Journal of Controlled Release 161 (2012) 290–306

Contents lists available at SciVerse ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Review

Therapeutics, imaging and toxicity of nanomaterials in the central nervous system

Antonio Nunes, Khuloud T. Al-Jamal [1], Kostas Kostarelos *

Nanomedicine Laboratory, Centre for Drug Delivery Research, UCL School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom

ARTICLE INFO

Article history:
Received 6 February 2012
Accepted 28 March 2012
Available online 4 April 2012

Keywords:
Nanomedicine
Brain
Neurodegenerative
Nanotechnology
Imaging

ABSTRACT

Treatment and diagnosis of neurodegenerative diseases and other CNS disorders are nowadays considered some of the most challenging tasks in modern medicine. The development of effective strategies for the prevention, diagnosis and treatment of CNS pathologies require better understanding of neurological disorders that is still lacking. The use of nanomaterials is thought to contribute to our further understanding of the CNS and the development of novel therapeutic and diagnostic modalities for neurological interventions. Even though the application of nanoparticles in neuroscience is still embryonic, this article attempts to illustrate the use of different types of nanomaterials and the way in which they have been used in various CNS applications in an attempt to limit or reverse neuropathological processes.

Contents

Abbreviations: CNS, central nervous system; BBB, blood brain barrier; CMT, carrier mediated transcytosis; AMT, absorptive mediated transcytosis; RTM, receptor mediated transcytosis; Pgp, P-glycoprotein; SC, spinal cord; EEG, electroencephalography; MEG, magnetoencephalography; CT, computer tomography; PET, position emission tomography; fMRI, functional magnetic resonance imaging; MRI, magnetic resonance imaging; SPIOs, supermagnetic iron oxide; USPIOs, ultra-small supermagnetic iron oxide; VSPIOs, very small supermagnetic iron oxide nanoparticles; RES, reticular endothelial system; CFS, blood–cerebrospinal fluid; Qdots, quantum dots; PEG, poly(ethylene glycol); PLA, poly(lactic acid); WGA, wheat germ agglutinin; CNT, carbon nanotubes; MWNT, multi walled carbon nanotubes; Pax, paclitaxel; Dox, doxorubicin; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; PBCA, poly(buthylcyanoacrylate); GBM, glioblastoma multiforme; PDT, photo-dynamic therapy; PLGA, poly(lactic-co-glycolic acid); CED, convection-enhanced delivery; PAMAM, polyamidoamine; PAA, polyacrylamide; ROS, reactive oxygen species; SOD, superoxide dismutases; BMM, bone-marrow-derived macrophage; $MPTP^{+}$, 1-methyl-4-Phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA, 6-hyroxydopamine; NGF, nerve growth factor; siRNA, small interfering RNA; HMGB-1, High Mobility Group Box-1; MCAO, middle cerebral artery occlusion; *h*-GDNF, human glial cell line-derived neurotrophic factor gene; FC_4S, hexasulfobutylated fullerenes; SWNT, single walled carbon nanotubes; SCI, spinal cord injury; SAPNS, self-assembling peptide nanofiber scaffold; IKVAV, isoleucine-lysine-valine-alanine-valine; LPS, lipopolysaccharide.

* Corresponding author at: UCL School of Pharmacy, University College London, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom.
E-mail address: kostas.kostarelos@pharmacy.ac.uk (K. Kostarelos).
[1] Present address: Institute of Pharmaceutical Sciences, King's College London, UK.

0168-3659/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2012.03.026

1. Introduction

Neurological diseases include an extended range of disorders that affect a large percentage of the world's population and according to epidemiological studies are expected to increase with the ageing of the population. Alzheimer's and Parkinson's diseases among others such as autism, traumatic brain injury, stroke, and schizophrenia are only a few of a large number of pathologies that have benefited from advances in basic neurology research in the last few decades. Although the genetic basis for many of these central nervous system (CNS) disorders is known, therapeutic efficacy is limited mainly by the selectivity of the blood brain barrier (BBB).

The BBB is considered the most important barrier to protect the brain, constituting a rate-limiting factor in the transport of drugs and genes into the CNS [1]. The BBB interfaces the blood with the extracellular compartment of the brain parenchyma and is mainly formed by polarised brain capillary endothelial cells sealed by complex tight junctions that restrict the paracellular transport of molecules into the brain [2] (Fig. 1). Paracellular transport is prevented as a result of high metabolic activity, low vesicular transport and lack of fenestrae. Additionally, the BBB is closely associated with astrocytes that form a network surrounding the blood vessels, providing biochemical support to the endothelial cells. Neuronal endings are also present, close to the endothelial cells. Pericytes make a further contribution to the BBB efficiency with their phagocytic capacity [2]. Large molecules, including recombinant proteins, monoclonal antibodies and nucleic acids, are not able to readily cross the BBB. Among small molecules, only around 2% have the ability to cross the BBB, and high lipid solubility also seems to be favoured [1,3].

The BBB is a dynamic system that allows a limited diffusion of exogenous compounds into the brain with specialised transport mechanisms for essential nutrients. Absorption of molecules across the BBB occurs through two mechanisms: passive and active transport, as described in Fig. 2. Passive transport includes para-cellular diffusion of hydrophilic compounds, and trans-cellular transport which is used by small lipophilic molecules (less 400–600 Da) to enter into the brain parenchyma. Active transport systems include: i) carrier mediated transcytosis (CMT) for relatively small molecules; ii) absorptive mediated transcytosis (AMT) for positively charged peptides; and iii) receptor mediated transcytosis (RTM) for certain proteins [2].

CMT is predominant in the transport of nutrients and the rate depends on the occupancy rate of the carrier. AMT does not involve any plasma membrane receptors. This pathway is triggered by electrostatic interactions between polycationic molecules and the negatively-charged plasma membrane. On other hand, RMT includes binding to specific membrane receptors, which are then internalised by endocytosis and released at the abluminal membrane of the brain capillary endothelium. Efflux mechanisms are also present in the BBB. The best known is the P-glycoprotein (Pgp) that has a high propensity for pumping out unwanted compounds, such as cytotoxic anticancer drugs and antibiotics, resulting in restricted accumulation of such molecules within the brain [2]. Because of the limited transport across the BBB, research has been directed towards uptake enhancement strategies both to expand available therapeutic interventions and improve their efficacy with minimum toxicological side effects.

The permeation of nanomaterials across the BBB is mainly related to procedures that involve their intravenous administration and will have pharmacological and neurotoxicological implications. Some studies aim to intentionally enhance the brain uptake of delivery systems for diagnostic or therapeutic purposes [4]. In that context, nanoparticles have been widely investigated as novel delivery systems able to increase translocation through the BBB into the brain parenchyma. Polymeric nanoparticles [5–17], solid lipid nanoparticles [14,18,19], liposomes [20–22], and dendrimers [23] are types of nanoscale systems that

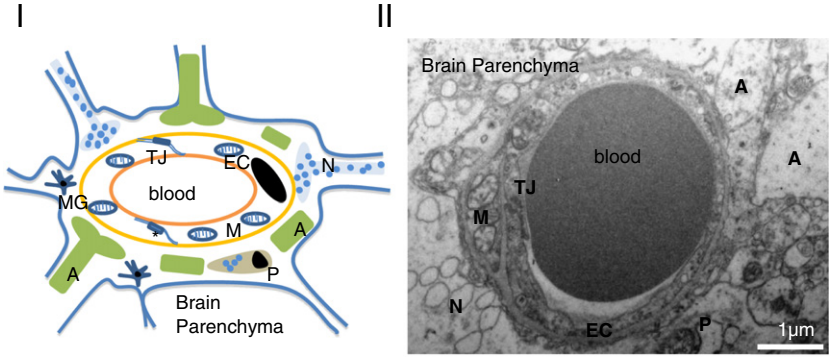

Fig. 1. Blood brain barrier structure. The image shows a diagram (I) and an electron micrograph (II) of the blood brain barrier (BBB). The main components of BBB are: EC—endothelial cells; A—astrocytes; MG—microglia; M—mitochondria; P—pericytes; N—neuron terminal/axon; and TJ—tight junctions. (Diagram (I) redrawn from [2]).

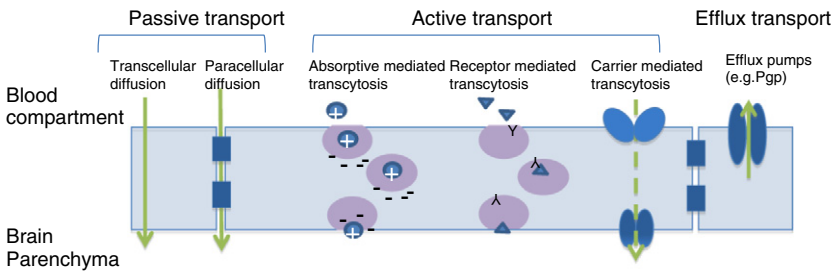

Fig. 2. Representation of specialised transport routes through the BBB (redrawn from [2]).

have been described in the literature for this purpose. Generally, targeting ligands have been conjugated to the nanoparticle surface in order to enhance BBB crossing. Despite all this work, there is still incomplete evidence that nanomaterials *per se* (*i.e.* without targeting ligands and without dissociation into their molecular constituents) can cross the BBB endothelium and reach the brain parenchyma [24,25]. Over the last decade, developments in imaging techniques have progressed rapidly, however the detection of intact nanomaterials inside the brain remains challenging. These difficulties are further corroborated with lack of sensitivity associated with the generally low concentration of nanomaterials in the brain [25], therefore identification of brain uptake usually relies upon indirect detection methods based on imaging probes, quantification of the transported drug molecules or behavioral assessment. Overall, elucidation of the kinetics of nanoparticle translocation across the BBB is imperative for our understanding on how nanomaterials behave *in vivo* in relation to the CNS. In the following sections even though BBB permeation will be mentioned, it will not be the sole focus of this review since it applies only to intravenous transport of nanomaterials. The scope of this article will be broader and attempt to discuss all previously reported imaging and therapeutic modalities using nanoparticles applied to the CNS pre-clinically and clinically.

2. Nanomaterials in the central nervous system

One of the major challenges for modern medicine is the development of effective strategies for the prevention, diagnosis and treatment of CNS pathologies. CNS tissue morphology along with the restricted anatomical access mentioned above, makes successful endpoints of diagnostic or therapeutic interventions challenging. Furthermore, the absence of an effective self-repair mechanism in the CNS results in irreversible functional problems that have a long-term impact on the patient's quality of life, as well as high medical and societal costs. Moreover, understanding of most neurological disorders is still lacking and this further precludes adequate diagnosis and treatment. The use of nanomaterials may contribute to our further understanding of the CNS and the development of novel therapeutic strategies for neurological interventions. According to Silva [26], their application in neuroscience is still at the early stages of development despite an impressive body of research that is emerging.

Nanomaterials have been used in neuroscience in an attempt to limit or reverse neuropathological processes. The advantages are due to their specific characteristics, such as the capacity to interact with biological systems with a high degree of specificity. Moreover, nanomaterials are thought to be able to stimulate and interact with target cells and tissues in controlled ways, inducing desired physiological responses with minimal side effects. The main neurological applications that nanotechnology research is contributing to are summarised in Table 1.

We will describe the majority of studies published today using nanomaterials and the neurotoxicological risks deriving from their application in the CNS. The use of nanomaterials as imaging agents will be discussed, pointing out their importance as precise diagnostic tools of the CNS. This will be followed by a description of the state-of-the-art application of nanomaterials in therapeutic modalities such as: neuro-oncology, neurodegenerative diseases and neuroprotection. Next, innovative strategies will be described employing nanomaterials for the treatment of traumatic CNS injuries and neural regeneration. Finally, studies concerning the neurotoxic profile of nanomaterials will be described and discussed. Fig. 3 schematically depicts the way different nanomaterials have been applied for diagnostic of therapeutic purposes of the CNT *in vivo*.

2.1. Nanomaterials for imaging in the CNS

Imaging of the CNS is an important tool for the study and monitoring of structural, biochemical and functional changes in the brain and spinal cord (SC). Advances in this field have led to a better understanding of the effect of cellular damage on the CNS function, and thus helped to improve the precision of neurological procedures and reduce their invasiveness. There are several arguments in favour of the development of imaging technologies. Firstly, only direct observation reveals the behaviour of cells in the brain. Secondly, the continuous observation of cells over time is more accurate and safe than deducing changes from a single observation in fixed tissues. Thirdly, observations of sequential pathological events could help establish causes and relationships, and thus better understand CNS disorders. And finally, imaging the same area before and after treatment can offer a good indication of the success of a particular treatment [27].

The current technologies used for imaging the CNS are very diverse. For example, in electroencephalography (EEG) and magnetoencephalography (MEG) electrodes are placed on the skull and record electrical signals from the brain surface. To record deeper, the skull must be opened and electrodes inserted into the brain mass, as in some therapeutic procedures. Microelectrode array technology was developed in order to enable simultaneous recording of different neurons. However, it is unsuitable for long term implantation. New approaches are currently under investigation using microelectrode arrays with biocompatible coatings to promote bio-specific cell adhesion. Although the preliminary results are positive and encouraging, the potential use of these biocompatible microarrays to image and monitor neural activity is considered to be very invasive [28]. Computer tomography (CT), position emission tomography (PET) and functional magnetic

Table 1
Neurological applications of nanomaterials.

• Brain activity monitoring devices
• Neurodiagnostics (imaging agents)
• Neuroprotective therapeutics
• Neuro-regenerative therapeutics
• Nanoscale tools to aid neurosurgical procedures
• Brain implants for electrophysiological interventions (*e.g.* nanoscale electrodes)
• Drug delivery/targeting systems for the CNS

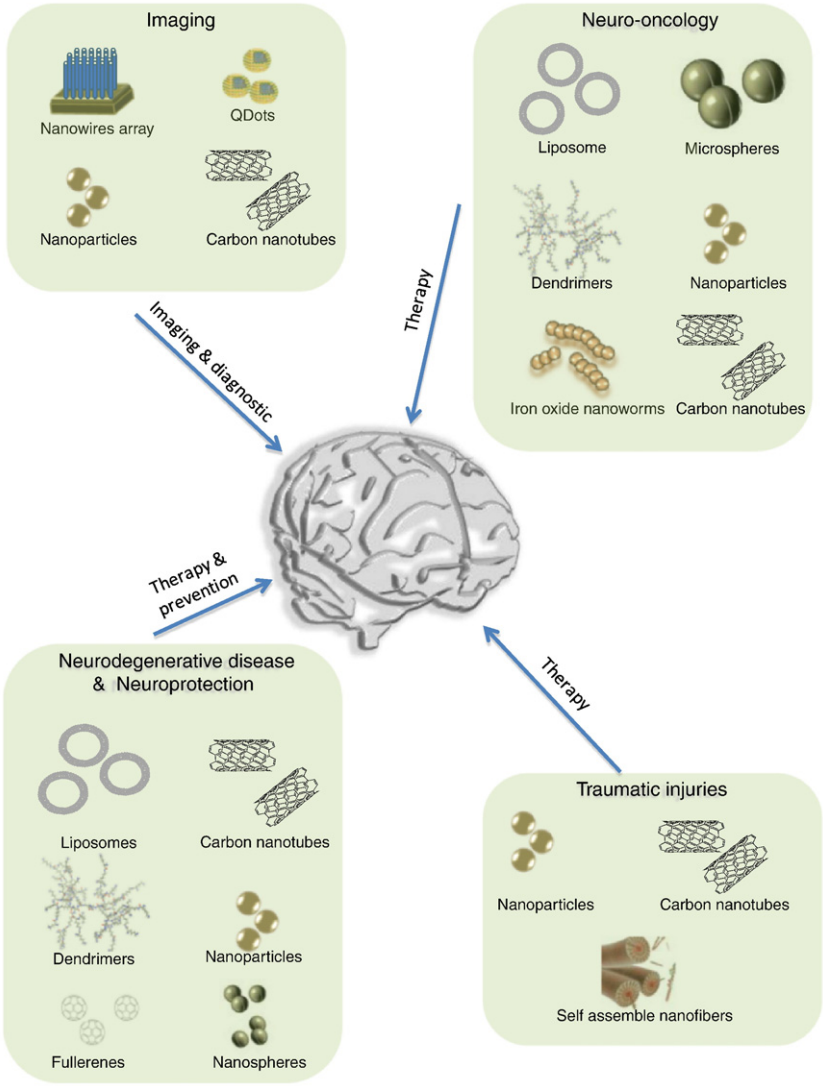

Fig. 3. Schematic representation of different nanomaterials and the main diagnostic and therapeutic applications on the CNS *in vivo*.

resonance imaging (*f*-MRI) have led to a better understanding of how neuronal circuits operate. Nevertheless, these imaging technologies have limitations, such as: lack of sensitivity (when compared to florescent modalities), reduced half-life after intravenous administration and restricted BBB permeation.

The use of nanotechnology for pre- and post-operative diagnostic imaging, as well as for real-time intra-operative visualisation, is expected to enhance the effectiveness of the diagnostic techniques, in particular for tumour patients. A summary of the most relevant studies published today using nanomaterials for imaging in the CNS *in vivo* is presented in Table 2 and will be further described below for each nanoparticle type.

2.1.1. Iron oxide nanoparticles

The potential of iron oxide nanoparticles as magnetic resonance imaging (MRI) contrast agents has been extensively studied, including for human use. They can be classified according to their diameter into several categories: i) supermagnetic iron oxide (SPIOs) nanoparticles with a mean diameter of more than 50 nm; ii) ultra-small supermagnetic iron oxide (USPIOs) nanoparticles with a diameter of 10–50 nm; and iii) very small supermagnetic iron oxide nanoparticles (VSPIOs) with a diameter of less than 10 nm [29]. Initial studies with commercially available magnetic iron oxide nanoparticles, such as Ferumostran-10 (USPIOs coated with dextran), Ferumoxides (SPIOs coated with Dextran) and Ferumoxytol (USPIOs coated with polyglucose sorbitol carboxymethyl ether) have not shown significant toxicity. It is generally accepted that magnetic iron oxide nanoparticles have attractive characteristics for their use as contrast agents. In particular: i) potential to permeate an intact BBB; ii) prolonged blood half-life; iii) minimum adverse-effects; and iv) the capability to be cleared by phagocytic cells [30]. Despite all the advantages, it is still difficult to differentiate the nanoparticle signal from the resident brain iron signal (for example following a cerebral haemorrhage caused by stroke or trauma).

Unlike other MRI agents, magnetic iron oxide nanoparticles can be imaged either by magnetic resonance or by light or electron microscopy, to allow their precise localisation within the neural tissue [29]. Following intravenous administration, supermagnetic iron oxide nanoparticles are captured by the reticulo-endothelial system (RES). The administration of Ferumoxtran-10 and Ferumoxytol for MRI contrast has been investigated in human patients with brain tumours both pre- and post-operatively [31,32]. Both studies were designed to compare the magnetic resonance signal of these magnetic iron oxide nanoparticles with that of gadolinium in patients with malignant brain tumours. Interestingly, intravenous administration of Ferumoxtran-10 or Ferumoxytol resulted in enhanced signals obtained from tumour areas that could not be imaged with gadolinium. This additional signal

Table 2
Studies using nanomaterials for imaging in the CNS.

	Nanomaterial	Model	Route	Aim and main conclusions	Ref
Nanoparticles	Iron oxide nanoparticles (Ferumoxtran or Ferumoxytol)	Patients with malignant brain tumours	Intravenous	• Comparison between the MRI signal of USPIO and gadolinium in patients with malignant brain tumours. • Ferumoxtran-10 and Ferumoxytol resulted in enhanced signals.	[31,32]
	Iron oxide nanoparticles (Ferumoxtran)	Patients with ischemic lesions	Intravenous	• Investigation of the cellular imaging of human ischemic stroke (Phase II). • Intravascular retention and lack of extravasation allowed better contrast between the vessel and adjacent tissue.	[33,118,119]
	Iron oxide nanoparticles (Endorem)	Rats with a cortical or spinal cord injury(SCI)	Intracerebral and Intravenous	• Investigation of iron oxide nanoparticles as an adjuvant for MRI to study the fate of trans-planted cell *in vivo* in rats with a cortical or SCI • Useful method for evaluating the migration and fate of stem cells in CNS.	[34]
	Iron oxide nanoparticles (dextran-coated SPIONs)	Mice with bilateral carotid artery occlusion (BCAO)	Intraperitoneal	• Investigation of additional biomarkers of angiogenesis-associated pericytes • Gene transcript-targeted MRI (GT-tMRI) non-invasively revealed neural progenitor cells during vascularisation.	[120]
	Iron oxide nanoparticles	Rat with glial brain tumour (U87MGdEGFR vIII)	Intravenous	• Investigation of brain tumour vessels in glioblastoma • Single domain antibody iron oxides targeted MRI contrast agent selectively binds to abnor-mal vessels.	[121]
	Iron oxide nanoparticles	Patients with spinal cord injury (SCI)	Lumbar puncture technique (LP)	• Iron oxide nanoparticles for MRI to study the fate of transplanted cells in patients with SCI. • Cells labelled with magnetic nanoparticles migrated into the injured site in patients with chronic SCI.	[35]
	Magnetite–dextran nanoparticles	Rat with glial brain tumour (A101.08)	Intra-carotid injection after disruption of BBB	• Investigation of magnetic nanoparticles as MRI agents for the diagnosis and treatment of brain tumours • Nanodispersed magnetite–dextran preparation penetrated into a rat brain tumour and peritumoural tissue	[119]
QDots	QDots (TAT conjugated)	Rat	Intravenous	• Investigation of the delivery of Qdots for imaging the brain tissue. • TAT peptide was necessary to overcome the BBB	[38]
	QDots coated with Serum	Wild-type mouse brain	Intravenous	• Investigation of the *in vivo* microangiography of deep brain capillaries and blood vessels in wild-type mouse brain after injection with Qdots. • Deep *in vivo* microangiography provided a new approach for visualisation microangiopathies, typical from Alzheimer's disease.	[39]
	QDots into a core of PEG-PLA nanoparticles (WGA conjugated)	BALB/c nude mice	Intranasal	• Study of the biodistribution of WGA-Qdots-nanoparticle following intranasal administra-tion in BALB/c nude mice • Targeted Qdots showed brain delivery by intranasal administration	[40]
	Amino(PEG) QDots	Rat with gliosarcoma (C6)	Intravenous	• Identification of neoplastic tissue within normal brain during biopsy and tumour resection • Qdots were visualised within the experimental brain tumour, outlining the tumour and potentially augmenting brain tumour biopsy and resection.	[37,122]
Dendrimers	PEGylated G-5 dendrimer conjugated to DOTA [Gd3+] (labelled with Cy5.5, Rodamine and Angiopet-2)	Mouse with glioblastoma (U87)	Intravenous	• Investigation of imaging brain tumour by dendrimer-based optical paramagnetic nanoprobe. • Tumour margins were successful delineated holding potential for preoperative brain tumour localisation and image-guided tumour resection during surgery.	[123]
Nanowires	Array of platinum nanowires	*N/A*	Intravenous	• Electrical recording of the activity of small groups of neurons by an array of platinum nanowires • Nanowires were guided to the brain through the circulatory system reaching specific targets.	[28]
Carbon nanotubes	Electrodes coated with MWNT	Rat and monkey	Intracranial insertion	• Development of CNT-based electrodes to record neuronal electrical events. • MWNT-coated electrodes improved the recording of neuronal electrical events *in vivo*.	[124]

enhancement is likely due to the prolonged plasma half-life of the iron oxide nanoparticles along with the efficient cellular trapping and accumulation.

Iron oxide nanoparticles have also been investigated in human patients with ischemic lesions. For example, a clinical Phase II study performed by Saleh et al. [33] investigated the use of Ferumostran-10 nanoparticles for imaging human ischemic lesions after stoke. Patients received Ferumostran-10 at the end of the first week after symptom onset and contrast enhancement was observed mainly at the periphery of the infracted brain region. This signal enhancement was linked to the iron-labelled macrophage infiltration, rather than to disruption of BBB. The advantage of this approach resulted from the intravascular retention and lack of extravasation, allowing better contrast between the vessel and the adjacent tissue for several minutes post-injection, with minimal adverse effects.

Moreover, supermagnetic iron oxide nanoparticles have been found to be useful in studying the fate of transplanted cells by MRI either in rat models [34] or human patients with chronic spinal cord injury [35]. New approaches exploiting the potential of these nanoparticles in combination with enhanced MRI could result in the development of novel strategies for the detection of a wide range of inflammatory CNS disorders, including stroke, epilepsy and CNS trauma [29]. Activated macrophages constitute an *in vivo* marker for both the diagnosis and the prediction of the disorder development, as they play a critical role in eliciting an immune response. While in healthy conditions the migration of immune cells to the brain is restricted, during a pathological event, they are able to cross the BBB and blood–cerebrospinal fluid (CFS) barrier and reach the brain parenchyma. Macrophages can be tracked using iron oxide nanoparticles and thus imaged by MRI. Due to the capacity of nanoparticles to target inflammatory tissues *via* macrophage tracking, a novel possibility for the characterisation of numerous inflammatory and degenerative diseases has emerged.

2.1.2. Quantum dots

The surgical management of brain tumours or other pathologies requires precise localisation within the brain parenchyma. Quantum dots (QDots) are optical semiconductor nanocrystals that can be conjugated with fluorescent tags using the same chemical approach applied in fluorophore immunocytochemistry. Compared to other fluorescence techniques, QDots offer higher signal detection due to reduced photobleaching. QDot technology represents a promising approach for CNS imaging, however their relatively poor stability and low BBB permeability have been limiting their *in vivo* application [36].

Certain disorders, such as brain tumours, are accompanied by migration of macrophages and microglia that are vital to the management of brain homeostasis. Once in the bloodstream, QDots can internalise within such cells and allow the microscopic identification and visualisation of brain tumours [37]. In the last decade, the use of QDot technology in the CNS has been enhanced by other strategies. For example, Santra et al. [38] administered (intra-arterially) QDots conjugated with TAT peptides—a membrane translocation peptide that helps to overcome the cellular membrane barrier to efficiently target the brain. Others have suggested the use of serum-labelled QDots for *in vivo* micro-angiography of deep brain capillaries and blood vessels in wild-type mouse brain [39]. In order to improve the stability of QDot systems, Gao et al. [40] have developed a multifunctional platform by encapsulating QDots in a core of poly(-ethylene glycol)–poly(lactic acid) (PEG–PLA) nanoparticles to reach the brain after intranasal administration. This system was conjugated with wheat germ agglutinin (WGA), combining both imaging and targeting platforms in the same system. Imaging of CNS with QDots is a useful tool for the visualisation of tumours during surgical procedures and may assist neurosurgeons. Such attractive prospects have to be followed by thorough evaluation of their safety profile when resident within the CNS.

2.1.3. Nanowires

Nanowires have been attracting attention as an innovative nanomaterial for the detection of CNS pathologies. Their ability to receive and deliver electrical impulses favours the detection of various pathologies. However, the procedure of inserting them through the skull and into the brain is quite invasive and can easily result in tissue damage. As an alternative, Llinas et al. [28] have developed platinum nanowires guided by blood circulation to the brain. This was based on the concept that the arterial pathways can guide larger catheter tubes to certain points of the body. They designed an entire array of nanowires connected to a catheter tube that could be guided to the brain through the circulatory system. Each nanowire recorded the electrical activity of neurons without invading the brain parenchyma. This nanoelectrode array is thought to be small enough to avoid interference with normal blood flow and nutrient exchange or disruption of brain activity. Future directions may involve the replacement of platinum nanowires with polymer coated nanowires, which can be biodegradable and therefore more suitable as short-term brain implants.

2.1.4. Carbon nanotubes

CNT-modified electrodes have led to significant improvements in the detection of electrophysiological signals following intracranial implantation [23]. Multi walled carbon nanotube (MWNT)-coated electrodes achieved better signal-to-noise ratio and higher sensitivity to spontaneous electrical neuronal activity *in vivo* (rodent and primate models). Such studies highlight the effectiveness of electrodes coated with CNT for the recording of neuronal electrical events *in vivo*.

2.2. Nanomaterials as therapeutics in nano-oncology

Treatment of brain tumours remains a great challenge despite the advances in tumour therapy and the increasing understanding of carcinogenesis. There is evidence of a critical correlation between early detection and positive prognosis, and consequently the success of the treatment. Nanomaterials have been emerging as potential vectors for the CNS due to their own structural advantages and possibility to further design them with the aim to cross the BBB. Table 3 shows a summary of the major *in vivo* studies published to date concerning the application of nanomaterials in the treatment of brain tumours.

2.2.1. Nanoparticles

Several chemotherapeutic drugs have been explored in the treatment of brain tumours using nanoparticles, such as paclitaxel (Pax) that exhibits activity against malignant gliomas and brain metastases. However, its use is limited by its low therapeutic index and because it is a substrate for the Pgp efflux pump. Nevertheless, some studies have described the incorporation of this drug into nanoparticles in order to enhance brain uptake and therapeutic efficacy [41,42]. Xin et al. [41] compared the commercially available formulation Taxol® with Pax-loaded nanoparticles (methoxy poly(ethylene glycol)–poly(ε-caprolactone) nanoparticles) and demonstrated an increase in brain uptake when the nanoparticle-based delivery system was used. This was likely due to the efficiency in overcoming the drug Pgp efflux pump at the BBB.

Other studies have used nanoparticle vectors for the delivery of doxorubicin (Dox) in the treatment of brain tumours. Dox is a chemotherapeutic drug that inhibits deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis; however due to its polarity, it has been shown to have limitations in crossing BBB after intravenous administration. The biodistribution of polysorbate-coated poly(buthylcyanoacrylate) (PBCA) nanoparticles carrying Dox after systemic administration has been studied by different groups. Couvreur et al. [43] showed that delivery of Dox using PBCA

Table 3
Representative studies using nanomaterials in neuro-oncology.

	Delivery system	Model	Route	Aim and main conclusions	Ref
Nanoparticles	Doxorubicin delivered by Polysorbate-80 coated PBCA nanoparticles	Rat intracranial glioblastoma (101/8)	Intravenous	• Investigation of the anti-tumour capacity of Dox-loaded nanoparticles. • Distribution of doxorubicin into the brain *in vivo* and therapy of brain tumours.	[45]
	Paclitaxel delivered by methoxy poly(ethylene glycol)-poly(ε-caprolactone) nanoparticles	Mouse intracranial gliosarcoma (C6)	Intravenous	• Investigation of the anti-tumour capacity of drug-loaded nanoparticles. • Nanoparticles enhanced anti-glioblastoma activity of paclitaxel.	[41]
	Paclitaxel delivered by PEGylated poly(trimethylene carbonate) nanoparticles	Mouse intracranial glioblastoma (U87)	Intravenous	• Nanoparticles enhanced the accumulation of paclitaxel in tumour tissue. • Survival rate of glioma-bearing rats was significantly prolonged after anti-glioblastoma therapy of paclitaxel nanoparticles.	[125]
	Paclitaxel delivered by magnetic nanoparticles	Rat intracranial glioma	Intravenous	• Anti-tumour capacity of paclitaxel-loaded superparamagnetic nanoparticles. • Survival rate of glioma-bearing rats was significantly prolonged after therapy with nanoparticles.	[42]
	pDNA (pORF-hTRAIL) delivered by cationic albumin-conjugated PEG-nanoparticles	Mouse intracranial gliosarcoma (C6)	Intravenous	• Systemic administration of pDNA loaded nanoparticles for gene therapy of glioma. • Tumour-specific nanoparticle delivery with induction of apoptosis and significant retardation of tumour growth.	[52]
	Dextran- and aminosilane-coated superparamagnetic iron oxide	Rat intracranial glioblastoma (RG2)	Intratumoural	• Investigation of the feasibility and tolerability of thermotherapy using magnetic nanoparticles on rats with GBM. • Thermotherapy with aminosilane-coated nanoparticles led to prolongation of survival rate over dextran-coated particles.	[50]
	Aminosilane-coated superparamagnetic iron oxide nanoparticles	Patients with GBM (*under clinical trial*)	Intratumoural	• Development of nanoparticle-based hyperthermia therapy for cancer treatment. • The clinical efficacy of intratumoural thermotherapy using magnetic nanoparticles in combination with fractionated stereotactic radiotherapy was demonstrated.	[47,49]
	PEGylated nanoparticles of polyacrylamide (PAA) core; photosensitizers; MRI contrast agents and molecular targeting groups	Rat intracranial gliosarcoma (C6)	Intratumoural	• Investigation of photodynamic nanoparticles for extracellular cancer therapy. • Improvement in residence time and tumour specific delivery, along with a significant increase in animal survival.	[53]
Nanoworms	Multifunctional iron oxide nanoworms: tumour-homing peptide (CGKRK), pro-apoptotic peptide ($_D$[KLAKLAK]$_2$)	Mouse intracranial glioblastoma (U87)	Intravenous	• Investigation of anti-angiogenic therapy by using a theranostic platform. • Targeted GBM tumours, providing effective therapeutic activity with diagnostic function.	[54]
Dendrimers	Methotrexate Polyamidoamine dendrimer conjugated to cetuximab	Rat intracranial glioblastoma (F98)	Intratumoural	• Investigation of the anti-tumour capacity of methotrexate-loaded dendrimers. • Specific tumours cells targeting and reduction of toxicity in normal brain tissue.	[63]
Microspheres	Mitoxantrone delivered by PLGA microspheres	Rat intracranial glioblastoma (RG2)	Intratumoural	• Mitoxantrone-loaded PLGA microspheres prevented glioma growth, minimising side effects.	[56]
	Imatinib mesylate delivered by PLGA microspheres	Mouse intracranial glioblastoma (U87)	Intratumoural	• Imatinib-microspheres were shown to significantly reduce tumour growth within two weeks post injection.	[57]
Liposomes	Sodium borocaptate delivered by immunoliposomes (transferrin antibody)	Mouse intracranial glioblastoma (U87)	Intravenous	• Investigations of therapeutic efficacy by boron neutron capture therapy. • Enhanced survival rate reported.	[59]
	Sodium borocaptate delivered by Immunoliposomes (EGFR antibody)	Mouse intracranial glioblastoma (U87)	Intravenous	• Investigations of therapeutic efficacy by boron neutron capture therapy. • High levels of sodium borocaptate were delivered into brain tumours.	[60]
	Irinotecan-loaded liposome (co-administered with Doxil®)	Rat intracranial glioblastoma (U251 and U87)	Intratumoural	• Therapeutic efficacy of co-administration between doxorubicin and irinotecan-loaded liposomes in the treatment of glioblastoma tumours. • Co-administration showed potential for brain tumour therapy.	[61]
	Non-PEGylated liposome encapsulated with both topoCED™ and gadoCED™	Rat intracranial glioblastoma (U87)	Intratumoural	• Therapeutic efficacy from co-administration of topoCED ™ with gadoCED™. • Real-time imaging of the liposome distribution, and a significant increase in the survival rate were reported.	[62]
	Doxorubicin delivered by PEGylated liposome (plaque-specific peptide AP-1antibody)	Mouse intracranial glioblastoma (GBM8401-luc)	Intravenous	• Investigation of applicability of repeated pulsed high-intensity focused ultrasound (HIFU) when treating GBM with liposomal doxorubicin. • Combination of repeated pulsed HIFU enhanced delivery of liposomal doxorubicin to the brain tumour and improved the anti-tumour effect.	[126]
	Co-delivery of Paclitaxel and pEGFP-hTRAIL gene by PEGylated Liposome-conjugated to Agiopet-2	Mouse intracranial glioma (U87)	Intravenous	• Investigation of paclitaxel delivery by PEGylated liposomes modified with angiopet-2 for the targeting and treatment of gliomas. • Dual targeting co-delivery system showed potential for glioma therapy in clinical application	[127]
Carbon nanotubes	MWNT coated with Pluronic PF-108	Mouse intracranial glioblastoma (GL261)	Intratumoural	• Evaluation of the uptake and toxicity of MWNT in a glioma model. • CNT injection led to a transient and self-limiting local inflammatory response.	[116]
	CpG oligonucleotide coated SWNT (chemically functionalised with PL-PEG)	Mouse intracranial glioma (GL261 and GL261)	Intratumoural	• Enhanced CpG uptake by tumour-associated inflammatory cells. • CpG immunotherapy eradicated glioma and protected against tumour re-challenge.	[128]
	Doxorubicin and PEGygylated MWNT-conjugated to Agiopet-2 (Dox and PEG were physically adsorbed to nanotubes)	Mouse intracranial glioma (C6)	Intravenous	• Investigation of targeted (with angiopet-2) PEGylated MWNT for targeting and treatment of gliomas. • Increased glioma targeting and improved survival reported.	[129]

nanoparticles leads to low heart accumulation. This is important since cardiotoxicity is one of the major drawbacks for the *in vivo* use of free Dox. Similarly, Gulyaev et al. [44] have demonstrated that PBCA nanoparticles coated with polysorbate-80 can deliver high concentrations of Dox into the brain after intravenous administration. Interestingly, the accumulation in RES organs has been shown to be lower compared to uncoated nanoparticles. The therapeutic efficacy of Dox-PBCA nanoparticles was also demonstrated. For example, a study performed by Steinigier et al. [45] showed that more than 20% of the animals (rats with intracranial glioblastoma) treated with intravenous administration of Polysorbate-80 coated PBCA nanoparticles survived for more than 180 days compared to only a 10–20 day survival rate in the control group (no nanoparticles).

The treatment of glioblastoma multiforme (GBM) is one of the most challenging problems in oncology and, despite new therapeutic approaches, efficacy remains elusive. Although multimodality methodologies are involved in the treatment of GBM (surgery, irradiation and chemotherapy), the average survival rate is only one year [46]. While chemotherapy is the first line of treatment for recurrent GBM, other alternative therapeutic approaches have been explored and have produced some promising results. For example, the ability of magnetic nanoparticles to induce hyperthermia has led to the development of a nanoparticle-based hyperthermia therapy [47–49]. This method relies on the direct introduction of iron oxide magnetic nanoparticles into the tumour and their subsequent induction of vibration by oscillation of a magnetic field, which generates heat. The feasibility and efficacy of intratumoural thermotherapy using magnetic nanoparticles (nanocancer® therapy) have been demonstrated previously in pre-clinical [50] and clinical studies [49]. The feasibility of this strategy was further verified in a post-mortem neuropathological study performed by Van Landeghem et al. [47] on GMB patients treated with magnetic aminosilate coated supermagnetic iron oxide nanoparticles. Most of the nanoparticles were aggregated and located in necrosis areas within the tumour, restricting their distribution to the injection site. The same group also demonstrated the clinical efficacy of intratumoural thermotherapy using magnetic nanoparticles in combination with fractionated stereotactic radiotherapy for the treatment of recurrent GBM [51].

Other strategies have focused on the delivery of cytotoxic genes by nanoparticles in order to treat brain tumours. One example in the literature reports the delivery of plasmid DNA (pORF-hTRAIL) conjugated to PEGylated albumin-PBCA nanoparticles [52]. Intravenous administration of this system resulted in the induction of apoptosis and significant retardation of intracranial glioma growth, demonstrating the potential use of this type of nanoparticles as a gene delivery system for the treatment of malignant tumours.

Tumour cells can also be irradiated through photo-dynamic therapy (PDT), where the drug is activated by light, causing cell death by oxidative damage. With this strategy, drugs are stimulated by the use of light only locally, resulting in insignificant damage of the healthy tissues, which constitutes an advantage over conventional treatments [53]. Recently, the efficacy of a multifunctional-based nanoparticle system containing photo-sensitizer agents and MRI enhancer agents has been investigated in an intracranial gliosarcoma model [53]. Improvement in residence time and tumour specific delivery was observed, along with a significant increase in animal survival from 5 days (control group) to 2 months (nanoparticle treated group), with an increased incidence of tumour elimination.

2.2.2. Iron-oxide nanoworms

GBM are among the most vascular tumours in the body and as such represent an attractive target for anti-angiogenesis therapy. In a recent work, Agemy et al. [54] showed GBM development to be arrested by anti-angiogenesis therapy using a theranostic platform based on nanoworm vectors. This multifunctional platform was incorporated by: i) a tumour homing peptide (CGKRK) which provided the target capacity of the system; ii) a pro-apoptotic peptide ($_D$[KLAKLAK]$_2$) with anti-tumoural activity; and iii) iron oxide nanoworms which enabled the imaging of GBM tumour in mice. Such a system provided both effective therapeutic activity and diagnostic capacity, encouraging further development of this vector towards clinical use.

2.2.3. Polymeric microspheres

Delivery of therapeutic agents to brain tumours has also been achieved through the use of microspheres. The administration of magnetic microspheres (magnetic neutral dextran and magnetic cationic aminodextran microspheres) has shown an ability to overcome BBB, enhancing brain uptake after carotid administration in intracranial glioma rat model [55]. Moreover, the clinical efficacy of polymeric microspheres on the glioma rat model (RG2 model) has also been investigated. Poly(lactic-co-glycolic acid) (PLGA) microspheres loaded with mitoxantrone – which is a potent drug against malignant glioma – have been shown to improve the brain delivery of this drug to the tumour sites, demonstrating capability to prevent glioma growth [56]. Similarly, imatinib mesylate, a tyrosine kinase inhibitor drug used as standard therapeutic for patients with chronic myelogneous leukaemia, has also been delivered using polymeric microspheres [57]. Microspheres loaded with imatinib mesylate were directly injected into the intracranial glioblastoma tumour (U87 model) and were shown to reduce by 79% the tumour growth at 14 days post injection. Overall, these studies indicated that microspheres could be used as an efficient therapeutic delivery system for brain tumours.

2.2.4. Liposomes

Gene therapy studies using cationic liposomes have been reported in the literature two decades ago, when Holt et al. [58] successfully transfected neurons in the embryonic brain of xenopus frogs by direct injection of liposome–DNA complexes. Since then, several liposome formulations have been investigated for brain cancer therapy. Doi A. et al. [59] developed a PEGylated liposome loaded with sodium borocaptate. This system was conjugated to transferrin and was found to deliver high concentrations of boron to brain tumours for boron capture neuron therapy, enhancing the survival of the glioma murine models. Very low levels of boron were accumulated in the non-tumour brain hemisphere, which points to the advantages of this approach. Similarly, Feng et al. [60] developed PEGylated liposomes targeted with EGFR antibody to deliver sodium borocaptate on glioma murine models (U87). This vector specifically delivered high levels of sodium borocaptate at glioma cells and surrounding areas for 24–48 h post injection.

Brain tumour therapy has also been investigated through the co-administration of different liposome formulation by convection-enhanced delivery (CED). For example, Krauze et al. [61] co-administered irinotecan-loaded liposome with Doxil® (PEGylated liposomes containing Dox) on different glioma rat models (U251MG and U87MG). A three-fold increase in survival rate was observed for the combinatory therapy over the control groups, demonstrating the potential of this strategy for brain tumour therapy. Furthermore, Granh et al. [62] tested the therapy efficacy of non-PEGylated liposomes loaded with topotecan, which had previously been shown to be an active drug against malignant glioma. These liposomes were co-administered with galodiamide-loaded liposomes on glioblastoma multiform rat models (U87 model). A real-time imaging of the liposome distribution was achieved, along with a significant increase in overall animal survival when compared to controls [62].

2.2.5. Dendrimers

Dendrimers have also been used because of their recognised high drug loading capacity and ability to encapsulate and solubilise hydrophobic drugs within the dendritic structure. Recently, a polyamidoamine (PAMAM) dendrimer containing the cytotoxic drug methotrexate and conjugated to cetuximab (specific to target EGFR) has been investigated

for their targeted capacity to achieve efficient tumour therapy in an intracranial glioma rat model (F-98 *EGFR*) [63]. The results have not shown significant differences between the efficacy of bioconjugated treated groups and controls (methotrexate alone), however, the advantage of this strategy lies in the reduced toxicity for normal brain tissue.

2.2.6. Carbon nanotubes

CNT have also been used in the treatment of brain tumours. Although the literature lacks studies based on intravenous administration for the treatment of brain tumours, therapeutic approaches based on the administration of CNT *via* intracranial injection have shown promising results. Zhao et al. [50] have recently shown that intra-tumoural injections of single-walled carbon nanotubes (SWNT) chemically functionalised with PEG_{2000} could enhance the internalization of CpG oligodeoxynucleotides in tumour-associated inflammatory cells. This approach led to inflammatory cytokine release by other primary monocytes and potentiated CpG immunotherapy in both intracranial glioma models tested (GL261 and GL261egfp models) eradicating the gliomas and protecting against tumour re-growth.

2.3. Nanomaterials as neuroprotective and therapeutic agents for neurodegenerative diseases

Neurodegenerative disorders are conditions related to the progressive and persistent loss of neurons and are often associated to ageing and a consequent decline in neurological functions. Applications of nanotechnology in the protection of CNS are expected to limit the effect of age-related neurodegenerative events such as oxidative stress that contributes to the ongoing tissue damage. The following table (Table 4) reviews the major applications to date of nanomaterials as neuroregenerative and neuroprotective systems.

2.3.1. Nanoparticles

The presence of transition metal ions within the brain (such as Cu_2^+ and Zn_2^+) is known to increase with age. Furthermore, the number of metal ions in Alzheimer's disease patients is often higher than that found in healthy patients. As a result, using chelating agents that could selectively bind, neutralise and remove these metals is seen as a potential option for the treatment of Alzheimer's disease. However, the limitations of this approach lie in the low BBB permeability of chelating agents. One way to overcome this is through the use of nanoparticles. Nanoparticles containing sodium salt conjugated to *d*-penicillamine were capable of solubilising Cu_2^+-amyloid-β aggregates in reduced environment *in vitro* [64]. Other chelating agents, such as deferoxamine and 2-methyl-N-(2-aminoethyl)-3-hydroxyl-4-pyridone, when conjugated to polysurbate 80-coated nanoparticles, have also demonstrated ability to chelate ions in brain sections of Alzheimer's patients [65]. Similarly, in a recent study a prototype nanoparticle–chelator conjugate (Nano-N2PY) has demonstrated the capacity to protect human cortical neurons from amyloid-β-associated oxidative damage by inhibiting the formation of β-amyloid aggregates [66]. Overall, nanoparticle-based therapy using chelating agents has been shown to increase their brain uptake, enhancing their bioavailability and decreasing their toxic side-effects. At the same time, this approach reduces the metal levels in neuronal tissue and therefore protects the brain from the harmful effect of the oxidative stress. More specifically, these approaches suggest that modulation of the amyloid-β aggregation constitutes a good therapeutic option for Alzheimer's disease or other neurodegenerative diseases associated with excess transition metals.

Moreover, the enhancement of reactive oxygen specimens (ROS) plays a critical role in many neurodegenerative diseases and has been linked to aggravated brain tissue damage. The brain damage can be further compromised because of the inefficient antioxidant defences elicited by the ischemic conditions of certain diseases, such as stroke or amyotrophic lateral sclerosis. Strategies involving exogenous delivery of ROS scavengers, such as superoxide dismutases (SOD), have been explored. However, their application is limited *in vivo*, because of their poor bioavailability and lack of BBB permeation. Reddy et al. [67] have demonstrated that PLGA nanoparticles loaded with SOD could be used as neuroprotective platforms up to 6 h after induction of oxidative stress by H_2O_2 in cultured human neurons. The potential of cerium and yttrium nanoparticles to reduce the oxidative stress by the inhibition of ROS production and the consequent reduction of neuronal death associated with γ-irradiation has also been demonstrated *in vitro* [68]. Others, using an *in vitro* model of acrolein-mediated cell injury, have demonstrated that hydralazine-loaded chitosan nanoparticles are also able to reduce membrane integrity damage, secondary oxidative stress and lipid peroxidation [69].

Chronic inflammation has been associated with neurodegenerative diseases, in particular Alzheimer's and Parkinson's. Inflammatory activity leads to excessive production of pro-inflammatory products and ROS, which could increase the neuronal cell death. Therefore, strategies that rely on the decrease of microglia-activated ROS productions have been explored as a therapeutic option to treat neurodegenerative diseases. Cells such as macrophages are able to uptake nanoparticles and release them in inflammatory sites. Following this idea, studies have been performed using bone-marrow-derived macrophage (BMM) systems to carry drug loaded nanoparticles for the treatment of neurodegenerative disorders [70]. Batrakova et al. [70] developed a nanoparticle system (with copolymer complex PEI–PEG) to deliver an enzyme (catalase) to injured brain regions in pre-induced Parkinson's disease models (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine ($MPTP^+$) model). BMM were incubated with the nanoparticles *in vitro* and then injected into mice. The cells containing catalase passed through the BBB and released the enzyme in an active form for a period greater than that obtained with catalase alone. The increased amount of the enzyme in the brain was found to be related to the decomposition of microglia hydrogen peroxide, suggesting the potential application of this system in the reduction of oxidative stress associated with neurodegenerative processes.

Others have further suggested that the modulation of inflammatory responses can reduce neuronal death and therefore deliver a positive outcome in the treatment of neurodegenerative diseases. One of the most promising examples described in the literature involves the use of VP025 (Vasogen inc., ON, Canada), which is a phosphatidylglycerol-based phospholipid nanoparticle currently enrolled in Phase-II clinical development. The therapeutic benefits derived from the use of VP025 nanoparticles have been demonstrated in Parkinson's disease model (6-hyroxydopamine (6-OHDA) model) [71]. Specifically, VP025 nanoparticles have shown potential to induce anti-inflammatory response of neural tissue, regulating cytokine productions and controlling inflammation reactivity of brain tissue.

Another strategy to improve the clinical efficacy on the treatment of neurodegenerative diseases is by increasing the drug or gene delivery to the brain. For example, through systemic administration, polymeric nanoparticles carrying nerve growth factor (NGF) [72] or urocortin [73] have been shown to enhance BBB penetration and elicit behaviour recovery of Parkinsonian models ($MPTP^+$ and 6-OHDA, respectively). Other studies, this time *via* intranasal administration, have also demonstrated that odorranalectin conjugated to PEG–PLGA nanoparticles could lead to functional and motor recovery of 6-OHDA Parkinson models [74].

Recently, nanospheres have been explored as neuroprotective agents on pre-clinical models of stroke. Karatas et al. [75] developed a transferrin targeted nanosphere loaded with caspase-3 inhibitor (Z-DEVD-FMK peptide) and showed their ability to deliver Z-DEVD-FMK peptide into the brain following intravenous administration. The results demonstrated a significant inhibition of caspase-3 activity both in the ischemic brain models and in the normal neonatal brain, suggesting their importance in the prevention of stroke.

Table 4
Studies using nanomaterials as neuroprotective and therapeutic agents for neurodegenerative diseases.

	Delivery system	Model	Route	Aim and main conclusions	Ref
Nanoparticles	Nanoenzyme: catalase immobilised into a cationic block copolymer (PEI/PEG)	Parkinson Disease ($MPTP^+$) mouse	Intravenous	• Investigation the used of BMM as a vehicle to carry therapeutic concentrations of catalase to the brain. • Reduced oxidative stress in Parkinson's Disease model.	[70]
	VP025 nanoparticles (phosphatidylglycerol-based phospholipid nanoparticles)	Parkinson Disease (6-OHDA) rat	Intramuscular	• Investigation of the neuroprotective effect of VP025 nanoparticles in Parkinson's Disease model (Phase II clinical trial). • Reduction of microglia activation in the substantia nigra of 6-OHDA-treated rats.	[71]
	*h*GDNF delivered by neurotensin polyplex (poly-l-lysine nanoparticles)	Parkinson Disease (6-OHDA) rat	Stereotactic (into substantia nigra)	• Investigation of transfection efficiency of *h*GDNF gene delivered by neurotensin polyplex into the surviving dopamine neurons of Parkinson's Disease model. • Biochemical, anatomical, and functional recovery of pre-induced Parkinson's Disease model.	[77]
	Nerve growth factor (NGF) delivered by polysorbate 80-coated PBCA nanoparticles	Parkinson Disease (MPTP +) mouse	Intravenous	• Investigation of the therapeutic effects of NGF against MPTP-induced neurotoxicity. • Reduction of the basic symptoms of Parkinsonism: oligokinesia, rigidity, and tremor.	[72]
	Urocortin delivered by PEG-PLGA nanoparticles (targeted with lactoferin)	Parkinson Disease (6-OHDA) rat	Intravenous	• Investigation of the therapeutic effect of Urocortin-loaded nanoparticles on Parkinson's disease model. • Attenuation of striatum lesion caused by 6-OHDA.	[73]
	Urocortin delivered by PEG-PLGA nanoparticles (targeted with odorranalectin)	Rat Parkinson's Disease (PD) (6-OHDA)	Intranasal	• Therapeutic efficacy of urocortin-loaded nanoparticles. • Functional and behavioural recovery reported.	[74]
	Cerium oxide nanoparticles	Mouse hippocampal model of ischemia	*N/A*	• Neuroprotective effects on *in vitro* model of ischemia. • Potential to prevent cellular ischemic cell death by reduced ROS production.	[130]
	Apomorphine delivered by glyceryl monostearate (GMS) solid lipid nanoparticles or polyethylene glycol monostearate (PMS) solid lipid nanoparticles	Rat PD (6-OHDA)	Oral	• Investigation of the bioavailability and therapeutic efficacy of solid lipid nanoparticles with different monostearate emulsifiers. • Targeting of brain striatum and PMS-based system better therapeutic efficacy on behavioural recovery.	[131]
Nanospheres	Z-DEVD-FMK delivered by PEGylated coated chitosan nanospheres (transferrin antibody)	Mouse stroke (MCAO)	Intravenous	• Therapeutic efficacy against pre-induced stroke or with cerebral developmental cell death. • Neuroprotection in normal neonatal and stroke models.	[75]
Dendrimers	Human (*h*)GDNF delivered by polyamidoamine PEGylated dendrimers targeted with lactoferin	Rat PD (6-OHDA or Rotenone)	Intravenous	• Neuroprotective effect in PD model. • Improvement of locomotor activity, reduction of dopaminergic neuronal loss and enhancement of monoamine neurotransmitter levels reported.	[78,79]
	HMGB-1 siRNA delivered by arginine-PAMAM dendrimers	Rat stroke (MCAO)	Stereotactic (into cortex)	• Investigation of siRNA efficiency in normal and ischemic rat brain	[76]

(continued on next page)

Table 4 (*continued*)

	Delivery system	Model	Route	Aim and main conclusions	Ref
				• Suppression of infarct formation in the post-ischemic brain.	
Liposomes	GDNF delivered by immunoliposomes (transferrin)	Rat PD (6-OHDA)	Intravenous	• Partial rescue of the nigral-striatal tract and behavioural recovery of PD symptoms.	[80]
	Haemoglobin encapsulated in PEGylated coated liposome (LEH)	Rat stroke (MCAO)	Stereotactic (cortex and basal ganglia)	• Suppression of the infarct area in rats with stroke.	[81,82]
	Vascular endothelium growth factor (VEGF) delivered by immunoliposomes (transferrin)	Rat stroke (MCAO)	Intravenous	• Investigation of VEGF to promote ischemic brain neuroprotection and angiogenesis • Decrease in infarct volume promoted neurovascularisation and improved neurophysiological function.	[132]
Fullerenes	C_{60} fullerene core attached to NMDA receptor antagonist (ABS-75)	Mouse Multiple Sclerosis (experimental autoimmune encephalitis)	Intraperitoneal	• Evaluation of receptor-specific antioxidant therapy, based on fullerenes in pre-induced chronically progressive multiple sclerosis model. • Reduction of MS progression accompanied by decrease of axonal and myelin loss in the spinal cord.	[84]
	Polyhydroxylated fullerol $C_{60}(OH)_{24}$	Cell-based PD (MPT^+) model in human SK-NMC neuroblastoma cells	*N/A*	• Potential to prevent mitochondrial dysfunction and oxidative damage.	[85]
	Hexa-sulfobutylated fullerenes (FC_4S)	Rat stroke (MCAO)	Intravenous	• Investigation of the free radical scavenging activity of FC_4S. • Effective suppression of the cerebral infarction volume.	[86,87]
	Carboxyfullerene	Rat stroke (MCAO)	Intravenous and intraventricular	• Investigation the ability of local administration of carboxyfullerene to protect cortical infarction in rat brain. • Attenuation of the oxidative injuries caused by ischemic stroke.	[88]
Carbon nanotubes	Acetylcholine delivery by SWNT (physically adsorbed)	Mouse model of Alzheimer disease	Gastrogavage (multiple) administration	• Therapeutic effects due to delivery of acetylcholine into lysosomes of neurons.	[133]
	Amine-functionalised SWNT	Rat Stroke (MCAO)	Intraventricular	• Investigation of the neuroprotective capacity of amine-modified SWNT in brain with ischemic injury. • Protection of neurons and enhancement of motor function.	[89]
	siRNA (siCaspase-3) delivered by amino-functionalised MWNT	Mouse stroke (Endothelin-1)	Stereotactic (cortex)	• siRNA silencing to rescue neuronal tissue in ischemic lesion. • Reduction of neuron apoptosis and promotion of behavioural recovery.	[91]

2.3.2. Dendrimers

Dendrimers have also found applications as neuroprotective modalities *in vivo*. For example, a recent study investigated the efficiency of biodegradable polycationic dendrimers (arginine ester of PAMAM dendrimer) in complexing small interfering RNA (siRNA)—specifically for the inhibition of High Mobility Group Box-1 (HMGB-1) [76]. HMGB-1 is a protein that plays a critical role as a mediator of local and systemic inflammation, activating various types of immune-related cells. Dendrimers were demonstrated to markedly reduce infarct volume in post-ischemic brains (middle cerebral artery occlusion (MCAO) model), highlighting their neuroprotective capacity.

Another possible therapeutic application of dendrimers in the CNS is in the treatment of Parkinson's. For example, poly-l-lysine dendrimers complexed with human glial cell line-derived neurotrophic factor gene (*h*-GDNF) have promoted the biochemical, anatomical and behaviour recovery of 6-OHDA pre-induced Parkinson's model (after direct injection in substantia nigra) [77]. These findings hold great promise, in particular as an effective treatment for reducing dopamine neurodegeneration. Similarly, Huang and co-workers [78,79] have developed PEGylated nanoparticles conjugated to lactoferrin and loaded with *h*-GDNF to treat different pre-induced Parkinson models (6-OHDA and rotenone-induced chronic model). Improvements in locomotor activity, along with reduction of dopaminergic neuron loss and enhancement of monoamine neurotransmitter levels were achieved after intravenous administration, which is seen as promising for gene therapy of chronic brain disorders.

2.3.3. Liposomes

Immunoliposomes (PEGylated liposomes with an antibody coupled) have been widely explored as a receptor mediated vector to deliver molecules inside the CNS. Recently, Xia et al. [80] have demonstrated that neurons of the nigrostriatal pathway could be rescued after single intravenous injection with immunoliposomes carrying GDNF plasmid and targeted with a transferrin antibody (OX-26). This approach was shown to improve behavioural symptoms of a pre-induced Parkinson's model (6-OHDA model). Nevertheless, the neuronal rescue was not complete, as the tyrosine hydroxylase enzyme activity in the striatum was only around 10% of the contralateral striatum.

Treatment of stroke has also benefited from the advances in liposome research in the last decades. PEGylated liposome formulations carrying haemoglobin have been successfully reported in the treatment of stroke (MCAO model), by the reduction of the infarct area and oedema associated to this ischemic pathology [81,82], after direct injection in the brain.

2.3.4. Fullerenes

One of the strategies proposed for the treatment of Alzheimer's disease is the inhibition of amyloid-β aggregates. It was in this context that intracerebroventricular injections of C_{60} fullerenes were administered, resulting in the improved performance of cognitive tasks in rats induced by amyloid-β peptide [83]. Fullerene-based therapy is a valuable option for the treatment of other neurodegeneration diseases, including multiple sclerosis, which is characterised by axon degeneration. Recent studies using a fullerene derivative (ABS-75) conjugated with glutamate receptor antagonists have demonstrated the capacity of the system to reduce the clinical progression of chronic multiple sclerosis (experimental autoimmune encephalitis murine model) [84]. This clinical recovery was attributed to a reduction of axonal loss and demyelination in the spinal cord.

Moreover, polyhydroxylated fullerenes have been shown to be a powerful radical scavenger, preventing mitochondrial oxidative damage in an acute cellular Parkinson model ($MPTP^+$ model induced in human neuroblastoma cells) [85]. The neuroprotective capacity of fullerenes has also been suggested by Huang and colleagues [86,87] who demonstrated that intravenous administration of hexasulfobutylated fullerenes (FC_4S) could effectively suppress infarct size on focal cerebral ischemia rat and gerbil models (MCAO model). Similar results were obtained in MCAO rat model, after intracerebroventricular administration with carboxyfullerene [88]. Despite some concerns regarding the possible *in vivo* biocompatibility of this material, all these studies provided evidence that supports the biomedical application of fullerenes in the treatment or prevention of neurodegenerative diseases.

2.3.5. Carbon nanotubes

CNT-based technology has also been investigated as an option for the treatment of neurodegenerative diseases. Lee et al. [89] investigated the potential use of amine-functionalised single walled carbon nanotubes (SWNT) (prepared by amidation reaction of pre-oxidised SWNT) to enhance the survival of neurons following ischemic injury in a MCAO stroke model. Intracerebroventricular injections of SWNT without any therapeutic molecule have been shown to enhance motor function recovery of the animals, however, the mechanism for such activity remains elusive [89,90]. Recently, our laboratories [91] have also demonstrated the effectiveness of amino-functionalised MWNT (by the 1, 3-dipolar cycloaddition reaction) in mediating the delivery of siRNA (specific to silence caspase 3) *in vivo*, promoting behavioral recovery in endothelin-1 stroke murine models. Unlike the former work performed by Lee at al. [89], in the latter no neuroprotective activity was attributable either to CNT alone or CNT complexed with scrambled siRNA. Nevertheless, a direct comparison between both studies is difficult to make because of significant differences in the protocols followed.

2.4. Nanomaterials in the treatment of CNS traumatic injury

Acute neurological incidents are devastating events that dramatically affect the quality of life of the patient [92]. Table 5 details the major studies selected from the literature using different nanomaterials in the treatment of traumatic injuries of the CNS.

2.4.1. Nanoparticles

The application of polymer-coated nanoparticles has been investigated as a novel therapeutic approach to promote behaviour recovery of different spinal cord injury (SCI) models. Evidence of substantial neuroregeneration and physiological recovery of damaged neural tissues was likely associated to the hydrophilic character of polymer chosen. PEG as well as other polymers such as chitosan, poloxamines or poloxamers are considered fusogens with a capacity to fuse and repair cellular membranes [92]. For example, electrophysiological recordings have revealed that PEG-coated silica nanoparticles could specifically target damaged spinal cord white matter, repairing damaged neuronal membranes and producing functional recovery in guinea pig spinal-cord injury model [93].

2.4.2. Self-assembling nanofiber scaffolds

The development of nano-engineering scaffolds has led to the creation of novel strategies for CNS repair. Self-assembling peptide nanofiber scaffold (SAPNS) has been shown to promote a favourable environment for the stimulation of cell-signalling pathways, a critical process in neuroregenerative medicine. SAPNS were found to support neuronal cells, allowing neurite outgrowth and functional synapse formation among neurons [94]. Furthermore, Ellis-Behnke et al. [95] have demonstrated that SAPNS not only create a permissive environment for the axons to regenerate, but also promote the connection of brain tissues, improving the functional recovery of CNS in a traumatic brain injury model (damaged visual model caused by midbrain lesion in hamsters).

Another neuro-regenerative strategy was based on the use of amphiphile molecules (isoleucine-lysine-valine-alanine-valine (IKVAV)) carrying neuronal progenitor cells. These peptides, under physiological ionic conditions, are designed to self assemble into a nanofiber scaffold network. Cells treated with these artificial nanofiber scaffolds showed extended and rapid differentiation into mature neuronal phenotypes

Table 5
Studies using nanomaterials for treatment of CNS traumatic injury.

	Delivery system	Model	Route	Aim and main conclusions	Ref
Nanoparticles	PEGylated-coated silica nanoparticles	Guinea pig SCI (crushed)	Intravenous	• Restoration of anatomical structure and physiological function. • Recovery of spinal cord conduction *in vivo*.	[93]
Self assembled nanofibers	Self-assembling peptide nanofiber: arginine, alanine, aspartate, and alanine ((RADA)16-I)	Hamster TBI (optical track lesion)	Intracortical (lesion site)	• Reconstruction of continuous tissue substrate after CNS injury. • Recovery of visual function.	[95]
	Self-assembling peptide nanofiber: isoleucine-lysine-valine-alanine-valine (IKVAV)	Mouse SCI (laminectomy)	Spinal cord (lesion site)	• Recovery from spinal cord injury. • Partial recovery of behavioural function.	[96]
Carbon nanotubes	SWNT chemically functionalised with PEG	Rat SCI (severing)	Spinal cord (lesion site)	• Promotion of tissue repair and functional recovery. • Modest improvement in hind-limb locomotor function.	[100]

when compared with controls. Interestingly, inhibition of astrocyte development following IKVAV treatment was also observed. Recently, IKVAV peptides were directly injected into an injured spinal cord model (FEJOTA mouse clips model) promoting regeneration of both motor fibres (ascendant and descendent sensory fibres) through the lesion site and improving the behavioural recovery of the animals [96].

2.4.3. Carbon nanotubes

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 [97]. Previous studies using different *in vitro* models to mimic neuronal tissue have illustrated the potential of CNT as substrates for neuronal growth, without compromising cell viability [98,99]. These studies highlight the possibility of using nanotubes as a compatible platform for neural tissue, without negatively impacting upon cell viability, whilst simultaneously promoting the formation of new synapses and developing functional neuronal circuits. Recently, the role of CNT in the treatment of traumatic CNS injuries has been recognised by Roman et al. [100]. Direct injection of SWNT (chemically functionalised with PEG) was shown to promote axonal regeneration at the SC of a rat model with SCI (lesion at T9 vertebral level). This study constitutes the first evidence to date that CNT are able to promote regeneration of damaged CNS tissues *in vivo*, opening up new perspectives in the field of neuroregenerative medicine.

2.5. Compatibility with the CNS and neurotoxicity of nanomaterials

The development of the biological application of nanomaterials in the last few decades has given rise to concerns regarding their possible toxicological effects. A clear understanding of how engineered nanoscale materials act upon living organisms, particularly within the CNS, is therefore crucial to the sustained development of neuro-nanomedicine. In this section, we attempt to highlight research using manufactured or engineered nanomaterials for medical purposes, without covering the interaction of combustion-derived nanoparticles (*e.g.* particulate matters, diesel exhaust particles, welding fumes) with the CNS.

2.5.1. Nanoparticles

The translocation of nanoparticles through BBB has been described in the literature, albeit with some conflicting data regarding the neurotoxicity of the material. On the one hand, nanoparticles have been associated with disturbance of the BBB [24,101]. For example, Oliver et al. [102] demonstrated that although intravenous administration of polysorbate-80 coated-PBCA nanoparticles carrying dalargine resulted in prolonged and potent analgesia effect, occasional mortality was also noted likely due to disruption of BBB. On the other hand, other studies have demonstrated that similar nanoparticles were able to translocate through BBB by specific mechanisms (endocytosis or transcytosis) and not by disruption of the barrier [103]. These contradictory results underline the importance of a full physicochemical characterisation of the materials and of the methodology used, as such divergence might be attributable to these factors.

Similarly, concerns regarding the neurotoxicity of metal nanoparticles have emerged due to their possible effects on the brain vasculature [104]. Chen et al. [104] demonstrated that the cerebral vasculature, in particular the expression of tight junction proteins (claudin-5 and occluding protein), may be affected after intravenous injection of aluminium oxide nanoparticles. The relationship between the type of metal nanoparticle and the induced neurotoxicity effects has also been investigated [105]. After intravenous administration of different metal nanoparticles (Ag, Cu or Al) in rats and mice, noticeable changes were observed in nerve cells, glial cells and myelin. This work demonstrated that silver nanoparticles induced more BBB disruption and caused more neuronal degeneration than other metallic nanoparticles. The same group also studied the influence of hyperthermia on the distribution of metal nanoparticles and their possible neurotoxicity [101]. Under these conditions, for all nanoparticles tested, an exacerbation of cognitive and motor dysfunction, as well as BBB disruption, was observed.

Another potential transport route to overcome the selectivity of the BBB that has been considered is intranasal administration. Several studies have shown that the olfactory nerve pathway can be a portal of entry to the CNS [24,106,107]. Some authors showed that inhaled nano-sized particles could accumulate in the nasal cavity, lung and brain of rats, causing harmful inflammation and risk of brain damage. Wang et al. [108] showed that iron oxide nanoparticles could reach the CNS through the olfactory nerve pathway, demonstrating for the first time higher induction of oxidative stress and nerve cell damage when compared to submicron-sized particles. The same group also reported that inhalation of titanium oxide nanoparticles in mice could induce neurological lesions, in particular swelling and disruption of mitochondrial membrane of microglia cells [109]. The shape of the nanoparticles can also influence their neurotoxicological profile, as a recent showed by Hutter et al. [110]. This study explored the *in vivo* microglia activation following intranasal administration of gold nanoparticles (coated with PEG) with different morphologies (spherical, rod and urchin). It was shown that rod and urchin geometries preferentially led to transient microglia activation, whereas spherical gold nanoparticles caused a small but detectable activation of microglia that only sustained over one week.

2.5.2. Quantum dots

Glial and neuron responsiveness after administration of QDots has also been described in the literature. Maysinger et al. [111] assessed the activation of astrocytes after intra-cerebral administration with different nanoparticles in GFAP-luciferase transgenic mice. Cerium oxide nanoparticles were not shown to induce astrocyte activation,

whereas non-PEGylated cadmium telluride nanoparticles led to robust and persistent activation of astrocytes. This work also included intracerebral injection of PEGylated QDots, which demonstrated a transient activation of astrocytes up to 7 days. The same group further investigated microglia activation induced by nanoparticles with different surface characteristics after intranasal administration [112]. The study used QDot (CdSe/ZnS) nanoparticles bearing cysteamine, PEG or lipopolysaccharide (LPS) and showed that only LPS-QDots induced microglia activation *in vivo*.

2.5.3. Nanowires

In the near future, nanowires are expected to be used in combination with microelectrodes for brain implantation of neuroprosthetic devices. In a recent study, the insertion of nanowires in the striatum of rats has been shown not to affect neuron viability, leading only to a transient activation of glial cells [113], thus offering evidence for optimism regarding their future application.

2.5.4. Fullerenes

Although the biological attributes of fullerenes as biomedical devices have been recognised, there are concerns regarding their neurotoxicity. In a recent study, intracerebral injections of C_{60} fullerenes led to abnormal changes of neurotransmitter levels (serotoninergic and dopaminergic) in several brain regions, suggesting that chronic exposure to fullerenes could affect brain homeostasis [114]. The same group also demonstrated that intracerebral injections of polyhydroxylated fullerenes have an acute but not permanent harmful effect on the CNS, in particular on the monoamine neurotransmission and locomotor activity [115]. Further studies need to be conducted in order to clarify this issue.

2.5.5. Carbon nanotubes

The literature has reported only a few *in vivo* studies illustrating the biocompatibility of CNT within the neuronal tissue. Van Handal et al. [116] reported the uptake and toxicity of pristine MWNT (coated with Pluronic F-108) after intra-tumoural injection within an intracranial glioma tumour model (GL261 model). It has been suggested that there is a preferential accumulation of MWNT in tumour macrophages in a dose dependent manner. Furthermore, the same group also showed that SWNT (chemically functionalised with PEG_{2000}) could deliver and promote the uptake of CpG oligodeoxynucleotide by tumour-associated inflammatory cells and (to a lesser extent) glioma cells, without showing major signs of toxicity. In both studies the direct intratumoural injection of the nanotubes was well tolerated, eliciting only a transient and self-limiting local inflammatory response. To date, the only *in vivo* study exploring the biocompatibility of CNT in brains of healthy animals was carried out by Bardi et al. [117]. In this work, pristine MWNT (coated with Pluronic F-127) were injected into the visual cortex of a mouse and no adverse toxicological effects were observed at the cellular level.

3. Conclusion

One of the major challenges for modern medicine is the development of an effective strategy for the prevention, diagnosis and treatment of CNS pathologies. The specificity of the CNS environment, along with its restricted anatomical access, provides serious obstacles to the successful outcome of diagnostics and therapeutics. In the last few years the application of nanomaterials in neuroscience has started to be explored with success, and new diagnostic and therapeutic options have emerged. The development of nanomaterials may be expected to lead to revolutionary therapeutic approaches, however, there are still fundamental gaps of knowledge regarding the pharmacological and toxicological profile of nanomaterials within CNS that need to be addressed.

References

[1] W.M. Pardridge, The blood–brain barrier: bottleneck in brain drug development, NeuroRx 2 (2005) 3–14.
[2] D.J. Begley, Delivery of therapeutic agents to the central nervous system: the problems and the possibilities, Pharmacol. Ther. 104 (2004) 29–45.
[3] W.M. Pardridge, Blood–brain barrier delivery, Drug Discov. Today 12 (2007) 54–61.
[4] S. Bhaskar, F.R. Tian, T. Stoeger, W. Kreyling, J.M. de la Fuente, V. Grazu, P. Borm, G. Estrada, V. Ntziachristos, D. Razansky, Multifunctional nanocarriers for diagnostics, drug delivery and targeted treatment across blood–brain barrier: perspectives on tracking and neuroimaging, Part. Fibre Toxicol. 7 (2010).
[5] V. Mishra, S. Mahor, A. Rawat, P.N. Gupta, P. Dubey, K. Khatri, S.P. Vyas, Targeted brain delivery of AZT *via* transferrin anchored pegylated albumin nanoparticles, J. Drug Target. 14 (2006) 45–53.
[6] J. Kreuter, R.N. Alyautdin, D.A. Kharkevich, A.A. Ivanov, Passage of peptides through the blood–brain-barrier with colloidal polymer particles (nanoparticles), Brain Res. 674 (1995) 171–174.
[7] U. Schroder, B.A. Sabel, Nanoparticles, a drug carrier system to pass the blood–brain barrier, permit central analgesic effects of iv dalargin injections, Brain Res. 710 (1996) 121–124.
[8] S.E. Gelperina, A.S. Khalansky, I.N. Skidan, Z.S. Smirnova, A.I. Bobruskin, S.E. Severin, B. Turowski, F.E. Zanella, J. Kreuter, Toxicological studies of doxorubicin bound to polysorbate 80-coated poly(butyl cyanoacrylate) nanoparticles in healthy rats and rats with intracranial glioblastoma, Toxicol. Lett. 126 (2002) 131–141.
[9] U. Schroeder, P. Sommerfeld, S. Ulrich, B.A. Sabel, Nanoparticle technology for delivery of drugs across the blood–brain barrier, J. Pharm. Sci.-Us. 87 (1998) 1305–1307.
[10] A. Friese, E. Seiller, G. Quack, B. Lorenz, J. Kreuter, Increase of the duration of the anticonvulsive activity of a novel NMDA receptor antagonist using poly(butylcyanoacrylate) nanoparticles as a parenteral controlled release system, Eur. J. Pharm. Biopharm. 49 (2000) 103–109.
[11] P. Calvo, B. Gouritin, I. Brigger, C. Lasmezas, J.P. Deslys, A. Williams, J.P. Andreux, D. Dormont, P. Couvreur, PEGylated polycyanoacrylate nanoparticles as vector for drug delivery in prion diseases, J. Neurosci. Methods 111 (2001) 151–155.
[12] B. Wilson, M.K. Samanta, K. Santhi, K.P.S. Kumar, N. Paramakrishnan, B. Suresh, Targeted delivery of tacrine into the brain with polysorbate 80-coated poly(n-butylcyanoacrylate) nanoparticles, Eur. J. Pharm. Biopharm. 70 (2008) 75–84.
[13] B. Wilson, M.K. Samanta, K. Santhi, K.P.S. Kumar, N. Paramakrishnan, B. Suresh, Poly(n-butylcyanoacrylate) nanoparticles coated with polysorbate 80 for the targeted delivery of rivastigmine into the brain to treat Alzheimer's disease, Brain Res. 1200 (2008) 159–168.
[14] Y.C. Kuo, F.L. Su, Transport of stavudine, delavirdine, and saquinavir across the blood–brain barrier by polybutylcyanoacrylate, methylmethacrylate-sulfopropylmethacrylate, and solid lipid nanoparticles, Int. J. Pharm. 340 (2007) 143–152.
[15] K. Michaelis, M.M. Hoffmann, S. Dreis, E. Herbert, R.N. Alyautdin, M. Michaelis, J. Kreuter, K. Langer, Covalent linkage of apolipoprotein E to albumin nanoparticles strongly enhances drug transport into the brain, J. Pharmacol. Exp. Ther. 317 (2006) 1246–1253.
[16] A. Zensi, D. Begley, C. Pontikis, C. Legros, L. Mihoreanu, S. Wagner, C. Buchel, H. von Briesen, J. Kreuter, Albumin nanoparticles targeted with Apo E enter the CNS by transcytosis and are delivered to neurones, J. Control. Release 137 (2009) 78–86.
[17] G. Tosi, L. Costantino, F. Rivasi, B. Ruozi, E. Leo, A.V. Vergoni, R. Tacchi, A. Bertolini, M.A. Vandelli, F. Forni, Targeting the central nervous system: *in vivo* experiments with peptide-derivatized nanoparticles loaded with Loperamide and Rhodamine-123, J. Control. Release 122 (2007) 1–9.
[18] C. Olbrich, A. Gessner, O. Kayser, R.H. Muller, Lipid-drug-conjugate (LDC) nanoparticles as novel carrier system for the hydrophilic antitrypanosomal drug diminazenediaceturate, J. Drug Target. 10 (2002) 387–396.
[19] J.X. Wang, X. Sun, Z.R. Zhang, Enhanced brain targeting by synthesis of 3′,5′-dioctanoyl-5-fluoro-2′-deoxyuridine and incorporation into solid lipid nanoparticles, Eur. J. Pharm. Biopharm. 54 (2002) 285–290.
[20] N.Y. Shi, Y. Zhang, C.N. Zhu, R.J. Boado, W.M. Pardridge, Brain-specific expression of an exogenous gene after i.v. administration, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 12754–12759.
[21] X.B. Zhang, Y. Xie, Y.G. Jin, X.P. Hou, L.Y. Ye, J.N. Lou, The effect of RMP-7 and its derivative on transporting Evans blue liposomes into the brain, Drug Deliv. 11 (2004) 301–309.
[22] Y. Xie, L.Y. Ye, X.B. Zhang, W. Cui, J.N. Lou, T. Nagai, X.P. Hou, Transport of nerve growth factor encapsulated into liposomes across the blood–brain barrier: *in vitro* and *in vivo* studies, J. Control. Release 105 (2005) 106–119.
[23] W.L. Ke, K. Shao, R.Q. Huang, L. Han, Y. Liu, J.F. Li, Y.Y. Kuang, L.Y. Ye, J.N. Lou, C. Jiang, Gene delivery targeted to the brain using an angiopep-conjugated polyethyleneglycol-modified polyamidoamine dendrimer, Biomaterials 30 (2009) 6976–6985.
[24] T.T. Win-Shwe, H. Fujimaki, Nanoparticles and neurotoxicity, Int. J. Mol. Sci. 12 (2011) 6267–6280.
[25] I. van Rooy, S. Cakir-Tascioglu, W.E. Hennink, G. Storm, R.M. Schiffelers, E. Mastrobattista, *In vivo* methods to study uptake of nanoparticles into the brain, Pharm. Res. 28 (2011) 456–471.
[26] G.A. Silva, Neuroscience nanotechnology: progress, opportunities and challenges, Nat. Rev. Neurosci. 7 (2006) 65–74.
[27] T. Misgeld, M. Kerschensteiner, *In vivo* imaging of the diseased nervous system, Nat. Rev. Neurosci. 7 (2006) 449–463.

[28] K.D.W., Rodolfo R. Llinas, Masayuki Nakao, Ian Hunter, Patrick A. Anquetil, Neuro-vascular central nervous recording/stimulating system: using nanotechnology probes, J. Nanopart. Res. 7 (2005) 111–127.
[29] J.S. Weinstein, C.G. Varallyay, E. Dosa, S. Gahramanov, B. Hamilton, W.D. Rooney, L.L. Muldoon, E.A. Neuwelt, Superparamagnetic iron oxide nanoparticles: diagnostic magnetic resonance imaging and potential therapeutic applications in neurooncology and central nervous system inflammatory pathologies, a review, J. Cereb. Blood Flow Metab. 30 (2009) 15–35.
[30] J.L. Winer, P.E. Kim, M. Law, C.Y. Liu, M.L.J. Apuzzo, Visualizing the future: enhancing neuroimaging with nanotechnology, World Neurosurg. 75 (2011) 626–637.
[31] E.A. Neuwelt, P. Varallyay, A.G. Bago, L.L. Muldoon, G. Nesbit, R. Nixon, Imaging of iron oxide nanoparticles by MR and light microscopy in patients with malignant brain tumours, Neuropathol. Appl. Neurobiol. 30 (2004) 456–471.
[32] E.A. Neuwelt, C.G. Varallyay, S. Manninger, D. Solymosi, M. Haluska, M.A. Hunt, G. Nesbit, A. Stevens, M. Jerosch-Herold, P.M. Jacobs, J.M. Hoffman, The potential of ferumoxytol nanoparticle magnetic resonance imaging, perfusion, and angiography in central nervous system malignancy: a pilot study, Neurosurgery 60 (2007) 601–612.
[33] A. Saleh, M. Schroeter, C. Jonkmanns, H.P. Hartung, U. Modder, S. Jander, *In vivo* MRI of brain inflammation in human ischaemic stroke, Brain 127 (2004) 1670–1677.
[34] E. Sykova, P. Jendelova, Magnetic resonance tracking of transplanted stem cells in rat brain and spinal cord, Neurodegener. Dis. 3 (2006) 62–67.
[35] F. Callera, C.M. de Melo, Magnetic resonance tracking of magnetically labeled autologous bone marrow CD34+ cells transplanted into the spinal cord *via* lumbar puncture technique in patients with chronic spinal cord injury: CD34+ cells' migration into the injured site, Stem Cells Dev. 16 (2007) 461–466.
[36] B. Kateb, K. Chiu, K.L. Black, V. Yamamoto, B. Khalsa, J.Y. Ljubimova, H. Ding, R. Patil, J.A. Portilla-Arias, M. Modo, D.F. Moore, K. Farahani, M.S. Okun, N. Prakash, J. Neman, D. Ahdoot, W. Grundfest, S. Nikzad, J.D. Heiss, Nanoplatforms for constructing new approaches to cancer treatment, imaging, and drug delivery: what should be the policy? NeuroImage 54 (2011) S106–S124.
[37] H. Jackson, O. Muhammad, H. Daneshvar, J. Nelms, A. Popescu, M.A. Vogelbaum, M. Bruchez, S.A. Toms, Quantum dots are phagocytized by macrophages and colocalize with experimental gliomas, Neurosurgery 60 (2007) 524–530.
[38] S. Santra, H. Yang, J.T. Stanley, P.H. Holloway, B.M. Moudgil, G. Walter, R.A. Mericle, Rapid and effective labeling of brain tissue using TAT-conjugated CdS:Mn/ZnS quantum dots, Chem. Commun. (Camb) 7 (2005) 3144–3146.
[39] M.J. Levene, D.A. Dombeck, K.A. Kasischke, R.P. Molloy, W.W. Webb, *In vivo* multiphoton microscopy of deep brain tissue, J. Neurophysiol. 91 (2004) 1908–1912.
[40] X. Gao, J. Chen, J. Chen, B. Wu, H. Chen, X. Jiang, Quantum dots bearing lectin-functionalized nanoparticles as a platform for *in vivo* brain imaging, Bioconjug. Chem. 19 (2008) 2189–2195.
[41] H.L. Xin, L.C. Chen, J.J. Gu, X.Q. Ren, Z. Wei, J.Q. Luo, Y.Z. Chen, X.Y. Jiang, X.Y. Sha, X.L. Fang, Enhanced anti-glioblastoma efficacy by PTX-loaded PEGylated poly(epsilon-caprolactone) nanoparticles: *in vitro* and *in vivo* evaluation, Int. J. Pharm. 402 (2010) 238–247.
[42] M. Zhao, C. Liang, A.M. Li, J. Chang, H.J. Wang, R.M. Yan, J.J. Zhang, J.L. Tai, Magnetic paclitaxel nanoparticles inhibit glioma growth and improve the survival of rats bearing glioma xenografts, Anticancer. Res. 30 (2010) 2217–2223.
[43] P. Couvreur, B. Kante, L. Grislain, M. Roland, P. Speiser, Toxicity of polyalkylcyanoacrylate nanoparticles II: doxorubicin-loaded nanoparticles, J. Pharm. Sci. 71 (1982) 790–792.
[44] A.E. Gulyaev, S.E. Gelperina, I.N. Skidan, A.S. Antropov, G.Y. Kivman, J. Kreuter, Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles, Pharm. Res. 16 (1999) 1564–1569.
[45] S.C. Steiniger, J. Kreuter, A.S. Khalansky, I.N. Skidan, A.I. Bobruskin, Z.S. Smirnova, S.E. Severin, R. Uhl, M. Kock, K.D. Geiger, S.E. Gelperina, Chemotherapy of glioblastoma in rats using doxorubicin-loaded nanoparticles, Int. J. Cancer 109 (2004) 759–767.
[46] P.Y. Wen, S. Kesari, Malignant gliomas in adults, N. Engl. J. Med. 359 (2008) 492–507.
[47] F.K.H. van Landeghem, K. Maier-Hauff, A. Jordan, K.-T. Hoffmann, U. Gneveckow, R. Scholz, B. Thiesen, W. Brück, A. von Deimling, Post-mortem studies in glioblastoma patients treated with thermotherapy using magnetic nanoparticles, Biomaterials 30 (2009) 52–57.
[48] A. Jordan, P. Wust, R. Scholz, B. Tesche, H. Fahling, T. Mitrovics, T. Vogl, J. Cervos-Navarro, R. Felix, Cellular uptake of magnetic fluid particles and their effects on human adenocarcinoma cells exposed to AC magnetic fields *in vitro*, Int. J. Hyperthermia 12 (1996) 705–722.
[49] K. Maier-Hauff, R. Rothe, R. Scholz, U. Gneveckow, P. Wust, B. Thiesen, A. Feussner, A. von Deimling, N. Waldoefner, R. Felix, A. Jordan, Intracranial thermotherapy using magnetic nanoparticles combined with external beam radiotherapy: results of a feasibility study on patients with glioblastoma multiforme, J. Neurooncol. 81 (2007) 53–60.
[50] A. Jordan, R. Scholz, K. Maier-Hauff, F.K.H. van Landeghem, N. Waldoefner, U. Teichgraeber, J. Pinkernelle, H. Bruhn, F. Neumann, B. Thiesen, A. von Deimling, R. Felix, The effect of thermotherapy using magnetic nanoparticles on rat malignant glioma, J. Neurooncol. 78 (2006) 7–14.
[51] K. Maier-Hauff, F. Ulrich, D. Nestler, H. Niehoff, P. Wust, B. Thiesen, H. Orawa, V. Budach, A. Jordan, Efficacy and safety of intratumoral thermotherapy using magnetic iron-oxide nanoparticles combined with external beam radiotherapy on patients with recurrent glioblastoma multiforme, J. Neurooncol. 103 (2011) 317–324.
[52] W. Lu, Q. Sun, J. Wan, Z. She, X.G. Jiang, Cationic albumin-conjugated pegylated nanoparticles allow gene delivery into brain tumors *via* intravenous administration, Cancer Res. 66 (2006) 11878–11887.
[53] R. Kopelman, Y.-E. Lee Koo, M. Philbert, B.A. Moffat, G. Ramachandra Reddy, P. McConville, D.E. Hall, T.L. Chenevert, M.S. Bhojani, S.M. Buck, A. Rehemtulla, B.D. Ross, Multifunctional nanoparticle platforms for *in vivo* MRI enhancement and photodynamic therapy of a rat brain cancer, J. Magn. Magn. Mater. 293 (2005) 404–410.
[54] L. Agemy, D. Friedmann-Morvinski, V.R. Kotamraju, L. Roth, K.N. Sugahara, O.M. Girard, R.F. Mattrey, I.M. Verma, E. Ruoslahti, Targeted nanoparticle enhanced proapoptotic peptide as potential therapy for glioblastoma, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 17450–17455.
[55] S.K. Pulfer, J.M. Gallo, Enhanced brain tumor selectivity of cationic magnetic polysaccharide microspheres, J. Drug Target. 6 (1998) 215–227.
[56] M. Yemisci, S. Bozdag, M. Cetin, F. Soylemezoglu, Y. Capan, T. Dalkara, I. Vural, Treatment of malignant gliomas with mitoxantrone-loaded poly (lactide-co-glycolide) microspheres, Neurosurgery 59 (2006) 1296–1303.
[57] O. Benny, L.G. Menon, G. Ariel, E. Goren, S.K. Kim, C. Stewman, P.M. Black, R.S. Carroll, M. Machluf, Local delivery of poly lactic-co-glycolic acid microspheres containing imatinib mesylate inhibits intracranial xenograft glioma growth, Clin. Cancer Res. 15 (2009) 1222–1231.
[58] C.E. Holt, N. Garlick, E. Cornel, Lipofection of cDNAs in the embryonic vertebrate central nervous system, Neuron 4 (1990) 203–214.
[59] A. Doi, S. Kawabata, K. Iida, K. Yokoyama, Y. Kajimoto, T. Kuroiwa, T. Shirakawa, M. Kirihata, S. Kasaoka, K. Maruyama, H. Kumada, Y. Sakurai, S. Masunaga, K. Ono, S. Miyatake, Tumor-specific targeting of sodium borocaptate (BSH) to malignant glioma by transferrin-PEG liposomes: a modality for boron neutron capture therapy, J. Neurooncol. 87 (2008) 287–294.
[60] B. Feng, K. Tomizawa, H. Michiue, S. Miyatake, X.J. Han, A. Fujimura, M. Seno, M. Kirihata, H. Matsui, Delivery of sodium borocaptate to glioma cells using immunoliposome conjugated with anti-EGFR antibodies by ZZ-His, Biomaterials 30 (2009) 1746–1755.
[61] M.T. Krauze, C.O. Noble, T. Kawaguchi, D. Drummond, D.B. Kirpotin, Y. Yamashita, E. Kullberg, J. Forsayeth, J.W. Park, K.S. Bankiewicz, Convection-enhanced delivery of nanoliposomal CPT-11 (irinotecan) and PEGylated liposomal doxorubicin (Doxil) in rodent intracranial brain tumor xenografts, Neuro Oncol. 9 (2007) 393–403.
[62] A.Y. Grahn, K.S. Bankiewicz, M. Dugich-Djordjevic, J.R. Bringas, P. Hadaczek, G.A. Johnson, S. Eastman, M. Luz, Non-PEGylated liposomes for convection-enhanced delivery of topotecan and gadodiamide in malignant glioma: initial experience, J. Neurooncol. 95 (2009) 185–197.
[63] G. Wu, R.F. Barth, W.L. Yang, S. Kawabata, L.W. Zhang, K. Green-Church, Targeted delivery of methotrexate to epidermal growth factor receptor-positive brain tumors by means of cetuximab (IMC-C225) dendrimer bioconjugates, Mol. Cancer Ther. 5 (2006) 52–59.
[64] Z. Cui, P.R. Lockman, C.S. Atwood, C.-H. Hsu, A. Gupte, D.D. Allen, R.J. Mumper, Novel D-penicillamine carrying nanoparticles for metal chelation therapy in Alzheimer's and other CNS diseases, Eur. J. Pharm. Biopharm. 59 (2005) 263–272.
[65] G. Liu, P. Men, P.L.R. Harris, R.K. Rolston, G. Perry, M.A. Smith, Nanoparticle iron chelators: A new therapeutic approach in Alzheimer disease and other neurologic disorders associated with trace metal imbalance, Neurosci. Lett. 406 (2006) 189–193.
[66] G. Liu, P. Men, W. Kudo, G. Perry, M.A. Smith, Nanoparticle-chelator conjugates as inhibitors of amyloid-[beta] aggregation and neurotoxicity: a novel therapeutic approach for Alzheimer disease, Neurosci. Lett. 455 (2009) 187–190.
[67] M.K. Reddy, L. Wu, W. Kou, A. Ghorpade, V. Labhasetwar, Superoxide dismutase-loaded PLGA nanoparticles protect cultured human neurons under oxidative stress, Appl. Biochem. Biotechnol. 151 (2008) 565–577.
[68] D. Schubert, R. Dargusch, J. Raitano, S.W. Chan, Cerium and yttrium oxide nanoparticles are neuroprotective, Biochem. Biophys. Res. Commun. 342 (2006) 86–91.
[69] Y. Cho, R. Shi, R. Ben Borgens, Chitosan nanoparticle-based neuronal membrane sealing and neuroprotection following acrolein-induced cell injury, J. Biol. Eng. 4 (2010) 2.
[70] E.V. Batrakova, S. Li, A.D. Reynolds, R.L. Mosley, T.K. Bronich, A.V. Kabanov, H.E. Gendelman, A macrophage-nanozyme delivery system for Parkinson's disease, Bioconjug. Chem. 18 (2007) 1498–1506.
[71] S. Crotty, P. Fitzgerald, E. Tuohy, D.M. Harris, A. Fisher, A. Mandel, A.E. Bolton, A.M. Sullivan, Y. Nolan, Neuroprotective effects of novel phosphatidylglycerol-based phospholipids in the 6-hydroxydopamine model of Parkinson's disease, Eur. J. Neurosci. 27 (2008) 294–300.
[72] K.B. Kurakhmaeva, I.A. Djindjikhashvili, V.E. Petrov, V.U. Balabanyan, T.A. Voronina, S.S. Trofimov, J. Kreuter, S. Gelperina, D. Begley, R.N. Alyautdin, Brain targeting of nerve growth factor using poly(butyl cyanoacrylate) nanoparticles, J. Drug Target. 17 (2009) 564–574.
[73] K.L. Hu, Y.B. Shi, W.M. Jiang, J.Y. Han, S.X. Huang, X.G. Jiang, Lactoferrin conjugated PEG–PLGA nanoparticles for brain delivery: preparation, characterization and efficacy in Parkinson's disease, Int. J. Pharm. 415 (2011) 273–283.
[74] Z.Y. Wen, Z.Q. Yan, K.L. Hu, Z.Q. Pang, X.F. Cheng, L.R. Guo, Q.Z. Zhang, X.G. Jiang, L. Fang, R. Lai, Odorranalectin-conjugated nanoparticles: preparation, brain delivery and pharmacodynamic study on Parkinson's disease following intranasal administration, J. Control. Release 151 (2011) 131–138.
[75] H. Karatas, Y. Aktas, Y. Gursoy-Ozdemir, E. Bodur, M. Yemisci, S. Caban, A. Vural, O. Pinarbasli, Y. Capan, E. Fernandez-Megia, R. Novoa-Carballal, R. Riguera, K. Andrieux, P. Couvreur, T. Dalkara, A nanomedicine transports a peptide caspase-3 inhibitor across the blood–brain barrier and provides neuroprotection, J. Neurosci. 29 (2009) 13761–13769.
[76] I.D. Kim, C.M. Lim, J.B. Kim, H.Y. Nam, K. Nam, S.W. Kim, J.S. Park, J.K. Lee, Neuroprotection by biodegradable PAMAM ester (e-PAM-R)-mediated HMGB1

siRNA delivery in primary cortical cultures and in the postischemic brain, J. Control. Release 142 (2010) 422–430.
[77] J.A. Gonzalez-Barrios, M. Lindahl, M.J. Bannon, V. Anaya-Martinez, G. Flores, I. Navarro-Quiroga, L.E. Trudeau, J. Aceves, D.B. Martinez-Arguelles, R. Garcia-Villegas, I. Jimenez, J. Segovia, D. Martinez-Fong, Neurotensin polyplex as an efficient carrier for delivering the human GDNF gene into nigral dopamine neurons of hemiparkinsonian rats, Mol. Ther. 14 (2006) 857–865.
[78] R. Huang, L. Han, J. Li, F. Ren, W. Ke, C. Jiang, Y. Pei, Neuroprotection in a 6-hydroxydopamine-lesioned Parkinson model using lactoferrin-modified nanoparticles, J. Gene Med. 11 (2009) 754–763.
[79] R. Huang, W. Ke, Y. Liu, D. Wu, L. Feng, C. Jiang, Y. Pei, Gene therapy using lactoferrin-modified nanoparticles in a rotenone-induced chronic Parkinson model, J. Neurol. Sci. 290 (2010) 123–130.
[80] C.F. Xia, R.J. Boado, Y. Zhang, C. Chu, W.M. Pardridge, Intravenous glial-derived neurotrophic factor gene therapy of experimental Parkinson's disease with Trojan horse liposomes and a tyrosine hydroxylase promoter, J. Gene Med. 10 (2008) 306–315.
[81] A.T. Kawaguchi, D. Kurita, H. Furuya, M. Yamano, Y. Ogata, M. Haida, Liposome-encapsulated hemoglobin alleviates brain edema after permanent occlusion of the middle cerebral artery in rats, Artif. Organs 33 (2009) 153–158.
[82] A.T. Kawaguchi, D. Fukumoto, M. Haida, Y. Ogata, M. Yamano, H. Tsukada, Liposome-encapsulated hemoglobin reduces the size of cerebral infarction in the rat — evaluation with photochemically induced thrombosis of the middle cerebral artery, Stroke 38 (2007) 1626–1632.
[83] I.Y. Podolski, Z.A. Podlubnaya, E.A. Kosenko, E.A. Mugantseva, E.G. Makarova, L.G. Marsagishvili, M.D. Shpagina, Y.G. Kaminsky, G.V. Andrievsky, V.K. Klochkov, Effects of hydrated forms of C-60 fullerene on amyloid beta-peptide fibrillization *in vitro* and performance of the cognitive task, J. Nanosci. Nanotechnol. 7 (2007) 1479–1485.
[84] A.S. Basso, D. Frenkel, F.J. Quintana, F.A. Costa-Pinto, S. Petrovic-Stojkovic, L. Puckett, A. Monsonego, A. Bar-Shir, Y. Engel, M. Gozin, H.L. Weiner, Reversal of axonal loss and disability in a mouse model of progressive multiple sclerosis, J. Clin. Invest. 118 (2008) 1532–1543.
[85] X. Cai, H. Jia, Z. Liu, B. Hou, C. Luo, Z. Feng, W. Li, J. Liu, Polyhydroxylated fullerene derivative C(60)(OH)(24) prevents mitochondrial dysfunction and oxidative damage in an MPP(+)-induced cellular model of Parkinson's disease, J. Neurosci. Res. 86 (2008) 3622–3634.
[86] S.S. Huang, S.K. Tsai, C.L. Chih, L.Y. Chiang, H.M. Hsieh, C.M. Teng, M.C. Tsai, Neuroprotective effect of hexasulfobutylated C60 on rats subjected to focal cerebral ischemia, Free Radic. Biol. Med. 30 (2001) 643–649.
[87] D.Y. Yang, M.F. Wang, I.L. Chen, Y.C. Chan, M.S. Lee, F.C. Cheng, Systemic administration of a water-soluble hexasulfonated C(60) (FC(4)S) reduces cerebral ischemia-induced infarct volume in gerbils, Neurosci. Lett. 311 (2001) 121–124.
[88] A.M.Y. Lin, S.F. Fang, S.Z. Lin, C.K. Chou, T.Y. Luh, L.T. Ho, Local carboxyfullerene protects cortical infarction in rat brain, Neurosci. Res. 43 (2002) 317–321.
[89] H.J. Lee, J. Park, O.J. Yoon, H.W. Kim, Y. Lee do, H. Kim do, W.B. Lee, N.E. Lee, J.V. Bonventre, S.S. Kim, Amine-modified single-walled carbon nanotubes protect neurons from injury in a rat stroke model, Nat. Nanotechnol. 6 (2011) 121–125.
[90] P. Higgins, J. Dawson, M. Walters, Nanomedicine: nanotubes reduce stroke damage, Nat. Nanotechnol. 6 (2011) 83–84.
[91] K.T. Al-Jamal, L. Gherardini, G. Bardi, A. Nunes, C. Guo, C. Bussy, M.A. Herrero, A. Bianco, M. Prato, K. Kostarelos, T. Pizzorusso, Functional motor recovery from brain ischemic insult by carbon nanotube-mediated siRNA silencing, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 10952–10957.
[92] Y. Cho, R.B. Borgens, Polymer and nano-technology applications for repair and reconstruction of the central nervous system, Exp. Neurol. 233 (2012) 126–144.
[93] Y. Cho, R. Shi, R. Borgens, A. Ivanisevic, Repairing the damaged spinal cord and brain with nanomedicine, Small 4 (2008) 1676–1681.
[94] T.C. Holmes, S. de Lacalle, X. Su, G. Liu, A. Rich, S. Zhang, Extensive neurite outgrowth and active synapse formation on self-assembling peptide scaffolds, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 6728–6733.
[95] R.G. Ellis-Behnke, Y.X. Liang, S.W. You, D.K. Tay, S. Zhang, K.F. So, G.E. Schneider, Nano neuro knitting: peptide nanofiber scaffold for brain repair and axon regeneration with functional return of vision, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 5054–5059.
[96] V.M. Tysseling-Mattiace, V. Sahni, K.L. Niece, D. Birch, C. Czeisler, M.G. Fehlings, S.I. Stupp, J.A. Kessler, Self-assembling nanofibers inhibit glial scar formation and promote axon elongation after spinal cord injury, J. Neurosci. 28 (2008) 3814–3823.
[97] P.A. Tran, L.J. Zhang, T.J. Webster, Carbon nanofibers and carbon nanotubes in regenerative medicine, Adv. Drug Deliv. Rev. 61 (2009) 1097–1114.
[98] V. Lovat, D. Pantarotto, L. Lagostena, B. Cacciari, M. Grandolfo, M. Righi, G. Spalluto, M. Prato, L. Ballerini, Carbon nanotube substrates boost neuronal electrical signaling, Nano Lett. 5 (2005) 1107–1110.
[99] M.P. Mattson, R.C. Haddon, A.M. Rao, Molecular functionalization of carbon nanotubes and use as substrates for neuronal growth, J. Mol. Neurosci. 14 (2000) 175–182.
[100] J.A. Roman, T.L. Niedzielko, R.C. Haddon, V. Parpura, C.L. Floyd, Single-walled carbon nanotubes chemically functionalized with polyethylene glycol promote tissue repair in a rat model of spinal cord injury, J. Neurotraum 28 (2011) 2349–2362.
[101] H.S. Sharma, A. Sharma, S. Hari Shanker, Nanoparticles aggravate heat stress induced cognitive deficits, blood–brain barrier disruption, edema formation and brain pathology, Prog Brain Res., Elsevier, 2007, pp. 245–273.
[102] J.C. Olivier, L. Fenart, R. Chauvet, C. Pariat, R. Cecchelli, W. Couet, Indirect evidence that drug brain targeting using polysorbate 80-coated polybutylcyanoacrylate nanoparticles is related to toxicity, Pharm. Res. 16 (1999) 1836–1842.
[103] J. Kreuter, P. Ramge, V. Petrov, S. Hamm, S.E. Gelperina, B. Engelhardt, R. Alyautdin, H. von Briesen, D.J. Begley, Direct evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs to the CNS *via* specific mechanisms requiring prior binding of drug to the nanoparticles, Pharm. Res. 20 (2003) 409–416.
[104] L. Chen, R.A. Yokel, B. Hennig, M. Toborek, Manufactured aluminum oxide nanoparticles decrease expression of tight junction proteins in brain vasculature, J. Neuroimmune Pharmacol. 3 (2008) 286–295.
[105] H.S. Sharma, S.M. Hussain, J.J. Schlager, S.F. Ali, A. Sharma, Influence of nanoparticles on blood brain barrier permeability and brain edema formation in rats, Acta Neurochir. Suppl. 106 (2010) 359–364.
[106] G. Oberdorster, Z. Sharp, V. Atudorei, A. Elder, R. Gelein, W. Kreyling, C. Cox, Translocation of inhaled ultrafine particles to the brain, Inhal. Toxicol. 16 (2004) 437–445.
[107] A.J.D. De Lorenzo, The olfactory neuron and the blood–brain barrier, Ciba Foundation Symposium — Internal Secretions of the Pancreas (Colloquia on Endocrinology), John Wiley & Sons, Ltd, 2008, pp. 151–176.
[108] 2. W.F., Bing Wang, Motao Zhu, Yun Wang, Meng Wang, Yiqun Gu, Hong Ouyang, Huajian Wang, Ming Li, Yuliang Zhao, Zhifang Chai, Haifang Wang, Neurotoxicity of low-dose repeatedly intranasal instillation of nano- and submicron-sized ferric oxide particles in mice, J. Nanopart. Res. 11 (2009) 41–53.
[109] J. Wang, C. Chen, Y. Liu, F. Jiao, W. Li, F. Lao, Y. Li, B. Li, C. Ge, G. Zhou, Y. Gao, Y. Zhao, Z. Chai, Potential neurological lesion after nasal instillation of TiO(2) nanoparticles in the anatase and rutile crystal phases, Toxicol. Lett. 183 (2008) 72–80.
[110] E. Hutter, S. Boridy, S. Labrecque, M. Lalancette-Hebert, J. Kriz, F.M. Winnik, D. Maysinger, Microglial response to gold nanoparticles, ACS Nano 4 (2010) 2595–2606.
[111] D. Maysinger, M. Behrendt, M. Lalancette-Hebert, J. Kriz, Real-time imaging of astrocyte response to quantum dots: *in vivo* screening model system for biocompatibility of nanoparticles, Nano Lett. 7 (2007) 2513–2520.
[112] M. Lalancette-Hebert, A. Moquin, A.O. Choi, J. Kriz, D. Maysinger, Lipopolysaccharide-QD micelles induce marked induction of TLR2 and lipid droplet accumulation in olfactory bulb microglia, Mol. Pharm. 7 (2010) 1183–1194.
[113] C. Eriksson Linsmeier, C.N. Prinz, L.M. Pettersson, P. Caroff, L. Samuelson, J. Schouenborg, L. Montelius, N. Danielsen, Nanowire biocompatibility in the brain—looking for a needle in a 3D stack, Nano Lett. 9 (2009) 4184–4190.
[114] T. Yamada, D.Y. Jung, R. Sawada, A. Matsuoka, R. Nakaoka, T. Tsuchiya, Effects intracerebral microinjection and intraperitoneal injection of [60]fullerene on brain functions differ in rats, J. Nanosci. Nanotechnol. 8 (2008) 3973–3980.
[115] T. Yamada, R. Nakaoka, R. Sawada, A. Matsuoka, T. Tsuchiya, Effects of intracerebral microinjection of hydroxylated-[60]fullerene on brain monoamine concentrations and locomotor behavior in rats, J. Nanosci. Nanotechnol. 10 (2010) 604–611.
[116] M. VanHandal, D. Alizadeh, L.Y. Zhang, B. Kateb, M. Bronikowski, H. Manohara, B. Badie, Selective uptake of multi-walled carbon nanotubes by tumor macrophages in a murine glioma model, J. Neuroimmunol. 208 (2009) 3–9.
[117] G. Bardi, P. Tognini, G. Ciofani, V. Raffa, M. Costa, T. Pizzorusso, Pluronic-coated carbon nanotubes do not induce degeneration of cortical neurons *in vivo* and *in vitro*, Nanomedicine 5 (2009) 96–104.
[118] K.G. Petry, C. Boiziau, V. Dousset, B. Brochet, Magnetic resonance imaging of human brain macrophage infiltration, Neurotherapeutics 4 (2007) 434–442.
[119] O. Mykhaylyk, A. Cherchenko, A. Ilkin, N. Dudchenko, V. Ruditsa, M. Novoseletz, Y. Zozulya, Glial brain tumor targeting of magnetite nanoparticles in rats, J. Magn. Magn. Mater. 225 (2001) 241–247.
[120] C.H. Liu, J.Q. Ren, Z. You, J. Yang, C.-M. Liu, R. Uppal, P.K. Liu, Noninvasive detection of neural progenitor cells in living brains by MRI, FASEB J. 26 (2012).
[121] B. Tomanek, U. Iqbal, B. Blasiak, A. Abulrob, H. Albaghdadi, J.R. Matyas, D. Ponjevic, G.R. Sutherland, Evaluation of brain tumor vessels specific contrast agents for glioblastoma imaging, Neuro Oncol. 14 (2012) 53–63.
[122] M.A. Popescu, S.A. Toms, *In vivo* optical imaging using quantum dots for the management of brain tumors, Expert. Rev. Mol. Diagn. 6 (2006) 879–890.
[123] H.H. Yan, J.Y. Wang, P.W. Yi, H. Lei, C.Y. Zhan, C. Xie, L.L. Feng, J. Qian, J.H. Zhu, W.Y. Lu, C. Li, Imaging brain tumor by dendrimer-based optical/paramagnetic nanoprobe across the blood–brain barrier, Chem. Commun. 47 (2011) 8130–8132.
[124] E.W. Keefer, B.R. Botterman, M.I. Romero, A.F. Rossi, G.W. Gross, Carbon nanotube coating improves neuronal recordings, Nat. Nanotechnol. 3 (2008) 434–439.
[125] X. Jiang, H. Xin, X. Sha, J. Gu, Y. Jiang, K. Law, Y. Chen, L. Chen, X. Wang, X. Fang, PEGylated poly(trimethylene carbonate) nanoparticles loaded with paclitaxel for the treatment of advanced glioma: *in vitro* and *in vivo* evaluation, Int. J. Pharm. 420 (2011) 385–394.
[126] F.Y. Yang, M.C. Teng, M. Lu, H.F. Liang, Y.R. Lee, C.C. Yen, M.L. Liang, T.T. Wong, Treating glioblastoma multiforme with selective high-dose liposomal doxorubicin chemotherapy induced by repeated focused ultrasound, Int. J. Nanomedicine 7 (2012) 965–974.
[127] X.Y. Sun, Z.Q. Pang, H.X. Ye, B. Qiu, L.R. Guo, J.W. Li, J.F. Ren, Y. Qian, Q.Z. Zhang, J. Chen, X.G. Jiang, Co-delivery of pEGFP-hTRAIL and paclitaxel to brain glioma mediated by an angiopep-conjugated liposome, Biomaterials 33 (2012) 916–924.
[128] D.C. Zhao, D. Alizadeh, L.Y. Zhang, W. Liu, O. Farrukh, E. Manuel, D.J. Diamond, B. Badie, Carbon nanotubes enhance CpG uptake and potentiate antiglioma immunity, Clin. Cancer Res. 17 (2011) 771–782.
[129] J. Ren, S. Shen, D. Wang, Z. Xi, L. Guo, Z. Pang, Y. Qian, X. Sun, X. Jiang, The targeted delivery of anticancer drugs to brain glioma by PEGylated oxidized

multi-walled carbon nanotubes modified with angiopep-2, Biomaterials 33 (2012) 3324–3333.
[130] A.Y. Estevez, S. Pritchard, K. Harper, J.W. Aston, A. Lynch, J.J. Lucky, J.S. Ludington, P. Chatani, W.P. Mosenthal, J.C. Leiter, S. Andreescu, J.S. Erlichman, Neuroprotective mechanisms of cerium oxide nanoparticles in a mouse hippocampal brain slice model of ischemia, Free Radic. Biol. Med. 51 (2011) 1155–1163.
[131] M.J. Tsai, Y.B. Huang, P.C. Wu, Y.S. Fu, Y.R. Kao, J.Y. Fang, Y.H. Tsai, Oral apomorphine delivery from solid lipid nanoparticles with different monostearate emulsifiers: pharmacokinetic and behavioral evaluations, J. Pharm. Sci. 100 (2011) 547–557.
[132] H. Zhao, X.J. Bao, R.Z. Wang, G.L. Li, J. Gao, S.H. Ma, J.J. Wei, M. Feng, Y.J. Zhao, W.B. Ma, Y. Yang, Y.N. Li, Y.G. Kong, Postacute ischemia vascular endothelial growth factor transfer by transferrin-targeted liposomes attenuates ischemic brain injury after experimental stroke in rats, Hum. Gene Ther. 22 (2011) 207–215.
[133] Y.G. Zhang, Z. Yang, Y.L.A. Yang, L. Sun, D. Han, H. Li, C. Wang, Pharmacological and toxicological target organelles and safe use of single-walled carbon nanotubes as drug carriers in treating Alzheimer disease, Nanomedicine 6 (2010) 427–441.