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REVIEW ARTICLE

Application of carbon nanotubes in neurology: clinical perspectives and toxicological risks

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Abstract Nanomedicine is an emerging field that proposes the application of precisely engineered nanomaterials for the prevention, diagnosis and therapy of certain diseases, including neurological pathologies. Carbon nanotubes (CNT) are a new class of nanomaterials, which have been shown to be promising in different areas of nanomedicine. In this review, the application of CNT interfacing with the central nervous system (CNS) will be described, and representative examples of neuroprosthetic devices, such as neuronal implants and electrodes will be discussed. Furthermore, the possible application of CNT-based materials as regenerative matrices of neuronal tissue and as delivery systems for the therapy of CNS will be presented.

Keywords Carbon nanotubes · Neurology · CNS neurotoxicity · Therapy · Neuroregeneration

Abbreviations

CNT	Carbon nanotubes
SWNT	Single-walled nanotubes
MWNT	Multi-walled nanotubes
pCNT	Pristine carbon nanotubes
ssDNA	Single-stranded DNA
CNS	Central nervous system
BBB	Blood–brain barrier
PEI	Polyethyleneimine
NGF	Nerve growth factor
BDNF	Brain-derived neurotrophic factor
PABS	Poly-m-aminobenzene sulphonic acid
PEG	Polyethylene glycol
Ca_2^+	Calcium
K^+	Potassium
ROS	Reactive oxygen species
PS-80	Polyoxyethylene sorbitan monooleate
SDS	Sodium dodecyl sulphate
SDBS	Sodium dodecylbenzene sulphonate
SC	Sodium cholate
LBL	Layer by layer
PDDC	Poly(diallyldimethylammonium chloride)
PAA	Poly(acrylic acid)
NSCs	Neural stem cells
SCI	Spinal cord injury
Ach	Acetylcholine

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Introduction

Nanomedicine is an emerging field that proposes the application of precisely engineered nanomaterials for the prevention, diagnosis and therapy of certain diseases, including neurological pathologies. Carbon nanotubes (CNT) are a new class of nanomaterials, which have been shown to be promising in different areas of nanomedicine (Bianco et al. 2005; Lacerda et al. 2006). The CNT

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structural backbone is exclusively composed of carbon atoms and exhibits exceptional properties, such as high electronic and thermal conductivity as well as great strength. Two main types of CNT have been explored in biomedicine: (1) single-walled nanotubes (SWNT), consisting of a single sheet of carbon benzene rings rolled up into a tubular structure; and (2) multi-walled nanotubes (MWNT) that consist of multiple concentric layers of carbon sheets. Their diameter varies from 0.4 to 2 nm for SWNT and 1.4 to 100 nm for MWNT, while their length may reach up to a few micrometres in both types of nanotubes (Fig. 1).

Even though as-produced CNT (pristine carbon nanotubes) are insoluble in most aqueous solvents, the development of functionalization chemistries of the nanotube surface led to a notable enhancement in aqueous dispersibility that has allowed their application in physiological environments including the central nervous system (CNS) (Georgakilas et al. 2002). Two main strategies have been described to enable the application of CNT under physiological conditions, namely non-covalent and covalent functionalization (Tasis et al. 2006). Non-covalent functionalization involves the coating of nanotubes with hydrophilic macromolecules and the introduction of repulsive forces (Shvartzman-Cohen et al. 2004). This has been achieved by coating or wrapping the CNT with surfactants (Moore et al. 2003), polymers (Shvartzman-Cohen et al. 2004), peptides (Dieckmann et al. 2003) or single-stranded (ss)DNA (Zheng et al. 2003). This approach allows the preservation of the aromatic structure of CNT without deleterious effects on their electronic characteristics. Covalent functionalization is the alternative chemical modification strategy of CNT surfaces via organic reactions (Georgakilas et al. 2002; Tasis et al. 2006). Charged groups may be attached onto the CNT backbone, creating electrostatic repulsive forces between the individualized tubes. Two main approaches have been described to achieve covalent functionalization of CNT, namely (1) sidewall covalent conjugation of functional groups and (2) oxidation of CNT and further functionalization. The sidewall covalent functionalization of organic functional groups may be achieved by a variety of chemical reactions, in particular by 1,3-dipolar cycloaddition of azomethine ylides. Firstly reported by Georgakilas et al. (2002), in this strategy amino functional groups are attached to the tips and sidewalls of CNT, and thus, a highly soluble material in aqueous solvent is obtained. Alternatively, oxidation leads to purified and shorter CNT. The carboxylic acid groups might be attached to the CNT backbone, which can be further derivatized into other types of functional groups (for example by esterification or amidation). In the last few years, the significant improvements in the dispersibility of nanotube dispersions have broadened dramatically the scope of their biological applications (Kostarelos et al. 2009), ranging from therapeutics and diagnostics for oncology (Podesta et al. 2009; Pantarotto et al. 2004; Bhirde et al. 2009) to neuroprosthetic devices (Keefer et al. 2008; Gabay et al. 2005; Lovat et al. 2005; Cellot et al. 2009).

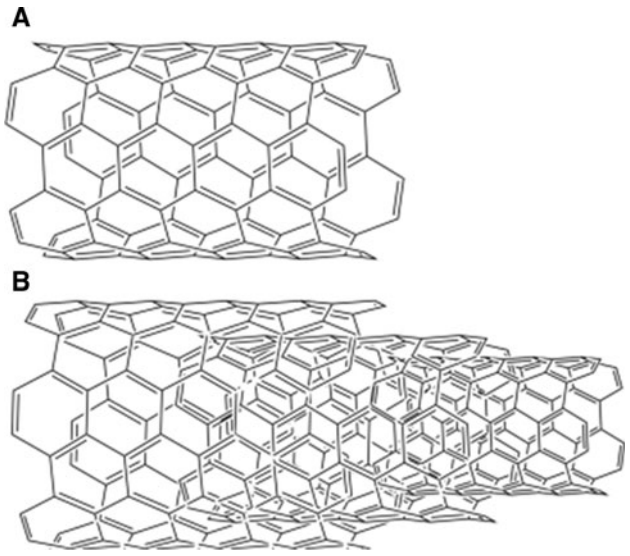

Fig. 1 The main types of carbon nanotubes used in biology. Schematic representation of: **a** single-walled nanotube (SWNT); and **b** multi-walled nanotube (MWNT)

Application of carbon nanotubes in neurology

Neurological diseases include an extended range of disorders that affect a large percentage of the world's population. Advances in nanomedicine are expected to have a major impact in neurological research, contributing to our further understanding of the CNS and the development of novel therapeutic strategies for neurological intervention (Gilmore et al. 2008; Modi et al. 2009). The use of carbon-based nanostructures, such as CNT, is one of the most attractive approaches for neurological applications. During the last decade, CNT have shown evidence of their electrical conductive capacity, strong mechanical properties and morphological similarity to neurites (Zhang and Webster 2009). Moreover, CNT structural features and dimensions are similar to many elements of the neural machinery (ion channels, signalling proteins and elements of the neuronal cytoskeleton). These characteristics may constitute an additional advantage by enhancing interactions at the molecular level and consequently better control over physiological activity and neuronal information processing (Kotov et al. 2009b; Cellot et al. 2009; Lovat et al. 2005; Mazzatenta et al. 2007). Table 1 summarizes the main rationale behind the use of CNT interacting with neuronal tissue and why that may be attractive for neurological research.

Table 1 Why use carbon nanotubes in contact with neuronal tissue?

Monitor electrophysiological activity of neural cells. Electrical properties of CNT can be tailored to match the charge transport characteristics of neuro-electrical interfacing;
Electrical stimulation and circuitry involved in the treatment of a variety of neurological pathologies (e.g. DBS);
Refractive nature of neurons to vector systems for chemical, genetic or other interventions;
Serious challenges with other vectors in overcoming the blood–brain barrier (BBB);
Limited therapeutic interventions for most neurological conditions;
Suitability of the mechanical and chemical properties of CNT for long-term implantation within neuronal tissue;
Biocompatibility and biodegradability of CNT within neural tissue

In this review, the application of CNT interfacing with the neuronal tissue and the CNS environment will be described, and representative examples of neuroprosthetic devices, such as neuronal implants and electrodes will be provided. Furthermore, the possible application of CNT-based materials as regenerative matrices of neuronal tissue and as delivery systems for the therapy of CNS will be presented. Figure 2 summarizes the areas in neurology where CNT have found some application, indicating the type of pre-clinical development (in vitro or in vivo) that has already appeared in the literature.

Carbon nanotube-neuron interface: interaction of CNT with neuronal electrical signalling

Previous studies using different in vitro models of neuronal tissue have explored the potential of CNT as substrates for neuronal growth, utilizing their capacity to integrate with neurons and enhance neuronal functions, as well as promote or facilitate re-establishment of connections among neurons (Matsumoto et al. 2007; Hui Hu et al. 2004; Lovat et al. 2005; Hui Hu et al. 2005; Mattson et al. 2000). Mattson et al. (2000) described for the first time how rat hippocampal neurons could grow on MWNT substrates (MWNT layer coated with the bioactive molecule 4-hydroxynonenal). These findings illustrated the compatibility of CNT as a substrate for nerve cell growth. Moreover, chemical modification of the CNT surface has been shown to affect neurite growth patterns and characteristics such as length, branching and number of growth cones. For example, neurons cultured on a positively charged multi-walled nanotube substrate (MWNT coated with polyethylenediamine) showed a large number of growth cones and neurite branches (Hui Hu et al. 2004). Similarly, SWNT chemically functionalized with cationic polymer polyethyleneimine (PEI) have been used as a substrate for neuronal cultures and have been shown to promote neurite outgrowth and branching, albeit to a smaller extent when compared with PEI substrate alone (Hui Hu et al. 2005). Matsumoto et al. (2007) also suggested that MWNT coated with growth factors (such as nerve growth factor (NGF), or brain-derived neurotrophic factor (BDNF)) had the capability to stimulate neuronal growth on the nanotube scaffold, controlling the differentiation and the survival of neurons.

Several laboratories have studied the interface between CNT and neurons, suggesting that this interaction was strongly modulated by the purity and three-dimensional organization of the CNT substrate. Lovat et al. (2005) demonstrated that a purified MWNT substrate was able to promote dendrite elongation and cellular adhesion of hippocampal cultured neurons. Similarly, Mazzatenta et al.

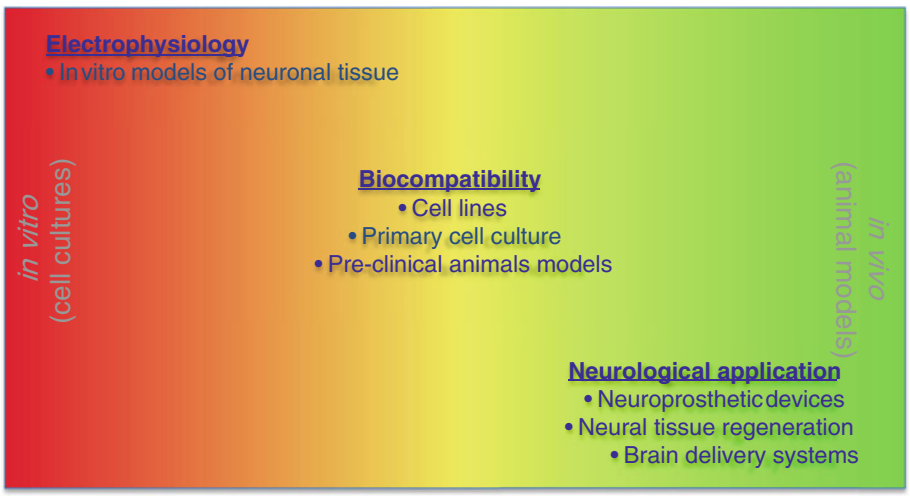

Fig. 2 Areas in neurology with reported applications utilizing carbon nanotubes

(2007) suggested that hippocampal neurons might grow and develop functional circuits when stimulated by a purified SWNT substrate. The growth of neuronal circuits observed was associated with the increase in the transmission of electrical signals within the neuronal network, due in part to the high electrical conductivity of CNT-based material that might affect neuronal information processing (Lovat et al. 2005; Cellot et al. 2009). Nonetheless, it remains unclear how neurons are able to reconstruct the functional network and rebuild active synapses when in contact with CNT substrates (Giugliano et al. 2008). In general, these studies propose the use of nanotubes as a platform compatible with the neural tissue able to maintain cell viability, while simultaneously promote the formation of neuronal circuits. In this way, offering possibilities for the engineering of novel neuroprosthetic devices in vivo.

Toxicity and biocompatibility of carbon nanotubes within the neuronal tissue

The success of the biological application of CNT in the CNS is closely dependent on their compatibility with that tissue. Even though the applications of CNT in neurology are at a very early stage, concerns regarding their short- and long-term neurotoxicity have emerged and will be addressed. The following sections consider some of the most relevant work (in vitro and in vivo) that has been described in the literature regarding the biocompatibility of CNT in the CNS, even though knowledge regarding the interaction and fate following cellular internalization of nanotubes within neuronal tissue remains scarce.

In vitro studies Ni et al. (2005) demonstrated for the first time that the incubation of hippocampal neuron culture with co-polymer (PABS and PEG) chemically functionalized SWNT did not affect the viability of the neurons. However, changes could occur on their morphology (enhanced neurite outgrowth and increased suppression of growth cones). The same group later reported that chemically functionalized SWNT with PEG (PEG–SWNT) could inhibit the cell depolarization–dependent influx of Ca^{2+}, a process that is known to regulate vesicle recycling of neurons and consequently change the rate of neurite elongation (Malarkey et al. 2008). This study also suggested that SWNT–PEG could preclude the membrane endocytosis of neurons, leading to the inhibition of depolarization-dependent Ca^{2+} channels, and consequently to a gradual decrease in the frequency of spontaneous post-synaptic currents. Other works have also reported modification in the activity of different ion channels, in particular inhibition on potassium (K^{+}) channels of PC12 cells after incubation with oxidized MWNT (Xu et al. 2009). These observations suggested that the electrophysiological properties of the neurons could be affected after passage of the electrical current through nanotube-based substrates. Such an effect was linked to the opening of ion channels, identical to traditional means of neuron excitation, where CNT-based substrates might promote an electrical coupling with neuronal cells (Gheith et al. 2006). Contrary to these results, Gaillard et al. (2009) demonstrated that chemically functionalized MWNT (amine functionalized by the 1,3-dipolar cycloaddition reaction on pre-oxidized MWNT) were highly biocompatible with neuronal cells. Amino-functionalized MWNT did not appear to alter the cell viability, neuronal morphology or normal function of primary neurons. Moreover, Gaillard and colleagues showed that the spontaneous activity of neurons remained normal after incubation with MWNT.

These contradictory results underline the importance for the detailed physicochemical characterization of the CNT material used, because such divergence might be attributed to the different types of CNT or functionalization. Another explanation could be due to the different conductivities of the CNT, a parameter that is not often properly characterized in the literature. For example, Malarkey et al. (2009) investigated how the conductivity of SWNT-based substrate could affect the neuronal growth and morphology. The results showed that only substrates in a narrow range of conductivity (<0.3 S/cm) were able to induce neuronal growth and neurite outgrowth, whereas with more highly conductive substrates this effect vanished.

Another concern that may contribute to the overall toxicity of the material is the presence of metal catalysts. Schrand et al. studied the biocompatibility of neuroblastoma cells after incubation with different carbon-based nanomaterials (including pristine MWNT and pristine SWNT). A high presence of impurities (catalyst) was deemed to have an impact on cell viability with an increase in reactive oxygen species (ROS) generation at higher concentrations of CNT (>50ug/ml) (Schrand et al. 2007). Nevertheless, one cannot exclude the lack of dispersibility from the interpretation of the result, as pristine nanotubes were dispersed in deionized water, which may have compromised the dispersibility and stability of the CNT when in contact with the cells. The critical role of the catalyst traces present in the CNT was highlighted in work performed by Jakubek et al. (2009) where it was demonstrated that yttrium traces released from SWNT (chemically functionalized with aryl-sulphonate) were responsible for the inhibition of neuronal Ca^{2+} ion channels. Recently, Vittorio et al. (2009) investigated the role of purity and surface oxidation of MWNT in the cytotoxicity of neuroblastoma (SHSY-5Y) cells. At short-term incubation (3 days), no signs of toxicity were observed; however, at long-term incubation (14 days), cytotoxicity was noted, which was attributed to the accumulation of impurities

(catalyst) within the cells as a consequence of better cellular uptake.

Another factor that may compromise the cytotoxicity of CNT is the dispersibility status and the type of surface functionalization of the material. Care needs to be taken in choosing the surfactant molecule used to improve the dispersibility of the nanotubes, as the possible toxic or lytic activity of the coating molecules could influence the overall cytotoxicity of the CNT. This was demonstrated by Dong and colleagues in a series of experiments which showed that whenever SWNT were dispersed in the presence of sodium dodecyl sulphate (SDS) or dodecylbenzene sulphonate (SDBS), a decrease in cell viability (human astrocytoma cells) was noted, whereas after dispersion with sodium cholate (SC) or ssDNA, cell proliferation was not affected (Dong et al. 2008, 2009). Similarly, Kateb et al. (2007) after the incubation of microglia (BV2) and glioma (GL261) cells with pristine MWNT coated with Pluronic F-108, found no cytotoxicity. However, those results also indicated transient changes in inflammatory cytokine profiles in both cell types (in particular IL-10, IL-1β and TNF-α), which were attributed to the presence of the F-108 molecules used to disperse the material. The same group, using SWNT chemically functionalized with PEG_{2000}, showed that this construct was able to enhance cellular uptake of CpG oligodeoxynucleotides without inducing additional toxicity in glioma cells (Zhao et al. 2011). Furthermore, Bardi et al. (2009) found that lower concentrations of Pluronic F-127, used for the coating of MWNT, could lead to the induction of apoptosis of cortical neurons, and more importantly, that apoptosis was significantly reduced in the presence of MWNT.

Noticeably, the safe use of CNT in contact with neuronal tissue is seen as closely dependent on their aqueous dispersibility (Kostarelos 2008, 2009; Smart et al. 2006). For example, Belyanskaya et al. (2009) incubated neuronal and glial primary cultures with a range of polyoxyethylene sorbitan monooleate (PS80)-coated SWNT possessing different dispersibilities. Interestingly, although none of the CNT caused neurite outgrowth, there was an impact on cell viability, closely dependent on the degree of dispersibility and concentration of the CNT solutions. Moreover, it has been demonstrated by others that unlike pristine or low functionalized MWNT, highly functionalized MWNT do not cause loss of cell viability in human astrocyte D384 cells (Coccini et al. 2010).

In vivo studies Understanding the prevailing interactions between CNT and neural cells in vivo is of major importance, especially in view of their potential use in neurology. However, so far, only a few studies have been reported regarding the biocompatibility of CNT within the brain tissue in vivo. VanHandal et al. (2009) investigated the uptake and toxicity of pristine MWNT coated with Pluronic F-108 in a GL261 intracranial tumour model. Their results showed a preferential accumulation of MWNT in tumour macrophages in a dose-dependent manner. The direct intratumoural injection of MWNT was well tolerated, eliciting only transient and self-limiting local inflammatory response. The same group later showed similar results, and this time, however, using SWNT chemically functionalized with PEG_{2000} to deliver CpG oligodeoxynucleotides (Zhao et al. 2011). In this study, SWNT were shown to enhance CpG uptake by tumour-associated inflammatory cells and glioma cells (to a lesser extent) without major signs of toxicity. The only in vivo study to date regarding the biocompatibility of CNT within the brain environment using healthy animals was carried out by Bardi et al. (2009). Pluronic F-127-coated MWNT were injected into the visual cortex of a mouse, with no observed adverse toxicological effects at the cellular level.

The majority of studies investigating the toxicity of CNT after interaction with neuronal tissue reported today can be divided into two groups, based on the in vitro or in vivo models they used. These studies are summarized in Table 2 where details regarding the type of CNT, the strategy of functionalization, the cell lines and animal models employed are described.

To summarize, it is difficult to make a direct comparison between these studies because different types of CNT, dose regimes, cell/animal models and duration of interaction were selected by the different groups. However, a unifying factor is that most of the studies using chemically functionalized CNT—a material that is generally associated with improved aqueous dispersibility—have presented promising results. Further understanding of the possible effects of CNT interacting with specific cell types, such as neural cells, will be crucial for the development of nanotube-based brain delivery vectors and novel neural engineering platforms.

Application of carbon nanotubes against CNS pathologies

Carbon nanotubes as neuroprosthetic devices

Conventionally, brain stimulation is performed using metal electrodes that are inserted deep into the brain. This approach, which has been used for the treatment of various neurological pathologies, such as control of motor movement disorders, utilizes electrodes to deliver a current in order to stimulate the neurons (Nicolelis and Lebedev 2006). However, electrical stimulation of the brain has limitations, in particular the activation of excessively large areas, the formation of fibrosis and the creation of heat inside the brain (Cho et al. 2010; Reichert et al. 2005). It is

Table 2 Neurotoxicity studies following the interaction of CNT with neuronal tissue in vitro and in vivo

Type of CNT	Type of functionalization	Functional group or dispersion agent	Cell type or animal model	Aim and main results	References
SWNT	Coated	Polyoxyethylene sorbitan monooleate (PS80)	Primary mixed neuron-glia culture obtained from spinal cord and dorsal root ganglia	Investigation of the influence of the degree of purity and state of aggregation on the cytotoxicity of MWNT Aggregation state of the CNT influenced the cell viability Glia cell population more affected by CNT treatment than neuronal cell population Within neurons, peripheral nervous system cells more reactive to CNT than central nervous systems cells	(Belyanskaya et al. 2009)
	Coated	Docecykl Sulphate (SDS), Sodium Docecylbenzene Sulphonate (SDBS), Sodium Cholate (SC) or ssDNA	*1321n1* Human astrocytoma cells	Investigation of the cytotoxicity of CNT dispersed in different surfactants Cytotoxicity dependent on surfactant selection SDS and SDBS led to toxicity SC and dsDNA did not cause toxicity	(Dong et al. 2008, 2009)
	Coated	Phospholipid-polyethylene glycol ($PL\text{-}PEG_{2000}$)	Murine glioma (GL261.gfp and GL261.luc)	Evaluation of the Internalization and cytotoxicity of glioma cells after incubation with PEG–SWNT carrying CpG oligodeoxynucleotides CNT-enhanced CpG uptake CNT-mediated CpG delivery potentiates monocyte activation inflammatory cells	(Zhao et al. 2011)
	Chemical functionalization	Copolymer: Poly-*m*-aminobenzene sulphonic acid (PABS) and polyethylene glycol (PEG)	Hippopampal neuronal culture	First study using CNT for the treatment of dissociated neuronal cultures Maintenance of neuronal viability Induction of morphological modifications in the neurons: Enhance neurite outgrowth and increased suppression of growth cones SWNT may affect Ca_2^+ dynamic in neurons, by the reduction in the depolarization-dependent influx of Ca_2^+	(Ni et al. 2005)
	Chemical functionalization (oxidation and PEGylation)	Polyethylene glycol (PEG_{600}) Dispersed in water	Hippocampal neuronal culture	Attempt to determine the mechanism behind the effects of SWNT-PEG on enhancement of neurite outgrowth CNT inhibited the membrane endocytosis of neurons, by inhibition of depolarization-dependent Ca_2^+ influx	(Malarkey et al. 2008)
	Chemical functionalization (oxidation and PEGylation)	Polyethylene glycol (PEG_{600}) Dispersed in water	Hippocampal neuronal culture	Investigation of how conductivity of the substrate affects the neuronal growth and morphology of the neurons Induction of neuronal growth only in substrates with a narrow range of conductivity (<0.3 S/cm)	(Malarkey et al. 2009)
SWNT	Chemical functionalization (aryl-sulphonate functionalization)	$–SO3^-$ Dispersed in electrophysiological solution containing CsCl, EGTA, EDTA, HEPES and MgATP	Human embryonic kidney cells that expressed neuronal channels	Assessment of possible effects of CNT on voltage-gated calcium ion channels Inhibition of neuronal calcium ion channels due to the yttrium traces released from SWNT	(Jakubek et al. 2009)

Table 2 continued

Type of CNT	Type of functionalization	Functional group or dispersion agent	Cell type or animal model	Aim and main results	References
MWNT SWNT	Coated	Dispersed in water	Neuroblastoma cells (cell type not mention)	Comparison of biocompatibility between CNT, carbon black (CB) and nanodiamonds (ND) Lack of purity of CNT associated with the toxicity observed	(Schrand et al. 2007)
MWNT	Coated	Pluronic F-108	Murine microglia (CV2) and Murine glioma (GL261)	Investigation of the internalization and toxicological effect of MWNT by microglia cells Uptake by microglia cells without toxicity No changes in cell proliferation were observed in the presence of CNT Transient changes in cytokines production (attributed to Pluronic F108)	(Kateb et al. 2007)
	Coated	Pluronic F-127	Primary murine cortical neurons	Investigation of the toxicity of MWNT in vitro Presence of CNT showed to reduce toxicity associated with Pluronic F-127	(Bardi et al. 2009)
	Coated and Chemical functionalization (oxidation)	–COOH All CNT dispersed in Pluronic F-127	Human neuroblastoma (SH-SY5Y)	Investigation of the influence of the degree of purity and surface oxidation on the cytotoxicity of different MWNT Cytotoxicity was dose and purity degree dependent	(Vittorio et al. 2009)
	Coated and Chemical functionalization (oxidation and amidation??)	–COOH –NH_2 All CNT Dispersed in Dulbecco's modified Eagle's medium	Human astrocyte D384	Investigation of the influence of the degree of functionalization on the cytotoxicity of MWNT Cytotoxicity of MWNT was modified by chemical functionalization in association with improvements of dispersibility	(Coccini et al. 2010)
MWNT	Chemical functionalization (oxidation)	–COOH Dispersed in Dulbecco's modified Eagle's medium	Undifferentiated pheochromocytoma PC12 cells	Investigation of the effect of CNT on the potassium (K^+) channels CNT suppress the K^+ channels activity Suppression of potassium K^+ channels not associated with an induction of oxidative stress	(Xu et al. 2009)
	Chemical functionalization (oxidation and 1,3 dipolar cycloaddition reaction)	–COOH –NH_3^+ Cell-adhesion peptide	Hippocampal neuronal culture	Investigation of the effect of MWNT peptides on different cell types CNT shown to have high biocompatibility with different cell types Maintenance of normal neuronal morphology, cell viability and basic cellular functions	(Gaillard et al. 2009)
SWNT	Chemical functionalization	Phospholipid-polyethylene glycol ($PL\text{-}PEG_{2000}$)	Intracranial glioma model (GL261.gfp and GL261.luc) in C57BL/6 and $CX3CR1^{GFP}$mice	Evaluation of CNT as CpG delivery vehicle in brain tumour models CNT-enhanced CpG uptake by tumour-associated inflammatory cells CNT-CpG improves survival of glioma-bearing mice	(Zhao et al. 2011)

Table 2 continued

Type of CNT	Type of functionalization	Functional group or dispersion agent	Cell type or animal model	Aim and main results	References
MWNT	Coated	Pluronic F-108	Intracranial glioma model (GL261) in C57BL/6	Evaluation of the uptake and toxicity of MWNT in glioma intracranial model	(VanHandal et al. 2009)
				Preferential accumulation of MWNT into tumour macrophages and to lesser extent in microglia	
				CNT injection led to a transient and self-limiting local inflammatory response	
	Coated	Pluronic F-127	Healthy mice (strain not mention)	Investigation of the in vivo biocompatibility of MWNT	(Bardi et al. 2009)
				MWNT were biocompatible, and no damage at the cellular structural level was observed	

important to emphasize that the challenge in brain-electrode interface research lies both in the reduction in electrical resistance to alternating currents (impedance of the electrodes) during the recording of signals and in the increase in the delivery of electrical charge whenever neurons are being stimulated.

In the last few years, nanotechnology has opened up new directions of research in order to create innovative neuroprosthetic devices able to enhance neuron stimulation. To improve the performance of the current electrodes, various surface modifications have been made to render the electrodes more sensitive to electrical signals and more efficient in charge transfer (Jan et al. 2009). The high electrical conductivity and excellent mechanical properties of CNT make them a desirable material for neuroprosthetic devices. However, the success of their application is closely dependent on the control of their interaction with neurons, in particular of neural excitability and changes in ionic conductance and synaptic transmission (Sucapane et al. 2009). For example, Gheith et al. (2005) demonstrated the biocompatibility, growth and differentiation of neuronal NG-108-15 neuroblastoma/glioma hybrid cells after interface with layer-by-layer (LBL)-assembled SWNT films made from poly(diallyldimethylammonium chloride) (PDDA) and poly(acrylic acid) (PAA). Further, the same group associated the capability of SWNT-polyelectrolyte films to electrically stimulate neural cells with the opening of voltage-activated cation channels, observed by traditional means of neuron excitation, demonstrating that SWNT-polyelectrolyte films do in fact have an electrical coupling with neuronal cells. Liopo et al. (2006) have similarly suggested that the conductive properties of CNT might be used to stimulate neurons. After the application of electrical current through SWNT films, the inward transmembrane current in neurons was recorded by whole cell patch clamping, and the results indicated no differences over those induced by direct electrode-mediated patch clamp. These findings demonstrated that SWNT-polyelectrolyte films were mechanically compatible with neural tissues and could be used as implants or repair devices for neurological-related injuries. Moreover, Gabay et al. (2005) developed multi-electrode arrays (MEA) to both electrically stimulate and record neurons, based on regular arrays of hydrophobic carbon nanotube islands (100 μm) that were grown in hydrophilic conductive substrates (SiO_2 or quartz substrates) to form ideal surfaces for neuronal adhesion. After incubation, neurons and glial cells showed a tendency to aggregate and accumulate in the CNT-coated regions, whereas cell density on CNT-free regions was very low. The study showed that attachment to CNT surface did not alter the normal functionality of the neurons. Overall, the self-assembly mechanism of the neurons on CNT clusters will constitute a potential platform to study

the neuronal adhesion and outgrowth features of neuronal cells.

The templating of electrodes with CNT has led to remarkable improvements in the detection of electrophysiological signals following intracranial implantation (Keefer et al. 2008). Keefer and colleagues (Keefer et al. 2008) in a series of experiments investigated the use of MWNT-coated electrodes for the preparation of brain–device interfaces. These electrodes achieved greater noise reduction and higher sensitivity to spontaneous electrical neuronal activity in vivo using two different animal models (rodents and non-human primates). Importantly, the study demonstrated the effectiveness of electrodes coated with CNT and their capacity to improve the recording of neuronal electrical events in vivo.

Carbon nanotubes for neural tissue regeneration

Acute neurological incidents are devastating events that affect the full spectrum of human society. Over the last two decades, despite a large number of studies that have been conducted and the exciting discoveries that have been made, the treatments are still limited, and more research is needed to explore the underlying mechanism of acute neurological disorders. The loss of brain and spinal cord cells is known to be associated with a large number of CNS pathologies, such as Alzheimer's, Parkinson's, stroke, heat stress, brain trauma and spinal cord trauma. There are two main strategies to promote the self-repair of damaged axonal connections: re-growth of axons and/or re-organization of the neuronal circuit. Therefore, the conditions for the success of regeneration engineering are that firstly, the neurons must be preserved, in order to promote a permissive growth environment, and secondly, after re-connection, the plasticity of the tissue needs to be promoted (Ellis-Behnke 2007).

In this context, neural stem cells (NSCs) have the potential to be differentiated into functional cell types, including neurons, astrocytes or oligodendrocytes, and thus to enhance neural tissue recovery (Jan and Kotov 2007; Reynolds and Weiss 1992). However, the challenge is to evaluate the effectiveness of the delivery and differentiation into favourable neuronal cell types, which will then contribute to the regeneration of the desired tissue type. Several studies have already shown the ability of CNT-based substrates to mediate the differentiation and electrical stimulation of NSCs (Jan and Kotov 2007; Kotov et al. 2009a). The NSCs have been shown to be biocompatible with CNT-based substrate, with levels of cell viability and the development of neural processes similar to those observed with the conventionally used growth substratum poly-L-ornithine (Jan and Kotov 2007). The effectiveness of carbon nanotubes to deliver NSCs into CNS-injured sites, and to support their differentiation into neurons, constitutes an essential requirement for the success of regeneration of damaged neural tissues.

Neural tissue engineering aims at the development of novel and improved biological scaffolds that restore, maintain and/or improve neural tissue functions. Due to their electrical and mechanical properties, along with neuronal biocompatibility, CNT are considered possible candidates for neural tissue repair (Tran et al. 2009). The contribution of CNT to the treatment of traumatic CNS injuries has recently been recognized in vivo. Roman et al. (2011) demonstrated that SWNT chemically functionalized with PEG were effective in the promotion of axonal regeneration in a rat model of SCI (at T9 vertebral level). The reduction in the lesion volume and the increase in the number of neurofilament fibbers in and around the lesion site, along with the partial induction of functional recovery of rat hindlimb, suggested the effectiveness of this approach. This constitutes the first evidence to date that CNT-based substrates are able to promote the regeneration of damaged CNS tissues in vivo, opening up new perspectives in the field of neuro-regenerative medicine.

Carbon nanotubes for therapy of the CNS

Only a few studies so far have been published on the use of CNT for brain drug delivery; however, this is likely to increase in the coming years. The emergence of CNT as a delivery vector for the CNS is based on their structural advantages, in particular good dispersibility in physiological solvents, large surface area, capability of being easily functionalized with drugs or imaging agents and biocompatibility with neural tissue. The first use of CNT for the treatment of CNS diseases was reported by Zhang et al. (2010), who utilized short pristine SWNT physically adsorbed with acetylcholine (SWNT-ACh) in Alzheimer's disease brains. This study suggested that doses of SWNT under 300 mg/kg, after gastrogavage administration, could ensure safe delivery of ACh into lysosomes of neurons, thus rendering the therapy more effective without compromising the toxicological profile of the material.

Another possible therapeutic application of CNT in the CNS is in the treatment of glioblastoma tumours, which are known for their recurrence even after aggressive multimodality treatment. The treatment of brain tumours remains a challenge despite advances in tumour therapy and the increasing understanding of carcinogenesis. The low permeability of anti-tumour drugs across the BBB when administered systemically has opened up new possibilities for CNT-based modalities. For example, Zhao et al. (2011) have recently demonstrated that the CNT delivery system significantly enhanced CpG oligodeoxynucleotides immunotherapy, eradicating the glioma and

protecting against tumour rechallenge (GL261 and $GL261_{egfp}$ models).

CNT-mediated therapy is a valuable option for the treatment of neurodegenerative diseases, including the treatment of stroke. For example, amine-functionalized SWNT (by amidation reaction of pre-oxidized SWNT) have been shown to improve the tolerance of neurons to ischaemic injury in MCAO stroke model (Lee et al. 2011). Interestingly, in this study, intracerebroventricular injections of amino-functionalized SWNT without any therapeutic molecule have been shown to protect neurons and enhance motor function recovery of the animals. However, the mechanism for such activity remains elusive (Lee et al. 2011; Higgins et al. 2011). More recently, a study performed by Al-Jamal et al. (2011) has illustrated the effectiveness of amino-functionalized MWNT in mediating the delivery of siRNA (able to silence Caspase 3) reducing apoptosis in the affected area and promoting behavioural recovery in an endothelin-1 stroke rodent model. Unlike in the former work performed by Lee et al. (2011), the intraparenchymal injections of MWNT-NH_3^+ and in complex with scrambled siRNA did not affect apoptosis or functional recovery from stroke, suggesting little neuroprotective activity of CNT alone. However, a direct comparison between the studies is difficult to make owing to significant differences in the methodology, such as CNT types, dimensions, manufacturer, dosage, location of injection and animal model.

Conclusion and final remarks

The most significant and representative studies that illustrated how CNT can modulate the molecular and cellular functions of the CNS, as well as their impact on neurotoxicity both in vitro and in vivo, have been discussed. Remarkable advances have already been achieved in the fields of neuroprosthetic device engineering and neuro-regeneration. Although it may still be too early to assert the long-term impact of CNT in clinical applications against CNS diseases, recent studies on various disease models have shown that the material constitutes a contender in the race to find new and more efficacious tools to achieve early diagnosis and treatment of debilitating neuropathological conditions.

Conflict of interest The authors declare that they have no conflict of interest.

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