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REVIEW

The neurotoxicity induced by engineered nanomaterials

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Dan Ge[1,2,*]
Qiqi Du[1,2,*]
Bingqing Ran[1,2]
Xingyu Liu[1,2]
Xin Wang[1,2]
Xuehu Ma[1]
Fang Cheng[1,3]
Bingbing Sun[1,2]

[1]State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116024, People's Republic of China; [2]Department of Chemical Engineering, Dalian University of Technology, Dalian 116024, People's Republic of China; [3]Department of Pharmaceutical Science and Technology, Dalian University of Technology, Dalian 116024, People's Republic of China

*These authors contributed equally to this work

Abstract: Engineered nanomaterials (ENMs) have been widely used in various fields due to their novel physicochemical properties. However, the use of ENMs has led to an increased exposure in humans, and the safety of ENMs has attracted much attention. It is universally acknowledged that ENMs could enter the human body via different routes, eg, inhalation, skin contact, and intravenous injection. Studies have proven that ENMs can cross or bypass the blood–brain barrier and then access the central nervous system and cause neurotoxicity. Until now, diverse in vivo and in vitro models have been developed to evaluate the neurotoxicity of ENMs, and oxidative stress, inflammation, DNA damage, and cell death have been identified as being involved. However, due to various physicochemical properties of ENMs and diverse study models in existing studies, it remains challenging to establish the structure-activity relationship of nanomaterials in neurotoxicity. In this paper, we aimed to review current studies on ENM-induced neurotoxicity, with an emphasis on the molecular and cellular mechanisms involved. We hope to provide a rational material design strategy for ENMs when they are applied in biomedical or other engineering applications.

Keywords: engineered nanomaterials, neurotoxicity, oxidative stress, inflammation, DNA damage, cell death

Introduction

Engineered nanomaterials (ENMs) exhibit extraordinary chemical, biological, optical, mechanical, and magnetic properties due to their small size and large surface-to-volume ratio. Until now, ENMs have been successfully applied not only in traditional manufacturing, eg, catalysis, energy, electronics, and personal care products, but also in emerging biomedical fields, eg, imaging, diagnosis, drug delivery, and cancer immunology.[1] With the extensive application of ENMs, their safety has raised wide public attention. In the process of ENM production, processing, transportation, usage, and disposal, people are obviously exposed to ENMs via several different routes including skin contact, inhalation, ingestion, and systemic administration.[2,3] After exposure, ENMs can be distributed to various organs, eg, heart, liver, spleen, lungs, and kidneys.[4–7] More importantly, more and more studies have demonstrated that ENMs could cross the blood–brain barrier (BBB) and further access the central nervous system (CNS), where ENMs could cause neurotoxicity.[5,8–13]

The BBB is a dynamic barrier separating blood and the CNS with the function of restricting the material exchanges between the blood and brain, and helping to maintain the homeostasis of the brain.[14,15] With special physicochemical properties, ENMs could pass through BBB via different routes, eg, receptor-mediated

Correspondence: Bingbing Sun
State Key Laboratory of Fine Chemicals, Dalian University of Technology, 2 Linggong Road, Dalian 116024, People's Republic of China
Email bingbingsun@dlut.edu.cn

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transcytosis, adsorptive-mediated transcytosis, transcellular diffusion, paracellular diffusion, and cell-mediated transcytosis after incidental exposure or systemic administration.[10,16,17] After crossing BBB, ENMs can interact with glial cells and neurons, which could potentially induce a series of disrupted outcomes in the neurological system.[12] Current studies have demonstrated that the physicochemical properties of ENMs, eg, chemical composition,[18] size,[19] shape,[20] surface coating,[21] charge,[22,23] and aggregation state,[24] can affect their neurotoxicity in several in vivo study models, eg, zebra fish, rats, and mice (Table 1) and in various cell types, eg, microvascular endothelial cells, glial cells, and neurons (Table 2).[12,25] However, ENM-induced neurotoxicity is not well understood owing to the diverse physicochemical properties of ENMs, and the complicated regulating signaling pathways in organisms.

In this paper, we aimed to review the neurotoxicity of ENMs in different study models. We focused on potential mechanisms of ENM-induced toxicity in different neurological systems. With these information, we hope to provide more knowledge for the establishment of structure-activity relationships (SARs) and safer design of ENMs.

How could ENMs access the CNS?

ENMs could access the blood or lymph nodes, and then translocate to different organs including lungs, liver, kidneys, heart, and even the brain which is well-protected by the BBB. It has been found that ENMs could be deposited in different regions of the brain, including olfactory bulb, hippocampus, cerebral cortex, and striatum.[26,27]

It was found that ENMs with specific characteristics could pass through the BBB via several different ways (Figure 1). 1) Transcellular diffusion. Solid lipid nanoparticles (NPs) with low molecular weight could pass through the BBB in this way.[28] 2) Paracellular diffusion. Silica NPs and reduced graphene oxide (rGO) could open paracellular spaces and access the CNS.[29,30] 3) Receptor-mediated transcytosis. ENMs with ligands such as transferrin, insulin, ANG, and ApoE, could be recognized by corresponding receptors on endothelial cells' surface and then cross the BBB through transcytosis. ANG conjugated carbon nanotubes could pass through BBB efficiently, probably with the help of LRP1 which is located on the brain capillary endothelium.[31] 4) Adsorptive-mediated transcytosis. Cationic albumin-conjugated pegylated NPs could deliver hTRAIL-encoding plasmid (pORF-hTRAIL) into the CNS through adsorptive transcytosis after intravenous administration.[32] Lactoferrin-modified cationic dendrimer-based NPs could be promoted to cross BBB through both receptor- and adsorptive-mediated transcytosis.[33] 5) Cell-mediated transcytosis. Macrophages could phagocytize ENMs in the blood, pass through BBB, and then release ENMs into the CNS.

In addition to transpassing the BBB directly through blood circulation, studies have indicated that ENMs could translocate to the olfactory bulb of the brain through the olfactory nerve or into the CNS via the trigeminal nerve when they are inhaled or intranasally instilled.[34–37] Neurons could also transport ENMs by retrograde and anterograde movement in axons and dendrites as well as perineural translocation.[36] Additionally, particles can be found in the brain of offspring when animals are exposed to ENMs. For example, both 70 nm silica (SiO_2) and 35 nm titanium dioxide (TiO_2) particles were found in the placenta, brain, and liver of offspring when the particles were injected into pregnant mice intravenously, suggesting ENMs could cross the placenta barrier in pregnant mice and further cause damage to offspring.[38]

Additionally, ENMs that have been applied in medical devices could have opportunities to interact directly with glial cells or neurons and cause neurotoxicity. For example, carbon nanofibers were considered to be a potential material in neuronal implants.[39] Gold (Au) NPs and silica-poly (ε-caprolactone) (Si-PCL/ICG) NPs were proposed to be used for laser-assisted tissue soldering in the brain, eg, cerebral bypass surgery.[40] Nano-electromechanical systems in the brain, eg, implantable silicon-based microelectrodes, are used in supervising and diagnoses.[41–43] Once these implants that are coated with ENMs are worn, potential toxicity may be inevitable.

Mechanisms of neurotoxicity induced by ENMs

Oxidative stress

Oxidative stress is commonly caused by over-production of ROS with disturbance of redox homeostasis.[44] The brain is especially vulnerable to oxidative stress due to the large amount of oxygen consumption, abundance of lipids and proteins, and low antioxidant defense activity.[45] ROS are usually produced at an early stage when cells interact with ENMs and oxidative stress is regarded as one of the main mechanisms of neurotoxicity induced by ENMs.[46–48] Thus far, ROS production through different sources have been documented (Figure 2).

Oxidative burst in microglia

It usually happens when microglia are activated by foreign stimuli. During this process, a large amount of superoxide

Table 1 Mechanisms of ENM-induced neurotoxicity in vivo

Exposure	NP type	NP properties	Model	Mechanisms	Results	Reference
IV	Iron oxide	10 nm	Adult male wild-type SD rats	Oxidative stress	Brain vasculature damage; a significant decrease in striatal dopamine and its metabolites	113
IV	Single-walled CNTs	Diameter: around 1.0 nm; length: around 1.38 μm	Male Kunming mice	Oxidative stress; oxidative stress-induced inflammation; NF-κB accumulation; enhanced release of pro-inflammatory mediators TNF-α and IL-1β; mitochondrial perturbation; apoptosis	Cognitive deficits and decreased locomotor activity; functional and pathological lesions in the mouse brains	114
Oral	Silver	Small-sized Ag NPs (22, 42, and 71 nm) and large-sized Ag NPs (323 nm)	ICR mice, male and female	Inflammation; small-sized Ag NPs induced increased B cell distribution and TGF-β levels in serum; IL-1, IL-6, IL-4, IL-10, IL-12, and TGF-β levels were increased in a dose-dependent manner	Size-dependent uptake of Ag NPs (NPs were distributed to the organs including brain, lung, liver, kidneys, and testes)	115
Oral	Titanium dioxide	5 nm	SD rats	Significant oxidative damage to nucleic acids and lipids in the offspring's brain	Induced depressive-like behaviors in adulthood	116
Oral	Titanium dioxide	Anatase structure; hydrodynamic diameter: 208–330 nm; positive potential	ICR female mice	Increased expression of TLR2 and TLR4; inflammation; necrosis; shrinkage of cell volume; nuclear irregularity and cellular degeneration; over-proliferation of all glial cells	Significant reductions in body weight; accumulation of titanium in the hippocampus; necrosis and abscission of perikaryon; impaired spatial recognition memory	62
IN	Silica	Average particle size: 80 nm; not uniform; near spherical	Young male rats of Wistar strain	Oxidative stress; activated p53 signal transduction pathway; morphological changes in mitochondria and ER; apoptotic neuronal cell death	Neuromuscular coordination and spontaneous locomotor activity altered; changes in biochemical, neurochemical, and ultrastructural profiles in CS region of rat brain	96
IN	Silica	15 nm (primary size); 156 nm in physiological saline; 92 nm in DMEM cell culture medium	SD adult rats	Oxidative stress in striatum; high TNF-α and IL-1β levels and significant reduction of DA activity in the striatum	Deposition in the striatum. No disorders in general behavior after 1 and 7 days' instillation	27
IN	Silver	Spherical; 22.8–25.9 nm in culture medium; negative zeta potential	Neonatal SD rats	Apoptosis	Obvious alterations in the morphology of granular layer; dose-related elevation of silver levels in cerebellum	51
IN	Titanium dioxide	Rutile-phase; needle-like or short rod-like;	CD-1 mice, female	Rutile TiO_2 NPs caused morphological changes to the neurons and disturbed monoamine neurotransmitter levels in the sub-brain regions	Accumulated TiO_2 NPs in the brain. No mortality or obvious health disturbances in mice	26

(*Continued*)

Table 1 (Continued).

Exposure	NP type	NP properties	Model	Mechanisms	Results	Reference
IP	Silica	Spherical, 20±6.34 nm	SD rats	Opened BBB paracellular spaces; oxidative stress and astrocyte activation in the brain	BBB permeability elevated; systemic inflammation	30
IP	Aluminum oxide	Oval shape; various sizes ranging from 30–60 nm	ICR female mice	Oxidative stress; production and aggregation of Aβ; disturbance of brain energy metabolism (reduction of AMPK)	Aluminum accumulation in brain; impairment of hippocampus-dependent memory; AD neuropathology; impaired spatial learning and memory deficit	80
IP	Titanium dioxide	<100 nm; average crystallite size: 44 nm	Male Swiss Webster mice	Oxidative stress; oxidative DNA damage and chromosomal damage; mutations in p53	Nano-TiO_2 accumulation selectively; dose-dependent toxicity	82
	Titanium dioxide	Primary particle sizes: 33.4±1.9 nm; hydrodynamic size in water: 149.4±1.3 nm	Larval zebra fish	Loss of DA; increased gene expression related to Lewy bodies	Cell death in hypothalamus; no significant increases in mortality; induced PD-like symptoms; decreased hatching time of zebra fish; disturbed locomotive activity; increased malformation rate	117
	GO	With a sheet thickness of 1.02±0.15 nm; lateral lengths: ranged from approximately 0.5 μm to several microns	Larval zebra fish	Oxidative stress; structural and morphological damage to the mitochondria; loss of DA neurons; CASP8-associated apoptosis; β-galactosidase-associated senescence; metabolic disturbance; the promotion of Lewy bodies (α-synuclein and ubiquitin)	Tail flexure and spinal curvature; PD-like symptoms; disturbance of locomotive activity	79
	Zinc oxide	Spherical or short-rod shaped; average size of the aggregates: 30–60 nm	Zebra fish embryos	Oxidative stress; upregulation of p53; reduction in the Bcl-2/Bax ratio; reduction in MMP; release of cytochrome C; activation of CASP9 and 3; apoptosis; DNA oxidative damage	Reduced rate of embryo hatching; significantly higher heart rates when the dose was 120 mg/L	92
	Iron oxide	Coated with cross-linked aminated dextran	Zebra fish	Apoptosis; enhanced mRNA levels of CASP8, 9, and jun genes	Decreased AChE activity; reduction in the exploratory performance; significantly higher level of ferric iron in the brain	118
	Fullerenes	Uncoated; 99.5% pure; stable 30–100 nm aggregates	Largemouth bass	Significant lipid peroxidation in the brain; oxidative stress	Toxicity was not obvious	119

Abbreviations: ENM, engineered nanomaterial; IV, intravenous; IP, intraperitoneal; IN, intranasal; CNTs, carbon nanotubes; NP, nanoparticle; ER, endoplasmic reticulum; BBB, blood–brain barrier; SD, Sprague Dawley; CS, corpus striatum; PD, Parkinson's disease; AD, Alzheimer's disease; DA, dopaminergic; AChE, acetylcholine esterase. AMPK, adenosine 5'-monophosphate activated protein kinase; ICR, institute of cancer research; DA, dopamine; GO, graphene oxide; Aβ, amyloid beta; MMP, mitochondrial membrane potential.

Table 2 Mechanisms of ENM-induced neurotoxicity in vitro

Model	NP type	NP properties	Cell type	Mechanisms	Results	Reference
BBB	Copper oxide	40 and 60 nm; strong aggregation	rBMECs	Increased levels of pro-inflammatory cytokines like PGE2, TNF-α, and IL-1β were associated with rBMEC permeability	Induced proliferation at low concentration but cytotoxitity at high concentration; enhanced barrier permeability	71
	Iron oxide	8 nm core; uncoated and oleic acid-coated	HCECs	For oleic acid-coated USPIO, poor internalization, a significantly decreased DNA synthesis and increased ROS was found. For uncoated USPIO, rapid deposition on the cell surface and a significant decrease in thiol levels. Significant DNA damage. Autophagy and lysosomal activation	Oleic acid-coated USPIO had higher cytotoxicity than the uncoated	99
	Silica	25 and 50 nm; fluorescent	HCECs	A significant decrease in DNA synthesis and a slight increase in ROS was found; 50 nm silica induced autophagy	50 nm silica had higher cytotoxicity	99
	Silica	Spherical, 20±6.34 nm	hBMEC/astrocytes	Oxidative stress and Rho/ROCK mediated pathway; microtubule destabilization; inflammation	BBB disruption	30
	Silver	25, 40, and 80 nm	rBMECs	Secretion of pro-inflammatory mediators (TNF-α, IL-1β, and PGE2)	Cytotoxicity and morphological changes of monolayer perforations; Increased rBMEC permeability; 25 nm has the biggest effect among 25, 40, and 80 nm nano-silver	19
	Silver	8 nm	bEnd.3 and ALT cells	Different toxic potency of Ag ions and Ag NPs. Ag NPs induced production of ROS	Damage of BBB integrity	70
	Titanium dioxide	21 nm core	HCECs	Increased ROS; a slight decrease in DNA synthesis and significant DNA damage	Significant cytotoxicity was induced	99
	Titanium dioxide	25.2 nm	Rat primary BECs+glial cells	Intense inflammatory response; a modulation of BBB functioning and repercussions on ABC transporter activities of glial cells	Accumulation of TiO_2 NPs and dysfunction of BBB	74
	Titanium dioxide	6 nm, 35 nm	bEnd.3 and ALT cells	Production of cytokine secretion (IL-4, IL-12, IL-13, IFN-γ, and TNF-α)	Damage of BBB integrity	70
Glial cells	Copper oxide	Diameter: 5 nm; dimercaptosuccinate-coated	Primary brain astrocytes	Intracellular liberation of copper ions from CuO-NPs; oxidative stress	Time-, concentration- and temperature-dependent accumulation of NPs; decreased cell viability	120

(*Continued*)

Table 2 (Continued).

Model	NP type	NP properties	Cell type	Mechanisms	Results	Reference
	Silica	150–200 nm; sphercal; different mass/volume	Primary rat microglial cells	Increased intracellular ROS and RNS; decreased TNF-α gene expression and increased pro-inflammatory genes' expression; detectable level of IL-1β.	Alteration of microglia functions	69
	Silver	3–5 nm; negative potential	ALT cells; BV-2 cells	Significant IL-1β secretion was induced in BV-2; induced gene expression of CXCL13 and GSS for inflammatory response and oxidative stress	Decreased cell proliferation of ALT but not BV-2	121
	Titanium dioxide	A mixture of the anatase (70%) and rutile (30%) forms; ~330 nm in media; positive surface charge	BV-2 cells	Oxidative stress, apoptosis, mitochondrial dysfunction, and inflammation (NF-κB) were induced	Time- and dose-dependent cytotoxicity was found	49
	Titanium dioxide	50 nm	C6 cells; U373 cells	Oxidative stress; morphological changes and damage of mitochondria; reduction in MMP	Cytotoxicity	122
	Titanium dioxide	P25, 3:1 mixture of anatase and rutile, 21 n; anatase, spherical, 50 nm; rutile, rod-like, 50 nm	Primary rat cortical astrocytes	Increased ROS generation and decrease in MMP	Mitochondrial damage and morphology changes; dose-dependent cytotoxicity; anatase crystalline phase was more toxic than rutile	123
	Titanium dioxide	Anatase (96%) and rutile (4%), 40–200 nm	C6 cells; U373 cells	Inhibitation of cell proliferation; induction of apoptotic death; depolymerization of Factin	Dose-related cytotoxicity; morphological changes	88
	Titanium dioxide	Anatase isoform; 15 nm	D384 cells; SHSY5Y cells	Alterations of the mitochondrial function; cell membrane damage	Dose- and time-dependent alterations of the mitochondrial function; SHSY5Y was more sensitive compared to D384	124
	Zinc oxide	45 nm, rod-shaped with smooth surfaces	Primary rat astrocytes	JNK pathway was involved in ROS-induced apoptosis in primary astrocytes; mitochondrial dysfuction was found	Significant cytotoxcity; cellular morphological modification	90
	Zinc oxide	38.52 nm	N9 cells	Altered intracellular calcium level, mitochondrial ROS, MAP kinases, cytochrome C, as well as CASP9 and 3; ATP depletion played a major role	Dose-and time-dependent toxicity	50
	Zinc oxide	Hexagonal prism-shaped; 50 nm	BV-2 cells	Oxidative stress; autophagy; PINK1/parkin-mediated mitophagy	Decreased cell viability	102
Neuron	Aluminum oxide	Oval shape; 30–60 nm	SHSY5Y cells; HT22 cells	Oxidative stress; DNA damage; activation of CASP3/7	Decreased cell viability; efficient internalization	80

(*Continued*)

Table 2 (Continued).

Model	NP type	NP properties	Cell type	Mechanisms	Results	Reference
	Copper	15–30 nm; weight fraction of Cu, CuO, and Cu_2O: 85.93%, 8.99%, and 5.08%	PC12 cells	Increased ROS; significant apoptosis	Concentration- and time- dependent cytotoxicity	125
	Graphene and SWCNTs	Graphene diameter: 100–110 nm; thickness: 3–5 nm. SWCNTs: diameter: 0.8–1.2 nm	PC12 cells	Concentration- and time-dependent ROS generation; apoptosis; increased LDH release; changed cell morphology	Concentration- and shape-dependent cytotoxicity; LDH levels were significantly higher for SWCNT than graphene	126
	GO	GO layer: thickness 0.6 nm, with sags and crests on the surface; rGO layer: thickness 0.9 nm, more fluent surface than GO	PC12 cells	Apoptosis and cell cycle arrest maybe due to ERK pathway regulation	Dose- and time-dependent cytotoxicity; GO was more toxic than rGO	85
	Iron oxide	A core size of 10 and 30 nm with a diameter of 8–10 nm surface coating; spherical	SHSY5Y cells	Activation of c-Abl (a pro-death compound), a molecular switch induced by oxidative stress; increased expression of neuronal α-synuclein (protein dysfunction); oxidative stress; mitochondrial integrity was affected; apoptosis	10 nm NPs had higher cytotoxicity than 30 nm ones	113
	Manganese	40 nm	PC12 cells	Oxidative stress	Nanoscale manganese can deplete DA, DOPAC, and HVA in a dose-dependent manner	127
	PAMAM dendrimers	5–6 nm; positive potential in water and negtive potential in media	PC12 cells; SHSY5Y cells	Increased ROS and autophagy flux	Cytotoxicity	106
	Silica	25±4 nm by TEM; 95.8 ±9.7 nm in suspended DMEM containing 10% FBS	PC12 cells	Aggregation of α-synuclein; impairment of UPS; autophagy	Cytotoxicity	100
	Silica	12.1 nm	SK-N-SH cells; N2a cells	Apoptosis and ROS release; morphological changes	Concentration-dependent cytotoxicity	128
	Silver	14 nm, spherical	PC12 cells	Apoptosis	Dose-dependent cytotoxicity. AgAc had higher cytotoxicity than nano-Ag, and there were silimar neurotoxic effects between two forms of silver	93

(*Continued*)

Table 2 (Continued).

Model	NP type	NP properties	Cell type	Mechanisms	Results	Reference
	Silver	10 nm or 75 nm, PVP or citrate coated	N27 cells	PVP-Ag increased intra-neuronal nitrite, activated ARE/NRF2, and CASP3/7; 75-nm PVP-Ag caused mitochondrial dysfunction, while 10 nm PVP-Ag affected NRF2	PVP-coated nano-Ag had higher cytotoxicity than citrate-coated ones	129
	Silver	3–5 nm; negative potential	N2a cells	Aβ deposition	Decreased cell proliferation	121
	Silver	85±5 nm; spherical	Human neurons (from dental pulp mesenchymal stem cells)	Apoptosis; damage of neuronal connections; changes of the gene expression involved in heavy metals' metabolism and cellular growth during oxidative stress conditions; impairment of mitochondrial function	Concentration-dependent cytotoxicity; changes of cell morphology and neurite outgrowth	130
	Silver	Spherical; 22.8–25.9 nm in culture medium and 21.7 nm 24.4 nm in aqueous solution; negative zeta potential	Rat CGCs	CASP3 mediated apoptosis; oxidative stress; increase of intracellular calcium	Significant dose-dependent cytotoxicity without cell membrane damage; Cell body shrinkage	51
	Single-walled CNTs	SWCNTs: diameter 0.7–1.6 nm, length 0.2–3 μm; SWCNT-PEG: diameter 2.5–4.5 nm, length 0.1–1 μm	PC12 cells	Concentration- and surface coating-dependent ROSproduction; genes involved in oxidoreductases and antioxidant activity, nucleic acid or lipid metabolism, and mitochondrial dysfunction were highly represented	Concentration-dependent and surface coating-dependent cytotoxicity; SWCNT-PEGs were less cytotoxic than uncoated SWCNTs	131
	Titanium dioxide	TiO_2-S: 25 nm, 100% anatase; TiO_2-D: 25 nm, 80% anatase and 20% rutile	SHSY5Y cells	Induced dose-dependent cell cycle alterations; genotoxicity	No effects on viability; effective internalization; higher uptake for TiO_2-S NPs than for TiO_2-D NPs; TiO_2-S NPs induced more cytotoxicity	86
	Titanium dioxide	Primary sizes: 33.4±1.9 nm;	PC12 cells	ROS generation; mitochondrial dysfunction	Dose-dependent cytotoxicity; reduced DA levels	132
	Titanium dioxide	Anatase TiO_2-NPs: 20 nm; rutile TiO_2-NPs: 20 nm	PC12 cells	Increased levels of LDH; increased ROS and apoptosis; activation of JNK- and p53-mediated signaling pathway; cell cycle arrest in G2/M phase	Concentration, size, and crystal structure-dependent cytotoxicity and membrane damage; anatase TiO_2 NPs were more toxic than rutile	91
	Titanium dioxide	A mixture of the anatase (70%) and rutile (30%) forms; ~330 nm in media; positive surface charge	N27 cells; primary cultures of embryonic rat striatum	For primary cultures of embryonic rat striatum, apoptosis was induced	For N27, no significant cytotoxicity; for primary cultures of embryonic rat striatum, neuronal loss occurred	49

(*Continued*)

Table 2 (Continued).

Model	NP type	NP properties	Cell type	Mechanisms	Results	Reference
	Titanium dioxide	21 nm	PC12	Apoptosis; enhanced intracellular ROS generation	Dose-dependent and time-dependent cytotoxicity	133
	Zinc oxide	A: rod-shaped, smooth surfaces, average size 47.1 nm, width 27.9 nm; B: NPs: rod-shaped, average size 18.5 nm, width 6.8 nm	SHSY5Y cells	Oxidative stress; apoptosis and cytoskeleton changes	Size-dependent cytotoxicity; cell morphology changes	134
	Zinc oxide	100 nm	SHSY5Y cells	Apoptosis and cell cycle alterations; DNA damage	Concentration- and time-dependent cytotoxicity	81
	Zinc oxide	Spherical, 35 nm; microsphere, 45 nm; hexahedral, prism-like, 2.5–6.0 μm in diameter and 18.0–60.0 μm in length; flower-like, 500–600 nm in diameter and several microns in length	RCS96	Apoptosis induction and G2/M phase cell cycle arrest; significant release of Zn-ions	Shape- and time-dependent neurotoxicity	20

Notes: hCMEC/D3, immortalized human brain capillary endothelial cells; bEnd.3, mouse endothelial cells; ALT, mouse brain astrocyte-like cell line; N9, mouse microglial cells; BV-2, mouse microglial cells; C6, rat brain glial tumor cells; U373, human glial cells; D384, human glial cells; N27, rat dopaminergic neurons; RSC96, rat Schwann cells; HT22, mouse hippocampal neuronal cells; SK-N-SH, human neuroblastoma Cell Line; N2a, mouse neuroblastoma cells.
Abbreviations: ENM, engineered nanomaterial; BBB, blood–brain barrier; PAMAM, polyamidoamine; CNTs, carbon nanotubes; NP, nanoparticle; GO, graphene oxide; rGO, reduced graphene oxide; SWCNT, single-walled carbon nanotube; SWCNT-PEG, SWCNTs functionalized with polyethylene glycol; CGCs, cerebellum granule cells; USPIO, ultra-small superparamagnetic iron oxide; GSS, glutathione synthetase; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; PVP, polyvinylpyrrolidone; UPS, ubiquitin-proteasome system; MMP, mitochondrial membrane potential; TEM, transmission electron microscope; rBMECs, rat brain microvessel endothelial cells; hBMEC, human brain microvessel endothelial cells; HCECs, human cerebral endothelial cells; BECs,brain endothelial cells; c-Abl, Abelson murine leukemia viral homolog 1; ARE, antioxidant response element; NRF2, nulear erythroid 2-related factor 2.

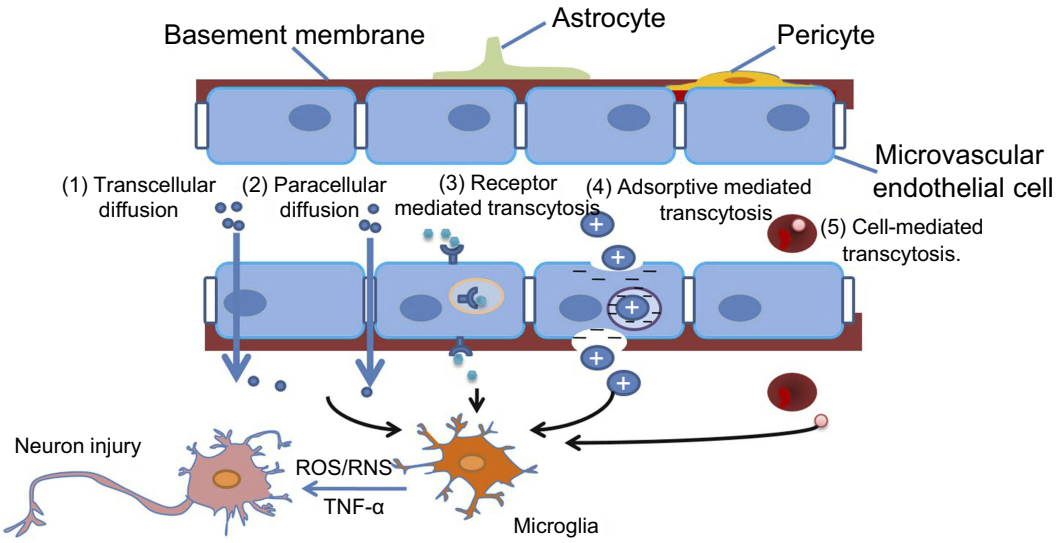

Figure 1 Possible ways in which engineered nanomaterials (ENMs) cross the blood–brain barrier (BBB) and the potential risks. ENMs with specific physicochemical properties could pass through the BBB by way of several different strategies.

Notes: 1) Transcellular diffusion. ENMs with low molecular weight, eg, solid lipid nanoparticles, can pass through the BBB in this way. 2) Paracellular diffusion. Some ENMs, eg, silica nanoparticles and reduced graphene oxide, can open paracellular spaces, and then get into the central nervous system (CNS). 3) Receptor-mediated transcytosis. ENMs with ligands like transferrin, insulin, ApoE, etc can be identified by corresponding receptors on the endothelial cells. 4) Adsorptive mediated transcytosis. ENMs with positive charges can be attracted by microvascular endothelial cells that are negatively charged. 5) Cell-mediated transcytosis. Macrophages with phagocytized ENMs in the blood could pass through the BBB, and release ENMs into the CNS.

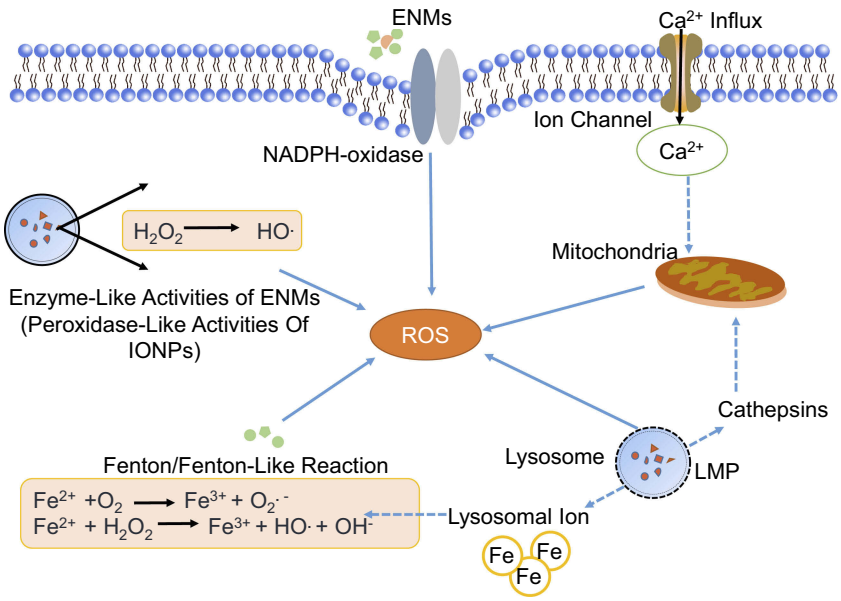

Figure 2 Mechanisms of ROS production induced by engineered nanomaterials (ENMs). ROS have different sources.

Notes: 1) Oxidative burst. ENM-activated microglia can produce ROS with the catalyzation of NADPH-oxidase. 2) Mitochondrial ROS. Disruption of electron transport chain will lead to significant increase of ROS. 3) Fenton or Fenton-like reaction. The transition metals, eg, iron, copper, chromium, and cobalt can mediate the formation of ROS like highly reactive hydroxyl radical ($OH^{\cdot}$) through Fenton reaction. 4) Enzyme-like activities of ENMs. For example, the INOPs could behave like peroxidase. (5) Lysosome membrane permeabilization (LMP). LMP could produce ROS through lysosomal iron or by causing mitochondrial membrane permeabilization and producing mitochondrial ROS.

Abbreviation: IONPs, iron oxide nanoparticles.

anion ($O_2^{\cdot -}$) is released through NADPH-oxidase mediated molecular oxygen (O_2) catalysis. $O_2^{\cdot -}$ could be further transformed to other forms of ROS, eg, hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\cdot}$), and peroxynitrites. Phenrat et al[48] found that when microglia were exposed to TiO_2 NPs (Degussa P25), there was immediate (<5 minutes) concentration-dependent intracellular H_2O_2 production through oxidative burst. The

particle size, aggregation state, surface coating, and sedimentation could influence the interaction between ENMs and cells that further affect oxidative burst-mediated ROS production. A study has demonstrated that large zero-valent iron (ZVI) NPs could agglomerate into micron-size particles and induce oxidative burst in BV-2 cells.[24]

Mitochondrial dysfunction

Mitochondria are a main source of ROS as a result of the electron transport chain (ETC) through which adenosine triphosphate (ATP) is generated. Mitochondrial disruption will lead to significant increase of ROS. Long et al[48,49] showed the existence of $O_2^{\cdot-}$ in mitochondria of BV-2 cells after TiO_2 (Degussa P25) exposure. Sharma et al[50] showed that ZnO NPs possibly induced mitochondrial ROS, which led to mitochondrial dysfunction, eg, decreased MMP and release of cytochrome C, and further induced microglial death. In addition, Yin et al[51] demonstrated that after exposure to silver (Ag) NPs, elevated calcium ions (Ca^{2+}) influx could cause oxidative stress by promoting mitochondrial depolarization in rat cerebellar granule cells.

Fenton/Fenton-like reaction

Transition metals, eg, iron, copper, chromium, and cobalt, could mediate the formation of ROS, eg, highly reactive hydroxyl radical ($OH^{\cdot}$), through Fenton reaction. Phenrat et al showed that ZVI NPs could act as electron donors and produce ROS through Fenton reaction in microglia. They also showed that iron oxide NPs (IONPs) could induce Fenton reaction by oxidizing ferrous iron to ferric iron, which contributed to the oxidative stress in astrocytes.[24,52]

Enzyme-like activities of ENMs

It was demonstrated that when human glioma U251 cells were exposed to dimercaptosuccinic acid-coated Fe_3O_4 and γ-Fe_2O_3 together with H_2O_2, both two types of NPs could localize in lysosomes and behave as peroxidase and catalyze H_2O_2 into $OH^{\cdot}$, which could further induce cytotoxicity.[53] On the contrary, GO quantum dots (QDs) showed catalase-like activity and decreased MPP^+-induced ROS generation in PC12 cells.[54]

Lysosome membrane permeabilization (LMP)

LMP is a known form of lysosome dysfunction and suggested as an indirect source of ROS. Firstly, LMP could produce ROS through lysosomal ferrous iron. Secondly, LMP could lead to mitochondrial membrane permeabilization and production of mitochondrial ROS by releasing proteases, eg, cathepsin B/D inside lysosomes.[55] Yang et al[56] found that when RAW264.7 macrophages were exposed to carbon nanohorn, LMP could be induced, which further led to the production of mitochondrial ROS.

However, it still remains unclear that how ENMs with different physicochemical properties could affect ROS generation via the previously mentioned mechanisms. Thus, more detailed studies are needed.

With the induction of oxidative stress, the subsequent consequences have been investigated. Firstly, oxidative stress is prone to enhancing the permeability of the BBB. Liu et al showed that, when brain microvessel endothelial cells (BMECs) were exposed to silica NPs, ROS were induced and considered as the mediators in increasing BBB permeability by down-regulating the tight junction proteins, eg, CLDN5 and OCLN, and further causing microtubule destabilization and triggering inflammatory response. By using N-acetyl cysteine, an antioxidant agent, ROS-induced BBB permeability was significantly inhibited.[30] Furthermore, ENM-induced ROS in microglia may damage surrounding neurons.[27,49,57,58] The study by Hsiao et al showed that BV-2 could produce large amounts of H_2O_2 after nano TiO_2 exposure. Co-culture of N2a neuroblastoma cells and BV-2 cells showed that N2a cells were significantly damaged when BV-2 cells were in direct contact with nano TiO_2. In contrast, direct exposure to nano-TiO_2 did not reduce the viability of N2a cells.[57] Another study by Long et al found that Degussa P25 induced the release of ROS and apoptosis in BV-2 microglia, but did not affect the cell viability of isolated rat dopaminergic neurons (N27). However, P25 caused neuronal loss of embryonic rat striatum containing both microglia and dopaminergic neurons, indicating the influence of microglia-generated ROS on neurons.[49]

Inflammation

Inflammation is a protective response in the body with the function of eliminating xenobiotic stimuli and repairing damaged tissue.[59] Neuroinflammation can be induced directly after ENMs get into the brain. It mainly occurs in microglia, the resident macrophages in the brain (Figure 3). Microglia are immune-competent cells, which usually remain quiescent and will respond to the destruction of brain homeostasis and entry of environmental toxins.[11] Upon ENM exposure, microglia could be activated and change their morphology from ramified to rounded macrophage-like ones and further produce pro-inflammatory cytokines, eg, interleukins, tumour necrosis factor protein families, and chemokines.[60,61] Knudsen et al found

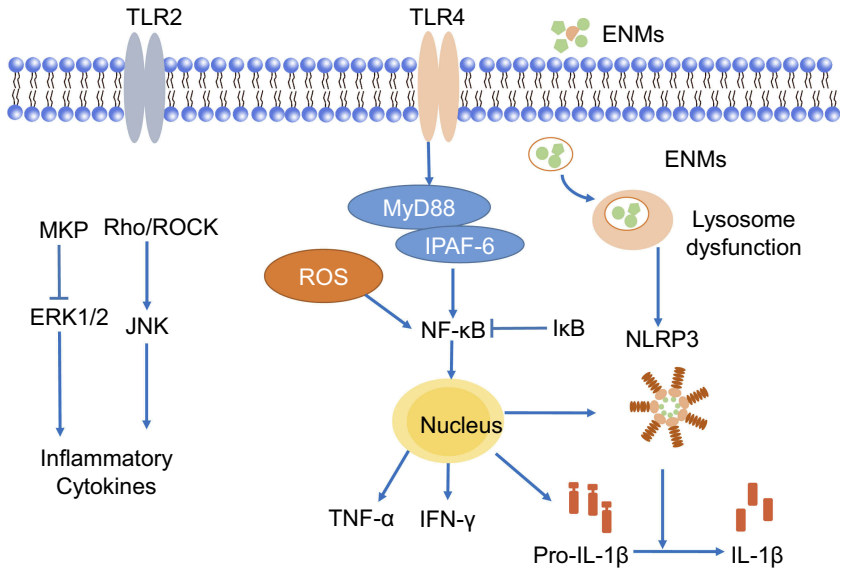

Figure 3 Possible mechanisms of releasing pro-inflammatory cytokines in microglia. Engineered nanomaterials (ENMs) can provoke TLRs like TLR2 and TLR4. TLR4 can further activate NF-κB pathway, which results in the release of inflammatory cytokines. ENMs can also activate ERK1/2 by inhibiting MKPs (MAPK phosphatases), and then cause the release of inflammatory cytokines.

that cationic PEGylated micelles and cationic liposomes could induce the focal filtration of phagocytes and activate microglia and astrocytes in the brain after an intracerebroventricular particle injection.[22]

ENMs, as a kind of stimuli, can provoke TLRs, eg, TLR2 and TLR4, of microglia that reside on the cell membrane. It was reported that nano-TiO_2 could induce TLR2 and TLR4 activation in the hippocampus after oral administration. The response of TLRs could further induce the activation of NF-κB through negatively regulating nucleic inhibitor of NF-κB (IκB) kinase and caused subsequent hippocampal inflammation in mice.[62] Hutter et al[63] showed that poly(ethylene glycol)-coated urchin/rod but not sphere Au NPs could upregulate TLR2 expression of microglia, suggesting effects of geometries on the activation of TLRs. However, detailed mechanistic studies are needed to elucidate the exact role of TLRs in neuroinflammation after ENM exposure.

NLRP3 inflammasome belongs to the NLR family, and is a multiprotein complex that could respond to exogenous stimuli. NLRP3 inflammasome could be directly activated by ENMs, and modulate the release of pro-inflammatory cytokines, eg, IL-1β.[64] Moquin et al[65] showed that lipopolysaccharides-QDs (LPS-QDs) could potentially induce mitochondrial ROS and lead to lysosome rupture, followed by NLRP3 inflammasome activation and cleavage of IL-1β in microglia. In addition, the uptake of large LPS-QDs contributed to the rupture of lysosomes. As many studies have already shown, ENMs could be taken up by microglia through macropinocytosis, phagocytosis, and clathrin-mediated endocytosis, and then translocate to lysosomes through endo-lysosome pathway for the activation of NLRP3 inflammasome.[57,66,67]

Neuroinflammation occurs not only in immune cells,[64] eg, microglia[68,69] in the brain, but also in microvascular endothelial cells,[19,70,71] astrocytes,[57] and neurons.[57] When encountering ENMs, common in vitro BBB models, BMECs or co-culture of BMECs and astrocytes/pericytes could produce pro-inflammatory cytokines. Trickler et al[71-73] found that CuO NPs and Ag NPs induced an increase of pro-inflammatory mediators IL-1β, TNF-α, and PGE_2 in rat BMECs and porcine BMECs, which affected the integrity of the BBB. ENM-induced pro-inflammatory cytokines and chemokines in BMECs could recruit immune cells in the blood and also promote the expression of CAMs, eg, E-selectin and ICAM-1, to facilitate the adhesion and transmigration of leukocytes through the BBB.[30,74] In addition, there was an immunoregulatory loop between BMECs and astrocytes after exposure of ENMs.[74] In short, astrocytes could integrate the paracrine inflammatory signals from BMECs, amplify them, and then return them to BMECs, thereby contributing to the destruction of the BBB.[30,75]

DNA damage

ENM exposure could induce DNA damage that could potentially lead to carcinogenesis or even impact offspring

(Figure 4).[76] Thus far, different types of DNA damage have been identified, including DNA cross-links, single/double strand breaks, and DNA adducts (Table 3).[77]

Studies have found that ENMs could induce DNA damage in two different ways, ie, primary DNA damage and secondary DNA damage. The former refers to the fact that ENMs could enter the cells and subsequently the nuclei, and further directly interact with DNA or DNA-related proteins.[78] For example, Ren et al[79] showed that GO sheet could translocate into cell nuclei of diencephalon in zebra fish larvae brain from water. Even if ENMs could not penetrate the nucleus, there still remains the opportunity that they could affect DNA in the process of mitosis. As to secondary DNA damage, it usually refers to the impact induced by ROS and inflammation. Shah et al[80] showed that nano-alumina could induce oxidative DNA damage in both in vitro models, ie, mouse hippocampal HT22 cells and human neuroblastoma SHSY5Y cells, and in vivo model, ie, brain of mouse models. Valdiglesias et al[81] showed that ZnO NPs could induce oxidative DNA damage and H2AX phosphorylation representing DNA double strand breaks in human SHSY5Y cells. El-Ghor et al[82] found that nano-TiO_2 induced both chromosomal damage and DNA damage in brain tissue due to ROS. In addition, Hawkins et al[83] found that cobalt and chromium (CoCr) NPs triggered release of IL-6 in a placental barrier model, and IL-6 led to DNA double strand breaks in differentiating neural progenitor cells.

In addition, studies have demonstrated that DNA damage could lead to cell cycle arrest to provide enough time for DNA repair, while inefficient DNA repair could induce apoptosis.[84] Kang et al[85] found that GO and rGO exposure could lead to two or more nuclei in PC12 cells and G0/G1 cell cycle arrest, further leading to apoptosis. Valdiglesias et al[86] found that TiO_2 NPs induced

Table 3 Assays for DNA damage and corresponding results

Test method	Results
SSCP	Mutation
Ames test	Mutation
HPRT forward mutation assay	Mutation
Chromosome aberration test	Chromosomal alterations
Comet assay	DNA single and double strand breaks
Cytokinesis-blocked micronucleus assay	Chromosomal damage
γ-H2AX staining	DNA double strand breaks
8-OHdG DNA adducts	DNA oxidative damage

Abbreviations: SSCP, single strand conformation polymorphism; HRPT, hypoxanthine–guanine phosphoribosyltransferase; H2AX, H2A histone family member X; 8-OHdG, 8-hydroxydeoxyguanosine.

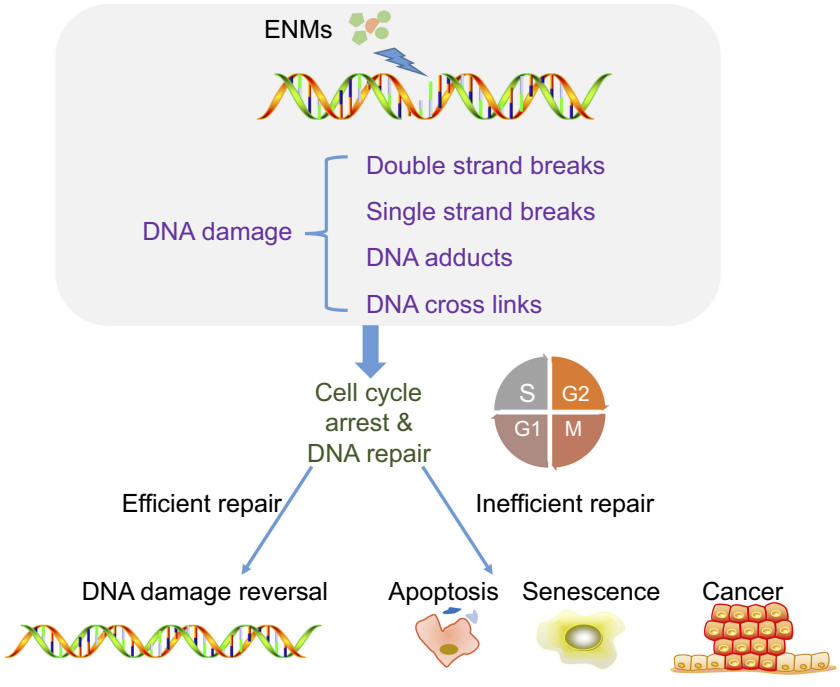

Figure 4 Mechanisms of engineered nanomaterials (ENMs)-induced DNA damage. ENMs could cause different types of DNA damage including DNA crosslinks, single/double strand breaks, and DNA adducts. DNA damage could lead to cell cycle arrest to provide enough time for DNA repair and inefficient DNA repair could induce apoptosis, senescence, and cancer.

micronuclei together with DNA strand breaks in SHSY5Y cells, and further, apoptosis was induced.

ENM induces differential cell death

Studies have suggested that different types of cell death are involved in the neurotoxicity of ENMs, such as apoptosis, necrosis, and autophagy (Figure 5).

Apoptosis and necrosis

Apoptosis is programmed cell death with the function of eliminating damaged cells and further maintaining the organism's homeostasis. It is the most common cell death found in ENM-induced neurotoxicity with the characteristics of cell shrinkage, nuclear condensation, and fragments.[87,88] In general, apoptosis can be induced by mitochondrial dysfunction (intrinsic pathway), death receptor (extrinsic pathway), and endoplasmic reticulum (ER) stress. Different caspases, eg, CASP8, 9, 3, are the primary molecular signals of apoptosis.

The underlying mechanisms of ENM-induced intrinsic apoptosis have been widely studied. MAPK molecules including p38 MAPK, JNK, and ERK are usually associated with intrinsic apoptosis.[89] In addition, P53 can be activated by ROS and DNA damage which may lead to apoptosis by inducing the transcription of pro-apoptotic genes like Bax. Xu et al[72] found that Ag NPs induced apoptosis through activating MAPK pathway, reducing anti-apoptotic Bcl-2 expression, and altering activity of mTOR in primary astrocytes. Wang et al[90] showed that ZnO NPs induced phosphorylation of JNK, ERK, and p38 MAPK in astrocytes, and that JNK played the major role in apoptosis. Wu et al[91] discovered that P53 promoted apoptosis together with JNK pathway in PC12 cells while they were treated with TiO_2 NPs. Zhao et al[92] found that ZnO NPs could lead to apoptosis in zebra fish embryos characterized by reduction in the Bcl-2/Bax ratio, decrease of MMP, release of cytochrome C, and activation of CASP9 and 3. In addition, Hadrup et al[93] showed that Ag NPs could induce both extrinsic and intrinsic apoptosis in PC12 cells based on the activation of CASP8 and 9. Another study also found that ZnO NPs induced the phosphorylation of ERK and p38, which further activated CASP9 and 8 respectively, and then apoptosis was induced.[50]

The ER is responsible for the synthesis, processing, and trafficking of protein, and the regulation of Ca^{2+}. ER stress could be induced by the change of Ca^{2+} homeostasis and misfolded proteins.[94] Ducray et al[95] found that two types of silica NPs, ICG/polycaprolactone (PCL)-rhodamine-doped NPs and Si-ICG/polycaprolactone-polylactic acid-rhodamine-doped NPs could be taken up by microglia and translocate in the ER when they were exposed to

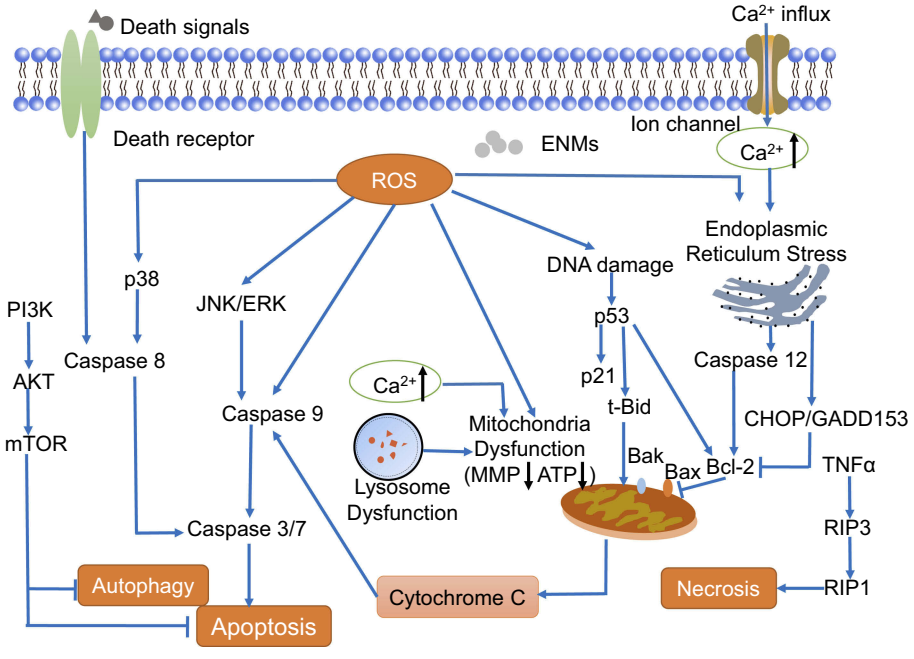

Figure 5 Mechanisms of engineered nanomaterials (ENMs)-induced cell death in neurotoxicity. Apoptosis is a caspase-dependent cell death, which has three main pathways including death receptor pathway, mitochondrial pathway, and endoplasmic reticulum stress pathway. Autophagy could be negatively mediated by PI3K-Akt-mTOR pathway. For necrosis, RIP3 responding to TNF family of cytokines binds to the kinase RIP1 and is crucial in the programmed necrosis pathway.

hippocampal slice cultures. This could cause a potential risk of inducing protein misfolding. Parveen et al[96] demonstrated that silica NPs could induce ER stress in the corpus striatum of young male Wistar rats. They showed significantly increased ER stress markers including CHOP, also known as GADD153, and CASP12, and further, Bax/Bcl-2 homeostasis was disrupted and apoptosis was induced.

In contrast, necrosis is caspase-independent with the characteristics of cytoplasmic swelling, expansion of organelles, and rupture of cell membrane. It further causes the outflow of intracellular content and induces inflammatory response.[97] Zhang et al[98] found that both necrosis and apoptosis were induced in the hippocampal cells in ICR mice when nano-alumina NPs were intranasally injected. More necrotic than apoptotic cell death was observed in hippocampal cells, and necrosis was further considered as a type of primary cell death contributing to the neurobehavioral defects in ICR mice.

Current studies have demonstrated that necrosis is not as common as apoptosis in the neurotoxicity induced by ENMs, however, more studies are needed to clarify the effects of necrosis induced by ENMs and the underlying mechanisms.

Autophagy

Autophagy is an intracellular mechanism for the degradation and reuse of cellular components, elimination of aberrant materials, and maintaining cellular homeostasis.[55] Autophagy occurs when ENMs get into endosomes in cells and form double-membrane autophagosomes, which fuse with lysosomes to produce autolysosomes for the degradation of ENMs.

Xue et al[99] demonstrated that TiO_2 NPs and oleic acid-coated IONPs could induce autophagy in human cerebral endothelial cells, which may involve oxidative stress and aggregations of ENMs. Xie et al[100] found that SiO_2 NPs induced autophagy in PC12 cells with elevated LC3-II and BECN1 levels. Further more, a mechanistic study showed that the autophagy was induced by inhibiting PI3K-protein kinase-B (Akt)-mTOR pathway. In addition, Wang et al[101] showed that cationic polyamidoamine (PAMAM) dendrimers could induce autophagy via Akt-mTOR pathway in human gloma cell lines.

Studies have demonstrated that autophagy plays both protective and detrimental roles in ENM-induced cytotoxicity. Wei et al[102] showed that ZnO NPs induced mitophagy, a form of autophagy which degrades dysfunctional mitochondria in BV-2 cells. They proved that mitophagy played a protective role in ENM-induced cytotoxicity. Hu et al[103] suggested that when zebra fish were exposed to GO, autophagy in the offspring played an important role in clearing α-synuclein and damaged ER. However, ENM-induced autophagy could also lead to toxicity.[104] Zhou et al[105] found that TiO_2 NPs could induce excessive autophagy in offspring of mice after prenatal exposure, based on the findings that there were significant increases of PI3K3C, BECN1, c-Jun, p-JNK, and microtubule-associated protein 1A/1B-light chain 3 (LC3), which led to the inhibition of dendritic outgrowth of hippocampal neurons. Wang et al[101] found that cationic PAMAM dendrimers induced autophagic flux in human gloma cell lines, which was suggested as the main source of their cytotoxicity. In addition, Li et al[106] also showed that the inhibition of PAMAM dendrimers-induced autophagy could improve the viability of SHSY-5Y cells.

Conclusion

We have comprehensively reviewed ENM-induced oxidative stress, inflammation, DNA damage, and cell death, all of which are considered as potential mechanisms of neurotoxicity. These mechanisms may work independently or they could interact with each other. For example, oxidative stress, as one of the most common mechanisms, is considered to trigger inflammation, DNA damage, apoptosis, and autophagy in some cases. DNA damage could also result in apoptosis. In addition, different types of cell death may share the same characteristics. For example, ATP depletion, ROS increase, and MMP decrease are associated with both apoptosis and necrosis. However, the specific roles of these mechanisms and their relationship with ENM-induced neurotoxicity remain elusive. Deeper exploration of the underlying mechanisms requires more efforts, and effective methodologies are required to establish a complete neurotoxicity evaluation system. More importantly, it is critical to establish SARs between ENMs and their biological effects. However, we still face the dilemma of lack of enough experimental data and sufficient knowledge about the interaction of nanomaterials and the biological system.[107–109] In addition, the neurotoxicity evaluations are mostly focused on inorganic nanomaterials, eg, metallic nanomaterials and carbon-based nanomaterials, and the studies on organic nanomaterials, eg, polymeric NPs and liposomes are limited.[110–112] Thus, more detailed studies are warranted for a better understanding of the

interaction of ENMs with neurological systems for safer and effective applications of ENMs.

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Disclosure

The authors report no conflicts of interest in this work.

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