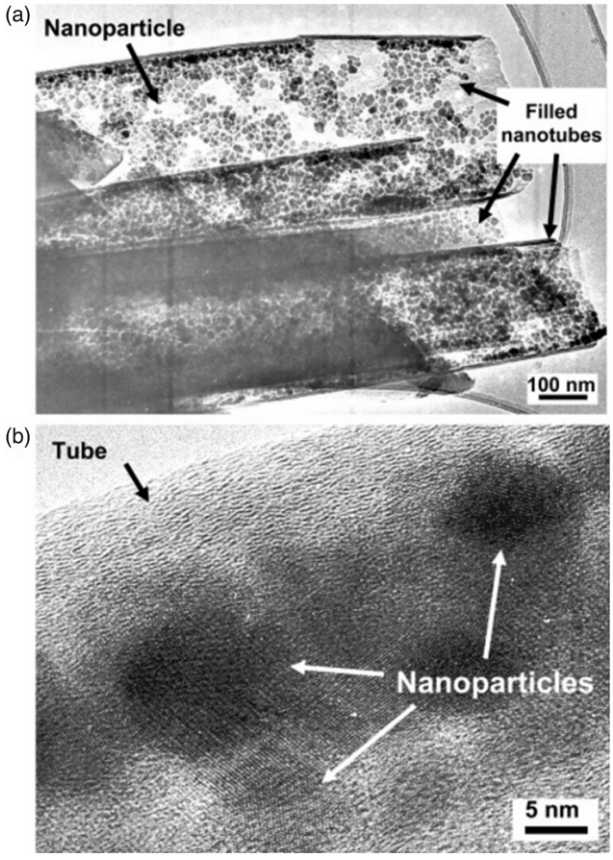

Figure 3. TEM images of MCNTs prepared by filling process. (a) MCNTs filled with ferrofluid in magnetic field. (b) High-resolution TEM image of a portion of the nanotube, filled with MNPs [34].

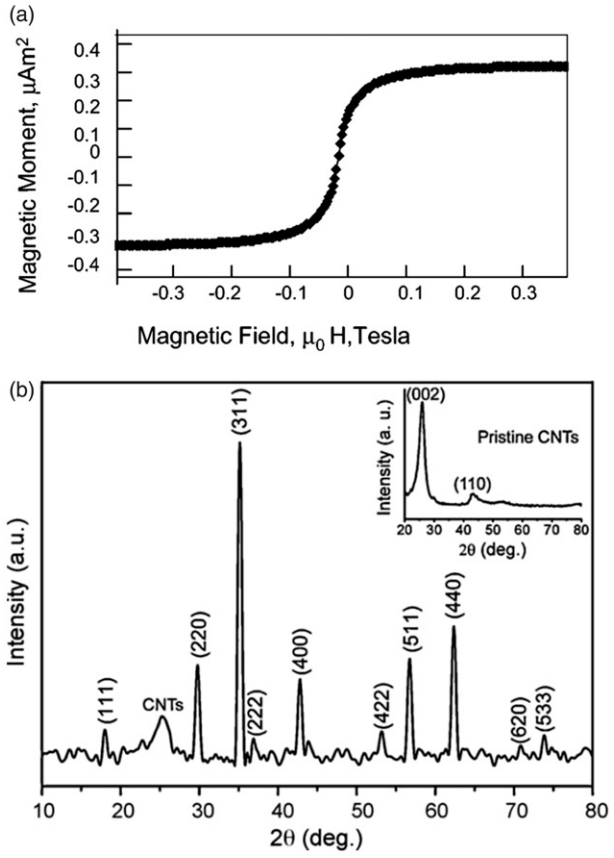

Figure 4. (a) Typical magnetization curve of a MCNT prepared by filling process [34]. (b) XRD pattern of CNTs/Fe_3O_4 hybrids obtained by self-assembly method (the inset shows the XRD pattern of treated CNTs) [67].

MCNTs consisting of a MWCNT core and Fe_3O_4 shell by in-situ thermal decomposition of triacetylacetonate, $FeCl_3$ or $Fe(CO)_5$ in 2-pyrrolidone containing pretreated MWCNTs. The pyrolysis approach features large-scale production, while offers very limited over the size and structure of the obtained products. The main disadvantage of this method is that much of carbon black, which is unwanted and is often formed by thermal decomposition or incomplete combustion of carbon hydrogen compounds. Moreover, the conduction of pyrolysis often requires inert atmosphere to avoid oxidation of the magnetic species. However, due to large-scale production, the pyrolysis approach will be more attractive once the above-mentioned disadvantages are overcome.

Template-based synthesis

Generally, in this method, magnetic particle formation and carbon source carbonization are carried out in a very confined space. Furthermore, this method can be used as a synthesis method for MCNT synthesis. Mesoporous silica and anodic aluminum oxide (AAO) are two main candidates as the hard template for nanomaterials synthesis. Jang et al. [73] designed a procedure which can synthesis CNTs enclosing magnetic iron oxide, using AAO template and precursor impregnation method. In their procedure, $FeCl_3$/poly(amic acid)/N-methyl pyrrolidone solution was dropped onto the AAO surface and capillary force took the solution into the AAO pores. By subsequent carbonization, iron containing CNTs formed in AAO channels and the MCNTs can be gained by dissolving AAO using hydrochloric acid. Using AAO template in order to synthesis, MCNT attracts more attentions by time [74]. Furthermore, polymer and surfactant are used as a structure-directing agent in soft template method, which have been under tremendous attention during last years. High quality of produced MCNTs is the major advantage of template-based method. Nevertheless, MCNTs' quality has a direct relation with the template structure, which is remain a great challenge to obtain it with preferred properties and structure. Moreover, another main challenge in this method is template elimination, which should be developed and controlled in a manner that minimizes product damage.

Functionalization

Unfortunately, all forms of CNTs have low dispersity in aqueous solutions and even in organic media and they have great resistant to wetting. However, functionalization can improve their solubility [75] and processability and thus, it will facilitate merging outstanding properties of CNTs with other materials. Based on CNT itself and desired results, there are different routes that can be utilize for functionalization. An accepted classification for functionalization techniques is based on their manner of action which consists of three groups including covalent functionalization, non-covalent functionalization (exohedral functionalization), and endohedral functionalization [76–80]. Encapsulation of MNPs into CNTs central hollow has many difficulties which limits the studies to endohedral preparation. On the contrary, the exohedral functionalization is more simple and adaptable with a diverse coating and numerous ligand chemistry [81].

Bioapplications

Drug delivery

Researchers and medical practitioners have access to a vast variety of drug delivery systems [82,83]. However, it is observed that, among all this variety of different drug delivery vehicles, carbon nanomaterials are resourceful alternative for transporting therapeutic molecules. The ultimate goal of drug delivery is to maximize bioavailability of therapeutic agents at the specific location in the body at the desired time [8]. Using a carbon nanomaterial as a drug delivery vehicle which has the ability to target specified tissue, *in vivo*, the overall dosage of a drug as well as its side effects can be simultaneously reduced. The employment of carbon nanomaterials can facilitate the drug deliverance to the specific location and accumulation inside tumours. Thus, loaded drug molecules can be delivered exactly into the cancerous tissue, while the normal tissue left unaffected by the drug. The ultra-high surface area of carbon nanomaterials can be functionalized and highly decorated with drugs including the anticancer drugs like doxorubicin (DOX), cisplatin, and methotrexate, [84,85] as well as anti-inflammatory drugs such as dexamethasone [86]. Moreover, these nanomaterials can efficiently increase the water solubility of hydrophobic small drug molecules and facilitate their release in the acidic conditions found within tumours [87]. Fortunately, the combination of drugs and carbon nanomaterials can rise the drug's efficacy and cytotoxicity. In addition to molecule drugs, carbon nanomaterials can be used as delivering agents in order to transport peptides, proteins, and other biologics into cells via endocytosis [88]. CNTs are most commonly used in drug delivery due to their unique spectroscopic properties, and because they can be readily functionalized and decorated with bioactive peptides, small molecule drugs, and proteins. It is very important to note that the therapeutic efficacy of the drug should not be altered after attaching to the CNT and also after crossing the cell membrane [6]. A great step to improve CNTs potential as a drug delivery vehicle was to combine them with MNPs. In one of the first attempts for delivering gemcitabine (GEM) with CNT, Yang et al. delivered a magnetic MWCNTs (MMWCNTs) carrying GEM to lymphatic vessels, in presence of an external magnetic field. MWCNTs were functionalized with poly acrylic acid and then decorated with magnetite NPs ($FeO.Fe_2O_3$) employing co-precipitation step method in the presence of Fe^{2+} and Fe^{3+}. In order to assess the efficiency of MMWCNT as a drug delivery agent, five groups of materials were experimented as follows; GEM (control), GEM carried by MMWCNT in presence of external magnet (MMWNTs-GEM-Magnet) and without (MMWCNT-GEM), and GEM carried by MACs (Nano activated carbon with magnetic particles) with external magnet (MACs-GEM-Magnet) and without (MACs-GEM). GEM concentration was measured in different times after injection in the left popliteal lymph nodes. It is obvious from Figure 5(a) that MMWCNTs-GEM-Magnet has the highest GEM concentration in all times

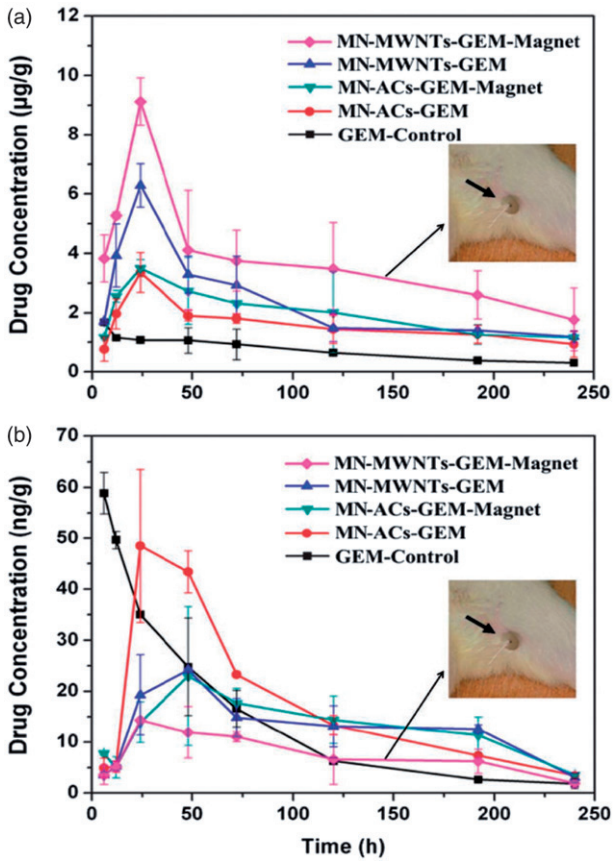

Figure 5. The curves of GEM concentrations at different times after administration of GEM, MMWNTs-GEM-Magnet MMWCNT-GEM, MACs-GEM-Magnet and MACs-GEM: (a) in the left popliteal lymph nodes and (b) in blood plasma [12].

that reached to its maximum amount 24 h after injection. On the other hand, using MMWNTs as a carrier, GEM concentration in blood plasma decreased extremely (Figure 5(b)) and no obvious accumulation was observed in the main body organs such as the liver, spleen, kidneys, heart, and lungs. As it is obvious, using MMWCNTs has a great positive effect on the drug delivery efficiency, and also lowers the drugs side effects [12]. Cytotoxicity profile and ease of transport across the cell membrane are two critical key factors that can be obtained with proper surface functionalization of CNTs [88].

In another study by Yang et al., the cytotoxicity of GEM (control), nano-sized activated carbon decorated with magnetic nanoparticles (MACs), MACs-GEM, MMWCNTs and MMWCNTs-GEM against SW1990, and BxPC-3 cancer cells were studied *in vitro*. The results after 48 h of incubation with a dosage series of mentioned groups proposing that the carrier (MMWCNTs) had relatively no toxic effect on the cancer cells. They also employed the lymph node metastasis model in order to assess the antitumour effect of MMWCNTs and MACs *in vivo*. They could not observe any volume reduction on the metastatic lymph node in MMWCNTs and MACs injected rats. Interestingly, administration of GEM also did not produce any decrease in lymph node volume. Conversely, MMWCNTs-GEM and MACs-GEM lessen lymph node volume remarkably, irrespective of whether the magnets exist or not (Figure 6) [89]. Fluorescein isothiocyanate (FITC)-labelled MCNTs (FITC-MCNT) uptake by the human monocytic leukemia cell line THP-1 under an external magnetic field was investigated by Gul et al. To this end, uptake efficiencies of FITC-MCNT were determined after 1, 3, and 6 h, under effect of an external magnetic field (both rotating and static) (see Figure 7(a)). As a result, 100% of FITC-MCNT uptake into THP-1 cells occurred within 1 h (Figure 7) [90].

One of the most beneficial properties of MCNTs is their pH-triggered drug release [91]. The DOX release from CNTs decorated with iron oxide MNPS at 37 °C in phosphate buffer solution at pH 5.3 (endosomal pH of cancer cell) and 7.4 (physiological pH) was investigated by Lu et al. As it is obvious from Figure 8, DOX release from MMWCNTs at pH 7.4 has a slow and controlled fashion (14% release in 192 h), while at pH 5.3 DOX release increased significantly (71% release in 192 h). This phenomenon was assigned to the improved hydrophilicity and solubility of DOX and also to weakened hydrogen bonds at lower pH values [92].

In order to enhance therapeutic efficacy of DOX and reduce its side effects, Al-Faraj et al. [93] have developed an iron oxide nanoparticle-tagged (ION-tagged) polyvinylpyrrolidone SWCNTs loaded with Dox and tested it on Luciferase (Luc2)-expressing 4T1 (4T1-Luc2) murine breast cancer (*in vivo*). They improved targeting specificity of the Dox-IONP-SWCNTs by conjugating it with endoglin/CD105 antibody. Bioluminescence imaging (BLI) performed on tumour-bearing mice showed the successful tumour growth for the primary injection site in the left inguinal mammary fat pad (Figure 9(a)). Following injection of SWCNT + CD105 and iron-tagged SWCNT + CD105 samples, the radiance efficiency revealed gradually increase and became more prominent with the progression of metastasis in the upper thoracic region (i.e. the lung) observed three-week post-tumour cells injection (i.e. seven-d post-SWCNTs injection). Following injection of free DOX drug, a reduction of bioluminescence was detected, and also metastasis showed less prominent progression. Based on results, following injection of drug-conjugated SWCNT + DOX along with higher extent in iron-tagged samples (i.e. SWCNT + CD105 + DOX) targeted to the primary tumour site magnetically, radiance efficiency was decrease noticeably along with inhibition of metastasis (Figure 9(b)) [93].

In another study performed by Afroze et al. [94], MWCNTs were functionalized by in-situ chemical precipitation of hydroxyapatite (HA) in order to enhance their magnetic properties (HA/f-MWCNTs). The results showed that HA/f-MWCNTs nanocomposites were hard ferromagnetic at room temperature. The authors claimed that HA/f-MWCNTs nanocomposites are capable of targeting the infected tissues and can be used as a chemo-hyperthermia therapy for treatment of cancerous cells in bone tissue. In another research, Zonghua et al. [95] decorated CNT's surface with Fe_3O_4 NPs and CdSe to fabricate CdSe@Fe3O4/CNTs. This novel nanocarrier offers outstanding properties such as targeting ability in presence of an external magnetic field, fluorescence for *in vivo* imaging and particle stability which shows its capability as a proper drug delivery system for cancer chemotherapies.

Gene delivery

Carbon nanomaterials, in particular, CNTs and graphene are frequently researched and utilized for gene delivery

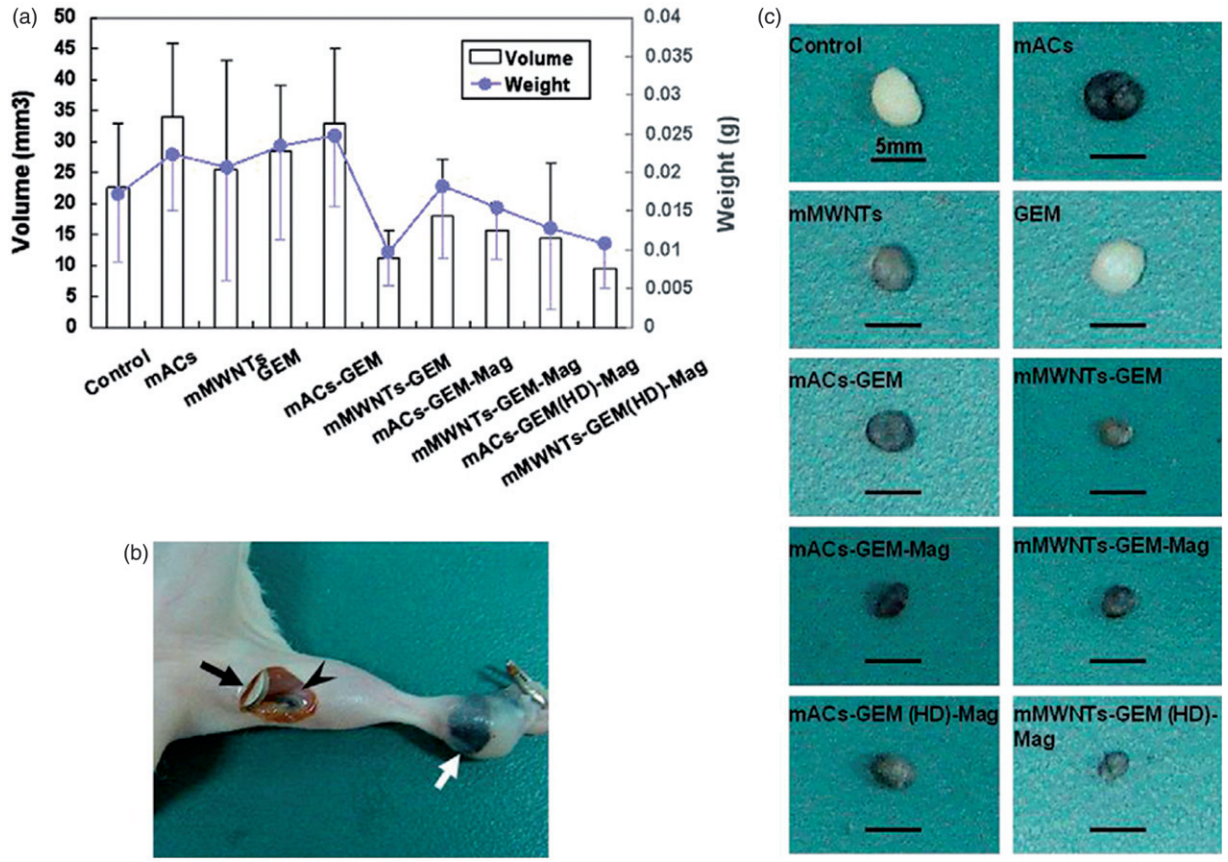

Figure 6. *In vivo* lymph node targeting of the MNPs. (a) Effects of different treatment groups on metastatic lymph node growth inhibition *in vivo*. (b) A representative photograph of the mouse to which MMWCNTs-GEM was subcutaneously administered under the magnetic field. The primary tumour (white arrow), blackened metastatic popliteal lymph node (arrowhead), and magnet (black arrow) were shown. (c) Photographs of popliteal lymph nodes isolated on day 15 from mice treated with saline, MACs/MMWCNTs, GEM, and various combinations with or without applying implanted *in vivo* magnets were shown. Scale bars are 5 mm [89].

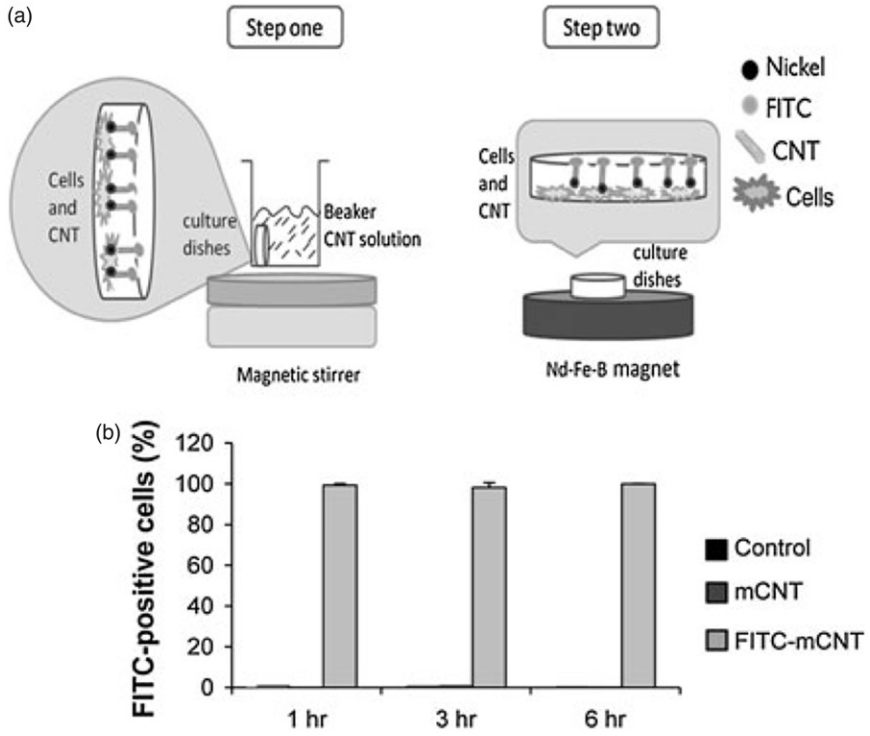

Figure 7. (a) Schematic illustration of FITC-MCNT uptake into THP-1 cells by external rotating and static magnetic fields and (b) % FITC-positive cells exposed to FITC-MCNT or MCNT only after 1, 3, and 6 h exposures [90].

applications in a similar manner to drug delivery. Treatment of various diseases by introducing nucleic acids into CNTs which can repair, replace, regulate, and add or delete a certain genetic target responsible for a particular disease, is attracted a great deal of interest; which forms our understanding of genetic processes and molecular biology [96]. Gene therapy introduces new disease treatment strategies, which necessitate development of non-toxic, biocompatible, and non-viral vectors for carrying therapeutic genes. The clinical applications of viral vectors are barricaded due to their immunogenicity concerns, limited genetic payload, and the poor targeting of particular cell populations. Nanovectors, such as CNTs, offer an encouraging alternative with additional benefits including the ease of access into a cell, an outstanding boost in the solubility and biocompatibility of nucleic acids and most importantly, improved protection for the loaded nucleic acids from cleavage and degradation [97]. Carbon nanomaterials can be functionalized to contain positive charges to bind and deliver the negatively charged DNA and siRNA. One of the greatest advantages of using carbon nanomaterials for gene therapy is their low intracellular toxicity even at very high concentrations, which is in contrast to commercial gene transfection agents. Besides, overall transfection rates for carbon nanomaterials are equal or more

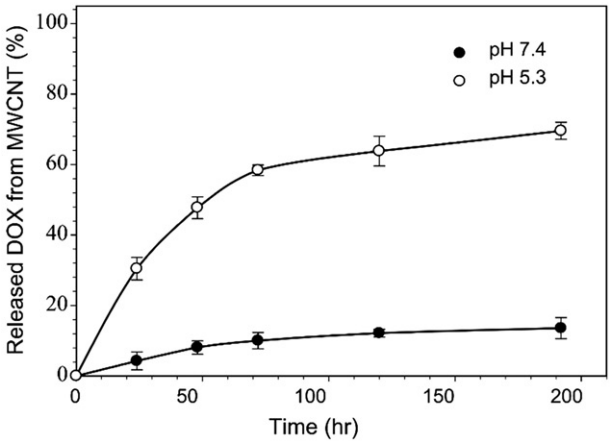

Figure 8. Drug release of DOX from FITC-MMWCNTs at pH 7.4 and 5.3 (37 °C) [92].

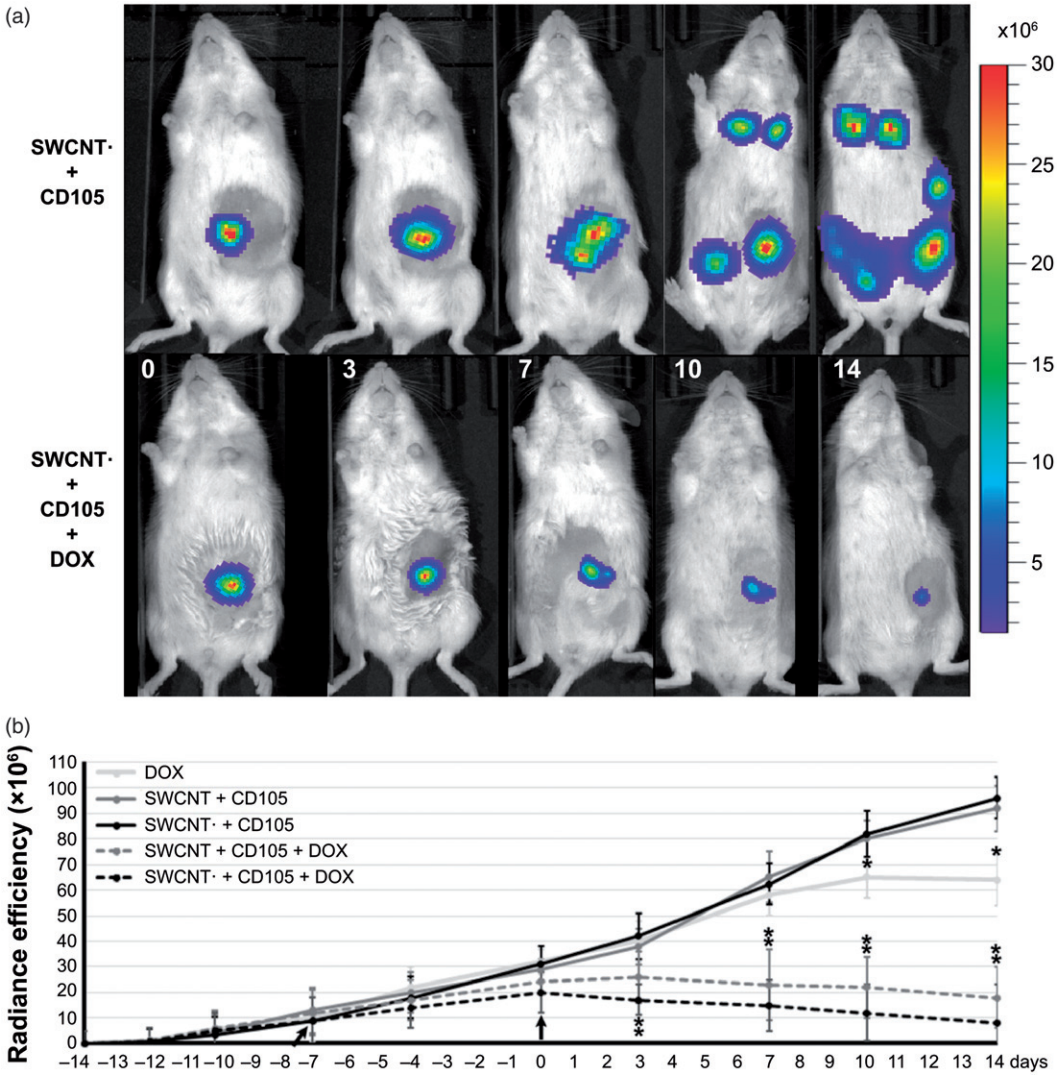

Figure 9. (a) BLI images of tumour-bearing mice at 0, 3, 7, 10, and 14 d after injection of either SWCNT·+CD105 (upper row) or DOX-loaded SWCNT·+CD105 (lower row) showing the progression of tumour and eventual metastasis following inoculation of 4T1-Luc2 breast cancer cells in the left inguinal mammary fat pad. (b) Quantitative assessments of radiance efficiency following intravenous injection of either free DOX or CD105- conjugated SWCNT samples with or without either iron tagging (·) or drug loading (DOX) accomplished to evaluate treatment-induced responses. Black arrows highlight the time of DOX or SWCNT injections [93].

than commercial gene transfection agents such as lipofectamine 2000; and also new approaches using these nanomaterial transporters for gene delivery have remarkably declined the required dosage and considerably improved transfection success rate [98–100]. There are new strategies which can be used in CNTs for increasing the efficiency of the delivery of exogenous DNA into cells; these CNTs are prepared to give a great improve to the efficacy of gene therapy. One of the innovative approaches is equipping CNTs with MNPs. Cia et al. reported a new method in which the nanotubes carrying ferromagnetic nickel, were coated with a DNA plasmid and driven into cells through a combination of variable and static magnetic field for which they named this new approach as "nanotube spearing". The residual nickel catalyst particles, which were enclosed in the tips of CNTs after their growth by CVD method, make nanotubes responsible to external magnetic field. These CNTs, carrying a genetic payload, that can be used to "spear" the cells and deliver their contents directly into the nucleus by adjusting the external magnetic fields. Carbon nanotubes internalization, naturally is through endosomal or lysosomal pathways and unfortunately, through these pathways many plasmids are hydrolyzed and thus destroyed before releasing their genetic payload. By employing MCNTs and nanotube spearing technique, DNA can be delivered to the cell nucleus, directly and DNA plasmids containing the sequence of enhanced green fluorescent protein (EGFP) delivering efficiency improved remarkably. Using CNTs alone, requires concentrations of ~1–5 μM, yet employing MCNTs and nanotube spearing technique, decreases required concentration to merely ~100 fM, and therefore increasing the molecular deliverance efficiency by 10^7 fold [14].

Hyperthermia

Applying an altering current (AC) magnetic fields to superparamagnetic NPs causes arbitrarily flips of magnetization direction between perpendicular orientations, leading to magnetic energy transfers to heat. This property can be used to rise the tumour tissues temperature, *in vivo*, in order to killing the pathological cells by mean of excessive heat. It is reported that tumour cells are more vulnerable to excessive temperature than healthy ones [101,102]. It has been shown that dextran coated magnetite and magnetite cationic liposomal NPs [103] can efficiently rise the tumour cell's temperature. In recent years, hyperthermia has been suggested as one of the important methods of cancer therapy [104]. There are some significant advantages in using MCNTs for hyperthermia. Firstly, it limits the heating to the tumour zone. Furthermore, the application of subdomain magnetic particles (nanometer-sized), the type which are used in MCNTs, has priority over multi-domain (sized) particles, because NPs absorb more power at AC magnetic fields [105]. In comparison with MNPs, because of their needle-like shape, MCNTs have better ability to penetrate into cells, which results in more effective hyperthermia result. Furthermore, carbon shield in MNP-filled CNTs prevents the MNPs and biological environment to interact with each other. Thus, MNPs and biological environment are being protected against oxidation and toxicity, respectively [91,106]. Klingerer et al. conducted a research on ferromagnetic Fe-filled MWCNTs in order to investigate the feasibility of their use in hyperthermia. They concluded that CNT filling with magnetic material makes them capable of hyperthermia uses and also the insertion of nuclear magnetic resonance (NMR) active substances permits their usage as markers and sensors [107].

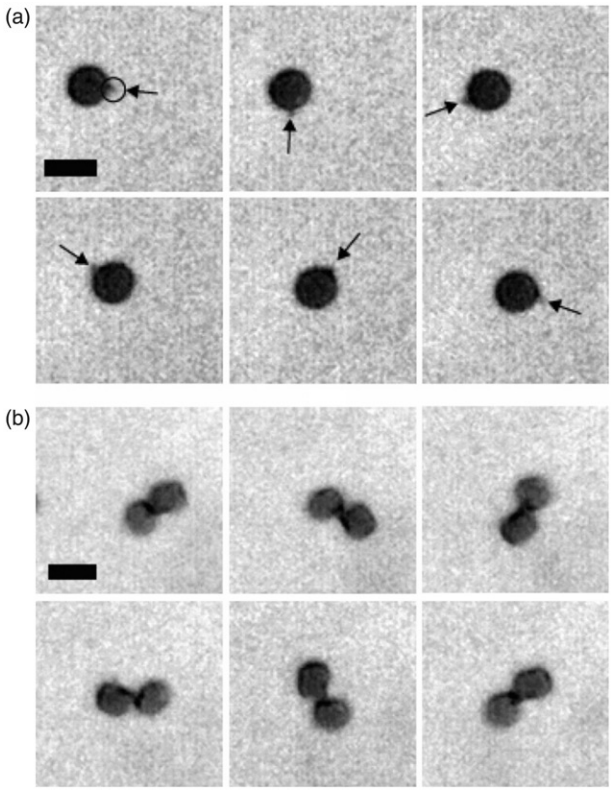

Figure 10. Rotational motion of sheep red blood cells. Magnetic nanotubes are attached to the cells. The images correspond to 0, 0.2, 0.4, 0.6, 0.8, and 1.0th cycle of the rotational magnetic field. The scale bars are 5 μm. (a) A sheep red blood cell. Magnetic nanotubes indicated by an arrow are attached to the cell. (b) Two sheep red blood cells bridged with magnetic nanotubes [15].

Cell separation and manipulation

The separation and detection of rare cells such as circulating tumour cells (CTCs) have a great importance in theranostic applications [108]. Detection of CTCs as potential metastasis biomarkers is very difficult either *ex vivo* (because of large blood volumes needed) or *in vivo* (time-consuming). Thus, the effectiveness of traditional technologies is quite low and the development of new strategies is essential [109]. MCNTs are potential candidates for using as separation and manipulation tools. They have many worthy properties including out-of-damage penetration to cells and out-of-body control by an external magnet which are very advantageous for this application [110]. Gao et al. accomplished a research on sheep red blood cells manipulation, employing MCNTs. He reported that blood cells in a phosphate solution can be rotated or conveyed selectively by changing the magnetic field direction. Moreover, two blood cells were manipulated due to bridging by the magnetic MCNTs (Figure 10) [15]. In a

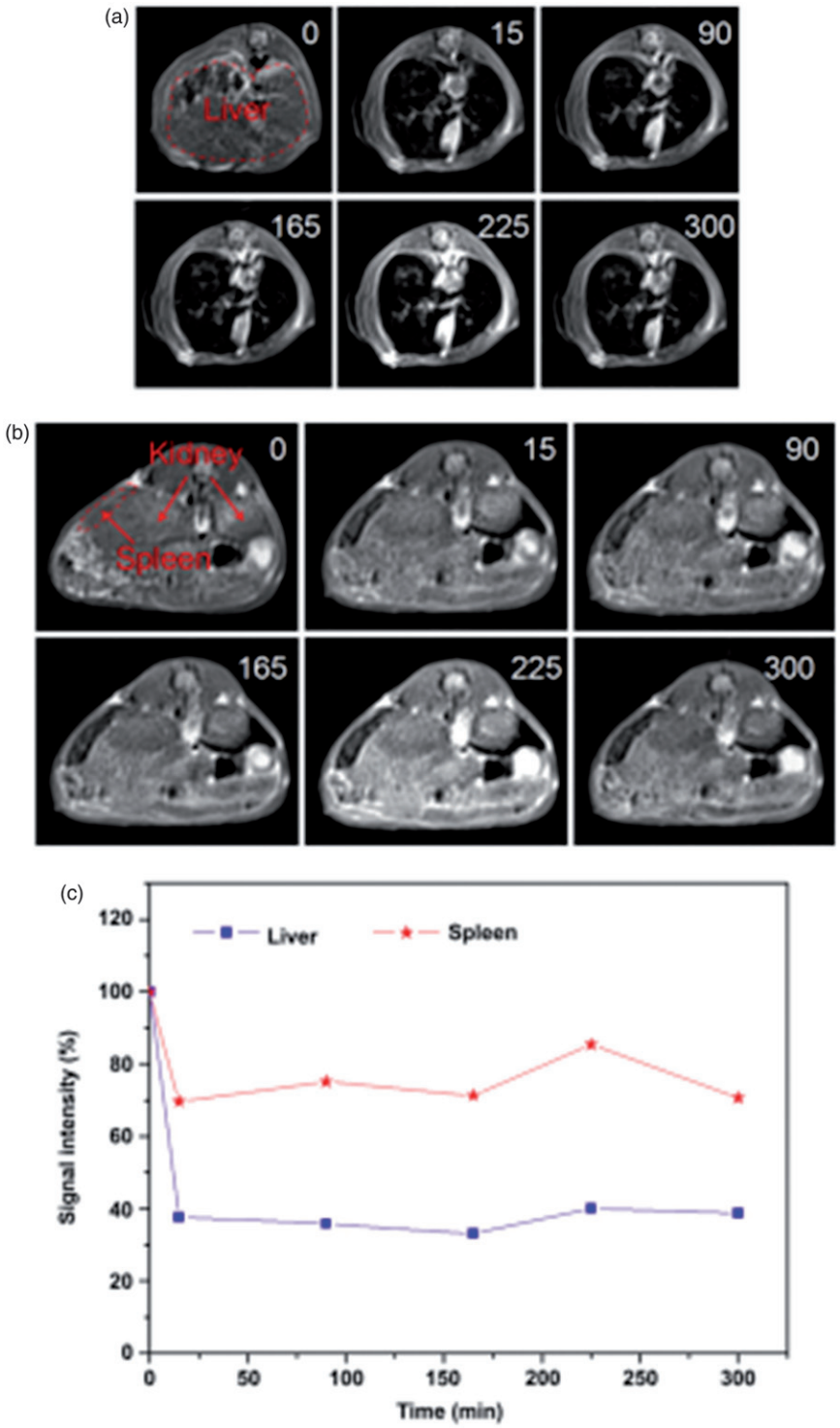

Figure 11. *In vivo* T2-weighted MRI images of (a) liver and (b) kidneys/spleen before and after administration of MMWCNT (post 15, 90, 165, 225, and 300 min), (c) time-dependence of the relative signal intensity in liver and spleen after the administration of MMWCNT [121].

research by Gul et al., MCNTs have been used in order to track haematopoietic stem and progenitor cell. They reported that MCNTs labelling method has no adverse effect on cell viability and would be a key technology which can be employed for labelling and tracking of HSPC *in vivo* [111]. In regard to the metallic MNPs attached into CNTs and their interactions with cells, CNTs can be applied for cellular manipulation. It is proved that MCNT-bound cells have measurable movement under an external magnetic field. By harnessing this behaviour, it is possible to barricade cell migration and thus metastasis establishment [112,113]. Vittorio et al. observed that applying MMWCNTs carrying NPs of Fe, Al, and Zn on PC12 rat pheochromocytoma cells has no adverse effect on cell viability and results in cell separation under an external magnetic field [114]. In the study of Shen et al., Fe_3O_4 decorated MWCNTs were non-covalently functionalized with phospholipid-polyethylene glycol (PL-PEG). Due to robust magnetic properties of produced

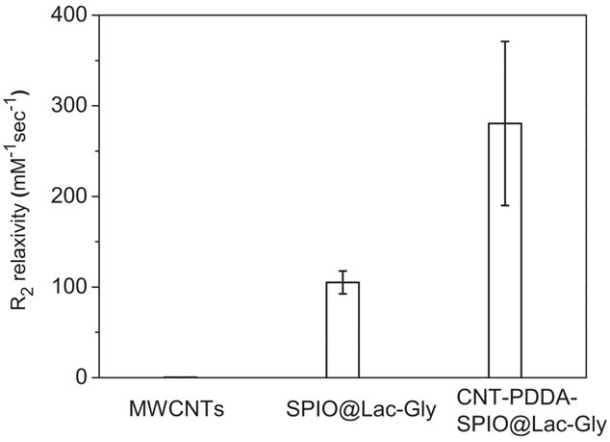

Figure 12. MWCNT, SPIO@Lac-Gly and CNT-PDDA-SPIO@Lac-Gly relaxivity results [124].

MMWCNTs, cells labeled by them were effectively manipulated and separated under an external magnetic field. As a result, a specific cell type can be targeted by PL-PEG-MMWCNTs, thus it can be captured, separated and manipulated. This NP examined *in vitro* and *in vivo* for their toxicity and based on the results, PL-PEG-MMWCNs causes no adverse effect [115].

MRI

Among all the clinical imaging techniques, MRI has the advantage of high-quality anatomical images with high spatial resolution. Instead of using ionizing radiation, MRI employs a high-intensity magnetic field that aligns the nuclear magnetization of hydrogen atoms of water molecules in body organs. Different water concentration among different tissues results in producing images from different organs. Common MRI contrast agents (CAs) are chemical compounds which are containing two different types of agents. The first type is paramagnetic metal ion (such as Gd^{3+} and Mn^{2+}) with larger effects on T_1 shortening (spin-lattice relaxation agents), and the second type is based on superparamagnetic materials such as IONs with larger effects on T_2 shortening (spin-spin relaxation agents), where T_1 and T_2 are the proton relaxation times. One of the biggest problems with common imaging CAs is their incapacity to cross the cell membrane. Using CNTs make these agents capable of intracellularly delivering for purpose of cell tracking by mean of selectively, in case of using biological targeting moieties. Imaging agents which are linked onto or packed into the CNTs can be internalized by cells [116] and fortunately, there is no need to cytotoxic transfection agents [117,118]. Results from separate researches on CNT carried T_1 weighted (GD^{3+}) and T_2 weighted superparamagnetic iron oxide (SPIO) agents revealed that desirable effects of this CNT-CAs in MRI (*in vivo* and *in vitro*) are mainly due to CNTs properties, or in better words, CNTs have some synergistic relations with CAs [119,120]. As mentioned above, superparamagnetic iron oxide nanoparticles (SPIONs) have also been used as MRI CAs. Synthetize of The MMWCNT containing SPIONs by a solvothermal method which produces a superparamagnetic CA at room temperature and a T_2-relaxivity nature was reported by Wu et al. In order to assess the *in vivo* contrast improving influence of MMWCNT, it was suspended in physiological solution and injected to mice. T_2-weighted MRI images of mice spleen, kidneys and liver before and after injection of MMWCNT is presented in Figure 11(a,b). In comparison with those without administration, after administration of MMWCNT, the liver and spleen were darkened considerably. Figure 11(c) shows a quantitative conformation for the time-dependent darkening of the MRI images in the mice's organs. These outcomes propose that the MMWCNT hybrids can be used as an appropriate negative (T_2-weighted) CA in MRI applications [121].

Maciejewska et al. reported the synthesis of MWCNT-Fe nanocomposites by floating catalyst CVD method. The MWCNT-Fe was then synthesized in order to produce oxidized (O^-) MWCNT-Fe (O-MWCNT-Fe) which resulted in enhanced hydrophilicity and as a result, O-MWCNT-Fe could be readily dispersed in water and cell culture medium. Water-dispersed O-MWCNT-Fes showed superparamagnetic-like behaviour. The obtained nanocomposites were employed as MRI CA for both types of samples (water or culture medium). The sample with 2 wt.% of Fe provided the strongest contrasting results and it is cytotoxic for HeLa cell lines was negligible. Therefore, O-MWCNT-Fe nanocomposites can be introduced as a promising CA for cancer imaging [122].

Wang et al. [123] used SWCNTs as substrate for Au NPs, SPIONs, and CdSe/ZnS quantum dots (QDs) to fabricate a multifunctional magnetic nanosystem for live cell imaging. Experimental results demonstrated that the designed nanosystem causes a negligible cytotoxicity and the cell viabilities were higher than 90% for different concentrations of NPs which is advantageous for live cell imaging applications; and also it was capable of producing strong fluorescence and surface-enhanced Raman scattering (SERS) signals, and its SPIONs magnetic properties could be employed for MRI imaging.

Liu et al. [124] have combined SPIONs with MWCNTs in order to produce an efficient MRI T_2-weighted CA with liver targeting capability. Poly(diallyldimethylammonium chloride) (PDDA)-coated MWCNTs were decorated by lactose-glycine (Lac-Gly) adduct targeting agent modified SPIONs. MWCNTs, SPIO@Lac-Gly NPs and CNT-PDDA- SPIO@Lac-Gly were screened by MRI scanner in water dispersed in agarose gel. The results revealed that CNT-PDDA-SPIO@Lac-Gly relaxivity R_2 ($1/T_2$) was more than two times higher than SPIO@Lac-Gly NPs, which is a result of higher SPION carrying capacity of CNT in comparison to SPION clusters (Figure 12). Moreover, The R_2 of CNT-PDDA-SPIO@Lac-Gly (R_2 ¼ 186 mM1 s1) showed meaningfully greater R_2 than that of the commercial iron oxide CA Feridex (R_2 ¼ 148 mM1 s1).

Figure 13 shows the mouse liver images which was injected with CNT-PDDA-SPIO@Lac-Gly. Based on results, CNT-PDDA-SPIO@Lac-Gly was taken up with liver and was internalized in tumour cells, which produced a strong contrast between tumour and healthy liver tissue [124].

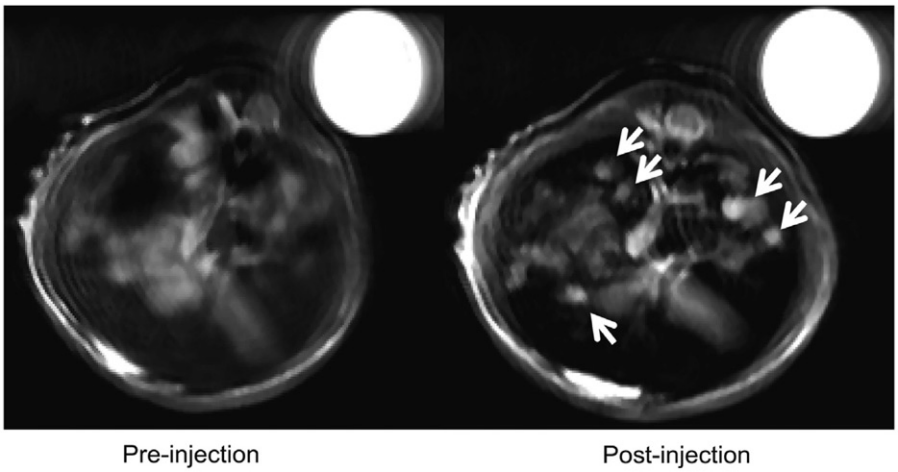

Figure 13. MRI images of mouse liver before and after injection of CNT-PDDA-SPIO@Lac-Gly (10 mg/kg) (white arrows indicate tumours) compared to internal standard (water, top right) [124].

Industrial applications

MCNTs, as important functional materials, have been investigated for potential applications in various fields of industry. Attraction of MCNTs for industrial applications is due to their flexibility for adding different and useful options on it that gives MCNTs the ability of adaptation to various applications. Some of the most common applications of MCNTs in industries are using as contamination absorbent, either for organic [125] or heavy metal contaminations [126], electrochemical sensor [127], catalyst [128], magnetic storage medium [129], and microwave absorbent [130]. Gong et al. have synthesized MMWCNT nanocomposite that was composed of multi-wall CNTs and IONs as an adsorbent for removal of cationic dyes from aqueous solutions [131]. Dyes are known as pollutants that reduce light penetration and photosynthesis, and some are considered toxic and carcinogenic for human health. On the other hand, heavy metal pollution has become one of the most serious environmental problems today. So, removal of heavy metal ions and radionuclides from waste solutions is an important environmental concern in waste management. Chen et al. produced MWCNT/iron oxide magnetic composites for adsorptions of Ni(II) and Sr(II) from wastewater. They have shown that adsorption capacity of the magnetic composites is much higher than those of MWCNTs and iron oxides and also, the solid magnetic composites can be separated from the solution by a magnetic process that it may be a promising candidate for solidification of heavy metal ions and radionuclides from large volumes of aqueous solution [132]. Another application of MCNTs is using them in electrochemical sensors. Qu et al. fabricated novel electrochemical-sensing sensor for the detection of hydrogen peroxide based on carbon nanotube/nano-Fe_3O_4 (CNT/Fe_3O_4) composite on electrodes. The magnetic nanocomposite could be loaded onto the surface of magnetic electrode with the aid of the Fe_3O_4 NPs [133].

Conclusions

In this paper, we highlighted the structure and properties of CNTs and MCNTs. In the following, bioapplication and industrial applications of MCNTs were discussed. Drug delivery and gene therapy are considered as most prominent applications of MCNTs in biotechnological systems. In recent years, the development of multifunctional nanosystem is easy to obtain and will surely result in increment of the number of *in vitro* and *in vivo* experiments using these nanosystems in the future. Hopefully, novel properties and improved performances of MCNTs proposes that they will satisfy the ever increasing need of safer and more efficient drug delivery vectors. Thanks to great progresses and developments in this area, the potential of these systems is claimed to be very high and the range of applications involves several biomedical and industrial fields. In the biomedical field, the most promising approach is the development of non-cytotoxic vectors with targeting ability exploited by the presence of MNPs that can be guided through selected tissues by an external magnetic field. Furthermore, the presence of MNPs can encourage the use of MCNTs as versatile systems for hyperthermia therapeutic strategies which is still not completely developed and widespread. In the industrial field, they are unique nanostructures that display the desirable properties of any other known material. They have special electronic and mechanical properties which lead to incredible forms of strength, and conductivity. Due to these qualities, the field of applications is almost endless. From reinforcements in composites, sensors and probes to energy storage, electrochemical devices and nanometer sized electronics, CNTs could revolutionize the world. Therefore, there is no doubt that the future for magnetic CNTs will be very bright.

Acknowledgements

The authors would like to thank the Department of Medical Nanotechnology, Faculty of Advanced Medical Science of Tabriz University of Medical Sciences for all the support provided.

Disclosure statement

No potential conflict of interest was reported by the authors.

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