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Review

Multifunctional carbon nanomaterial hybrids for magnetic manipulation and targeting

Ngoc Do Quyen Chau [a], Cécilia Ménard-Moyon [a], Kostas Kostarelos [b], Alberto Bianco [a,*]

[a] CNRS, Institut de Biologie Moléculaire et Cellulaire, Laboratoire d'Immunopathologie et Chimie Thérapeutique, 67000 Strasbourg, France
[b] Nanomedicine Laboratory, Faculty of Medical & Human Sciences and National Graphene Institute, University of Manchester, AV Hill Building, Manchester M13 9PT, United Kingdom

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ABSTRACT

Nanosized materials and multifunctional nanoscale platforms have attracted in the last years considerable interest in a variety of different fields including biomedicine. Carbon nanotubes and graphene are some of the most widely used carbon nanomaterials (CNMs) due to their unique morphology and structure and their characteristic physicochemical properties. Their high surface area allows efficient drug loading and bioconjugation and makes them the ideal platforms for decoration with magnetic nanoparticles (MNPs). In the biomedical area, MNPs are of particular importance due to their broad range of potential applications in drug delivery, non-invasive tumor imaging and early detection based on their optical and magnetic properties. The remarkable characteristics of CNMs and MNPs can be combined leading to CNM/MNP hybrids which offer numerous promising, desirable and strikingly advantageous properties for improved performance in comparison to the use of either material alone. In this minireview, we attempt to comprehensively report the most recent advances made with CNMs conjugated to different types of MNPs for magnetic targeting, magnetic manipulation, capture and separation of cells towards development of magnetic carbon-based devices.

1. Introduction

Carbon nanomaterials (CNMs) have shown great potential in biomedical applications, mainly due to their unique chemical and physical properties [1–5]. Carbon nanotubes (CNTs) and graphene are two of the most widely used CNMs due to their physical and chemical stability as well as their high surface area-to-weight ratio. In the field of nanomedicine, they are used as platforms for the immobilization of nanoparticles (NPs) [6,7] and as versatile carriers for a variety of bioactive molecules [8–10]. CNMs are also endowed with characteristic optical properties, such as fluorescence and Raman scattering, making them useful for sensing applications and a variety of imaging modalities such as magnetic resonance, near-infrared fluorescence, photoacoustic tomography, photothermal and Raman imaging [4,5,11–13].

Magnetic nanoparticles with appropriate physicochemically tailored surface properties, colloidal stability and biological behavior have been used in drug delivery, hyperthermia, magnetic resonance imaging (MRI), biosensing, biochemical separations and bioanalysis [14–17]. The combination of CNMs and different types of MNPs has recently attracted interest in biomedical applications [18–21]. In particular, CNM/MNP hybrids exhibit advantageous and often synergistic properties arising from their combination and molecular interactions [22,23]. For instance, in sensing applications, the association of NPs with graphene renders greater catalytic and conducting properties, enhancing their sensitivity and selectivity in comparison to graphene- or NP-based sensors alone [24]. Endowing CNMs with magnetic properties thanks to the association with MNPs is opening many opportunities for future biomedical applications.

In this minireview, we describe the association of CNMs, mainly CNTs and graphene, and MNPs for various biological applications. Specifically, we will focus on CNM/NP hybrids for magnetic targeting in multifunctional drug delivery and imaging, for biosensing, for magnetic molecular extraction, magnetic manipulation, capture and release of cells. We will also discuss the ability of CNT/NP hybrid for magnetic manipulation and fabrication of biomedical devices.

2. Magnetic targeting

The combination of CNMs and NPs has led to the generation of novel systems that are finding a wide range of applications in

* Corresponding author.
E-mail address: a.bianco@ibmc-cnrs.unistra.fr (A. Bianco).

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biomedicine due to their versatile magnetic properties. Advanced multifunctional magnetic CNM-based vectors bearing fluorescent moieties [*e.g.* fluorescein isothiocyanate (FITC)], proteins (*e.g.* transferrin), targeting ligands [*e.g.* folic acid (FA)] or therapeutic drugs [*e.g.* doxorubicin (DOX)] have been used not only in targeted therapies, but also in imaging and theranostics. In the context of therapy, CNM/MNP hybrids have shown great promise as drug carriers and have been exploited for targeting cancer cells.

The superparamagnetic graphene oxide (GO)-Fe_3O_4 NP hybrid loading DOX for controlled targeted drug delivery was first reported in 2009 [25]. This hybrid, with or without DOX, aggregated under acidic conditions, and was then reversibly redispersed under basic conditions. More interestingly, after aggregating at low pH, the GO/Fe_3O_4 hybrid can be dragged under the application of an external magnetic field. Thus, the transfer efficiency of drug vectors could be greatly improved by magnetic field guidance. This also indicated that at acidic pH, some functional groups on the GO, such as carboxylic acid groups, even after loading with a large amount of Fe_3O_4 NPs and DOX were still free for efficient formation of hydrogen bonding, making this hybrid a promising pH–triggered targeted carrier. Due to its pH–triggered magnetically controlled capabilities, this hybrid was functionalized with different biomolecules or drugs such as folic acid [26,27] and 5-fluorouracil (5-FU) [28], for specific multi-targeting or multi-drug loading and delivery. Indeed, it is known that the loading and release of DOX depends on the hydrogen bonding interaction with multifunctionalized GO [26]. At acidic conditions, protonation of amine groups on DOX can break the hydrogen bonding between DOX and GO, leading to a quick release of the drug. Hence, the multifunctionalized GO was able to first transport the drugs to the targeted tumor cells by the force of an external magnetic field localized at the site of the tumor, and then the drug-loaded carriers were taken up by the tumor cells.

In the case of CNTs, the functionalization of iron-decorated multi-walled CNTs (MWCNTs) with a targeting moiety (FA) and drugs (such as DOX) has also been reported [29,30]. For instance, FA/FITC/MWCNT-Fe has been used to deliver DOX in human cervical cancer HeLa cells [29]. Thanks to the adsorption of iron NPs on both the inner and outer surface of the MWCNTs, the nanocarriers were guided to the location of cancer cells by applying an external magnetic field. Then, the conjugates were specifically targeted to FA receptors that were overexpressed on cancer cells.

In addition to CNTs and GO, multifunctional carbon nanohorns (CNHs) have also been combined with MNPs. By using an external magnetic field, the targeted vectorization of CNHs loaded with

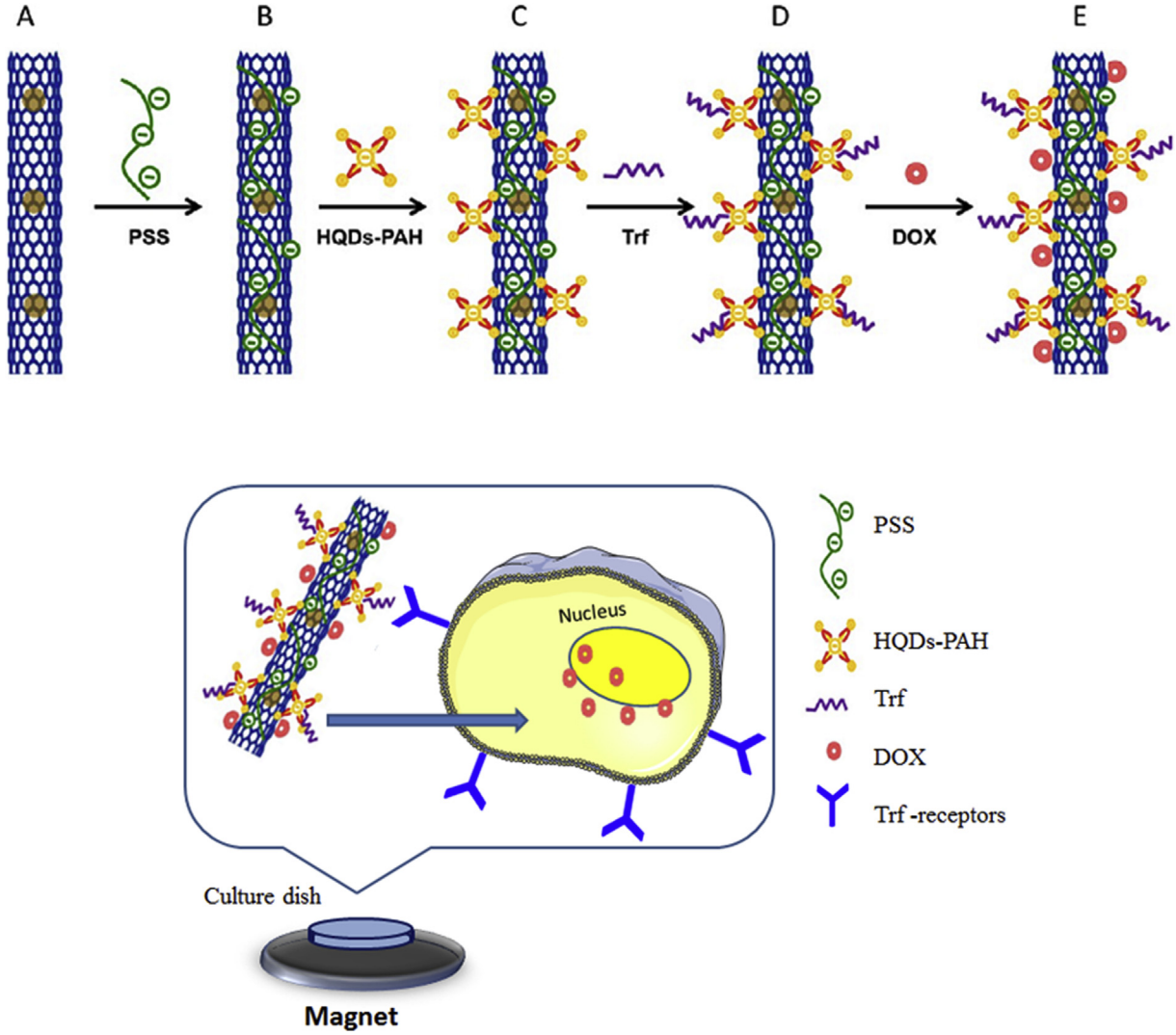

Fig. 1. Top: Preparation of water-dispersible DOX-Fe_3O_4@CNT-HQD-Trf conjugates. (A) Fe_3O_4@CNT; (B) PSS-coated Fe_3O_4@CNT; (C) Fe_3O_4@CNT-HQD; (D) Fe_3O_4@CNT-HQD-Trf; (E) DOX-Fe_3O_4@CNT-HQD-Trf. Adapted from Ref. [33]. Bottom: Schematic representation of *in vitro* (DOX) delivery and release using Fe_3O_4@CNT-HQD-Trf hybrids in the presence of a magnetic field. PSS: poly(sodium 4-styrenesulfonate); HQD-PAH: SiO_2-coated quantum dots-poly(allylamine); Trf: transferrin.

magnetite (Fe_3O_4) NPs and functionalized with polyethyleneimine (PEI) into HeLa cells was significantly enhanced [31].

As CNM/NP hybrids have been shown to display interesting properties for therapy and imaging, theranostic applications could be figured out. DOX was loaded onto superparamagnetic GO iron oxide hybrid (IONP, iron oxide nanoparticle) functionalized with polyethylene glycol (PEG) (GO-IONP-PEG-DOX complex). This conjugate was used for magnetically targeted drug delivery and photothermal therapy (PTT) due to the strong optical absorbance of GO from the visible to the near-infrared (NIR) region. The PTT treatment resulted in the selective killing of cancer cells in highly localized regions. The T_2 contrast agent properties of the IONPs were exploited for MRI in tumor-bearing mice [32].

A multifunctional nanoplatform has also been developed by conjugating SiO_2-coated CdTe quantum dots (HQDs, hybrid quantum dots) with Fe_3O_4-filled MWCNTs to achieve simultaneous cancer-targeted optical imaging *in vitro* and magnetically guided-drug delivery (Fig. 1; top) [33]. The MWCNTs prevented the magnetite NPs from aggregation, enhanced their chemical stability, improved the drug loading, and avoided magnetite nanocrystals-induced quenching of the fluorescence of the HQDs. A dual targeted drug delivery system was developed by coating transferrin (Trf) on the surface of CNTs to effectively transport DOX into HeLa cells under the application of an external magnetic field (Fig. 1; bottom).

In another work, a multifunctional theranostic platform incorporating Fe_3O_4 MNPs for enhanced magnetic targeting and MRI has been also synthesized [34]. The targeting peptide-modified magnetic graphene-based mesoporous silica (MGMSPI) demonstrated the potential of DOX-loaded MGMSPI for MRI, dual-targeting recognition (magnetic targeting and receptor-mediated active targeting), and chemo-photothermal therapy into a single system for visualized-synergistic therapy of glioma.

In addition to the combination with IONPs and gold NPs [35], graphene has recently been combined with other types of NPs for PTT of cancer cells, such as superparamagnetic zinc ferrite spinel ($ZnFe_2O_4$). The results indicated that high efficiency of magneto-PTT was obtained by the localization of the magnetic nanomaterials guided by the magnetic field. Hence, the heating effects on cancer cells upon NIR irradiation were increased with the use of minimum concentrations of reduced GO (rGO) and $ZnFe_2O_4$–rGO [36].

Furthermore, CNM/NPs have also shown interesting results in lymphatic targeting, with the delivery of chemotherapeutic drugs to the lymph nodes being of great interest. MWCNTs coated with phospholipids (PEG and FA in their terminal groups), with a layer of Fe_3O_4 NPs and chemotherapeutic agents (such as 5-FU and cisplatin) incorporated into the inner cavity of the nanotubes were taken up by lymphatic vessels and delivered to regional lymph nodes under the action of an external magnetic field [37]. These MWCNTs were retained within the draining lymph nodes for several days acting as depots of released chemotherapeutics. Similarly, Fe_3O_4-MWCNTs coated with poly (acrylic acid) (PAA) were used to deliver gemcitabine (GEM) into the lymph nodes with high efficiency under the guidance of a magnetic field in order to eradicate lymph node-localized metastasis [38,39]. Overall, such studies showed that the hybrids provided an effective means to maintain chemotherapeutic drugs locally at the lymph nodes.

Moreover, CNM/MNP hybrids have been explored for diagnosis and imaging, in particular for the detection of cancer cells. The first study on the efficient cellular MRI using Fe_3O_4-GO has been reported in 2011 with the significantly improved T_2-weighted MRI contrast thanks to the aggregation of Fe_3O_4 NPs onto the GO sheets [40].

In another work, chitosan (CHI)-functionalized magnetic rGO (CMG) was used as platform for simultaneous targeted cancer chemotherapy and gene therapy. The CHI/graphene was loaded with superparamagnetic iron oxide NPs (SPIONs) as T_2 contrast agent for MRI and with DOX as anticancer drug. The DOX-loaded CMG was encapsulated with a reporter DNA sequence. The conjugates were evaluated for DOX and DNA delivery both *in vitro* and in tumor bearing mice. The results demonstrated that the conjugate DOX-CMG-GFP-DNA (GFP: green fluorescent protein) was extensively distributed throughout the tumor tissue. The DOX and GFP concentrations increased with time, but no expression was observed in other organs. The toxicity of DOX-CMG NPs was also investigated by monitoring the body weight changes of mice after a single intravenous administration. No loss in the body weight of mice treated with DOX-CMG, compared to the loss of 14% in two weeks in case of mice receiving free DOX [41].

In the context of cellular imaging thanks to the magnetic targeting, Fe_3O_4-PEG-FITC-CNTs were designed with high dispersibility in aqueous medium, synergistic magnetism for targeting by applying the force of an external magnetic field, and fluorescent capacity. Due to the presence of Fe_3O_4 through NIR laser irradiation and upon application of an external magnetic field, this nanosystem could exhibit the potential in phototherapy and hyperthermia effects in cells and tissue [42].

Recently, a novel fluorescent, surface enhanced Raman scattering (SERS) encoded and magnetic nanoprobe for live cell imaging was designed by using single-walled carbon nanotubes (SWCNTs) as scaffold decorated with gold NPs (Au NPs), SPIONs, and QDs [43]. Here, Au NPs serve as SERS substrate and QDs as fluorescent agent. The adsorption of Au NPs, QDs and SPIONs on to SWCNTs was based on electrostatic interactions. Strong fluorescence and SERS signals were produced under different excitation wavelengths. The magnetism provided by the SPIONs allowed performing magnetic field-guided dual mode imaging of live cells.

In conclusion, in the field of magnetic targeting, the association of CNMs with MNPs has proved to be attractive and promising for various potential applications in theranostics such as guiding to target sites under application of an external magnetic field, imaging, drug delivery, or hyperthermia cancer therapy. Table 1 summarizes the variety of CNM/NP hybrids and their multimodal uses.

3. Magnetic manipulation of cells

In the field of cell-based cancer therapy, nanosystems made of magnetic CNTs have been considered promising tools as they allow to combine their high cellular uptake efficiency with magnetic responsiveness. Based on the metallic impurities entrapped into CNTs during the synthesis process and their interactions with cells, CNTs have been used for cellular manipulation. Functionalized MWCNT-bound cells proved to cause progressive cell displacement towards the most intensive magnetic field zones under a permanent constant magnetic field. This behavior can be exploited to prevent cell migration leading to formation of metastasis [44,45].

The interactions of CNTs with cells without compromising their viability and favoring the differentiation induced by an external magnetic field has been also investigated using MWCNTs with the impurities of 3% Fe, Al, and Zn on PC12 rat pheochromocytoma cells. The results showed the ability to move towards the magnetic source of both undifferentiated and differentiated PC12 cells cultured in CNT-containing solution [46].

One of the first controllable, stable and highly loadable magnetic CNTs has been synthesized by exploiting the electrostatic interactions between magnetic IONPs and polyelectrolyte-grafted MWCNTs [47]. The magnetic cells were prepared by assembling the magnetic CNTs onto sheep red blood cells in a phosphate buffer solution. These cells were selectively rotated or conveyed by

Table 1
Description of the different approaches developed for magnetic targeting using CNM/NP hybrids.

Type of CNM	Type of NP	Functionalization	*In vitro* model	*In vivo* model	Applications	Ref
MWCNTs	Iron	FA; DOX and FITC	HeLa cells	/	Magnetic targeting	[29]
MWCNTs	IONP	FA-PAA; DOX and FITC	U87 human glioblastoma cells	/	Fluorescence bioimaging and magnetic chemotherapy	[30]
CNHs	Fe_3O_4	PEI	HeLa cells	/	Cellular uptake	[31]
GO	SPION	PEG and DOX	4T1 murine breast cancer cells	4T1-tumor-bearing BALB/c mice	MRI; magnetic chemotherapy and magnetic PTT	[32]
MWCNTs	Fe_3O_4, Cd/Te QDs	DOX, SiO_2 coated QDs, Trf	HeLa cells and HEK293 human kidney cells	/	Magnetically guided-drug delivery and fluorescence imaging	[33]
Graphene	Fe_3O_4	Mesoporous silica; IL-13 peptide; PEG and DOX	Glioma cells (U251) and normal cells (1800)	Glioma-bearing mice	MRI; chemotherapy and PTT	[34]
GO	IONP and AuNPs	PEG-FA	Human carcinoma KB and murine breast cancer cells 4T1	Tumor-bearing mice	MRI; X-ray imaging and PTT	[35]
GO	$ZnFe_2O_4$	/	Prostate cancer cells LNCaP	Human glio-blastoma tumors	Magneto–PTT	[36]
MWCNTs	Fe_3O_4	PAA-GEM	Human pancreatic cancer cell lines BxPC-3 and SW1990	BALB/c mice	Cancer lymph node metastasis treatment	[39]
GO	SPION	Chitosan; DOX and p-DNA	A549 human lung epithelial cells and C42b human prostate cancer cells	Prostate tumor-bearing TRAMP mice	MRI; chemotherapy and gene therapy	[41]
MWCNTs	Fe_3O_4	PEG and FITC	MCF7 human breast cancer cells	/	Fluorescence bioimaging and MRI	[42]
SWCNTs	Au NPs, SPIONs, and QDs	SiO_2	Human breast cancer cells SKBR3 and MCF7	/	Magnetic field guided fluorescence and SERS dual mode imaging	[43]

changing the magnetic field direction. Moreover, two blood cells were manipulated because of the bridging by the magnetic MWCNTs (Fig. 2).

Later, it has been reported that MWCNTs could interact with neuroblastoma cells and induce cell displacement towards the magnetic source under the effect of a permanent dipole magnet [45]. The displacement mechanism is still not clear; it is either due to the uptake of the nanotubes (by endocytosis or pinocytosis) or their attachment to the cell membrane. However, this discovery can be considered the starting point for further investigation of the characterization of magnetic properties of CNTs in order to clarify the interactions between cells and CNTs and to control displacement of cells in selective cancer therapy.

Purified functionalized SWCNTs containing residual metallic impurities were used to magnetically manipulate NIH3T3 mouse fibroblast cells thanks to the efficient cellular internalization of CNTs. Cells containing the magnetic functionalized SWCNTs were attracted and aligned towards the direction of a magnetic field. This study has opened new opportunities for non-invasive drug delivery as well as targeted cell therapy [48].

Furthermore, the decoration of metal oxide onto CNTs has shown promising applications. In the study of Shen et al., MWCNTs-Fe_3O_4 were non-covalently functionalized with phospholipid (PL)-PEG. Thanks to the strong magnetism of MWCNTs-Fe_3O_4, cells labeled with the hybrid were efficiently manipulated and separated under a magnetic field. Therefore, by targeting specific cell types, the PL-PEG-MWCNTs-Fe_3O_4 can help to achieve the targeted cells for capture and separation. Additionally, by labeling this hybrid with FITC, it was used as fluorescent probe for cell imaging. The effectiveness of MWCNTs-Fe_3O_4 for *in vitro* and *in vivo* PTT of cancer by NIR irradiation was also investigated. The tumor volume was efficiently reduced under the treatment using the combination of PL-MWCNTs-Fe_3O_4 and laser. The inhibition of tumor growth in mice led also to a prolonged survival of the animals. Moreover, the toxicity induced by CNTs was also investigated. The internal organs were normal in all live mice after the treatment, with no evidence of nanotube-induced damage or inflammation. The results exhibited that combination of MWCNTs-Fe_3O_4 and NIR is a powerful medical technique for PTT of tumors *in vivo* [49]. In another work, it has been shown that only a little concentration (micromolar scale) of iron was sufficient to achieve magnetic manipulation of cancer cells after the uptake of the MWCNT/NP hybrids [50].

In an alternative study, it has been demonstrated that the magnetic response of MWCNTs based on iron/nickel impurities and the consequent rotation of the polymer-coated MWCNTs impacted the integrity of cell membrane. In addition to the enhancement of cell membrane poration, the tumor cells were also ablated. These findings indicated the possibility of magneto-cell-lysis as a means of image-guided *in situ* ablation of tumors. These results open up new ways for further potential clinical applications related to tumor cell poration for targeted cancer chemotherapy and mechanical ablation of tumors [51].

Recent studies presented contrast results. It has been shown that manipulation of MWCNTs with iron oxide and/or cobalt-based NPs could spatially and temporally control their interactions with cells without any effect on cell viability [52]. In particular, when exposed to a rotating magnetic field, the CNTs were shown to rotate on the cell membrane. More interestingly, after 30 min of magnetostirring, the initial shape and morphology of cells were maintained, in contrast to previously reported experiments [51]. Additionally, the cells were able to divide following magnetic stimulation.

Overall, not only MNP/CNTs can be easily manipulated by a magnetic field, but also the cells that internalize them. Thus, these magnetic nanotubes can be used as magnetic tools to reach a precise target site, to control orientation, to improve cellular uptake and/or to drive cell motion.

4. Capture and separation of cells

Numerous studies have reported the use of magnetic CNMs for magnetic separation and purification of low-abundant biomolecules, including proteins [53–56], antigens [57], phosphopeptides [58,59], and DNA, from complex biological samples [60].

The magnetic properties of CNM/NP hybrids have also been exploited to capture and separate cells from a pool of cells or from blood. Fe-filled MWCNTs conjugated to a monoclonal antibody (Cetuximab) that selectively binds the epidermal growth factor receptor (EGFR) have been used as scaffolds for magnetization of cancer cells (Table 2) [61]. EGFR is a plasma membrane receptor

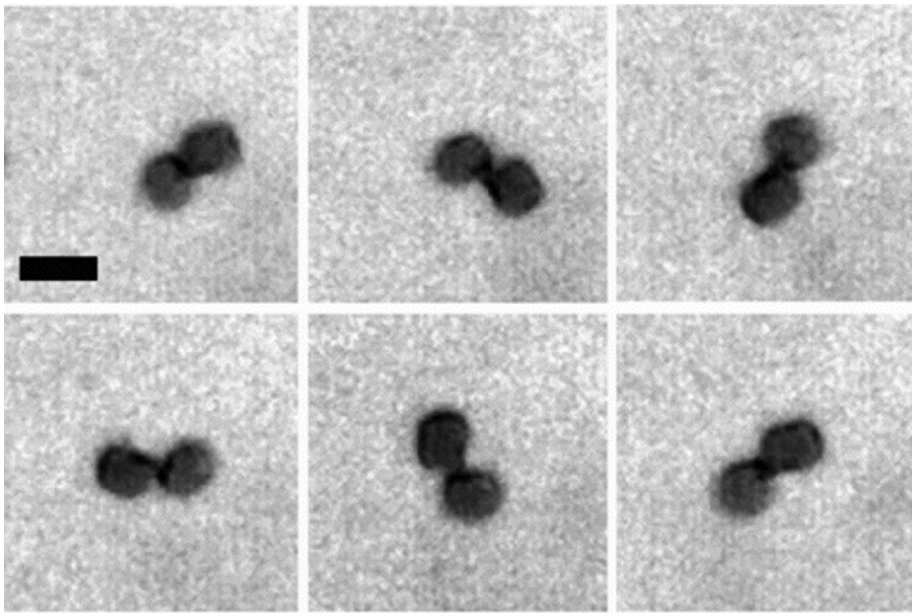

Fig. 2. Rotational motion of two sheep red blood cells bridged by magnetic nanotubes attached to the cell surface. The snapshots correspond to 0, 0.2, 0.4, 0.6, 0.8 and 1.0th cycle of the rotational magnetic field. The blood cells were rotated in the clockwise direction. The scale bar is 5 μm. Reproduced with permission [47]. Copyright 2006, American Chemical Society.

over-expressed in several cancer cells. *In vitro* magnetic filtration of a mixture of cell lines that overexpress or not the receptor led to a selective removal of the EGFR-positive cells (Fig. 3). The application of an electromagnetic radiation inducing magnetic fluid hyperthermia showed a high selectivity towards the suppression of these type of cells that internalized the tubes. Nevertheless, the killing efficiency was modest (~25%), but it could be enhanced by improving the content and distribution of Fe inside the MWCNTs.

In an alternative approach, a nickel micropillar device coated with graphite oxide/magnetite NPs functionalized with anti-epithelial cell adhesion molecule (EpCAM) antibody has been designed for magneto-controllable capture and release of cancer cells [62]. The EpCAM antigen is overexpressed on the membrane of most tumors. The micropillars were aligned in a microfluidic chip by the application of an external magnetic field. The high density packing of antibody-modified magnetite NPs/graphite oxide on the micropillars increases the local concentration of antibody under an external magnetic field. Colorectal carcinoma HCT116 cells were captured at a rate above 70% in culture medium and superior to 40% in blood sample. The cells were then released from the micropillars upon the removal of the applied magnetic field with relatively high viability, which would allow subsequent biological analysis. This magnetic micropillar device could be exploited for the detection of low-abundant cells, which is very promising for the development of novel techniques of cell-based diagnosis.

The isolation and analysis of rare cells are currently highly needed in various fields, for instance in cancer therapy for the capture of extremely rare circulating tumor cells (CTCs) from blood samples [63]. CTCs are potential biomarkers for the development of cancer metastasis, but their detection is very challenging due to large blood volumes necessary for *ex vivo* microchip methods and time-consuming *in vivo* diagnosis. In addition, blood contains billions of red blood cells. Therefore, the efficiency and specificity of traditional technologies are rather low and the development of new strategies for capture and release of targeted cells is required, such as microfluidic techniques [64]. Nevertheless, the non-invasive release of captured cancer cells for subsequent analysis is still a bottleneck due to strong affinity between cells and surfaces. Approaches exploiting MNPs can overcome this issue by manipulating cells upon the application of a local external magnetic field [65,66]. Indeed, the combination of MNPs and gold-plated SWCNTs (named golden CNTs) [67] has been proposed for the capture of CTCs in the bloodstream of mice upon exposure to a magnet and subsequent photoacoustic imaging [68,69]. The MNPs with Fe_2O_3 core were functionalized with a fragment of the urokinase plasminogen activator that targets specifically the urokinase plasminogen activator receptors, highly expressed on several cancer cells but found only at low levels in healthy cells. The MNPs also served as photoacoustic contrast agent thanks to the intrinsic absorption of the maghemite core. In addition, the golden CNTs conjugated with FA were used as a second contrast agent for photoacoustic imaging and for duplex molecular targeting of CTCs. The use of dual magnetic-photoacoustic flow cytometry technology allowed the detection of CTCs in the bloodstream in real time *in vivo*. Even if the capture of all the CTCs *in vivo* is very difficult to achieve, this strategy allows early cancer diagnosis and opens many opportunities. Indeed, the captured CTCs could be extracted by microsurgery to perform biological investigation. They could be then ablated by laser or bypassed to prevent metastasis.

GO quantum dots (GOQDs) coated with magnetite NPs have also been used for the capture of CTCs [70]. In this study, the magnetic GOQDs were PEGylated and an anti-Glypican-3 (GPC3)-antibody was conjugated to the amine-functionalized PEG. The multifunctional GOQDs were used for separation of GPC3-expressed Hep G2 hepatocellular carcinoma tumor cells and enrichment from an infected blood sample. The capture efficiency was 91% from infected blood containing 10 tumor cells/mL of blood in a 15 mL sample, which was clinically-relevant. The removal of the cancer cells was monitored by two-photon luminescence. Because of strong quantum confinement and edge effects, the GOQDs possess a very high two-photon absorption cross section, which is ~3 orders of magnitude higher than organic dyes.

In addition to capture and separation of CTCs, the magnetic CNMs have been used for the removal and *in vivo* magnetic enrichment of circulating bacteria cells (CBCs) from infected blood [71]. Bacteremia, which is one of the most severe types of

Table 2
Description of the different approaches developed for the capture and separation of cells using magnetic CNMs, and for molecular delivery and extraction and cellular probing using magnetic CNT-based devices.

Type of CNM	Type of NP	Functionalization	*In vitro* model	*In vivo* model	Applications	Ref
MWCNTs	Fe	Cetuximab antibody	A431 cells	/	Selective removal of cells	[61]
Graphite oxide	Fe_3O_4	EpCAM antibody	Colorectal cancer cell line HCT116	/	Capture and release of cancer cells	[62]
Golden nanotubes	Fe_2O_3	FA, fragment of the urokinase plasminogen activator	Breast cancer cells MDA-MB-231	Human breast cancer xenografted mice	Photoacoustic detection and capture of CTCs	[68]
GO quantum dots	Fe_3O_4	Anti-GPC3 antibody	Hep G2 hepatocellular carcinoma tumor cells	/	Capture of CTCs	[70]
Golden CNTs	Fe_3O_4 and gold nanorods	Anti-*S. aureus* protein A and anti-lipoprotein antibodies	*S. aureus* strain UAMS-1	Bacteremia model mice	Photoacoustic detection and photothermal eradication of CBCs	[71]
GO	Iron core-gold shell NPs	Anti-GD2 antibody	Malignant melanoma cell line UACC903	/	SERS detection and separation of malignant melanoma cells	[74]
CNTs	Fe_3O_4	T-cell growth factor IL-2	$CD8^+$ T-cells	Mice inoculated with melanoma cells expressing the ovalbumin antigen (B16-OVA)	T-cell therapy (cancer immunotherapy)	[75]
CNTs	Ni	p-DNA	Neurons, splenic B cells, and B lymphocytes	/	Molecular delivery into cells	[79]
MWCNTs	Ni (layer)	/	Human embryonic kidney cancer HEK293 cells	/	Molecular extraction in single live cells	[80]
MWCNTs	Fe_3O_4 and gold	Fluorescent liquid or polymer NPs	HeLa and human osteosarcoma cells	/	Intracellular probing and delivery	[82]

infections, can lead to sepsis. The worldwide mortality rate associated to sepsis is comparable with that from cardiovascular diseases. The development of new antimicrobial agents is time-consuming and expensive; it is also stopped to be a priority for pharmaceutical industries, although this is coming more and more relevant as the life expectancy of population is continuously increasing. The physical destruction of bacterial pathogens is an alternative therapeutic strategy currently explored by different research groups [72,73]. Spectrally tunable golden CNTs and gold nanorods, as well as silica-coated magnetite NPs have been used for *in vivo* photoacoustic detection and photothermal eradication, respectively, of *Staphylococcus aureus* in tissue and blood using NIR light. To impart specificity, the different NPs were functionalized with antibodies specific for *S. aureus* protein A and/or lipoprotein that are both highly expressed in *S. aureus* and absent in mammalian cells, for the purpose of molecular targeting of this bacterial strain. This strategy could find potential applications for fast

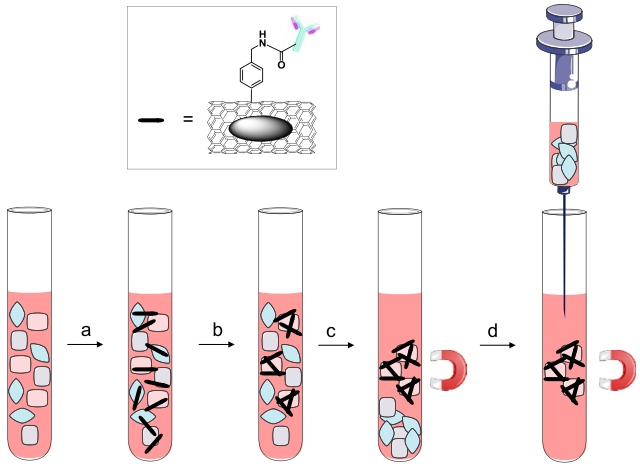

Fig. 3. Magnetic sorting of targeted cells: a) addition of iron filled CNTs functionalized on their sidewall with an antibody, b) antibody-antigen binding between the surface receptors of targeted cells and CNTs, c) magnetic attraction using a permanent magnet, d) removal of the non-targeted cells by aspiration. The different components in the figure are not on scale. The figure was adapted from Ref. [61].

and non-invasive diagnosis of skin infections and disinfection of surgical instruments without the need of antimicrobial agents. As perspectives, an extracorporeal bypass could potentially be developed for humans. Indeed, the injection of MNPs at the entrance of the bypass, followed by the magnetic removal of both CBCs and MNPs before entering the systematic circulation, could be used for purging of infected blood during sepsis.

Alternatively, the development of highly sensitive label-free assays is also a great challenge for early stage detection of CTCs as they are present at extremely low abundance (1–10 cells in 10^5 to 10^6 peripheral blood mononuclear cells). In this context, multifunctional plasmonic-magnetic GO has been designed for the ultra-sensitive and label-free detection of malignant melanoma UACC903 cells in blood [74]. The surface of GO was decorated with iron core-gold shell NPs functionalized with anti-GD2 antibody. The tumor-associated ganglioside GD2 is expressed in most melanomas. The gold shell allowed SERS plasmon enhancement. This plasmonic-magnetic GO-based SERS assay was highly sensitive for the detection of UACC903 cells as low as 10 cells per mL. The malignant melanoma cells were separated from the whole blood by using a bar magnet.

Finally, a CNT-polymer composite has been designed and used as artificial antigen-presenting cell substrate for expanding T-cells [75]. The stimulation of T-cells against tumor targets is one of the new strategies used in cancer immunotherapy. But, efficient methods for *ex vivo* expansion and infusion of tumor-specific T-cells from the blood of patients are lacking, which limits the development of cell-transfer therapy. Nonetheless, the design of devices for T-cell expansion and enrichment could overcome this issue. It has been demonstrated that CNTs are promising scaffolds due to their high specific surface area and physico-chemical properties, leading to enhanced cell–cell interactions and long-term cell cultures [76–78]. It has been demonstrated that the surface topology of CNTs could mediate a clustered presentation of T-cell stimuli, peptide-loaded major histocompatibility complex class-I, and the co-stimulatory ligand anti-CD28 [75]. For this purpose, magnetite and the T-cell growth factor interleukin-2 (IL-2) were encapsulated in poly(lactide-co-glycolide) NPs. The number of T-cells obtained was enhanced to a level using a thousand-fold less soluble IL-2 under conventional culture conditions. The

expanded T-cells were then magnetically separated from the CNT-polymer composite thanks to the presence of the co-encapsulated MNPs, which allowed easy isolation of activated T-cells for adoptive transfer. After these steps, the isolated T-cells were injected peritumorally into a murine model for melanoma, resulting in a significant tumor growth delay. Thus, this CNT-polymer composite containing MNPs results as a suitable platform to generate a high number of cytotoxic T-cells for cancer immunotherapy with the help of magnetic force.

5. Magnetic carbon nanotube-based devices for molecular delivery or extraction, and cellular probing

Vertically aligned CNTs with ferromagnetic catalyst nickel particles embedded in their tips have been used to transfect genetic materials into cells [79]. DNA plasmids (pDNA) encoding for enhanced green fluorescent protein (EGFP) sequence were immobilized on the nanotube surface. The CNTs penetrated the cell membranes driven by a magnetic field. This nanotube spearing effect allowed efficient delivery of pDNA in hard-to-transfect cells such as B cells and primary neurons with high viability. The transduction efficiency was equivalent to that of viral approaches. On the contrary, the expression of EGFP was not detected when Lipofectamine 2000 was used as control carrier. The technique is less invasive compared to other techniques, such as electroporation or electric field-mediated gene delivery. Other macromolecules like siRNA, proteins, or peptides could also be delivered into mammalian cells. This nanotube spearing approach could be used in the case of transfection issues using non-viral techniques, in particular *in vivo* for gene therapy, tissue engineering and drug delivery.

In contrast, magnetic MWCNTs have been used for extraction of biomolecules from living cells without affecting their viability and proliferation [80]. CNTs has the capacity to cross the plasma membrane through passive diffusion, like a nanoneedle [3,81]. Straight-aligned CNTs coated with a nickel layer were functionalized with an electropolymerized tyrosine-based hydrophilic film. The magnetic CNTs were sneaked into and out of the cells under a magnetic force. While passing through the cytoplasm, they adsorbed green fluorescent protein molecules on their surface. The main methods for extraction of molecules from living cells usually require cell lysis to release intracellular molecules, which does not allow repetitive sampling at successive time points. However, the advantage of this strategy relies on non-invasive molecular sampling, opening opportunities to investigate cellular processes at the single-cell level using magnetic nanomaterials.

Finally, a CNT-based cellular endoscope has been designed to probe the intracellular environment and transport fluids inside cells without inducing any damage [82]. The endoscope was made of a conventional glass micropipette mounted at the tip with a MWCNT filled with magnetite NPs (Fig. 4). Gold NPs were attached at the nanotube tip to enable intracellular measurements by SERS. The application of a magnetic field allowed to remotely move the endoscope to precise locations, for transferring fluorescent liquid or polymer NPs, and for simultaneous optical, fluorescence, and electrochemical diagnostics at the single organelle level. The mitochondria membrane polarization and the frequency and level of Ca^{2+} influx were monitored. In addition, Ca^{2+}-containing cytosol was extracted from the cell by aspiration into the endoscope after membrane penetration, without any disturbance to the cell metabolism. The minimally invasive intracellular probing offered by this CNT-based endoscope represents a major breakthrough in cellular endoscopy, with potential applications to study the functions of single organelles, and also in drug delivery. The main advantage of this novel type of endoscope is the presence of CNTs that endow the endoscope with electrical conductivity, mechanical strength, and cylindrically shaped tips. Overall, these few examples illustrate the potential of magnetic CNT for the design of devices like endoscopes for molecular delivery or extraction, and cellular probing.

6. Perspectives

Carbon-based nanomaterials are becoming important elements for the innovation in the field of nanobiotechnology and nanomedicine. The recent advances have been possible thanks to the development of multifunctional biocompatible systems. As illustrated by the examples described in the above sections, intense efforts have been devoted to the design of hybrids between CNMs and NPs endowed of magnetic properties. The combination of these two types of nanomaterials is opening the doors to the new multimodal systems and strategies in therapy, imaging and diagnosis of different diseases, and in particular cancer. CNMs and MNPs can be integrated all-in-one becoming innovative tools for magnetic targeting, magnetic manipulation, capture and separation of cells and development of magnetic CNM-based devices. Magnetic manipulation and control of the hybrid systems loaded with therapeutic molecules and guided to the site of action under a magnetic field allow to improve the therapeutic efficacy of a treatment and exploit their multimodal characteristics. For a safe translation of such hybrids, still in a nascent stage of development, into clinical relevant tools, biocompatibility and biodegradability

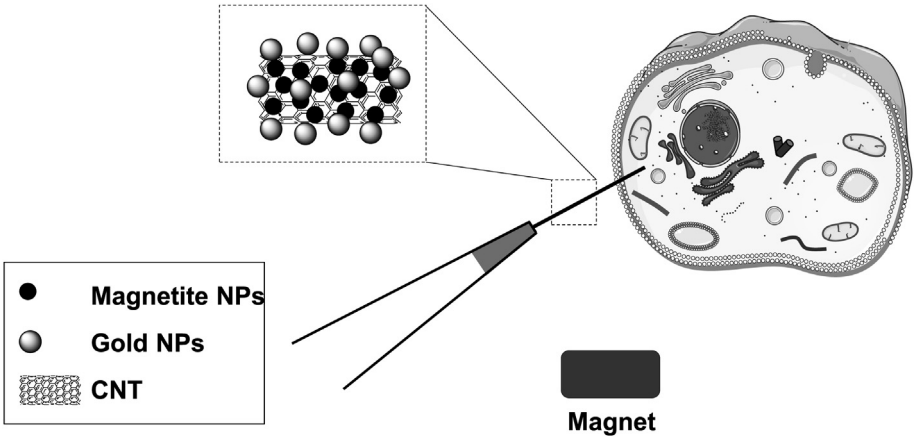

Fig. 4. Diagnosis at the single cell level: insertion of a glass micropipette mounted at the tip with a MWCNT filled with magnetite NPs and decorated on the surface with gold NPs to enable intracellular measurements by SERS upon the application of a magnetic field. The different components in the figure are not on scale.

of the nanomaterials need to be carefully evaluated. CNTs in particular have raised concerns about their toxicity. However, appropriate functionalization strategies that limit their pathogenic risks have been proposed [83–85]. The choice of the chemistry approaches and the control of the functional groups are fundamental to render the nanotubes biocompatible and biodegradable [83]. Other types of CNMs like graphene related materials look safer and much less biopersistant [86,87]. However, the determination of the advantages and benefits over the risks posed by CNMs are fundamental aspects that have to be taken into consideration in the development of new multifunctional systems. The examples reported in this minireview are certainly interesting although they remain limited to testing at the cellular level. Few preclinical animal models have been explored to assess their efficacy and the results are encouraging. Finally, the complexity of such systems needs to be carefully considered as the design and control of these hybrids become more and more challenging as the degree of their complexity increases.

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