Review

Nanomaterials and Neurodegeneration

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The increasing application of nanotechnology in various industrial, environmental, and human settings raises questions surrounding the potential adverse effects induced by nanosized materials to human health, including the possible neurotoxic and neuroinflammatory properties of those substances and their capability to induce neurodegeneration. In this review, a panel of metal oxide nanoparticles (NPs), namely titanium dioxide, silicon dioxide, zinc oxide, copper oxide, iron NPs, and carbon nanotubes have been focused. An overview has been provided of the in vitro and in vivo evidence of adverse effects to the central nervous system. Research indicated that these nanomaterials (NMs) not only reach the brain, but also can cause a certain degree of brain tissue damage, including cytotoxicity, genotoxicity, induction of oxidative stress, and inflammation, all potentially involved in the onset and progression of neurodegeneration. Surface chemistry of the NMs may play an important role in their localization and subsequent effects on the brain of rodents. In addition, NM shape differences may induce varying degrees of neurotoxicity. However, one of the potential biomedical applications of NMs is nanodevices for early diagnostic and novel therapeutic approaches to counteract age related diseases. In this context, engineered NMs were promising vehicles to carry diagnostic and therapeutic compounds across the blood–brain barrier, thereby representing very

Abbreviations: AChE, acetilcholinesterase; AD, Alzheimer's disease; Ag NPs, silver nanoparticles; AgNO3, silver nitrate; ALS, amyotrophic lateral sclerosis; AMNPs, amorphous silica nanoparticles; APP, ß-amyloid precursor protein; Aß1–42, amyloid-ß1–42 peptide; BACE1, beta-secretase 1 gene; bax, BCL2-associated X protein; BBB, blood–brain barrier; bcl-2, B-cell lymphoma 2 protein; casp8, caspase-8 gene; casp9, caspase-9 gene; CBF, cerebral-blood flow; CNS, central nervous system; CNT, carbon nanotubes; Comt, catechol-O-methyltransferase gene; COX-2, Cyclooxygenase-2 gene; CuO NPs, copper oxide nanoparticles; DA, dopamine; DAPI, 4',6-diamidino-2-phenylindole; DMSA, dimercaptosuccinic acid; DNA, deoxyribonucleic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; ERK, extracellular-signal-regulated kinase; Fe2O3 NPs, maghemite nanoparticles; Fe3O4 NPs, magnetite nanoparticles; FITC, fluorescein isothiocyanate; GADD45, growth arrest and DNA damage-inducible 45; GFAP, glial fibrillary acidic protein; Gpr37, G protein-coupled receptor 37 gene; Gpx1, glutathione peroxidase 1 gene; GSH, glutathione; GSH-PX, glutathione peroxidase; 5-HT, 5-hydroxytryptamine; H2O2, hydrogen peroxide; HD, Huntington's disease; hGDNF, human glial cell-derived neurotrophic factor gene; HO-1, heme oxygenase 1; HVA, homovanillic acid; IL-10, interleukin-10; IL-1ß, interleukin-1 beta; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; IONPs, iron oxide nanoparticles; LDH, lactate dehydrogenase; JNK, c-Jun N-terminal kinases; Jun, transcriptional factor AP-1; Maoa, monoamine oxidase A gene; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; MIP-1α, macrophage inflammatory protein 1 alpha; MMP-9, matrix metallopeptidase 9; MNPs, magnetic nanoparticles; MRI, magnetic resonance imaging; mRNA, messenger RNA; MWCNT, multi-walled carbon nanotubes; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NE, norepinephrine; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NMDAR, N-methyl-d-aspartate receptors; NO, nitric oxide; NOS, nitric oxide species; NPs, nanoparticles; Nrf-2, NF-E2-related factor 2; NSC, neural stem cells; p21, cyclin-dependent kinase inhibitor 1; p53, tumor protein p53; Park2, Parkinson's disease (autosomal recessive, juvenile) 2 gene; PD, Parkinson's disease; PEG, polyethylene glycol; PGE2, prostaglandin E2; PI, propidium iodide; PLGA, polylactic-co-glycolic acid; RNA, ribonucleic acid; ROS, reactive oxigen species; SiO2 NPs, silicon dioxide nanoparticles; SN, substantia nigra; Snca, synuclein gene; SOD1, superoxide dismutase-1 gene; SOD, superoxide dismutase; SPIONs, superparamagnetic iron oxide nanoparticles; SWCNT, single-walled carbon nanotubes; Th, tyrosine hydroxylase gene; TiO2 NPs, titanium dioxide nanoparticles; TNF-α, tumor necrosis factor-alpha; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; Txnrd1, thioredoxin reductase 1 gene; USPIONs, ultrasmall superparamagnetic iron oxide nanoparticles; ZnO NPs, zinc oxide nanoparticles.

The authors declare no conflicts of interests.

Grant sponsor: European Commission's Seventh Framework Programme (FP7/2007–2013); Grant number: No 280716 (Sanowork [www.sanowork.eu]).

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Received 14 August 2014; provisionally accepted 7 November 2014; and in final form 13 November 2014

DOI 10.1002/em.21931

Published online 00 Month 2014 in
Wiley Online Library (wileyonlinelibrary.com).

timely and attractive theranostic tools in neurodegenerative diseases. Therefore, a careful assessment of the risk–benefit ratio must be taken into consideration in using nanosized materials. Environ. Mol. Mutagen. 00:000–000, 2014.

Key words: nanomaterials; nanoparticles; neurotoxicity; oxidative stress; neurodegenerative diseases; nanodevices

INTRODUCTION

Nanomaterials are small molecules with distinct biological activity that have been progressively and increasingly applied in various industrial and medical settings over the last 30 years [Robertson et al., 2010; Schröfel et al., 2014; ShannahAn et al., 2012]. However, despite great progress in nanotechnologies, comparatively little is known to date on the negative effects that exposure to NMs may have on the human brain, including the potential induction of pathways leading to neurodegeration [Cupaioli et al., 2014]. Although many NMs exhibit potential benefits for diagnostic and therapeutic purposes, some of these molecules can exert unfavorable effects, suggesting that the beneficial and harmful effects should be compared prior to their application to humans [Iqbal et al., 2013]. Indeed, NMs can enter the human body through several routes, including absorption through the skin or the digestive tract, inhalation, and blood injection. NMs may cross the blood–brain barrier (BBB) to reach the central nervous system (CNS), where they have been suspected to impair several molecular pathways and contribute to neurodegeration [Iqbal et al., 2013; Cupaioli et al., 2014].

Neurodegenerative diseases are a heterogeneous group of either hereditary or sporadic conditions all characterized by progressive nervous system dysfunction resulting from the degeneration of selected neurons in the CNS. Some of the most well known neurodegenerative diseases are Alzheimer's disease (AD) and other dementias, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) [Migliore and Coppedè, 2009]. Despite the heterogeneous nature of neurodegenerative diseases, the application of recent genome-wide and -omics approaches has provided novel insights into the critical molecular pathways of those disorders, revealing that aggregation and accumulation of misfolded proteins, mitochondrial dysfunction, oxidative stress, oxidatively damaged DNA and impaired DNA repair, apoptosis, impaired autophagy-lysosomal activities, inflammation and microglia activation, perturbation of vesicle trafficking and synapse dysfunction, RNA processing and protein degradation pathways, as well as epigenetic deregulation of gene expression, are common pathways in neurodegenerative diseases [Coppedè and Migliore, 2010; Giordano et al., 2013; Golde et al., 2013; Ramanan and Saykin, 2013; Vanderweyde et al., 2013; Amor et al., 2014; Bäumer et al., 2014].

Recent evidence indicates that NPs can impair dopaminergic and serotoninergic systems, the former being relevant for PD and the latter for AD pathogenesis, and can cause changes of neuronal morphology and cell death. In addition, NPs can also contribute to neurodegeneration by inducing mitochondrial dysfunction, redox imbalance and apoptosis, autophagy and impaired lysosomal activity, cytoskeletal damage and vesicle trafficking perturbations, neuroinflammation, and microglia activation [Iqbal et al., 2013; Cupaioli et al., 2014]. Furthermore, in vitro evidence suggests that engineered NMs are able to induce changes in the expression of genes involved in DNA methylation pathways, as well as global changes in epigenetic marks such as DNA methylation and histone tail modifications, all potentially involved in human complex disorders, including neurodegeneration [Stoccoro et al., 2013].

Conversely, since biodegradable NMs can be engineered to load drugs, contrast agents, and cellular or intracellular component targeting moieties, they have emerged as potential alternatives for tracking and treating human diseases, including neurodegenerative disorders [Marrache et al., 2013]. Nanoparticulate drug carriers are able to cross the BBB by virtue of their size, surface potential, or surface coatings, and are currently under investigation for effective delivery of pharmaceuticals or contrast agents active in the treatment and detection of AD and other neurodegenerative diseases [Garbayo et al., 2013; Oesterling et al., 2014]. Indeed, during the past decade, nanotechnology has been widely considered as a promising tool for theranosis (diagnosis and therapy) of neurodegenerative diseases [Amiri et al., 2013]. The aim of this review is to critically discuss available in vitro and in vivo data on the potential neurotoxic effects of NMs in the context of neurodegeneration, with a focus on the induction of cytotoxic and genotoxic effects, oxidative stress, and inflammatory pathways. Moreover, we also provide some examples of the potential beneficial uses of NMs in the context of diagnosis and treatment of neurodegenerative diseases.

CYTOTOXIC, GENOTOXIC, OXIDATIVE, AND INFLAMMATORY POTENTIAL OF NMS: IMPLICATIONS FOR NEURODEGENERATION

Due to their unique physico-chemical properties (i.e., small size, large surface area, composition, and functionalization) several types of metallic NPs are able to cross

the BBB and interact with the CNS components. However, despite the large number of both in vitro and in vivo investigations performed so far, the interactions between NMs and the CNS are still not completely understood and their toxic potential is still unclear. The majority of the data available in the literature report that metallic NPs induce toxic effects to the target cells or to the exposed animals, and the toxicity is mainly triggered via oxidative stress. The evidence that NMs induce cytotoxicity and genotoxicity, as well as oxidative stress and inflammation in various cell lines representative of body compartments such as the respiratory system, the intestine, and the immune system, amplifies the need for comprehensive studies on the neurotoxicity and the neurodegeneration induced by NMs engineered for the screening, diagnosis, and therapy of CNS diseases. Moreover, the evidence that the CNS is a potential susceptible target for nanosized materials and that NMs can penetrate there through the olfactory bulb and deposit in the hippocampus [Oberdörster et al., 2004; Wang et al., 2008] further emphasizes the need for studies on the potential neuronal effects of NMs. Retention of particles in the CNS, neurotoxicity, apoptosis, and oxidative stress, as well as changes in gene expression and neuropathological lesions are the most investigated parameters. The next sections discuss the evidence available on the most widely used NMs in industrial or biomedical applications, and provide a summary of in vitro (Table I) and in vivo (Table II) studies.

Titanium Dioxide Nanoparticles

Titanium dioxide nanoparticles (TiO_2 NPs) are one of the most frequently used NPs in industrial applications, ranging from paints to ceramics and from food to cosmetics. Therefore, investigation of risks associated with occupational exposure to TiO_2 NPs is of pivotal interest. However, the neurotoxic potential of these NPs has only recently been examined.

Among the first studies to demonstrate the cytotoxic effects of TiO_2 micro- and nanoparticles on human neural cells (U87 astrocytoma cells) as well as in human fibroblasts (HFF-1 cells), were Lai et al. [2008]. Both TiO_2 microparticles (1–1.3 μ particle size) and nanoparticles (<25 nm) were able to induce cell death in both human cell types, with mechanisms including apoptosis, necrosis, and possibly apoptosis-like and necrosis-like cell death types [Lai et al., 2008]. Subsequently, Márquez-Ramírez et al. [2012] demonstrated that the in vitro proliferation of murine C6 and human U373 glial cells was linearly inhibited in the presence of 40–200 nm TiO_2 NPs, with apoptosis induced 96 hr after exposure. Moreover, TiO_2 NPs were internalized in cytoplasmic vesicles and induced morphological changes, observable after just 24 hr of incubation [Márquez-Ramírez et al., 2012]. The toxicity of TiO_2 NPs to the same C6 and U373 cells was further confirmed by Huerta-García et al. [2014]. They used immunostaining to observe the morphological changes exerted by titania, including impairment of the integrity of mitochondria. In addition, severe changes in the redox-state of the cells and in lipid peroxidation, accompanied by increased levels of glutathione peroxidase, catalase, and superoxide dismutase, demonstrated that TiO_2 NPs induced oxidative stress in rat and human microglial cells [Huerta-García et al., 2014]. The ability of TiO_2 NPs to induce oxidative stress had already been previously reported in the murine microglial cell line BV-2, where the release of free radicals occurred less than 5 min after exposure to subtoxic concentrations of Degussa P25 nanoparticles [Long et al., 2006]. In addition, the prolonged (2 hr) release of reactive oxygen species (ROS) suggested that TiO_2 NPs interfered with the mitochondrial apparatus of BV-2 cells [Long et al., 2006]. Further investigations were performed to test the ability of TiO_2 NPs to cause inflammation and microglia-mediated neurotoxicity. Xue et al. [2012] demonstrated that exposure of Sprague-Dawley rat freshly isolated microglia cells to TiO_2 NPs enhanced the release of nitric oxide (NO) via upregulation of the expression of inducible nitric oxide synthase (iNOS), both at the mRNA and protein levels. Moreover, the inflammation produced by TiO_2 NPs led to increased expression of monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein 1 alpha (MIP-1α), while secretion of TNF-α, IL-1ß, and IL-6 was significantly enhanced upon exposure to titania. Finally, to test if TiO_2 NPs were able to initiate microglia-mediated neurodegeneration, the rat embryonic pheochromocytoma cell line PC12 was incubated with supernatants of the exposed microglial cells. The inflammatory cytokines TNF-α, IL-1ß, and IL-6 contained in the supernatant from TiO_2 NP-treated microglia impaired the viability of PC12 and severely suppressed the expression of the tyrosine hydroxylase (*Th*) gene, which is involved in the dopamine (DA) secretion in the CNS [Xue et al., 2012].

The impact of the crystalline structure of TiO_2 NPs on neurodegeneration has also been explored. Since TiO_2 NPs can mainly occur in the anatase and rutile forms, a comparison of the effects of these two crystalline structures was performed on PC12 [Wu et al., 2010] and SHSY5Y [Valdiglesias et al., 2013b] neuronal cells. Anatase TiO_2 NPs were more efficient than rutile NPs at generating a concentration-dependent decrease of cell viability in PC12 cells. Similarly, membrane damage evaluated via the lactate dehydrogenase (LDH) release assay was greater in the presence of anatase TiO_2 NPs. At high doses (200 μg/mL) ROS production was significantly higher in PC12 exposed to anatase TiO_2 NPs than to rutile, with similar results observed for the cellular levels of glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA). Furthermore, annexin

TABLE I. Neurotoxic Effects of Engineered Nanoparticles In Vitro

Nanoparticle	Cell system	NP characteristics	Effects	References
Titanium dioxide	BV-2 murine microglial cells	Degussa P25 (a mixture of the anatase (70%) and rutile (30%) forms of TiO_2, <100 nm particle size)	TiO_2 NPs induced rapid (<5 min) and prolonged (2 hr) release of ROS. Interference with the mitochondrial machinery	Long et al., 2006
	U87 astrocytoma cells. HFF-1 human fibroblasts	Anatase TiO_2 microparticles (1–1.3 μ particle size) or nanoparticles (<25 nm)	Dose-related cytotoxicity (apoptosis, necrosis, apoptosis and necrosis-like cell death). Both TiO_2 microparticles and nanoparticles, are equally effective in human neural cells and fibroblasts	Lai et al., 2008
	PC12 rat embryonic pheochromocytoma cells	Anatase (20 nm in size) and rutile (20 nm in size) TiO_2-NPs. and micrometer-TiO_2 particles (1 μm)	Concentration-dependent cytotoxicity and membrane damage. ROS, GSH, SOD, and MDA increased production. Apoptosis and necrosis; cell cycle arrested in G2/M phase. Anatase TiO_2 NPs more toxic than rutile. Micron-sized TiO2 did not exhibit any toxic response	Wu et al., 2010
	C6 murine glial cells and U373 human glial cells	Anatase (96%) and rutile (4%) forms of TiO2, 4–200 nm particle size	Dose-related cytotoxicity. Induction of apoptotic events. Internalization on TiO_2 NPs in membrane-bound vesicles. Morphological changes	Márquez-Ramírez et al., 2012
	Primary rat microglial and PC12 rat embryonic pheochromocytoma cells	TiO_2, 20 nm particle size	Oxidative stress via increased NO release. Increased secretion of TNF-α, IL-1ß, and IL-6. Increased expression of MCP-1 and MIP-1α	Xue et al., 2012
	SHSY5Y human neuroblastoma cells	Anatase (100%) and a mixture of anatase (80%) and rutile (20%) forms (2% nm in size) TiO_2-NPs	No cytotoxicity nor morphological alterations. Time- and dose-dependent internalization. Pure anatase TiO_2 NPs altered cell cycle and induced apoptosis/necrosis. Micronuclei formation and primary oxidative DNA damage	Valdiglesias et al., 2013
	C6 murine glial and U373 human glial cells	Anatase (96%) and rutile (4%) forms of TiO2, ≤50 nm particle size	Cytotoxicity. Morphological changes. Increased ROS, lipid peroxidase, GSH-PX, CAT, and SOD	Huerta-García et al., 2014
Silicon dioxide	Primary rat microglial cells	Amorphous SiNPs embedded with a fluorescent dansylamide dye, spherical in shape and about 150–200 nm in diameter	No cytotoxicity. SiO_2 NPs internalization in membrane-bound vesicles. Oxidative stress	Choi et al., 2010
	PC12 rat embryonic pheochromocytoma cells	SiO_2 NPs of differentsizes (20 and 50 nm)	Concentration-dependent cytotoxicity. Dose-related GSH depletion and enhanced ROS production. Cytoplasmic accumulation of SiO_2 NPs agglomerates. Morphological changes	Wang et al., 2011a
	Primary rat microglial cells	SiO_2 NPs (20 nm in diameter)	Enhanced secretion of TNF-α, IL-1ß and IL-6 pro-inflammatory cytokines	Xue et al., 2012
	SK-N-SH human neuroblastoma and N2a mouse neuroblastoma cells	SiO_2 NPs, 12.1 nm mean particles size	Concentration-dependent cytotoxicity, apoptosis and ROS release. Morphological changes. SiO_2 NPs dispersed in the cytoplasm of SK-N-SH cells. SiO_2 NPs stored intracellularly in vesicles in N2a cells	Yang et al., 2014

TABLE I. (continued).

Nanoparticle	Cell system	NP characteristics	Effects	References
Zinc oxide	NSC mouse Neural Stem Cells	ZnO NPs with zincite crystal structure, of different size (10, 30, 60, and 200 nm)	Concentration-dependent, but no size-dependent toxic effects. Altered morphology and induction of apoptosis. Significant release of Zn-ions	Deng et al., 2009
	PC12 rat embryonic pheochromocytoma cells	ZnO NPs, <50 nm particle size	Mitochondrial impairment and reduced cell viability. Internalization of ZnO NPs in membrane-bound vesicles	Kao et al., 2012
	RCS96 rat Schwann cells	ZnO NPs with different architecture: spherical (35 nm in diameter); microsphere (45 nm in diameter); hexahedral, prism-like (∼2.5 to 6.0 μm in diameter and ∼18.0 to 60.0 μm in length.); flowers-like (∼500 to 600 nm in diameter and several microns in length)	Shape- and time-dependent neurotoxicity. Apoptosis induction and G2/M phase cell cycle arrest. Significant release of Zn-ions	Yin et al., 2012
	SHSY5Y human neuroblastoma	ZnO NPs, 100 nm mean particles size	Cytotoxicity: apoptosis and cell cycle alterations and genotoxicity (micronuclei, H2AX phosphorylation and primary and oxidative DNA damage), in a concentration- and time-dependent manner	Valdiglesias et al., 2013a
Copper oxide	PC12 rat embryonic pheochromocytoma cells	Cu NPs, 90 nm particle size	Reduced levels of DA, DOPAC and HVA. Downregulation of *Gpx1* gene. Upregulation of *Txnrd1*, *Snca*, and *Maoa* genes	Wang et al., 2009
	rBMEC rat brain microvessel endothelial cells	Cu NPs, 40 and 60 nm particle size	Size-related cytotoxicity. Increased levels of PGE2, TNF-α and IL-1ß. Enhanced barrier permeability	Trickler et al., 2012
Silver	PC12 rat embryonic pheochromocytoma cells	Ag NPs, 15 nm particle size	Upregulation of *Gpx1* gene. No variations of the genes associated to DA metabolism and Parkinson's pathogenesis	Wang et al., 2009
	Rat brain microvessel endothelial cells co-cultured with astrocytes	Ag NPs	Ag NPs crossed the BBB and accumulated in endothelial cells	Tang et al., 2010
	Primary rat brain microvessel endothelial cells	Ag NPs, 25, 40, or 80 nm particle size	Dose- and size-dependent Ag NPs internalization. Cytotoxicity and morphological changes with monolayer perforations. Size- and time-dependent increase of TNF-α and IL-1ß levels. Size-selective impairment of the barrier permeability	Trickler et al., 2010
	Rat cortical cells	Ag NPs, 20 nm particle size	Loss of cytoskeleton structure and F-actin and ß-tubulin degradation. Inhibition of neuronal extension and cytotoxicity	Xu et al., 2013
	SHSY5Y human neuroblastoma and D384 human astrocytoma cells	Ag NPs, 20 nm particle size	Dose-related impaired mitochondrial metabolism and membrane damage. Dose-related inhibition of colony forming efficiency. Significant release of Ag-ions	Coccini et al., 2014
	Primary porcine brain microvessel endothelial cells	Ag NPs, 25, 40, and 80 nm particle size	Increased PGE2, TNF-α and IL-1ß levels. BBB leakage. Enhanced barrier permeability	Trickler et al., 2014
	CGC primary rat cerebellar granule cells	0.2% polyvinylpyrrolidone (PVP)-coated Ag NPs <100 nm	Calcium imbalance. Activation of NMDAR. Oxidative stress by ROS production. Impaired mitochondrial membrane potential	Ziemínska et al., 2014

TABLE I. (continued).

Nanoparticle	Cell system	NP characteristics	Effects	References
Iron oxide	Primary rat cerebellar cortex astrocytes	Nanosized iron oxide superparamagnetic particles (Fe_3O_4 orγ-Fe_2O_3).	Severe reduction of cell viability and cell adhesion. No damage to the cell membrane integrity	Au et al., 2007
	PC12 rat embryonic pheochromocytoma cells	Anionic Fe_2O_3 nanoparticles with surface coating (DMSA); nanoparticle diameters between 5 and 12 nm.	Impaired cell viability. Morphological changes	Pisanic et al., 2007
	Primary rat astrocyte-rich cultures	DMSA-coated Fe NPs, 60 nm average diameter	Temperature-dependent IONPs uptake	Geppert et al., 2009
	OLN-93 spontaneously transformed primary rat brain oligodendroglial cells	Citrated-coated Fe NPs	Cellular Fe-content significantly increased in a concentration dependent manner. IONPs stored in membrane-bound perinuclear vesicles	Hohnholt et al., 2010
	Primary rat astrocyte-rich cultures	DMSA-coated Fe NP, 60 nm average diameter	Time- and dose-dependent IONPs internalization	Geppert et al., 2011
	Primary rat astrocyte-rich cultures	DMSA-coated Fe NP, 60 nm average diameter	IONPs stored in membrane-bound perinuclear vesicles. Fe-ions sequestered by proteins to protect cells from cytotoxic effects	Geppert et al., 2012
	Primary rat astrocyte-rich cultures	DMSA-coated Fe-NP, 60 nm average diameter	Time-, concentration- and temperature-dependent uptake of IONPs by endocytotic processes	Hohnholt et al., 2012
	Primary rat astrocyte-rich cultures	DMSA-coated Fe NPs, 60 nm average diameter	Time-, concentration- and temperature-dependent uptake of IONPs. IONPs accumulation enhanced by the presence of a magnetic field	Lamkowsky et al., 2012
	Primary rat cerebellar cortex astrocytes	Fe_3O_4 NPs coated with polyethyleneimine and tagged with rhodamine B isothiocyanate (Fe_3O_4-PEI-RITC), mean core size 24.3 ± 5.7 nm	No cytotoxic effects. Rapid and extensive IONPs uptake	Yiu et al., 2012
	Primary rat microglial cells	Fe_3O_4, 45 nm average	Increased expression levels of TNF-α, IL-1ß and IL-6. No changes in NO, MCP-1 and NF-κB production	Xue et al., 2012
Carbon nanotubes	Primary chicken embryos mixed neuronal and glial cells	SWCNT-agglomerates (SWCNT-a), with a diameter of approximately 100 nm SWCNT-bundles (SWCNT-b), with a diameter of approximately 20 nm.	Agglomerated SWCNT induced cytotoxicity	Belyanskaya et al., 2009
	PC12 rat embryonic pheochromocytoma cells	Long single-walled carbon nanotubes (LSWCNT: Outer Diameter 1–2 nm, Length 20 μm), short single-walled carbon nanotubes (SSWCNT: OD 1–2 nm, Length:0.5–2 μm).	SWCNT induced time- and concentration-related cytotoxicity. Decreased mitochondrial membrane potential. Oxidative stress via ROS and lipid peroxidation increase. Time- and dose-dependent decreased SOD, GSH-Px, CAT, and GSH. Cell cycle arrested in G2/M phase. Dose-dependent apoptotic rate	Wang et al., 2011b
	PC12 rat embryonic pheochromocytoma cells	SWCNTs, OD 1–2 nm, length 20 μm	Time- and dose-dependent apoptotic cell death. Formation of ROS. Decreased levels of lipid peroxide. Increased levels of GSH, SOD, GSH-Px, and CAT. Reduced mitochondrial membrane potential. Activation of caspase-3. Vitamin E protects cells from the toxicity induced by SWCNT	Wang et al., 2012
	PC12 rat embryonic pheochromocytoma cells	Fe-low and Fe-high MWCNTs based on their metal impurities, outer diameter 2–50 nm, length 50 μm	Cytotoxicity. Cytoskeleton disruption. Effects amplified by the presence of metal impurities such as iron	Meng et al., 2013
	Co-cultures of primary embryonic rat hippocampal neurons and glial cells	Substrates containing polypyrrole and/or SWCNTs	Impaired cell viability. Neuroprotective effects in the presence of polypyrrole	Hernández-Ferrer et al., 2014

TABLE II. Neurotoxic Effects of Engineered Nanoparticles In Vivo

Nanoparticle	Animal model	Administration	NP characteristics	Effects	References
Titanium dioxide	CD-1 mice, female	Intranasal	Four types rutile-phase TiO_2 NPs (<5 μm;<100 nm; hydrophilic and with silica-coated surface)	Hydrophobic rutile TiO_2 NPs accumulated in the cerebral cortex and striatum and cause morphological changes to the neurons. Hydrophilic rutile TiO_2 NPs were not internalized in the brain but induced reduced NE levels in hippocampus, cerebral cortex, cerebellum and striatum	Zhang et al., 2011
	Swiss albino mice, male	Oral	Mixture of rutile and anatase TiO_2 NPs (<75 nm)	Enhanced DA and NE levels. Oxidative stress with increased ROS	Shrivastava et al., 2014
	Wistar rats, pregnant	Intragastric	Anatase (10 nm)	Reduction of cell proliferation in the hippocampus and impaired learning and memory in offspring	Mohammadipour et al., 2014
Silicon dioxide	SD rats	Intranasal	SiO_2 NPs (15 nm)	Internalization of SiO_2 NPs in the brain. Oxidative stress. Inflammation	Wu et al., 2011
	SD rats, male	Intraperioneal injection	SiO_2 NPs (50–60 nm)	BBB disruption. Neuronal damage. Behavioral impairment	Sharma et al., 2013a
	Zebrafish (*Danio rerio*)	Exposure in water	SiO_2 NPs (15- and 50-nm)	Size-dependent behavioral changes. Parkinson's like behavior	Li et al., 2014
	Balb/c mice	Intraperitoneal injection	Mesoporous hollow silica nanoparticles (MHSNs) (110 nm)	Size-dependent migration through the BBB of PEGylated silica. Time-related NPs accumulation in the brain	Liu et al., 2014
Zinc oxide	SD rats, male	Intranasal	ZnO NPs (<50 nm)	ZnO NPs translocation in the olfactory bulb and in the brain	Kao et al., 2012
	Swiss mice, male	Intraperitoneal injection	ZnO NPs (20–80 nm)	Impairment of synaptic responses. Disrupted spatial memory	Xie et al., 2012
	SD rats, male	Oral	ZnO NPs (40 nm)	ZnO NPs translocation in the brain	Cho et al., 2013
Copper	SD rats, male	Intraperitoneal injection	Cu NPs (50–60 nm)	Neuronal alterations. Brain dysfunction. Cognitive impairment	Sharma and Sharma, 2007
	SD rats, male	Intraperitoneal injection	Cu NPs (50–60 nm)	BBB damage. Brain edema formation	Sharma et al., 2009
	SD rats, male	Intraperitoneal injection	Cu NPs (50–60 nm)	Neuronal cell damage, glial cell activation, loss of myelinated fibers. Brain edema formation	Sharma et al., 2010
	Wistar rats	Intraperitoneal injection	CuO NPs (10–70 nm)	Oxidative damage in hippocampus. Altered cognitive functions (poor performance of animals in behavioral tests)	An et al., 2012
Silver	C57BL/6N mice, male	Intraperitoneal injection	Ag NPs (25 nm)	Altered expression of oxidative stress and antioxidant genes. ROS enhancement. DNA damage	Rahman et al., 2009
	SD rats, male	Intraperitoneal injection	Ag NPs (50–60 nm)	BBB leakage. Brain edema formation. Glial activation. Reduced cerebral blood flow. Loss of myelinated fibers	Sharma et al., 2009
	ICR mice, male and female	Oral	Small-sized Ag NPs (22, 42, and 71 nm) and large-sized Ag NPs (323 nm)	Size-dependent Ag NPs internalization (small-sized NPs were distributed to the organs including brain, lung, liver, kidney, and testis). Increase of TGF-ß levels in serum by small-sized Ag NPs	Park et al., 2010
	Wistar Hannover Galas rats, female	Oral	Ag NPs (~14 nm)	Ag NPs and Ag-ions uptake in the brain. Significant release of Ag-ions	Loeschner et al., 2011

TABLE II. (continued).

Nanoparticle	Animal model	Administration	NP characteristics	Effects	References
	Wistar rats, male	Intravenous injection	Ag NPs (20 and 200 nm)	Time-related Ag NPs uptake20 nm Ag NPs better internalized than 200 nm Ag NPs	Dziendzikowska et al., 2012
	C57BL/6J mice, male	Inhalation	Ag NPs (25 nm)	Translocation of Ag NPs into the olfactory bulb and lateral brain ventricles	Genter et al., 2012
	Wistar rats, female	Oral	Ag NPs (14 nm)	Increased DA and 5-HT levels. Significant release of Ag-ions	Hadrup et al., 2010
	SD rats, male	Oral	Ag NPs <20 nm, noncoated and <15 nm PVP-coated	Uptake and biopersistence of Ag NPs into the brain. PVP-coated particles show limited ion dissolution	van der Zande et al., 2012
	SD rats, male	Intraperitoneal injection	Ag NPs (50–60 nm)	Size-related BBB disruption. Age-related BBB damage. Neuronal NOS upregulation. Neuronal impairment	Sharma et al., 2013b
Magnetic	ICR mice, male and females	Intraperitoneal injection	PVP-stabilized cobalt ferrite silica-overcoated. [MNPs@SiO_2(RITC)]	IONPs passed through the BBB	Kim et al., 2006
	ICR mice, male and females	Inhalation	Fluorescent magnetic nanoparticles (50 nm)	IONPs passed through the BBB	Kwon et al., 2008
	ICR mice, male and females	Oral	Fe3O4 MNPs (~20 nm)	IONPs passed through the BBB	Wang et al., 2010
	SD rats, male	Intraneural injection	Four IONPs of different surface and core chemistries: DMSA-Fe_2O_3, DMSA-Fe_3O_4, PEG-Fe_3O_4 and PEG-Au-Fe_3O_4, roughly spherical at 8–10 nm	Macrophages, monocytes and lymphocytes accumulation at sites of injection. Increased levels of ERK, caspase-3, MMP-9, HO-1 and IL-1ß	Kim et al., 2013
	SD rats, male	Inhalation	Fe_3O_4 NPs (30 nm)	IONPs deposited in olfactory bulb, striatum and hippocampus. Oxidative stress via upregulation of GSH, H_2O_2, SOD and MDA	Wu et al., 2013
	ICR mice, male	Inhalation	α-Fe_2O_3 and γ-Fe2O3 NPs (~22 and ~31 nm respectively)	Brain pathological alteration, microglial proliferation, activation and recruitment in hippocampus and striatum, especially in olfactory bulb	Wang et al., 2011
	Zebrafish (*Danio rerio*)	Oral	Superparamagnetic iron oxide nanoparticles (dextran-coated)	Induction of apoptosis. Brain accumulation of IONPs. Enhanced mRNA levels of *casp-8*, *casp-9* and *jun*	de Oliveira et al., 2014
Carbon nanotubes	Wistar rats, male	Intravenous injection	Gadolinium-catalyzed SWNTs (Gd-SWCNTs). Average diameter 2.05 nm, length 500–1.5 μm	Accumulation of SWCNT into the cerebral cortex. No inflammation. No altered tissue morphology	Avti et al., 2013
	C57BL/6J, female	Injections at specific stereotactic locations in the motor cortex	MWNT shortened (by oxidization) and amino-functionalized (oxMWNT-NH3) and only amino-functionalized (MWNTNH3)	Presence of both functionalized MWCNT in astrocytes, microglial and neuronal cells. Induction by both types of MWNT of a transient enhancement of TNF-α, IL-1ß, IL-6 and IL-10. Microglia and astrocytes activation with increased levels of GFAP and CD11b	Bardi et al., 2013
	C57BL/6J, male	Inhalation	MWCNT	Time-dependent accumulation of MWCNT in brain	Mercer et al., 2013
	C57BL/6J $p53^{+/-}$ mice, male and female	Intravenous injection	CNT	MWCNT passed the blood-placental barrier. Brain deformity and malformations via an indirect neurotoxic mechanism. No evidence of MWCNT into the brain of the pups	Huang et al., 2014

V-FITC and PI staining showed that apoptotic and necrotic PC12 cells increased significantly with anatase titania, but flow cytometry demonstrated that both crystalline forms were able to arrest the cell cycle in G2/M phase. Western blot analysis confirmed that anatase TiO_2 NPs were more potent than rutile in activating apoptosis and cell cycle checkpoint proteins: the expression of JNK, p53, p21, GADD45, as well as bax and bcl-2 was higher following exposure to anatase NPs than to the rutile ones [Wu et al., 2010].

In contrast to the above, crystalline forms of TiO_2 NPs had no impact on cytotoxic effects in the human neuroblastoma SHSY5Y cell line [Valdiglesias et al., 2013b]. MTT test and neutral red uptake showed that up to 24 hr exposure to 0–150 μg/mL pure anatase and P25 (80:20 anatase:rutile) TiO_2 NPs did not impair the viability of SHSY5Y. Moreover, no morphological alterations were observed and electron microscopy studies showed that TiO_2 NPs were internalized in a time- and concentration-dependent manner, although pure anatase TiO_2 NPs were slightly more efficiently taken up than P25. Additionally, as previously observed in PC12 cells [Wu et al., 2010], pure anatase TiO_2 NPs altered the SHSY5Y cell cycle and induced apoptotic and necrotic events, while no effects were observed in cells treated with P25. Interestingly, both types of TiO_2 NPs enhanced the formation of micronuclei and increased primary, but not oxidatively, damaged DNA observed with the comet assay [Valdiglesias et al., 2013a, b].

Since TiO_2 NPs induced in vitro neurodegeneration, in vivo studies are critically important to further investigate the toxic potential of these NPs. To this end, Zhang et al. [2011a, b, c] focused their attention on the neurological lesions in the brain of female CD-1 mice induced by intranasally instilled TiO_2 NPs of various size and surface coating. The results indicated that surface properties play a role in the neurodegenerative mechanisms of TiO_2 NPs: after 30 days exposure, hydrophobic TiO_2 NPs accumulated significantly in the cerebral cortex and in the striatum, while microsized and nano-hydrophilic (silica-coated) titania did not differ from the unexposed animals. Moreover, hydrophilic TiO_2 NPs caused morphological changes of neurons in the cerebral cortex. There was also a significant decrease in norepinephrine (NE) levels in the hippocampus, cerebral cortex, cerebellum, and striatum after hydrophilic TiO_2 NPs instillation, whereas hydrophobic titania did not alter the monoamine neurotransmitter levels in sub-brain regions [Zhang et al., 2011a, b, c]. Shrivastava et al. [2014] exposed Swiss albino mice for 21 days to a single oral dose of TiO_2 NPs and observed enhancement of DA and NE levels. They also detected oxidative stress conditions with increased ROS and reduced SOD production, suggesting that TiO_2 NPs are neurotoxic. It is, therefore, interesting to note that mutations of superoxide dismutase 1 (*SOD1*) cause familial forms of ALS [Rosen et al., 1993].

Finally, an interesting study was recently performed in pregnant Wistar rats that received intragastric TiO_2 NPs (100 mg/kg body weight) daily from gestational days 2–21. Exposure to TiO_2-NPs significantly reduced cell proliferation in the hippocampus, and impaired learning and memory in offspring [Mohammadipour et al., 2014].

The TiO_2 NPs assessed in the above in vitro and in vivo experiments differed according to their crystalline form (anatase or rutile), surface characteristics, or dose used. Similarly, the endpoints measured (cell viability, inflammation, oxidative stress markers, cytogenetic effects) also differed greatly, complicating direct comparisons between studies. Nevertheless, both forms (anatase and rutile) have been shown to be able to induce neurotoxicity at various levels, with the anatase form generally more active than the rutile form. Taken together the above findings indicate that single neurons, microglial cells, and the whole CNS (including brain regions critical for the onset of neurodegenerative diseases) are potentially susceptible targets for TiO_2 NPs.

Silicon Dioxide Nanoparticles

Another type of metal oxide used in industry and proposed for drug and gene delivery is silicon dioxide nanoparticles (SiO_2 NPs). The mechanism of toxicity of SiO_2 NPs is linked to the overproduction of ROS and to the activation of pro-inflammatory responses [Liu and Sun, 2010; Park and Park, 2009]. For example, SiO_2 NPs stimulated the secretion of the pro-inflammatory cytokines TNF-α, IL-1 ß, and IL-6 in freshly isolated rat microglial cells, but they were not able to stimulate the secretion of NO, MIP-1α and MCP-1, or NF-κB, which are known to be involved in the induction of inflammation-related genes and in microglia activation [Xue et al., 2012]. Using primary microglial cells from Sprague-Dawley pups, Choi and collaborators reported that SiO_2 NPs were stored intracellularly within phagocytic membrane-bound vesicles, and that silica induced a significant release of ROS and nitric oxidative species (NOS) accompanied by an increased *COX-2* gene expression, although the cell viability was not affected [Choi et al., 2010]. Moreover, exposure of PC12 neuronal cells to SiO_2 NPs revealed a concentration-dependent decrease in cell viability, depletion of GSH, and enhanced ROS production. In this study, silica nanoparticles were internalized as agglomerates in the cytoplasm and induced significant morphological changes, resulting in cells that appeared small and fragmented and that had reduced ability to grow neurites, therefore impeding the development of intercellular contacts and the formation of mature cells [Wang et al., 2011a, b]. Exposure to low doses (10 μg/mL) of 15 nm SiO_2 NPs similarly led to morphological alterations and concentration-dependent cytotoxicity in human SK-N-SH and mouse Neuro2a (N2a), two common neuroblastoma

cell lines. By electron microscopy, SK-N-SH cells were shown to be able to internalize silica particles throughout the cytoplasm, while the particles were found to be stored within vesicles in N2a cultures [Yang et al., 2014]. In addition, treatment of SK-N-SH and N2a cells caused ROS release and significant dose-dependent apoptosis, as shown by nuclear and TUNEL staining. Interestingly, Yang et al. [2014] reported that SiO_2 NPs (mean particles size 12.1 nm) increased the deposition of intracellular ß-amyloid peptide ($Aß_{1–42}$) due to both the upregulation of ß-amyloid precursor protein (APP) and the downregulation of the amyloid-ß-degrading enzyme neprylysin, suggesting a possible role for SiO_2 NPs in the development of AD. Indeed, according to the amyloid cascade hypothesis of AD, changes in APP and/or $Aβ_{1–42}$ homeostasis foster the assembly of Aβ peptides into progressively higher order structures, from dimers all the way up to the insoluble plaques, which finally deposit in the brain; these events are sufficient to initiate the pathological and clinical changes of the disease [Hardy and Selkoe, 2002].

The use of SiO_2 NPs in many applications and their potential use for drug and gene delivery, makes it essential to conduct further studies on possible biological effects. This is particularly important given that in vitro studies have shown that SiO2 NPs are cytotoxic [Eom and Choi, 2009; Akhtar et al., 2010], induce oxidative stress [Napierska et al., 2009; Zhang et al., 2011a, b, c; Ahmad et al., 2012; Ahamed, 2013] and inflammatory responses [Panas et al., 2013; Kusaka et al., 2014; Mendoza et al., 2014] in many cell types, including cells representative of the CNS [Choi et al., 2010;Wang et al., 2011a, b; Xue et al., 2012; Yang et al., 2014]. Furthermore, SiO_2 NPs have been reported to induce inflammation [Lee et al., 2011; Morishige et al., 2012; Brown et al., 2014], as well as pulmonary [Choi et al., 2008; Zhao et al., 2014] and hepatic [Nishimori et al., 2009; Liu et al., 2012] toxicity in vivo.

Wu et al. [2011] exposed SD rats to intranasal instillation of SiO_2 NPs and observed significant brain accumulation of nanoparticles, oxidative stress (increased H_2O_2 and MDA and significant decrease in GSH), and increased TNF-α and IL-1ß levels, indicative of inflammation. Interestingly, when a deeper analysis of the content of silica in the different brain regions was performed, it was possible to establish a ranking of SiO_2 NPs accumulation that corresponded to olfactory bulb > striatum > hippocampus > brain stem > cerebellum > frontal cortex [Wu et al., 2011]. In Balb/c mice polyethylene glycol-coated silica nanoparticles (PEG-SiO_2 NPs) crossed the BBB, showing size-dependent variation in transport efficiency. At short exposure times (15 min), 50 and 100 nm PEG-SiO_2 NPs were poorly able to migrate through the BBB but their uptake significantly increased after 60 min. Smaller (25 nm) PEG-SiO_2 NPs, in contrast, were already significantly taken up after a 15 min incubation, and their migration across the BBB was further enhanced after 1 hr [Liu et al., 2014]. In addition to BBB disruption and neuronal damage, SiO_2 NPs have also been associated with behavioral impairment in rats [Sharma et al., 2013a]. Similarly, silica nanoparticles disturbed the neural behavior of zebrafish *Danio rerio* in a size-dependent manner, as 15 nm SiO_2 NPs significantly changed the color preference of the animals and caused PD-like behavior, neither of which were observed for 50 nm particles [Li et al., 2014].

Overall, both in vitro and in vivo data indicate that SiO_2 NPs can pass through the BBB. The increased production of ROS and of pro-inflammatory responses, which seem a common feature of SiO_2 NPs, can adversely affect different cell types. The major emerging finding is that transport efficiency of SiO_2 NPs across BBB was found to be size-dependent, with increased particle size resulting in decreased efficiency. Greater concern is therefore justified when considering neurotoxicity associated with small sized silica nanoparticles in biomedical applications and occupational exposure in large-scale production.

Zinc Oxide Nanoparticles

Zinc oxide nanoparticles (ZnO NPs) are of industrial interest because of their exceptional optoelectronic, piezoelectric, ferromagnetic and optical properties. Moreover, ZnO NPs have been used in sunscreens, biosensors, food additives, and pigments [Ji and Ye, 2008]. Due to their antiseptic activity, they also have potential applications in combatting bacteria-related infections and diseases. Although some data on the toxic potential of ZnO NPs are available in the current literature, little is known about their neurotoxic effects. Deng et al. [2009] showed that in neural stem cells (NSC), ZnO NPs whose nominal mean size diameter ranged between 10 and 200 nm impaired cell viability in a dose-dependent manner. In contrast, size did not play a role in inducing toxic effects, as a comparison of the differently sized nanoparticles did not result in any significant difference in terms of cell viability. Electron microscopy analysis and nuclear staining were used to demonstrate that ZnO NPs induced apoptosis in NSC cells [Deng et al., 2009]. Nevertheless, the authors speculated that the cytotoxicity and apoptosis induced by ZnO NPs in NSC cells might result from the zinc ions dissolved either in solution or intracellularly. This hypothesis was supported also by the fact that the internalized ZnO NPs were not detectable by electron microscopy [Deng et al., 2009]. ZnO NPs neurotoxicity was further evaluated in RSC96 rat Schwann cells comparing four different hierarchical structures: monodispersed spherical ZnO NPs of 35 nm size, hollow ZnO microspheres (2.7 μm), prism- (ca., 2.5–6.0 μm in diameter and ca., 18–60 μm in length) and flower-like (500–600 nm in diameter and several microns in length)

structures [Yin et al., 2012]. Results demonstrated that prism- and flower-like ZnO NPs did not induce cytotoxic effects after 12 hr exposure, while significant impairment of the viability of RSC96 cells was observed at 48 hr. Similarly, spherical monodispersed ZnO NPs and zinc microspheres exerted concentration- and time-dependent cytotoxicity. Moreover, they significantly enhanced apoptotic events, and G2/M cell cycle arrest was observed when RSC96 cells were exposed for 12 hr to 80 μg/mL ZnO nanoparticles and microspheres [Yin et al., 2012]. Interestingly, analysis of the levels of zinc ions performed in culture media at increasing time points revealed that the observed time-related ion levels enhancement was the result of a leaching process occurring during the incubation period, which suggested that the cytotoxic effects observed in RSC96 rat Schwann cells were also due to the ionic fraction in the culture environment and not exclusively to the nanoparticulated fraction [Yin et al., 2012].

By confocal microscopy, Kao et al. [2012] observed that ZnO NPs were internalized in membrane-bound vesicles in PC12 neuronal cells and caused the reduction of cell viability and mitochondrial impairment. An extensive study on Zn NPs was performed in the human neuroblastoma SHSY5Y cell line, testing several concentrations and exposure times and employing a battery of cytotoxicity and genotoxicity assays. The internalization of the Zn NPs was assessed by flow cytometry but it was not possible to demonstrate that ZnO NPs enter the neuronal cells. However, a wide range of cytotoxic effects were induced including apoptosis and cell cycle alterations, as well as genotoxic effects (micronuclei, H2AX phosphorylation, and primary and oxidatively damaged DNA), in a dose- and time-dependent manner [Valdiglesias et al., 2013a].

In vivo, Kao et al. [2012] showed that ZnO NPs intranasally administered to Sprague-Dawley rats (6 hr exposure) translocated into the olfactory bulb and the synaptosomes, as clearly shown by electron microscopy micrographs. The translocation of ZnO NPs across the BBB and into the CNS was further confirmed by Cho et al. [2013]; after 13 weeks of repeated oral administration, enhanced ZnO NPs levels were measured in rats brain compared with the untreated group, although the uptake was not dose-related. Additionally, ZnO NPs were reported to disrupt spatial memory and to significantly impair the synaptic responses of Swiss male mice with depressive-like behavior [Xie et al., 2012].

In many experimental systems in which Zn NPs were able to induce neurotoxic effects, there was often no evidence for NP uptake by the cells, and no effect of NP size on cytotoxicity. Instead, a time-dependent increase of Zn^{2+} concentration in the culture media was sometimes found, most likley due to decomposition of ZnO NPs and the subsequent release of ions, as already reported. However, the question of whether the increase of intracellular ions is due to the NPs being taken up by cells, or to NP dissolution in medium, still remains to be resolved [Vandebriel and De Jong, 2012].

Copper Oxide Nanoparticles

Copper, an essential trace element vital for the life and the development of organisms, is known to be involved in neurodegenerative disorders such as Menkes [Kodama et al., 201], Wilson's [Lorincz, 2010], AD, HD, and PD [Desai and Kaler, 2008; Rivera-Mancia et al., 2010; Greenough et al., 2013; Montes et al., 2014], acting through the induction of oxidative stress [Halliwell and Gutteridge, 1984] and the activation of microglial cells and inflammation [Zhang et al., 2011b]. Although it is important to understand how nanosized copper oxide (CuO NPs) can induce neurotoxicity, to date few investigations have been performed. Wang et al. [2009] investigated expression changes in genes associated with the dopaminergic system and their correlation with DA depletion in PC12 cells. Treatment with CuO NPs significantly reduced the content of DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in PC12 cells, and induced downregulation in the expression of the redox-status gene glutathione peroxidase 1 (*Gpx1*) and upregulation of thioredoxin reductase 1 gene (*Txnrd1*). In addition, CuO NPs upregulated the expression of the monoamine oxidase A (*Maoa*), which is related to DA metabolism, and of the alpha-synuclein gene (*Snca*) associated with the pathogenesis of neurodegeneration in PD [Wang et al., 2009]. Indeed, PD results from loss of neuromelanin-containing dopaminergic neurons in the *substantia nigra* (SN), with the presence of eosinophilic, intracytoplasmic inclusions (termed Lewy bodies) and containing aggregates of α-synuclein as well as other substances. Furthermore, human *SNCA* mutations cause autosomal dominant PD, and *SNCA* polymorphisms or epigenetic changes of *SNCA* gene expression are believed to contribute to sporadic forms of the disease [Thomas and Beal, 2011]. An additional study on rat brain microvessel endothelial rBMECs cells showed that low concentrations of 40 and 60 nm CuO NPs increased the cellular proliferation while 50 μg/mL Cu-NPs were cytotoxic, and the extracellular concentration of the proinflammatory mediators Prostaglandin E2 (PGE2), TNF-α, and IL-1β were significantly increased [Trickler et al., 2012]. Moreover, Trickler et al. [2012] reported that the enhanced permeability of rBMEC upon exposure to CuO NPs suggests that the NPs can be neurotoxic and damage the BBB even at low doses.

To better investigate their involvement in the etiology of neurodegenerative disorders, CuO NPs were studied in vivo. CuO NPs of approximately 50–60 nm mean diameter were able to induce brain dysfunction in rats which, after 7 days of exposure, exhibited mild cognitive

impairment and cellular alterations in the brain [Sharma and Sharma, 2007]. Additionally, intraperitoneal, intravenous, intracarotid or intracerebroventricular administration of CuO-NPs significantly altered BBB function in several regions of the brain and spinal cord 24 hr after administration, and a marked decrease in local cerebral blood flow (CBF) and severe brain edema was observed in brain areas associated with BBB leakage [Sharma et al., 2009]. Moreover, Sharma et al. [2009] observed that the injured brain areas exhibited neuronal cell damage, glial cell activation, heat shock protein upregulation and loss of myelinated fibers, and that these changes were more evident in mice compared with rats. Furthermore, by means of Evans blue leakage, it was possible to show that brain edema formation took place in rats after intravenous, intraperitoneal and intracerebral administration of CuO NPs, and that the most severely damaged areas were the ventral surface of brain and the proximal frontal cortex, whereas the dorsal surfaces of cerebellum showed mild to moderate damage [Sharma et al., 2010]. CuO NP treatment also led to detrimental effects on the cognitive functions of Wistar rats, highlighted by poor performance of animals in behavioral tests. The occurrence of an imbalance in oxidation–antioxidation homeostasis, and of neuronal damage in the hippocampus, suggested the induction of oxidative damage and neuronal apoptosis [An et al., 2012]. Despite the scarcity of available studies, mainly carried out in a few experimental centers, CuO NPs seem neurotoxic both in vitro and in vivo. Of particular concern is the finding that nano-CuO can induce brain dysfunctions and affect the abilities of learning and memory in rodents.

Silver Nanoparticles

Due to its bactericidal properties and its role as an imaging contrast agent, silver nanoparticles (Ag NPs) are promising tools for biomedical applications. It is well known that the CNS is sensitive to silver [Carpenter, 2001], and that Ag can be retained in the CNS for long periods of time [Panyala et al., 2008] and induce neuronal degeneration and BBB malfunction. The ability of Ag NPs to translocate into the brain by crossing the BBB was reported in 2010 by Tang et al. Using an in vitro coculture model composed of rat brain microvessel endothelial cells and astrocytes, Ag NPs were observed to pass the BBB by transcytosis and accumulate in endothelial cells, as shown by electron microscopy [Tang et al., 2010]. In freshly isolated rat brain microvessel endothelial rBMEC cells, 25–40–80 nm Ag NPs accumulated in a dose- and size-dependent manner, and at high concentrations (25–50 $\mu g/cm^3$) induced an impairment of the cell viability. Furthermore, size-related morphological changes and formation of perforations in the monolayer were observed in rBMECs [Trickler et al., 2010]. In a follow-up study using confluent porcine brain microvessel endothelial cells, Trickler et al. [2014] observed that 25–40–80 nm Ag NPs induced pro-inflammatory responses by enhancing the extracellular levels of PGE2, TNF-α and IL1ß, in addition to causing BBB leakage and significantly higher permeability.

Loss of cytoskeleton structure with degradation of beta-tubulin and F-actin was observed in primary rat cortical cells exposed to Ag NPs (20 nm mean size diameter), and phase contrast images showed that Ag NPs inhibited neuronal extension, neuritic overlap, and impaired the viability of the rat cortical cells [Xu et al., 2013]. Size- and time-dependent TNF-α and IL-1ß secretion were detected, while PGE2 was not released in the presence of 40 and 80 nm Ag NPs. In addition, Ag NPs selectively affected the permeability of rBMECs: small Ag NPs (25 nm) induced an increased permeability of fluorescein across rBMECs, whilst 40 nm Ag NPs only slightly damaged the integrity of the barrier, and 80 nm particles did not exert any effect [Trickler et al., 2010]. In PC12 cells, expression changes in genes associated with the dopaminergic system were analyzed following exposure to 15 nm Ag NPs: *Gpx1* was the only upregulated gene, whereas there was no change in the expression of genes related to DA metabolism (*Th*, *Maoa*, and *Comt*) or genes (*Gpr37*, *Snca* and *Park2*) associated with the pathogenesis of neurodegeneration in PD [Wang et al., 2009]. In human-derived SHSY5Y neuroblastoma and D384 astrocytoma cells, exposure to 20 nm Ag NPs revealed that at short exposure times (4–48 hr), Ag NPs induced dose- and time-dependent impairment of mitochondrial metabolism and cell membrane damage. Similarly, longer exposures (10 days) of SHSY5Y and D384 cells treated with increasing concentrations of Ag NPs showed dose-dependent reduction in colony forming efficiency.

Since Ag NPs are known to release silver ions in solution, a comparison with $AgNO_3$ was performed. Cytotoxicity was more severe when SHSY5Y and D384 cells were incubated in the presence of $AgNO_3$ compared with Ag NPs at both short (4–48 hr) and at long (10 days) time points [Coccini et al., 2014]. Ziemínska et al. [2014] investigated the role of Ag NPs in the induction of excitotoxicity, a pathological process by which nerve cells are damaged and killed by excessive stimulation of neurotransmitters such as glutamate. Excitotoxicity is linked to alterations of intracellular calcium levels and deregulation of intracellular calcium signaling pathways, leading to ROS production, mitochondrial dysfunction, and ultimately cell death. To this end, primary cultures of rat cerebellar granule cells exposed to Ag NPs activated the glutamatergic N-methyl-d-aspartate receptors (NMDAR) and induced calcium imbalance, changes in mitochondrial membrane potential and significant ROS production, thus suggesting that Ag NPs have neurotoxic potential [Ziemínska et al., 2014]. Interestingly, Ziemínska et al.

[2014] showed that the toxic effects exerted by Ag NPs were attenuated in the presence of MK-801, a non-competitive inhibitor of NMDAR.

In vivo studies have demonstrated that Ag NPs accumulate in liver [Kim et al., 2008, 2010] and lungs [Sung et al., 2009; Song et al., 2013], but Ag NPs are also able to translocate into the CNS. In fact, 25 nm Ag NPs were detected by autometallography in the olfactory bulb and in the lateral brain ventricles of C57BL/6J mice [Genter et al., 2012], and mass spectrometry showed size-related internalization of Ag NPs in young ICR mice, with 22–71 nm particles distributed into the brain, whereas 300 nm Ag NPs were not detected in the tissue after 14 days of oral administration [Park et al., 2010].

Ag NPs (50–60 nm) administered into systemic circulation or brain ventricular spaces of rats and mice showed severe BBB leakage, formation of brain edema and decrease in local CBF, as well as glial activation and loss of myelinated fibers [Sharma et al., 2009]. Size-dependent BBB breakdown, NOS upregulation, neuronal damage, and glial fibrillary acidic protein upregulation was observed in inbred male Sprague-Dawley rats: small Ag NPs (20–30 nm) induced more severe damage in young (9–10 weeks old) and old (30–35 weeks old) rats compared with mid-age (18–20 weeks) animals, and the effect was significantly reduced in the presence of 50–60 and 130–150 nm Ag NPs [Sharma et al., 2013b]. The evidence that very young and old rats showed the most severe neurodegeneration led Sharma et al. [2013b] to suggest that children and elderly might be more susceptible to Ag NPs-induced brain damage.

Altered expression of mouse oxidative stress and antioxidant genes was observed in different regions of C57BL/6N mice exposed by injection to Ag NPs, suggesting that 25 nm Ag NPs are able to induce oxidative stress and oxidatively damaged DNA, and could be involved in the development of neurotoxicity and the pathogenesis of neurodegenerative disorders [Rahman et al., 2009].

Silver NPs in solution are known to release Ag-ions that induce significant toxicity as reported in vitro [Singh and Ramarao, 2010; Hamilton et al., 2014] and in vivo [Radniecki et al., 2011; Yang et al., 2012; Visnapuu et al., 2013]. It is therefore pivotal to understand if the neurotoxic potential of Ag NPs is due to the nanosized fraction or to the silver ions, which leached into solution. When the neurotoxicity induced by Ag NPs and Ag-ions was compared, interesting results were reported. Hadrup et al. [2012] observed that in female Wistar rats, 28 days of 14 nm Ag NPs and silver ions oral administration induced an increase in DA levels; in contrast, 5-hydroxytryptamine (5-HT) was enhanced exclusively following exposure to Ag NPs whereas noradrenaline was upregulated only following exposure to silver ions. Similar effects were also reported in Wistar Hannover Galas rats; animals were exposed by repeated oral administration for 28 days and the analysis of homogenates revealed that both nanosized and ionic silver accumulated in the brain with comparable distribution [Loeschner et al., 2011]. Moreover, silver was detected in brains of 28 days exposed Sprague-Dawley rats and, while it was eliminated from liver and spleen, a biopersistance of silver was observed in the brain [van der Zande, 2012]. Interestingly, Ag NPs also were detected in $AgNO_3$ exposed animals, supporting the evidence that nanoparticles can originate from Ag-ions in vivo and thus explaining the fact that Ag NPs and Ag salts exhibited similar distribution and clearance [van der Zande, 2012]. Additionally, Dziendzikowska et al. [2012] showed that at short and mid-term exposures (24 hr and 7 days) the brain was the organ with the lowest concentration of silver, while a significant increase was measured after 28 days Ag NPs intravenous administration in Wistar rats, demonstrating that Ag NPs displayed time-dependent deposition in the brain.

Therefore, based on findings in animals, Ag NPs seem able to distribute and accumulate over time in many organs, including the brain. Increasing evidence suggests that Ag NP-induced neurotoxic effects may occur via silver ions that are released from the particle surface, as happens for other metal oxide NPs. A size-dependent effect was found in vitro and in vivo (small-sized AgNPs were more active). Moreover, a higher susceptibility in the age groups most vulnerable (in the younger or older animals) has been found in vivo.

Magnetic Nanoparticles

The use of magnetic nanoparticles (MNPs) has become an area of increasing interest in biomedicine. MNPs have unique features, such as their reaction to a magnetic force, that can be utilized in drug targeting and cell sorting. Moreover, MNPs have gained interest because of their potential use as contrast agents for magnetic resonance imaging (MRI) and as heating mediators for hyperthermia and cancer therapy [Ito et al., 2005]. However, their potential neurotoxicity has been poorly investigated. Au et al. [2007] exposed astrocytes from the cerebral cortices of newborn Sprague-Dawley rats to 10 μg/mL iron oxide superparamagnetic particles (Fe_3O_4 or γ-Fe_2O_3) and reported that although the cell membrane integrity was not affected, viability and cell adhesion were significantly impaired. Anionic magnetic nanoparticles (AMNPs) were shown to severely affect the viability of PC12 neuronal cells, and caused morphological alterations such as reduced microtubules protrusion, reduced formation of actin microfilaments within the soma, and loss of organized actin in the cellular body, thus inducing PC12 cells to assume a spheroidal shape [Pisanic et al., 2007]. In rat primary microglia cells, while NO and MCP-1

production and NF-κB binding activity were comparable to the untreated control cells, Fe_3O_4 NPs were found to exert a mild increase in the expression of the pro-inflammatory cytokines TNF-α, IL-1ß, and IL-6, indicating that other inflammatory signaling pathways may act independently of NF-κB activation [Xue et al., 2012]. However, since significant cytotoxicity in PC12 cells was not observed following incubation with the supernatant from Fe_3O_4 NPs-treated microglia, Xue et al. [2012] concluded that the proinflammatory activity exerted by iron NPs was not sufficient to cause neurotoxicity and neurodegeneration.

The interaction of iron oxide nanoparticles (IONPs) with astrocytes has been extensively investigated, and Hohnholt et al. [2013] reviewed the main results. Astrocytes play an important role in the CNS because they regulate the metal homeostasis in the brain [Tiffany-Castiglioni and Qian, 2001; Dringen et al., 2007; Jones, 2012] and protect the brain from metal toxicity and oxidative stress [Hirrlinger and Dringen, 2010; Macco et al., 2013]. Time- [Geppert et al., 2011], concentration- [Geppert et al., 2011; Hohnholt et al., 2013; Lamkowsky ey al., 2012] and temperature-dependent [Geppert et al., 2009; Lamkowsky et al., 2012] accumulation of IONPs was shown in cultured murine astrocytes, and IONPs were observed to stably remain in the cells without inducing cytotoxicity [Lamkowsky et al., 2012; Yiu et al., 2012]. Furthermore, the resistance of astrocytes to IONPs cytotoxic effects was suggested to be dependent on the fact that particles are stored in intracellular vesicles and are not freely dispersed in the cytosol [Hohnholt et al., 2010; Geppert et al., 2011, 2012], but also the sequestration of IONPs-leached ions by proteins (such as ferritin) has a protective effect to the cells [Geppert et al., 2012].

The in vivo uptake and the potential adverse effects of IONPs in brain have been reviewed by Petters et al. [2014]. They highlighted that although IONPs are able to cross the BBB [Kim et al., 2006; Kwon et al., 2008; Wang et al., 2010, 2011a, b] and induce activation and the proliferation of microglial cells in the olfactory bulb, it remains unclear as to under which conditions IONPs migration occurs, and which regions of the brain are targeted by the particles.

Wu et al. [2013] demonstrated that after 7 days of intranasal instillation, 30 nm Fe_3O_4 NPs differentially deposited in the brain of SD rats; olfactory bulb, striatum and hippocampus were the regions where IONPs mostly accumulated compared with brain stem, cerebellum, and frontal cortex, and the clearance of Fe_3O_4 NPs from the brain was slow, as striatum and hippocampus still retained more than half of IONPs up to 14 days post-instillation. In addition, Fe_3O_4 NPs increased the levels of the oxidative damage markers GSH, H_2O_2, SOD, and MDA in the striatum, thus emphasizing the neurotoxic potential of magnetic NPs [Wu et al., 2013]. Intraneural injection of maghemite (Fe_2O_3) and magnetite (Fe_3O_4) NPs coated with dimercaptosuccinic acid (DMSA) and PEG into the sciatic nerve of Sprague-Dawley rats resulted in an accumulation of macrophages, monocytes, and lymphocytes at the injection sites, together with increased levels of ERK, caspase-3, IL1ß, matrix metallopeptidase 9 (MMP-9) and heme oxygenase 1 (HO-1), confirming that IONPs are able to induce oxidative stress, inflammation and apoptotic events [Kim et al., 2013]. The accumulation of IONPs and the induction of apoptosis was also demonstrated in the brain of zebrafish, where increased levels of ferric iron and enhanced mRNA levels of caspase-8 (*casp8*), caspase-9 (*casp9*) and transcriptional factor AP-1 *jun* were detected [de Oliveira et al., 2014].

It is established that magnetic NPs are able to pass through the BBB and enter the CNS, and that ROS production is one of the main mechanisms by which they induce toxicity in the CNS. A very recent review taking into account a wide range of toxic effects induced by IONPs, including neurotoxicity, indicate that surface coatings and particle size seem to be crucial for the observed IONPs-induced effects [Valdiglesias et al., 2014].

Carbon Nanotubes

Carbon nanotubes (CNT) are a class of NMs whose structure is exclusively composed of carbon atoms and which display high electronic and thermal conductivity. CNTs can occur in two main types: single-walled carbon nanotubes (SWCNT) consisting of a single sheet of carbon benzene rings rolled up into a tubular structure; and multi-walled carbon nanotubes (MWCNT) consisting of multiple concentric layers of carbon sheets. The use of CNTs in biomedicine has grown, due in part to their improved aqueous dispersibility resulting in some functionalized forms being water dispersible (e.g., carboxylated MWCNT [Ntim et al., 2012]). Nevertheless, our understanding of the interactions between CNTs and the CNS, both in vitro and in vivo, remains limited, and their potential short- and long-term neurotoxicity is still unclear.

SWCNTs were reported to induce time- and dose-dependent impairment of cell viability and membrane damage in PC12 neuronal cells, as well as decrease the mitochondrial membrane potential. Moreover, SWCNT induced the formation of ROS, enhanced the levels of lipid peroxide and decreased SOD, glutathione peroxidase, catalase and GSH in a time- and dose-dependent manner [Wang et al., 2011b, 2012]. Additionally, PC12 cells exhibited condensed chromatin, fragmented nuclei and a block of the cell cycle in G2/M phase, indicating that apoptotic events were enhanced by the exposure to SWCNT [Wang et al., 2011b]. These effects were prevented by pre-incubation with vitamin E [Wang et al.,

2012]. CNTs have been proposed as substrates for neuron growth, and in some experiments have shown toxicity in cell culture. In order to reduce their toxicity it may be possible to modify SWCNT surfaces to make the contact between cells and nanotubes less close. This can be achieved by enveloping the CNT molecule with surfactants or polymers, such as polypyrrole (PPy). While the viability of co-cultures of primary embryonic rat hippocampal neurons and glial cells was impaired in SWCNT substrates, toxicity was lower for the PPy-SWCNT-substrates [Hernández-Ferrer et al., 2014]. Even the different degrees of agglomeration of SWCNTs can influence neurotoxicity. In primary mixed neuronal and glial cells from chicken embryos spinal cord or dorsal root ganglia, agglomerated SWCNTs significantly decreased the DNA content and reduced the amount of glial cells, whereas bundled SWCNT had only mild effects [Belyanskaya et al., 2009].

CNTs can retain metal impurities. To test the role of these impurities in inducing neurotoxicity, MWCNT with increasing concentrations of iron (Fe-MWCNT) were investigated in PC12 cells. The results showed that highly impure Fe-MWCNT impaired cell viability, increased cytoskeleton disruption, diminished the ability to form mature neurites and influenced the neuronal dopaminergic phenotype in NGF-treated rat pheochromocytoma cell line PC12 cells [Meng et al., 2013].

MWCNTs functionalized with amino groups were injected into C57/Bl6 mice. The MWCNTs were internalized in microglia, astrocytes and neurons, and stimulated a transient induction of the pro-inflammatory cytokines TNF-α, IL-1ß, IL-6, and IL-10 at early time points (<16 hr) [Bardi et al., 2013]. Moreover, the oxidation of nanotubes induced significant levels of glial fibrillary acidic protein (GFAP) and CD11b in the areas of injection, indicating that astrocytes and microglia were locally activated by MWCNT [Bardi et al., 2013]. In Wistar rats, gadolinium (Gd-SWCNT) and iron (Fe-SWCNT) single-walled carbon nanotubes were found to accumulate as aggregates in the cerebral cortex of the brain without altering the tissue architecture nor inducing inflammation [Avti et al., 2013]. The ability of MWCNT to translocate into the brain was further demonstrated using C57BL/6J mice, where monodispersed MWCNT accumulated in the brain after 12 days of inhalation in a time-related manner [Mercer et al., 2013]. Moreover, 50 nm MWCNTs were reported to induce brain deformity via an indirect mechanism: MWCNTs crossed the blood-placental barrier of $p53^{+/-}$ pregnant C57BL/6J mice and induced crown-shaped tissue malformations of the brain, but they did not migrate through the BBB as demonstrated by the fact that CNT did not accumulate in fetal brains [Huang et al., 2014]. Although CNTs have shown much promise in many applicative fields, including biomedicine and neurobiology, a limited number of studies are available on their neurotoxicity both in vitro and in vivo. Toxicological studies performed in vivo have often evaluated the specificity of many tissues and organs, but the nervous system was almost never included. Of current interest is research to identify safer types of CNTs, which should then be tested in vivo over medium to long periods of exposure.

USE OF NMS IN DIAGNOSIS AND TREATMENT OF NEURODEGENERATIVE DISEASES

Although different types of evidence have shown that many NMs can induce toxic mechanisms and cause cytotoxicity, genotoxicity, inflammation, and oxidative stress in vitro and in vivo, the design, development and synthesis of engineered NMs for biomedical applications is a very dynamic field. It is expected that engineered NMs will be used in the screening, diagnosis and treatment of diseases. However, use of NMs in the diagnosis and treatment of neurodegenerative diseases implies that NMs migrate through the BBB, which is known to be tightly regulated and presents a very low rate of transcytotic vesicles and acts as a restrictive paracellular diffusion barrier, protecting the neural tissue from toxins and toxicants [Wolburg and Lippoldt, 2002]. The ability of NMs specifically engineered for the diagnosis and the treatment of neurodegenerative diseases to cross the BBB and enter the CNS depends on the physico-chemical properties of NMs, on their composition and on their functionalization [Kreuter, 2004]. The use of lipidic (liposomes, nanoemulsions, and nanocapsules), polymeric (micelles, dendrimers, nanogels, and polymeric particles), and inorganic (quantum dots and iron oxide) NMs for CNS targeting, diagnostic, and therapeutic purposes was recently reviewed [Modi et al., 2009, 2010; Garbayo et al., 2013; Rocha, 2013; Cupaioli et al., 2014] and a number of suitable and promising nanocarriers have been identified (Fig. 1). Therefore, we only mention some of the many recent examples highlighting the potential application of nanotechnology in diagnosis and treatment of neurodegenerative diseases.

Due to their lipophilic nature, which allows them to cross the BBB by passive diffusion [Brasnjevic et al., 2009; Redzic et al., 2011], in the recent past nanolipidic structures have been coupled to drugs and used for the treatment of AD and PD. For instance, the encapsulation of rivastigmine, an inhibitor of acetylcholinesterase (AChE) and butyrylcholinesterase, into liposomes showed potential therapeutic effects in an aluminium chloride-induced Alzheimer's rat model. The administration of rivastigmine-loaded liposomes to $AlCl_3$-treated rats normalized expression of *BACE1* (the gene coding for the β-secretase which cleaves APP producing Aβ peptides), *AChE* (coding for the enzyme acetylcholinesterase which inactivates the neurotransmitter acetylcholine by

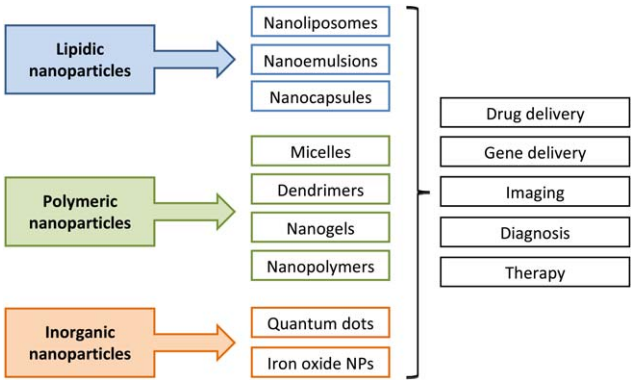

Fig. 1. The variety of materials of nanometric size summarized here may be useful for the diagnosis and treatment of neurodegenerative diseases. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

catalyzing its hydrolysis to choline and acetic acid), and *IL1B* gene (coding for a member of the interleukin 1 cytokine family, mediator of the inflammatory response). In contrast, co-treatment with rivastigmine solution caused a significant down-regulation of these genes [Ismail et al., 2013]. To overcome the poor bioavailability and solubility of curcumin, a pleiotropic molecule with anti-inflammatory and anti-oxidant activity, nanoliposomes loaded or functionalized with curcumin have been designed. In vitro, curcumin liposomes showed very high affinity [Mourtas et al., 2011] for $A\beta_{1-42}$ and inhibited its aggregation [Taylor et al., 2011]. Moreover, Lazar et al. [2013] demonstrated that mono-dispersed curcumin-conjugated nanoliposomes are biocompatible and bind selectively to $A\beta_{1-42}$ deposits. In vitro these nanolipidic structures were not toxic to HEK human embryonic kidney and human neuroblastoma SHSY5Y cells and down-regulated the secretion of the amyloid peptide. Ex vivo they were reported to strongly bind to $A\beta_{1-42}$ deposits in post-mortem brain tissue of AD patients, and in vivo they specifically stained $A\beta_{1-42}$ in APPxPS1 mice, a transgenic animal model of AD expressing mutant APP and presenilin 1, both involved in $A\beta_{1-42}$ production. Furthermore, anti-apoptotic and neurotrophic effects were demonstrated in a rat model of PD by using liposomal-formulated curcumin targeting histone deacetylase [Chiu et al., 2013].

Polymeric nanoparticles are stable NPs that are characterized by high drug loading capacity and their ability to protect the loaded drug against degradation, facilitating its delivery to the CNS [Behan et al., 2001]. Poly(n-butylcyanoacrylate) nanoparticles coated with 1% polysorbate 80 were shown to be more efficient in delivering rivastigmine into the brain of male Wistar rats than the free drug [Wilson et al., 2008]. Orally administered Tween80-coated polylactide-co-glycolide (PLGA) NPs containing estradiol resulted in significantly higher brain estradiol levels after 24 hr as compared with uncoated ones in an ovariectomized rat model of AD [Mittal et al., 2011]. Moreover, the conjugation of polyethylene glycol-polylactide-polyglycolide nanoparticles (PEG-PLGA NPs) with lactoferrin was shown to facilitate NP internalization in brain endothelial cells in vitro, and to enhance NPs accumulation in an in vivo mice model of PD [Hu et al., 2011]. Also in vivo, delivery of the human glial cell line-derived neurotrophic factor gene *hGDNF* by loading it into lactoferrin-modified PEG-PLGA NPs and injecting it repeatedly into a PD rat model was shown to improve locomotor activity, reduce dopaminergic neuronal loss and enhance monoamine neurotransmitter levels [Huang et al., 2009].

Among the inorganic NPs, IONPs are widely used in therapeutic and diagnostic applications. IONPs, which depending on their size can be classified in superparamagnetic iron oxide (SPIONs, 60–150 nm in diameter) and ultrasmall superparamagnetic iron oxide (USPIONs, 10–40 nm), have a Fe-core and can be coupled to organic materials and drugs. USPIONs chemically coupled with $A\beta_{1-42}$ were proposed as poorly invasive diagnostic tools for the in vivo detection of amyloid plaques by magnetic resonance microimaging [Yang et al., 2011]. Due to their increased relaxivity leading to an improvement in the contrast of the image during MRI and in vitro binding to ß-amiloid aggregates, SPIONs were proposed as ultra-sensitive nanoprobes for AD imaging [Zhou et al., 2014]. Several examples of nanovehicles to carry monoclonal antibodies against $A\beta_{1-42}$ into the brain have been recently developed as theranostic tools, some of them also being able to carry conjugated drugs to the Aß deposits [Poduslo et al., 2011; Agyare et al., 2014; Jaruszewski et al., 2014]. Similarly, quantum dots proved to be highly efficient to detect the potential AD biomarker apolipoprotein-E [Morales-Narváez et al., 2012], and SWCNTs were reported to be able to deliver acethylcholine in the brain of Kunming mice [Yang et al., 2010].

The treatment of neurodegenerative diseases is a major challenge, both because suitable drugs have not yet been identified for most diseases, and because of the limited access of bulky molecules, such as peptides and proteins, through the BBB. To overcome the latter problem, a growing number of nanotechnology-based delivery systems have ben proposed that are likely to be useful for either the diagnosis or treatment of neurodegenerative disorders. Many approaches are being tested with promising results, which go beyond the limited number of examples shown here. However, research into these materials is in its infancy. Among the important issues to be taken into consideration are the affinity between the drug and the nanobiocarrier (whereas there are drugs still to be identified), and the subsequent removal of the nanodevices from the brain.

CONCLUDING REMARKS

The ever-growing use of NMs in several human settings, including medical applications, raises the question

of the safety of humans employed in the manufacturing of those materials and consumers of NMs-containing products. In this regard, several authors have suggested that NMs can be toxic to various human organs and systems, including the CNS, thereby potentially contributing to the onset of human complex pathologies such as neurodegenerative diseases. On the other hand, the increasing number of elderly people in both developed and developing countries, coupled with the fact that there is actually no available treatment to halt the progression of most neurodegenerative conditions, lead to projections that those disorders will soon represent a serious health and socio-economic concern, reinforcing the demand for early diagnostic tools and novel therapeutic approaches. Nanotechnology has the possibility to impact both sides of this same coin. NMs may contribute to the onset and progression of several human pathologies due to the toxic properties. Alternatively, the physico-chemical properties of NMs make them important in the delivery of either diagnostic or therapeutic compounds to the site of disease lesion that might be difficult to reach with other methodologies.

In this review we presented an overview of studies that assess the impact on the nervous system of some of the most widespread nanoparticles. For in vitro approaches various cell models have been used for the assessment of neurotoxicity and of other related effects, representing the main cell types composing the brain: neurons and neuroglial cells (oligodendrocytes, astrocytes, microglia) or Schwann cells, responsible for the myelination of axons, or endothelial cells, which compose the BBB. The main cell lines employed were non-neuronal tumor cell lines such as pheochromocytoma (PC12) cells and neuronal tumor cell lines represented for instance by the human neuroblastoma SHSY5Y. In other cases primary cells obtained from mouse brain were used (mainly glial). For in vivo studies many of the best known mammalian models (rat and mouse) have been employed, as well as the invertebrate zebrafish (*Danio rerio*), including a transgenerational model. Among the in vivo experiments, different routes and times of administration have been employed. Almost all the reported studies clearly demonstrate the potential for several NMs to reach the CNS and induce toxic effects. Moreover, many NMs may interfere with pathways including oxidative stress, genotoxicity, apoptosis, inflammation, and microglia activation, which are common to most of the human neurodegenerative disorders, suggesting that they are able to contribute to neurodegeneration.

However, it is hard to compare the studies, both for the various cell models used, and for the different NMs employed, which can differ in relation to chemical and physical properties. For example, particle size can influence the behavior of the particle, that is, microsized NPs are typically less active than nanosized ones. For instance, micron-sized TiO_2 did not induce toxicity in PC12 cells, in contrast to nanosized TiO_2 [Wu et al., 2010]. Also smaller Ag NPs produced stronger inflammatory responses correlated with increased cerebral microvascular permeability compared with the larger ones in primary rat brain microvessel endothelial cells [Trickler et al., 2010]. Likewise, the influence of the size was shown in vivo; in different animal models (mouse and rat) smaller nano-sized Ag NPs internalize better and may cause BBB damage, organ toxicity and inflammatory response, in a size-dependent manner [Park et al., 2010; Dziendzikowska et al., 2012; Sharma et al., 2013b]. Conversely, in some cases the small size did not aggravate toxic effects, such as TiO_2 when tested in human astrocytoman cells [Lai et al., 2008] or ZnO, when tested in NSC mouse neural stem cells [Deng et al., 2009].

Even the NM shape can exert an influence on neurotoxicity: ZnO nanoparticles and microspheres displayed significant cytotoxic effects on RSC96 rat Schwann cells in dose- and time-dependent manners, while no or low cytotoxic effect was observed when the cells were treated with the prism-like and flower-like ZnO [Yin et al., 2012]. The surface modification of the NMs also seems to play a role on their effects on the brain, as shown in the in vivo studies of Zhang et al. [2011a, b, c] where mice were intranasally instilled with four different types of TiO_2 NPs varying in size and coating. Hydrophobic particles without coating resulted in less neurotoxicity than hydrophilic particles with silica surface coating. Particular concern should be devoted to metallic substances, which have the potential to be taken up through airborne exposure and enter the brain directly via retrograde transport through the olfactory nerve. In the brain, NMs may induce inflammation, apoptosis and oxidative stress as accumulating evidence strongly suggests that ROS generation and the induction of oxidative stress is a major toxicological paradigm for engineered metal oxide nanoparticles. For all the NMs considered in this review it has been demonstrated that NMs deposited in the nasal epithelium of animals may enter the brain via the olfactory bulb. Another portal of entry of NMs to the brain is from the systemic circulation. Neurotoxic effects have also been demonstrated through other routes of administration employed in the in vivo studies (e.g., oral, intraperitoneal).

Considering all of the studies conducted so far, what emerges is a deficiency in the use of models and standardized methods. Standardized approaches are desirable in NM research to ensure that the data can be used effectively in risk assessment. The field can be improved by introducing a concern-driven strategy for NMs potentially at risk or employed for specific purposes in the area of CNS [Oomen et al., 2013]. Moreover, it should be necessary to take into account (as much as possible) the biopersistence and accumulation of NMs, as well as their fate within critical tissues. In addition, the solubility

should be taken into account when metal NPs are investigated, highlighting the importance of including proper controls in the experimental design, in order to discriminate between the toxicity triggered by the ionic part and the effects induced by the particles themselves. It is desirable to develop new models, in line with the 3Rs principle (by using fewer animals, but obtaining more information at the same time), as well as exploiting the potential of emerging technology that employs iPSCs (induced pluripotent stem cells, increasingly used as cell model in vitro for neurodegenerative diseases), and the inclusion of new endpoints (such as epigenetic marks).

Collectively the data indicate an obvious need for a better assessment of the human risk of disease following exposure to NMs, including a clarifying of our understanding on the impact of those NMs on the human body, and their potential aggregation, accumulation, and targets. This is particularly true for those compounds designed for clinical applications or to be in direct contact with human tissues, for which a careful assessment of the risk-benefit ratio is compulsory. Drs. Migliore, Uboldi, Di Bucchianico and Coppedè together researched, designed, and wrote this review article.

AUTHOR CONTRIBUTIONS

Drs. Migliore, Uboldi, Di Bucchianico and Coppedè together researched, designed, and wrote this review article.

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