Neurotoxicity of nanoscale materials

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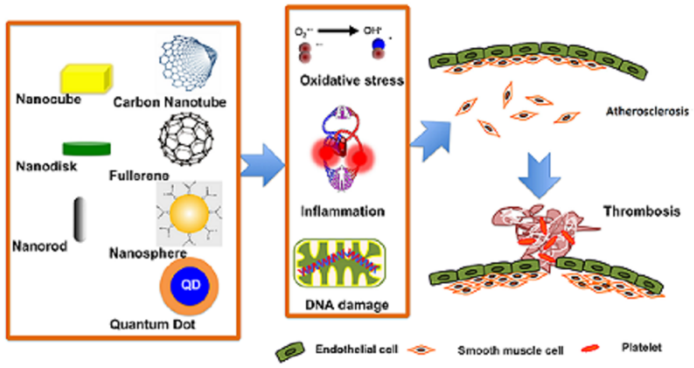
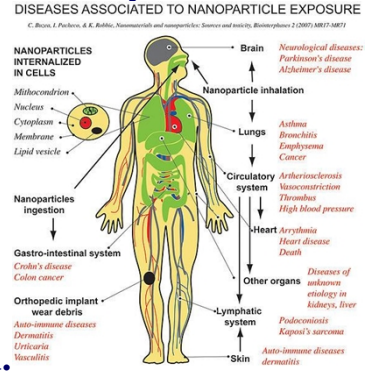


Nanotechnology Core Facility, Office of Scientific Coordination, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR 72079, USA b School of Public Health, Shanxi Medical University, 56 XinJian South Road, Taiyuan 030001, China article info Article history: Received 30 September 2013 Accepted 28 December 2013 Available online 4 February 2014 Keywords: Bloodebrain barrier Nanomaterial Neurotoxicity Oxidative stress

Abstract

Nanotechnology has been applied in consumer products and commercial applications**, showing a significant impact on almost all industries and all areas of society.** Significant evidence indicates that manufactured nanomaterials and combustion-derived nanomaterials **elicit toxicity in humans exposed to these nanomaterials**. The interaction of the engineered nanomaterials with the nervous system has received much attention in the nanotoxicology field. In this review, the biological effects of metal, metal oxide, and carbon-based nanomaterials on the nervous system are discussed from both in vitro and in vivo studies. **The translocation of the nanoparticles through the bloodbrain barrier or nose to brain via the olfactory bulb route, oxidative stress, and inflammatory mechanisms of**



nanomaterials are also reviewed.

Introduction

People working in certain industries, for example, automobile, aerospace, various activities such as combustion, welding, and biomedical applications electronics and communications, and chemical and paint industries are at high risk of being exposed to a large amount of NPs [1e10]. As NPs persist in the environment, people living in those environments are at **higher risk of NP exposure**. Nanotechnology involves creating and applying engineered materials at the nanoscale to take advantage of these specific properties. Humans have been exposed to many nanoparticles (NPs) originating from
Copper, zinc, iron, cerium, silver, gold, iron, manganese, titanium, aluminum, silica, and other carbon-based nanomaterials are some of the NPs to which humans are exposed significantly and may cause several health-related problems **including neurotoxicity**.--As a rapidly growing emerging science, **nanotechnology has shown a significant impact on almost all industries and all areas of society**. Nanomaterials, defined by the National Nanotechnology **Initiative, have at least one dimension in the range of 1-100 nm**. Due to their small size, the properties of nanomaterials differ from those of their bulk materials, **showing unique chemical, physical, optical, and electrical properties**.

Comment [O]: **NP= NanoParticles**

Journal of food and drug Analysis

In recent years, a significant number of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, or Huntington's disease have been diagnosed and treated. The **increased amount of environmental pollutants, including NPs, may be responsible for increasing the number of these neurodegenerative diseases.** The role of the bloodebrain barrier (BBB) is crucial in understanding NP toxicity in the brain. **BBB separates blood from cerebrospinal fluid in the central nervous system (CNS). The BBB is an extended plasma membrane that contains tight junctions between the adjacent endothelial cells of the cerebral capillaries. The permeability properties of the BBB are of interest** [1,11]. Unlike noncerebral capillaries, the cerebral endothelium does not have vesicles for macromolecular transport. Astrocytic end feet cover most (85%) of the cerebral capillary endothelial cells and they also contain a thick basement membrane [12]. **The presence of such complex combinations of astrocytes, cerebral capillaries and basement membrane strongly supports the BBB function** [11,13], even though establishing the clear cut roles of the basal lamina and/or astrocytic end feet in maintaining BBB permeability needs further study. **When NPs reach the circulation, they may interfere with the function of the endothelial cell membrane. The effect of NPs on the cell membrane may be due to their direct toxicity, or indirectly, they may induce some cascade mechanism that disrupts the tight junctions in the BBB or alters the permeability of the membrane. It has been shown that intravenous, intraperitoneal, or intracerebral administration of Ag, Cu, or Al NPs (50-60 nm)**

reportedly disrupts the BBB, as indicated by staining with albumin-bound Evans blue [14]. Vesicular transport may also be stimulated by NPs in order to gain access to the CNS microenvironment to exert toxic effects in the CNS. The unique size and surface modification of NPs could deliver drugs or therapeutic agents to the brain in the development of nanomedicine. Additional research is, however, necessary in order to understand fully how NPs are translocated from the blood to the brain across the BBB. **Nanomaterials could enter the human body by different routes including inhalation, dermal penetration, ingestion, and systemic administration, by which NPs may be accumulated in different tissues and organs including the brain** [15,16]**. It has been indicated that the olfactory nerve pathway may serve as a portal of entry for NPs into the CNS in humans who are environmentally or occupationally exposed to airborne NPs** [17e19]. De Lorenzo [18] showed that when silver-coated colloidal gold particles (50 nm) were intranasally instilled in squirrel monkeys, the NPs anterogradely **moved in the axons of the olfactory nerve to the olfactory bulbs. Olfactory epithelium that has been exposed to manganese, cadmium, nickel, and cobalt nanomaterials can translocate the nanomaterials to the brain via olfactory neurons** [20e25]. Therefore, full understanding of the neurotoxicity of these nanomaterials may lead to the design of safer therapeutics and reduce the side effects of these nanomaterials in future. Having a greater surface area than their bulk counterparts, metal oxide NPs are used in various fields such as water treatment, medicine, cosmetics, and engineering, and provide superior performance in their applications. **Unfortunately, almost no federal or state laws have specifically established regulations for the manufacture, transportation, use, sale, or disposal of nanomaterials** [26]. For metal oxide NPs, their widespread application**, small size, and large specific surface area endow them with high chemical reactivity and intrinsic toxicity, and their health effects in living creatures, especially on the nervous system**, have been of concern. **Metal oxide NPs are capable of translocating along the olfactory nerve pathway to the brain after intranasal instillation, and accumulating in the olfactory bulb, cortex, and cerebellum.** Moreover, NP deposition in the brain can **stimulate oxidative stress, inflammatory responses, and pathological changes**. These observations have provided evidence that metal oxide NPs can reach the brain and cause a certain degree of tissue damage. **Metal oxide toxicity can also be induced by dissolved metal ions from the oxides.** Brunner et al [27] studied the toxicity of NPs in human and rodent cell lines. **They divided the tested NPs into soluble and insoluble NPs, and showed that the toxicity of soluble NPs was from the soluble metal ions released from NP dissolution prior to or after the NPs entered the neural cells**. Considering the unique physicochemical properties, including small size effect, large specific surface area, and high biological surface reactivity, NPs might induce the **neurotoxicological behavior and effects in organisms**.

Comment [O]: This is one of the reasoms never to buy any product with a nano delivey system or method or products that say colloidal but are in fact NANO~ these are nano particles irrespective if they come in a bottle or pill and that the food supply is also being sprayed with nano is

Comment [O]: This would include everyone since we aare all either eating dfrinking or breatheing these particles and these

Comment [O]: occurring or performed in the normal or forward direction of conduction or flow occurring along nerve cell processes

Comment [O]: Pathological ☐ extreme in a way that is not normal or that shows an illness or mental problem ☐ *medical* : relating to or caused by

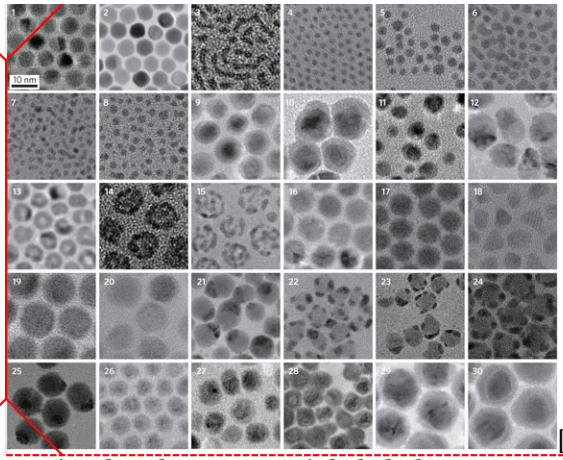
Comment [O]: The charged particles from the NANO MATERIAL will have a negative impact

2. Neurotoxicity and mechanism of nanomaterials

2.1. Titanium dioxide NPs

Among several metal-based NPs, those originating from titanium have been used widely and in large quantities. Titanium dioxide (TiO_2) is the most common compound of titanium that has found a variety of uses in our lives. TiO_2 is a white, odorless, **water-insoluble** material **that was believed** to have low toxicity [28e31]. TiO_2 is a relatively stable, nonflammable material that is found naturally in the form of various ores such as rutile, anatase, and brookite. TiO_2 can also be extracted from an iron-containing mineral ($FeTiO_3$) known as ilmenite [32e36]. TiO_2 possesses certain physiochemical properties that make it useful for multiple applications. Corrosion resistance, biocompatibility, mechanical strength, whitening property, opacity, and photocatalytic, optical, and electrical activity are some of the attractive properties that have paved the way for large-scale applications of TiO_2 [37]. The National Nanotechnology Initiative of America classifies **nanoparticulate TiO_2 particles as one of most widely manufactured NPs globally** [38]. Industrially, 80% of TiO_2, including its **nanoparticulate form (globally), is used to produce paints, varnishes, plastic, and papers**. Besides these applications, nanoparticulate TiO_2 has major uses in developing various products such as **cosmetics, foodstuffs, toothpaste, sun blocks**, printing ink, car materials, rubber, cleaning products, materials for industrial photocatalytic applications including solar cells, **and catalysts for remediation of organic matter in wastewater** [39]**. Toxicity of nanosized TiO_2 has yet to be completely understood despite its widespread uses. Recent toxicological studies have indicated harmful effects of TiO_2 NPs in biological systems, which is of major concern [40]. It has been** recently recognized that TiO_2 may be carcinogenic to humans if inhaled [31]. As a result, it is of great importance to understand the risks and hazards including neurotoxicity associated with nanoparticulate TiO_2 exposure and its dose-dependent response [41]. Irrespective of the different forms of TiO_2, exposure route and particle size, it has been found that **TiO_2 NPs translocate to different parts of the brain [39,42e46]. The NPs accumulate in this organ and induce structural changes in the neuronal architecture**

Comment [O]: **Where direct exposure will enter the system**



[39,43,45]. As mentioned previously, when NPs are inhaled, they can translocate to the CNS using the olfactory nerve as a means of entry. Several studies in mice have indicated that rutile NPs can translocate to the brain and accumulate throughout the organ, primarily in the hippocampus regions [39,43,45]. Such a neuronal translocation pathway of **TiO2 NPs may be responsible for neurotoxicity**. TiO2 NPs **when instilled intratracheally in mice accumulate in the brain via the blood circulation and penetration of the BBB**. This type of accumulation is **responsible for inducing tissue damage** [42]. **Accumulation of nanoparticulate TiO2 in the brain induces release and metabolism of neurotransmitters such as norepinephrine and 5-hydroxytryptamine** [39,43,45,46]. After intranasal exposure of TiO2 NPs, enhanced levels of the above-mentioned compounds were detected [43]. However, a **decrease in response was detected when anatase TiO2 NPs were administered intragastrically** [45]. **Reduced levels of homovanillic acid, dopamine, 5hydroxyindole acetic acid, and 3,4-dihydroxyphenylacetic acid were detected when TiO2 NPs were administered intranasally or intragastrically [43,46]. Enhanced catalase and acetylcholinesterase activity was detected during intranasal instillation of rutile [39] and intragastric administration of anatase TiO2 NPs [46]. Acetylcholine, glutamic acid, soluble protein carbonyl, and nitric oxide content were also increased by such NP treatments**. When anatase TiO2 NPs were intraperitoneally injected, increased nitric oxide **but decreased acetylcholine and glutamic acid were detected** [44]. Hu and colleagues [46] **showed that the levels of sodium, potassium, magnesium, calcium, iron, and zinc in the brain were changed after nanoparticulate TiO2 exposure**. **In that study, the treated mice had impaired spatial recognition memory, which could be linked to the disturbed homeostasis of neurotransmitters, trace elements, and enzymes in the brain** [46]. **Proteomic analysis showed differentially expressed proteins in the brain in response to TiO2 NP exposure, even though no NPs were detected in the tissue** [47]. Oxidative-stress-related damage with a consequent change in the balance between oxidative and antioxidative activities was observed

Comment [O]: **Translocation into lymph node, blood and urinary excretion of INP1 (left) and INP3 (right) using real-time NIR fluorescence imaging. Each point represents the mean ± s.d. of *n***

Comment [O]: **Norepinephrine is synthesized and released by the central nervous system, and also by a division of the autonomic nervous system called the sympathetic nervous**

Comment [O]: **Norepinephrine is synthesized from the amino acid tyrosine by a series of enzymatic steps in the adrenal medulla and postganglionic neurons of the sympathetic nervous**

Comment [O]: **5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter. Biochemically derived from tryptophan,[9] serotonin is primarily**

both in vitro [48e50] and in vivo [39,42,44,45,47]. **Levels of malondialdehyde, an oxidative marker, increased after intranasal instillation [39,44] of TiO2 NPs. A similar effect was also found with intraabdominal injection and intratracheal instillation of TiO2 NPs in mice** [42]. **Reactive oxygen species (ROS) such as superoxide [42], hydrogen peroxide [42,45], and hydroxyl radical [42] were also found to be increased in animals treated with TiO2 NPs. Increased cytokine levels, which are indicative of inflammatory effects in the brain**, were detected in animals treated with TiO2 NPs [44,51]. TiO2 NPs (P25 Degussa TiO2 and rutile forms) when injected intraperitoneally in mice induce **an increase in lipopolysaccharides, and alter the mRNA levels of interleukin IL-1b and tumor necrosis factor (TNF)-a, as well as IL-1b protein. Lipopolysaccharide induction** was necessary to cause this phenomenon, which suggests the importance **of a trigger element or a possible synergistic role in tissue responses to nanoparticulate TiO2**. The embryotoxic role of TiO2 was also studied by maternal intravenous injection of TiO2 NPs, which yielded no characterized TiO2 NPs [52], and by subcutaneous injection of TiO2 NPs in the anatase form [53e55]. In the case of subcutaneous injections, TiO2 **accumulation was found in the offspring cerebral cortex and olfactory bulb.** A large number of olfactory bulb cells were found to be positive for markers of apoptosis [53]. **Altered gene expression was detected for prenatal TiO2 NP exposure**, which was involved in **cell death, brain development, and the response to oxidative stress in newborn pups** [54]. Finally, the influence of prenatal **TiO2 NP exposure on the dopaminergic system was established as increased levels of homovanillic acid, dopamine, 3,4dihydroxyphenylacetic acid, and 3-methoxytyramina hydrochloride in the prefrontal cortex and neostriatum of exposed mice** [55]. **These findings indicate that TiO2 NPs can be carried from the mother to the fetal brain, which ultimately has a toxic effect on fetal brain development, leading to several nervous system disorders**. More in-depth studies are necessary in order to understand fully the toxic effect of TiO2 NPs on neurons in various stages of life, including during pregnancy and early stages of development.

Zinc oxide NPs

Like TiO2, another metal-based **NP is zinc oxide (ZnO)**, which has broad uses and applications. **ZnO is also white, thermally stable**, and a naturally occurring material. **It can be used to develop sunscreens, biosensors, food additives, cement, rubber, ceramics, pigments, plastic, catalysts, and electronic materials. ZnO shows antibacterial activities** and in recent years studies have also focused on the effect of **nanoparticulate ZnO on various microorganisms** [56,57]. In recent years, ZnO toxicity has been demonstrated both in vitro and in vivo in various mammalian cells. Dissolved Zn from the NPs is responsible for the toxicity. **ROS were detected in these studies and may have been responsible for the inflammatory effects**

associated with ZnO toxicity. The neurotoxic effect of ZnO has not been studied much. In one of the early works, **neurotoxicity of different-sized ZnO NPs (10-200 nm) in mouse neural stem cells (NSCs) was investigated**. As determined by cell viability studies, ZnO NPs showed dose-dependent toxic effects towards NSCs. However no size dependent toxic effects on NSCs were found in this study [58]. **Using confocal microscopy, transmission electron microscopy, and flow cytometry, apoptotic cells were detected and analyzed in this toxicity study.** Like previous studies, **the results indicate that ZnO NP toxicity originates from the dissolved Zn O in the culture medium or inside the cells** [58]. The effects of ZnO NPs on voltage-gated sodium and potassium pumps and action potential generation have been studied by Zhao et al [59]. The study on isolated rat **hippocampal CA3 pyramidal neurons demonstrated that ZnO NP solution was able to generate neuronal injury by inducing depolarization through activation of voltage-gated sodium channels, and led to higher Naþ influx and intracellular accumulation of Naþ and Ca2þ, release of glutamate, and neuron excitability.** ZnO **NPs are also able to induce neuronal apoptosis by depleting intracellular K level due to increased ion efflux** [59]. An in vivo toxicity study involving rats showed **that intraperitoneal ZnO altered synaptic plasticity, which changed spatial learning and memory ability** [60]. In that study, 20-80-nm ZnO NPs (4 mg/kg body weight) twice weekly for 8 weeks were administered to rats. ZnO NPs synthesized using the solegel method and starch as a template have been tested for in vitro cytotoxicity in neuro2A cells. **A dose-dependent toxicity profile was obtained, whereas nontoxic effects were seen at a concentration < 6 mg/mL [61]. More studies have shown that the antibacterial activity or adverse effects of ZnO NPs are partly due to the generation of ROS** [62-69], or **causing membrane damage through the direct NPecell membrane interaction or generation of ROS** [56,65], or release of Zn2þ ions in the ZnO NP suspensions [27,67]. **Studies in mammals have suggested that oral exposure of ZnO NPs causes an increase in blood viscosity and pathological lesions in the stomach, liver, kidney, pancrea, and spleen** [70]. However, the potential hazards of high concentrations of manufactured nanoscale ZnO on the CNS need further investigation.

Manganese oxide NPs

Manganese is an important metal. It is a trace element and necessary for survival. In plants in photosystem II, a manganese-containing metal cluster is responsible for oxygen generation from water activity and there are several enzymes that use manganese for their activity [71]. Manganese has found several other uses in our lives. Manganese is a major component of making different types of steel and cast iron [72]. Manganese chloride is used in batteries, disinfectants, dyes, paint driers, and dietary supplements. Oxides of manganese, such as manganese oxide (MnO), are used in colored glass, ceramics, paints, textile printing, fertilizers, and in food supplements and additives. Manganese dioxide (MnO_2) is used in

Comment [O]: **This explains the use of copper and the requirements of copper needing to be increased to assist in the displacing of the ZNP which is also accumalating within the cells**

Comment [O]: **This would explain the over reaction of people and there moods and anxiety issues as well due to the turning on the glutamate activity this is where Taurine and Glyscine would come in and Magnesium and Potassium to offset the nanotoxicolgy going on in the brain**

Comment [O]: **Strips the potassium by increasing sodium ion channels so you would need more potassium to offset this and to maintain brain and heart functions**

batteries and may also be generated from the welding of manganese alloys. Use of manganese-containing welding rods is a major source of occupational exposure to welders. **Manganese tetroxide (Mn_3O_4) may be generated in situations where other oxides of manganese are heated in** air [73]. Methylcyclopentadienyl manganese tricarbonyl is used as an antiknocking agent in some unleaded gasolines. The compound is **released to the environment during fuel combustion in the form of manganese sulfate, phosphate, and oxides**. Farm workers who work with Maneb (manganese ethylenebis-dithiocarbamate) **may also be exposed to a significant amount of manganese** [74]. **As manganese is known for its neurotoxicity**, toxicity studies associated with manganese-containing nanomaterials provide a useful test case in the evaluation of nanomaterial toxicity [75]. The occupational disease associated with **manganese exposure and toxicity is known as manganism**. **The disease in later stages resembles Parkinson's disease** [76]. **It has been found that if manganese is inhaled in water-soluble and water-insoluble forms, it is translocated to the brain, crossing the BBB via the olfactory nerve pathway [77]. It has been found that, among many metals, manganese is preferentially taken up via the olfactory nerve route** [21,78]. **After nasal exposure to manganese oxide NPs (MnO, MnO_2, Mn_2O_3, and Mn_3O_4), the concentration of manganese in the olfactory bulb, striatum, frontal, and other brain regions is increased**. Macrophage inflammatory protein-2, glial fibrillary acidic protein, and neuronal cell adhesion molecule mRNA is also increased in the olfactory bulb. **The results indicate that the olfactory neuronal pathway is efficient for translocating inhaled manganese oxide as solid ultrafine particles to the CNS and can result in inflammatory changes** [24]. Although absorption of manganese in the lungs is dependent on particle size and solubility [24,79], **for neuronal manganese uptake and further translocation into the CNS, dissolution of manganese is not necessary**. As mentioned earlier, major sources of ultrafine manganese oxide particles include the iron and steel industries, battery production, ferroalloy production, and power plant and coke oven combustion emissions [80]. **Use of glass, paints, and ceramics may also provide major sources of manganese oxide. Methylcyclopentadienyl manganese tricarbonyl is presently used in gasoline, mainly in Canada and Australia [81,82], and decomposition and oxidation of 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) during combustion may release manganese oxide of nanoparticulate size into the environment**. In all of these cases, the most likely route of **human exposure is through inhalation**. Toxicity of various manganese oxide nanomaterials has been investigated in a neuronal precursor cell model. The Promega Cell Titer Aqueous One Solution Cell Proliferation (MTS) assay was used to evaluate mitochondrial function in living cells and the lactate dehydrogenase (LDH) assay was used to quantify the **release of the enzyme as a result of damage to the cell membrane**. Both assays indicated that **manganese toxicity was dependent on the type of manganese oxides and their concentration. State of cell differentiation also contributed to varying NP toxicity.** Manganese oxide

Comment [O]: **Indicating that nanomanganese is transferable irrespective of size volume or density**

NPs are responsible for the generation of ROS and cell death due to apoptosis, as revealed by flow cytometry. During cell division, exposure to manganese oxide NPs results in elevated levels of the transcription factor nuclear factor NF-kB. Such enhanced levels of NF-kB mediate the cellular inflammatory response [83]. In another study, Hussain et al [84] investigated the effect of **manganese oxide NPs (40 nm) on dopamine production in PC12, neuronal phenotype cells. Manganese oxide NPs induced depletion of dopamine and its metabolites dihydroxyphenylacetic acid and homovanillic acid in PC12 cells, with a similar mechanism as Mn2þ**[84]. In an in vivo study, adult male Wistar rats were exposed to **MnO2 NPs of w23 nm diameter**. The experiment was a model study to understand the inhalational risks associated with MnO2 NPs. MnO2 NPs were instilled into the trachea for several weeks in daily doses of 2.63 mg/kg and 5.26 mg/kg. The endpoints of functional neurotoxicity (open field behavior and electrophysiology) and general toxicity (body and organ weights) were investigated. Animals treated with MnO2 did not gain weight after 6 weeks exposure. **High levels of manganese were detected in brain and blood samples of the treated animals after 9 weeks exposure**. **The open field behavior of treated rats showed decreased ambulation and rearing, and increased local activity and immobility were observed**. Electrophysiological studies of animals treated for 9 weeks **indicated a shift in spontaneous cortical activity to higher frequencies,** lengthened cortical **evoked potential latency, and slowed nerve conduction**. Many of these neurofunctional and general parameters were significantly correlated with the tissue manganese levels**. It can be concluded that the instilled manganese in the NP form was absorbed and the NPs were responsible for the neurotoxic effects**[85]. The acute oral toxicity of MnO2 NPs and MnO2 bulk particles in female albino Wistar rats was investigated [86]. MnO2 NPs (45 nm) exhibited higher absorption and tissue distribution compared with MnO2 bulk particles. **The histopathological analysis revealed that MnO2 NPs caused alterations in the liver, spleen, and brain**. The neurotoxicity of 45-nm MnO2 NPs in the brain and red blood cells, as determined through acetylcholinesterase activity, was significantly inhibited at doses of 1000 mg/kg and 500 mg/kg. MnO2 NPs (45 nm) **disrupted the physicochemical state and neurological system of the animals through alterations in ATPases** via the total NaþeKþ, Mg2þ, and Ca2þ levels in the brain. Toxicity of Mn3O4 NPs was investigated in ST-14 rat striated neuroblasts, a neuronal precursor cell model, using the MTS assay to evaluate mitochondrial function in living cells and the LDH assay to quantify the release of the enzyme as a result of damage to the cell membrane [87]. Both assays showed that the toxicity of Mn was dependent on the type of manganese oxide NPs and their concentration, as well as the state of cell differentiation. Following exposure to manganese oxide NPs, ROS were generated, and flow cytometry experiments **suggested that cell death occurred through apoptosis. During exposure to manganese oxide nanomaterials, increased levels of the transcription factor NF-kB (which mediates the cellular inflammatory response**) were observed.

Comment [O]: **NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls transcription of DNA, cytokine production and cell survival. NF-κB is found in almost all animal cell types and is involved in**

Comment [O]: **In the brain, dopamine functions as a neurotransmitter—a chemical released by neurons (nerve cells) to send signals to other nerve cells. The brain includes several distinct dopamine pathways, one of which plays a**

Comment [O]: **Things that would inhibit Manganese These Substances may Interfere with Manganese**

Minerals

Excessive consumption of Calcium may

Silver NPs

death and oxidative stress in human skin carcinoma and fibrosarcoma cells [94]. The same group have also reported that **Ag NPs can enter cells, causing DNA damage and apoptosis in liver cells and fibroblasts** [95]. **Cell viability is decreased when alveolar macrophages and lung epithelial cells are treated with Ag NPs** [96]. In vitro studies have shown Ag NP toxicity in neural-like cell lines, such as PC12 cells, which is a rat cell line with a neuronal-like phenotype [97]. **It has been shown that Ag NPs could come across through and be accumulated in brain microvessel vascular endothelial cells. An in vitro BBB model composed of primary rat brain microvessel vascular endothelial cells, it has been shown crossing and accumulation capability of silver nanoparticles** [98]. **Ag NPs can induce inflammation and affecte the integrity of this BBB model, and be readily translocated to the brain** [99]. **Ag NPs can also induce BBB damage, astrocyte swelling, and neuronal degeneration [100]. Ag NPs can translocate to the brain using the nasopharyngeal system as a gateway during inhalation exposure** [17]. In vivo studies by Liu and coworkers have shown the effects of Ag NPs on hippocampal synaptic plasticity and spatial cognition in rats. Their studies have revealed that intranasally administered **Ag NPs induce impairment of hippocampal function** [101]. These results suggest that **Ag NPs cause neurotoxicity in humans and other animals.** More recently, a significant finding indicated **that 7nm Ag NPs decreased motor activity and body weight in a time- and dose-dependent manner after intravenous injection, suggesting that the nervous system may be targeted by Ag NPs** [102]. Yin and coworkers tried to establish the mechanism of Ag NP neurotoxicity both in vitro and in vivo using rat cerebellar granule cells. Their studies indicated that **Ag NPs, depending on the caspase-activation-mediated signaling, drastically decreased the survival of primary neuronal cells through apoptosis coupled to oxidative stress** [103]. Silver is a bright, silvery white, soft metal that has been used for thousands of years. Silver ornaments, utensils, and art work have been around for a long time. Silver has monetary value and silver coins and jewelry are considered as valuables. Silver is used in large quantities as catalysts, mainly in the production of ethylene oxide. It is also used industrially for conductors, mirrors, and photographic applications. One of the interesting properties of silver is its antibacterial and antifungal activity. As a result, the use of nanoparticulate silver is one of the fastest growing areas of commercial NP applications [88]. Due to their excellent antibacterial properties, silver NPs have been used in food services, building materials, textile industry, medical instruments, personal care products, and washing machines [89]. Silver NPs (Ag NPs) are used as room sprays, deodorants, wall paints, and laundry detergents, and are also used for indoor air purification and water detoxification [90,91]. **As a result of these widespread uses and exposure of silver NPs to humans, it is likely that Ag NPs enter the body and accumulate in various tissues and organs** [92].

Comment [O]: **Brain Damage—Another Hoax on the benefit of nano silver**

Comment [O]: **The hippocampus is a small organ located within the brain's medial temporal lobe and forms an important part of the limbic system, the region that regulates emotions. The hippocampus is associated mainly with memory, in particular long-term memory. The organ also plays an important role in spatial navigation. Damage to the**

Previous research has indicated that Ag NPs can accumulate in several organs, which includes the kidney, liver, testis, lung, and brain [93]. In vitro studies have shown that **Ag NPs are capable of inducing toxicity in cells derived from a variety of tissues, including liver, skin, vascular system, lungs, and reproductive organs**

Iron oxide (FeO, Fe2O3, Fe3O4) NPs

Iron oxide or superparamagnetic iron oxide nanoparticles (SPIONs) have become one of the most favorable and exciting choices in both the industrial and biomedical fields, due to their superparamagnetic property and other physicochemical characteristics unique to nanomaterials. SPIONs (Feridex) are small NPs composed of a Fe3O4 (magnetite) or Fe2O3 (maghemite) core. Although maghemite is naturally ferromagnetic, **with the decreasing size (< 30 nm), it becomes superparamagnetic.** Their potential application ranges from biomedical imaging (magnetic resonance imaging, positron emission tomography, or ultrasound as contrast agent), gene and drug delivery, tissue regeneration, hyperthermia in cancer treatment, catalysis, and magnetic storage [104]. They are extensively used specifically for brain imaging or braintargeted drug and gene delivery, due to their ability to move across the BBB [105]. SPIONs are metal oxide NPs that have been clinically approved, **although recently they have been taken off the market** [106,107]. In spite of their desirable traits, there is a critical need to investigate their toxicity both in vivo and in vitro. SPIONs have already been shown to have **potential toxicity that can lead to altered gene expression, actin modulation, interference with cell cycle regulation and signaling pathways, excessive ROS generation, and disruption of iron homeostasis** [108]. According to the recent findings, environmental factors are a major contributor to the development of neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease [109]. Peters et al [110] **have emphasized the significance of oxidative stress generated by NPs in the brain, along with the evaluation of the possible connection between long-term NP exposure and neurodegenerative disease**. With increased use of Fe3O4 NPs in industry and biomedical sciences, **the risk related to occupational exposure has escalated considerably.** Involvement of ultrafine particulate materials in polluted air **leads to protein fibrillation**. Fibrillation of specific proteins, for example, Ab42 and a-synuclein, may play a role in the development of Alzheimer's disease and Parkinson's disease [111]. SPIONs have further been shown to form a corona with plasma proteins. **This corona can lead to several toxic side effects because the initial cellular interaction of magnetic nanoparticle (MNP) changes lead to downstream modification of cellular and tissue interaction** [112,113]. In 2007, Pisanic et al [114] used PC12 cells as a quantifiable in vitro model system to study the toxic effect of anionic Fe3O4 MNPs in a dosedependent manner. In that study, it has been established that when PC12 cells were exposed to the anionic MNPs at an increasing concentration ranging from 0.15mM to 15mM iron, they lost their **viability and were unable to generate normal neurite growth in the**

Comment [O]: **biophysicochemical properties of NPs, which define their affinity for protein monomers, unfolded monomers, oligomers, critical nuclei, and other prefibrillar states---.**
Amyloidosis
- **Systemic**
  - **Build**
  - **up of amyloid deposits**
- **Organ**
  - **specific amyloidosis**
- **E.g. Alzheimers disease, Parkinsons disease…**
- **Protein re/mis**
  - **folding and aggregation**
- **General feature of all proteins ('the other side of folding')?**
- **From native/soluble to non**
  - **native/cytotoxic, massive insoluble**

presence of nerve growth factor. They have concluded that the anionic **MNPs were possibly interfering with transcriptional regulation and protein synthesis, for example, Growth associated protein (GAP)-43 leading to cellular death and phenotypic changes**. In 2009, Wang et al [115] discussed the ability of submicron level Fe_3O_4 NPs to be transported to the brain via the olfactory nerve pathway, leading to oxidative-stress-related damage in the brain. **They also discussed changes in the ultrastructure of the olfactory bulb nerve cells.** Recently, Wu et al [116] have focused on the neurotoxicity of iron oxide NPs in the rat brain (in vivo). The study investigated the effect of uptake and retention of Fe_3O_4 NPs in rat brain hippocampus and striatum, including oxidative injuries. T**he olfactory bulb, striatum, and hippocampus seemed to be the main sites for Fe_3O_4** NP deposition after intranasal instillation [117]. **Approximately 80% of NPs were still found in the striatum at 7 days after instillation and about 50% were found in both the striatum and hippocampus after 14 days. The striatum in the instillation groups exhibited comparatively more susceptibility to oxidative stress, as indicated by increased levels of H_2O_2 and decreased Glutathione peroxidase (GHS-PX) activity in the control group at 7 days after exposure. The group also investigated the effect of Fe_3O_4 NPs in PC12 cells in vitro. The PC12 cells showed dose-dependent cytotoxicity, as measured by LDH release and MTT assay, demonstrating membrane disruption and mitochondrial enzyme activity, respectively.** The oxidative stress was also evident **by the reduced GSH-PX and superoxide dismutase activity**, and increased ROS level and **lipid peroxidation**. Fe_3O_4 NPs also had a substantial cytotoxic effect on PC12 cells by modulating the cell cycle and inducing apoptosis. JNK is usually activated by oxidative stress and **modulates apoptosis, neurodegeneration, cell cycle control, and cellular proliferation** [118]. The cells also exhibited phosphorylation of p53 protein at ser15 position and elevated levels of bax and bcl-2 proteins upon exposure to NPs. It has been demonstrated that intranasally instilled **Fe_2O_3 NPs are transported into the brain via the olfactory route [119], and additional investigation has been made of the size-related effect. After a single intranasal exposure of 21-nm Fe_2O_3 NPs, there was a significant increase in iron content in almost all the brain regions, from the olfactory bulb, hippocampus, cerebral cortex, and cerebellum to the brainstem** [120]. However, a single intranasal exposure of 280-nm Fe_2O_3 NPs led to a significant increase in iron content only in the olfactory bulb and hippocampus, with no significant alteration of iron content in other brain regions. At 30 days after instillation of 280nm Fe_2O_3 NPs in mice, the iron content in the olfactory bulb and hippocampus also increased but was lower than that in mice treated with 21-nm Fe_2O_3 NPs. **It is widely known that brain iron accumulation is associated with the oxidative stress induced by the formation of the highly reactive OH via the Fenton reaction [121e123]**. The excess iron in the brain suggests an association with the oxidative stress response. The generation of ROS is a well established paradigm to explain the toxic effects of NPs [40]. It has been demonstrated that intranasal exposure of iron oxide NPs causes a certain degree of oxidative stress

Comment [O]: **these are the highest or most potent antioxidants in the immune system and when iron nano is added is showing a drastic reduction in them due to the fact the system is trying to rid the density of the iron and as a result is overloaded and depleted**

Comment [O]: **You would almost think that the nanotech is by design made to induce brain damage and brain trauma ~ disconnect from the body and to create the inability to think or remember**

response in mouse brain [119]. Significant oxidative stress responses in the brain of mice have also been observed after intranasal exposure of 21-nm and 280-nm Fe_2O_3 NPs [124]. **Alterations of iron and zinc levels in the brain are more evident in mice exposed to nano-sized than submicron-sized Fe_2O_3**. Furthermore, the strong positive correlation between the iron and zinc levels in the sub-brain regions may contribute to the understanding of zinc homeostasis in the brain after Fe_2O_3 particle inhalation. **The biomarkers of oxidative stress, activity of nitric oxide synthases, and release of monoamine neurotransmitters in the brain have been studied as well [115]. It was shown that significant oxidative stress was induced by the two sizes of Fe_2O_3 NPs**. The activities of GSH-PX, copper, zinc superoxide dismutase, and constitutive nitric oxide synthase were significantly elevated and the total glutathione and glutathione/glutathione disulfide ratio were significantly decreased in the olfactory bulb and hippocampus after treatment with nano- and submicron-sized Fe_2O_3 particles. **The nano-sized Fe_2O_3 generally induced greater alteration and a more significant dose effect response than the submicron particles did**. Transmission electron microscopy showed that nano-sized Fe_2O_3 treatment induced some **ultrastructural alterations in nerve cells, including neurodendron degeneration, membranous structural disruption, and increased lysosomes in the olfactory bulb, dilation in the rough endoplasmic reticulum, and increased lysosomes in the hippocampus.** The results indicated that intranasal exposure of Fe_2O_3 NPs could induce more severe **oxidative stress and nerve cell damage in the** brain than the larger particles did. **Fe_3O_4 NPs also exert cytotoxic effects by influencing the cell cycle and apoptosis** [116]. For example, cells are arrested at the G2/M phase after 24 hours exposure to NPs. Arrest at the G2/M phase provides time for these cells to instigate DNA repair and delay cell death. **However, cells with impaired DNA repair processes enter apoptosis**. The study indicates that Fe_3O_4 NPs are deposited and retained in the striatum after intranasal instillation, and the NPs may then cause oxidative damage in the striatum. The results of in vitro studies on dopaminergic neurons have demonstrated that Fe_3O_4 NP exposure decreases cell viability and induces marked oxidative stress. Furthermore, Fe_3O_4 NPs mediated apoptosis signaling pathway included JNK and c-Jun phosphorylation, p53 phosphorylation, Bax upregulation, Bcl-2 downregulation, and apoptosis.

Comment [O]: **NANO Iron causes severe nerve damage –reduces the glutathione and SOD**

Copper and copper oxide NPs

Copper is one of the essential trace elements for energy production in biological systems. **Copper is a requirement for the synthesis of different enzymes, including cytochrome c oxidase, superoxide dismutase, tyrosinase, lisyl oxidase, and cupro-protein** [125,126]. **Copper is also responsible for stimulating the production of neurotransmitters such as epinephrine and norepinephrine in the brain and can be found there at a high concentration** [127]. However, at higher than normal levels, unbound copper become toxic to the human body

because it disrupts homeostasis. Its redox activity can give rise to ROS, leading to oxidative stress and modification of protein, lipid, and nucleic acid [128,129]. Compounds of copper such as copper oxide (CuO) NPs have found a broad use in various areas. CuO NPs are used in inks, lubricants, coatings, semiconductors, heat transfer fluids, antimicrobial preparations, and intrauterine contraceptive devices [130]. Copperbased NPs are used as lubricant additives because they reduce friction and wear, and worn surfaces can be repaired by an addition of copper NPs in lubricants. As more copper NPs are currently in use, it is likely that human exposures to copper NPs will increase gradually. **Due to their nanolevel size, CuO NPs are capable of crossing the BBB and pose a threat to the CNS**. Studies have shown that copper **NPs can cause BBB dysfunction, swelling of astrocytes, and neuronal degeneration once introduced into the bloodstream** [1,131]. Li et al [132] showed neurotoxicity of CuO NPs in a dose-dependent manner in H4 neurogiloma cells using an automated image analysis technique. **Primary cultures of dorsal root ganglion of neonatal rat pups were investigated to measure neurotoxicity of copper NPs of varying size and concentration** by Prabhu et al [133]. After exposured to 10-100mM copper NPs (40 nm, 60 nm, and 80 nm) for 24 hours**, the neurons started to develop vacuoles and became detached from the substratum**. **They also exhibited disruptive neurite growth.** LDH and MTT assays have also shown the significant toxicity of **copper NPs, and the smaller size is associated with higher toxicity**. The whole-cell patch-clamp technique was used to study the influence of CuO NPs on voltage-dependent potassium current in acutely isolated rat CA1 pyramidal neurons of the hippocampus [134]. Although the CuO NPs did not have a significant effect on the outgoing potassium current, they did inhibit the delayed rectifier potassium current at a relatively high concentration. CuO NPs shifted the inactivated curve of rectifier potassium current negatively but did not show any significant effect on transient outgoing potassium current. These blockades of the potassium current might inhibit the normal functional activity of nerve cells. In another study, Trickler et al [135] **has determined the effect of copper NPs on induction of proinflammatory mediators, followed by their influence on rat brain microvessel endothelial cells**. **At a low dosage, the copper NPs enhanced cellular proliferation, whereas at a high concentration, they started to express toxicity. NP exposure increased prostaglandin E2 release. Extracellular levels of TNF-a and IL-1b were considerably higher in the exposed cells**. **This resulted in the disruption of cerebral microvasculature by increasing its permeability**. According to Karlsson [136], nano-CuO is highly toxic when compared with other metal oxide NPs. However, few studies have investigated the direct effects of nano-CuO on neurotoxicity and the potential mechanisms involved in these effects. A study explored the potential neurotoxicity of nano-CuO on ion channels of neuron, voltage-dependent sodium current (INa) in rat hippocampal slices with whole cell patch-clamp technique [137]. **The results showed that nano-CuO inhibited the peak amplitude of INa, which might have decreased intracellular Naþ concentration due to decreased Naþ influx**. This could inhibit the exchange of Naþ for Ca2þ by

Comment [O]: A vacuole (/ˈvækjuːoʊl/) is a membrane-bound organelle which is present in all plant and fungal cells and some protist, animal[1] and bacterial cells.[2] Vacuoles are essentially enclosed compartments which are filled with water containing inorganic and organic molecules including enzymes in solution, though in certain cases they may contain solids which have been engulfed. Vacuoles are formed by the fusion of multiple membrane vesicles and are effectively just larger forms of these.[3] The organelle has no basic shape or size; its structure varies according to the needs of the cell. The function and significance of vacuoles varies greatly according to the type of cell in which they are

NaþeCa2þ exchangers [138]. **The exchanger was shown to generate inward current during the repolarization phase of the action potential [139], thus, the effect on INa could contribute to the change in action potential shape by nano-CuO. It is well established that voltage-gated sodium current (VGSC) plays a role in neurotransmitter release** [140]. Thus, the effects of nano-CuO on INa also mean that modulation may produce functional effects on neurotransmission in the CNS. It has been shown that nano-CuO produces a hyperpolarizing shift in the activation curve. The S4 segment in a subunit of VGSCs contains 4-8 positively charged residues at three residue intervals. They serve as voltage sensors and initiate the voltage-dependent activation of VGSCs by moving outward under the influence of the electric field [141,142]. **The results suggest an effect on the S4 segment of the activation gating, resulting in conformational changes of the channel**. The findings also confirm that the effects of nano-CuO on hippocampal neurons are mediated through activation of ROSeINaeaction potential signaling cascades and are independent from the G-protein pathway. These results show the primary mechanisms underlying nano-CuO-induced INa amplitude inhibition and improve our understanding of nanoCuO neurotoxicology. To determine the potential neurotoxicity of CuO NPs, human SH-SY5Y neuroblastoma and H4 neuroglioma cells were exposed at a concentration range of 0.01-100 mM for 48 hours [132,143]. The data indicated that exposure of CuO NPs induced differential toxic effects in both SH-SY5Y and H4 cells, and the cells had dose-dependent toxic responses to the CuO NPs. The toxic effects of CuO NPs were also investigated in a semiadherent, fast-growing, mouse neuroblastoma cell line (N2A cells), to provide a better understanding of the toxicological risks of CuO NPs in future nanotechnology developments [144]. N2A cells were less sensitive to CuO NP effects than other cultured cells were. The lower sensitivity may have been due to the agglomeration of CuO NPs in the culture medium, which resulted in an average particle size > 300 nm. **Agglomeration of CuO NPs reduced surface-specific effects specific to nanoscale materials, and increased the contribution of particle solubilization in the toxic response** induced in N2A cells. **Agglomerated CuO NPs induced both cytotoxic and genotoxic effects in N2A cells**.

Aluminum oxide (alumina, Al2O3) NPs

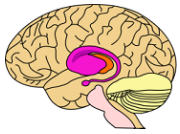
In recent years, the areas of nanotechnology and nanomedicine have expanded rapidly, aluminum oxide (alumina) NPs, having good electric and abrasive properties, are widely used as abrasive agents or insulators in motor vehicles, electronics, energetics, exterior coatings, personal care products, scratch-resistant coatings, alloys, and sensors [145]. **This has led to increased human exposure to aluminum oxide NPs (nano-alumina).** An in vivo study in ICR mice aimed to investigate the effects of nano-alumina, with a focus on the effects on neurobehavioral defects and possible mechanisms of action. It showed that **nano-alumina induced apoptosis via increased caspase-3 gene expression and impaired spatial learning behavior, which suggests that mitochondrial impairment plays a key role in the**

neurotoxicity of nano-alumina [146]. The research could help to understand the underlying mechanisms of toxicity of nano-alumina, particularly with respect to neurobehavioral function. The authors declared that **impairment of the mitochondria played an important role in the neurotoxicological effects of nano-alumina and might be a direct cause of neurobehavioral defects**. The possible neurotoxic effects of nano-alumina and bulk alumina have been compared in nematodes [147]. The relatively large surface area of nano-alumina compared with bulk alumina might also explain the differences in toxicity between nano-alumina and bulk alumina. **The decrease in locomotive behavior in nematodes chronically exposed to nano-alumina was associated with both an increase in ROS generation and disruption of ROS defense mechanisms.** Drosophila was used as another model organism to explore the effects of nano-alumina on the CNS [148]. The rhythmic and electrophysiological activities in the antennal lobe of Drosophila were recorded using patch clamps. Fifteen minutes after application of nano-alumina, **the average frequency of spontaneous activity was significantly decreased** compared with that of the control groups. The results indicate that **nano-alumina might have adverse effects on the CNS** in Drosophila. The hypothesis that nano-alumina can affect the BBB and induce endothelial toxicity has been proposed [149]. In the first series of experiments, **human brain microvascular endothelial cells were exposed to nano-alumina and control NPs in dose- and time-responsive manners**. Treatment with nano-alumina markedly **reduced human brain microvascular endothelial cell viability, altered mitochondrial potential, increased cellular oxidation**, and decreased tight junction protein expression as compared to treatment with control NPs. Alterations of **tight junction protein levels were prevented by cellular enrichment with glutathione**. In the second series of experiments, rats were infused with nano-alumina at a dose of 29 mg/kg and brains were stained for expression of tight junction proteins. **Treatment with nano-alumina resulted in marked fragmentation and disruption of integrity of** claudin-5 and occludin. The results indicate that the **cerebrovasculature could be affected by nano-alumina**. In addition, the data indicate that alterations of mitochondrial function might be the underlying mechanism of nanoalumina toxicity. As far as the assessment of toxicological properties of nanoparticles is concerned, it is important to know whether cultured neural cells take up NPs, and if so, what mechanisms are involved [150]. **Ultrastructural examination has shown that nano-alumina penetrates the cell membrane and that some particles are engulfed by the cells and mainly accumulated in the cytoplasm**. It has been demonstrated that the NPs entering the cells are likely to have an effect on cellular function. **Bulk-alumina-treated cells show apoptotic characteristics,** whereas **nano-alumina-treated cells demonstrate both apoptotic and necrotic morphological changes**. Photomicrographs **show that the vesicles with individual particles and aggregates remain in the cytoplasm and the nucleus**. According to transmission electron micrographs**, NPs form aggregates inside the lysosomal vesicles and their internalization in lysosomal bodies is**

arranged in a perinuclear fashion. The presence of an elevated amount of lysosomes might reflect enhanced phagocytosis of exogenous particles. Microglia and astrocytes are dominant glial and major immune cells in the CNS. They are sensitive to changes in the microenvironment of the CNS and are rapidly **activated in almost all conditions that affect normal neuronal functions**. Activation of microglia and astrocytes in the cortex and hippocampus following peripheral administration of nanoalumina have been analyzed in SpragueeDawley rats [151]. There was significant glial activation induced in rat brain after nano-alumina administration.

Silicon dioxide (silica) NPs

Silica (SiO_2) NPs have been developed for mechanical polishing, additives to food and cosmetics, and have various applications in biomedical fields, including diagnosis, optical imaging, targeted drug delivery for the CNS, cancer therapy, and controlled drug release for genes and proteins. In particular, being considered more biocompatible than other imaging NPs, silica NPs are emerging as ideal materials for medical applications. For applications of potential drug delivery, imaging, and diagnostics in the CNS, silica NPs are also being modified or used for coating or stabilization of other optical materials. However, to date, little is known concerning the potential adverse effects on the brain associated with exposure to silica NPs**. Research has indicated that silica NPs via intranasal instillation enter the brain and show a distinct pattern of biodistribution, and are especially deposited in the striatum**,



except for the olfactory bulb [152]. **Such an accumulation could result in oxidative stress, inflammatory changes, and functional damage of the striatum**. In addition, silica NPs appeared to induce **depleted dopamine in the striatum, and the main contribution was downregulation of tyrosine hydroxylase protein**. In vitro studies on dopaminergic neurons have demonstrated **that silica NPs have marked cytotoxic effects and oxidative stress activity against PC12 cells** [152]. Furthermore, activation of the p53 pathway is involved in the mechanism of the silica-NP- induced G2/M arrest and apoptosis**. Additionally, the decrease in dopamine levels is most likely attributable to the reduction of dopamine synthesis.** The authors **have claimed that although extrapolation** of the animal effects to humans remains a challenge, **their results for the neurotoxic effect on rat brains could be suggestive of human exposure**, because different species may respond differently to the same substance. **Another study demonstrated that exposure to 300 ppm silica NPs in differentiating cells showed less cytotoxicity than in undifferentiated cells** [153]. Silica NPs at 100 ppm had no significant effect on the viability of either undifferentiated or differentiating neuroblastoma (SH-SY5Y) cells. Neurite outgrowth in differentiating cells after 48 hours exposure to 100 ppm silica NPs was not significantly changed. **Thus, silica NPs appeared to**

Comment [O]: **Functionally, the striatum coordinates multiple aspects of cognition, including motor and action planning, decision-making, motivation, reinforcement, and reward perceptio**

Comment [O]: **This would explain the possibility of a T4 deficiency since the tyrosine and selenium and iodine are part of this nd dopamaine uptake and use**

have no effects in the early initiation of neurites. Although the production of ROS was not induced, **neurotoxicity induced by silica NPs may be the result of increased DNA damage, apoptosis, and cell cycle arrest in undifferentiated and differentiating cells, which is independent of neuronal differentiation of SH-SY5Y cells.**

Carbon-based nanomaterials

Owing to their unique chemical and physical properties, carbon-based nanomaterials have a potential use in a variety of biomedical applications, including early diagnosis of cancer, imaging, targeted photothermal therapy, drug delivery, and tissue engineering. **Based on the shape, organic carbon-based nanomaterials are categorized as carbon nanotube, fullerene, graphene, or carbon NPs. Carbon nanotubes are onedimensional forms of graphitic material and are present in many forms, depending on the number of graphene sheets used: single-walled carbon nanotubes, double-walled carbon nanotubes, and multi-walled carbon nanotubes with diameters of 1-2 nm and lengths of 0.05-1 mm.** Graphene has similar chemical composition and crystalline structure with a flat sheet with a single layer or multilayer graphene with several layers. The fullerenes (C60) are named after Richard Buckminster Fuller as buckminsterfullerene, or the "bucky ball". **This allotrope of carbon consists of 60 carbon atoms joined together to form a cage-like structure**. C60 is soluble in aromatic solvents (e.g., toluene or benzene), but insoluble in water and alcohol. However, C60 can be functionalized (e.g., with eOH, eCOOH, or eNH2) to increase its hydrophilicity. By contrast, aqueous fullerene aggregates can be generated by mixing pure C60 in water or through solvent extraction. Some fullerenes have been shown to inhibit human immunodeficiency virus (HIV) activity through inhibiting an HIV-associated protease, an essential enzyme for viral survival. It has been reported that some fullerenes can interact with biological membranes to elicit antimicrobial action, antitumor activity, enzyme inhibition, DNA photo cleavage, and neuroprotective activity via antioxidant actions. At present, fullerenes are commercially used in products including fuel cells, semiconductors, and product coatings, for example, bowling ball surfaces. **Studies of carbon nanomaterials have indicated the potential neurotoxic effects after inhalation or systemic exposure**. Oberdorster and co-workers [17] showed that inhalation ¨ of elemental 13C NPs of 36 nm by rats, **which were exposed for 6 hours whole-body exposure**, **led to a significant and persistent increase in the accumulation of 13C NPs in the olfactory bulb, and the NP concentration gradually increased**. A recent study has shown that **different shapes of carbon nanomaterials elicit different toxicity in neuronal culture models**. Specifically, pure graphene is less toxic than highly purified single-walled carbon nanotubes in a concentration dependent manner after 24 hours exposure of PC12 cells, involving the apoptosis pathway [154]. Subsequently, the impact of surface functionalization on the toxicity of carbon nanotube has been demonstrated

using the same culture model. Carbon nanotubes with surface-coating polyethylene glycol are **less toxic** than uncoated carbon nanotubes, by measuring mitochondrial function and membrane integrity. A mechanistic study has shown **that oxidative stress is involved in this toxic pathway, with surface coating playing an important role [155]. It has been reported that 14-nm carbon black particles might translocate to the olfactory bulb through olfactory neurons, resulting in the activation of microglial cells, which induces proinflammatory cytokines and chemokines, suggesting an inflammatory response** [156]. Additional systematic evaluations and mechanistic in vivo studies are needed to understand the effect of surface coating on the biocompatibility of these carbon-based nanomaterials prior to use in humans.

Future perspectives

Physical and chemical characterization is considered to be the key element in assessing the neurotoxicity of nanomaterials. The nanomaterials used in the study require a comprehensive physicochemical characterization before during, and after the biological testing models are exposed to nanomaterials. **As mentioned previously, the size, size distribution, purity, shape, crystal structure, composition, surface coating, surface charge, and surface reactivity may result in a different distribution, accumulation, and transport of the nanomaterials to the target organs, as well as across the BBB**. Research findings are meaningless for hazard identification in the absence of adequate evaluation of the physical and chemical properties of nanomaterials. For example, **impurities that contaminate the nanomaterials being tested may contribute most to neurotoxicological responses**. **The dissolution of metal ions from metal oxide nanomaterials may play an important role in neurotoxicity. The size or surface charge of nanomaterials might change the biokinetics of the nanomaterials, resulting in different pharmacological or toxicological actions in biological systems. However, batch-to-batch inconsistency is a major challenge when nanomaterials are produced by different manufactures/laboratories. The exposure dose level should be carefully considered when laboratory animals or in vitro models are exposed to nanomaterials**. The practically exposure level to human should be used as a reference when calculateing the relevant dose exposed to the animals or in vitro models. This will support studies for understanding the dosimetry in the nervous system. **The characteristics of the nanomaterials should also be considered in physiologically based pharmacokinetic modeling to better predict the environmental hazard of the nanomaterials**. To date, the data gap of well-designed neurotoxicity assessment of nanomaterials still exists, and further in vivo studies will be considered as an urgent demand in the future**. Appropriate dose eresponse research should be considered in neurotoxicological studies**. Recent inhalation studies have shown that the surface area or particle number, instead of the nanomaterials mass, is considered

Comment [O]: Does nor imply safe just means not as dangerious or is less hazardous

Comment [O]: The other issues with nano is the integration with other nano partiles which can integrate and alter the initial chemical structure and as a result when falling to the ground or environmental exposure or a mixture of air pollutamts already in the atmosphere engage with biological and pathological will also play a roll in the impact of the damage and morphology of the nano with the genetic code or dna of other organic life

Comment [O]: The reason why this is so is because of the unregulated policy is due to the 1.6 trillion profits and the fact this is a total weaponized tech

as the major dosimetry unit in term of the dose-response relationship**. Cellular or target organ dose will provide a better understanding of the neurotoxicological responses, because the physical properties might change quickly in the biological system under the experimental conditions**. Sensitive and specific methods need to be developed to quantify the nanomaterials, including metal NPs or carbon-based nanomaterials. The nanomaterials may interfere with the enzymatic assay during the measurement of neurotransmitters (such as acetylcholine or dopamine) using traditional methods. Therefore, the traditional approaches using chemicals should be carefully validated because they are used in nanoneurotoxicological studies.

[21] [1] Sharma HS. A special section on nanoneuroscience: nanoneurotoxicity and nanoneuroprotection. J Nanosci Nanotechnol 2009;9:4992e5. [2] Song Y, Tang S. Nanoexposure, unusual diseases, and new health and safety concerns. ScientificWorldJournal 2011;11:1821e8. [3] Zhang R, Bai Y, Zhang B, et al. The potential health risk of titania nanoparticles. J Hazard Mater 2012;211e212:404e13. [4] Kulvietis V, Zalgeviciene V, Didziapetriene J, et al. Transport of nanoparticles through the placental barrier. Tohoku J Exp Med 2011;225:225e34. [5] Jachak A, Lai SK, Hida K, et al. Transport of metal oxide nanoparticles and single-walled carbon nanotubes in human mucus. Nanotoxicology 2012;6:614e22. [6] Konczol M, Ebeling S, Goldenberg E, et al. Cytotoxicity and genotoxicity of size-fractionated iron oxide (magnetite) in A549 human lung epithelial cells: role of ROS, JNK, and NFkappaB. Chem Res Toxicol 2011;24:1460e75. [7] Peters A, Ruckerl R, Cyrys J. Lessons from air pollution epidemiology for studies of engineered nanomaterials. J Occup Environ Med 2011;53:S8e13. [8] Eisen EA, Costello S, Chevrier J, et al. Epidemiologic challenges for studies of occupational exposure to engineered nanoparticles; a commentary. J Occup Environ Med 2011;53:S57e61. [9] Heng BC, Zhao X, Tan EC, et al. Evaluation of the cytotoxic and inflammatory potential of differentially shaped zinc oxide nanoparticles. Arch Toxicol 2011;85:1517e28. [10] Sharma HS, Muresanu DF, Patnaik R, et al. Superior neuroprotective effects of cerebrolysin in heat stroke following chronic intoxication of Cu or Ag engineered nanoparticles. A comparative study with other neuroprotective agents using biochemical and

morphological approaches in the rat. J Nanosci Nanotechnol 2011;11:7549e69. Lanone S, Boczkowski J. Biomedical applications and potential health risks of nanomaterials: molecular mechanisms. Curr Mol Med 2006;6:651e63. Kreuter J, Alyautdin RN, Kharkevich DA, et al. Passage of peptides through the blood-brain barrier with colloidal polymer particles (nanoparticles). Brain Res 1995;674:171e4. Sharma HS, Ali SF, Hussain SM, et al. Influence of engineered nanoparticles from metals on the blood-brain barrier permeability, cerebral blood flow, brain edema and neurotoxicity. An experimental study in the rat and mice using biochemical and morphological approaches. J Nanosci Nanotechnol 2009;9:5055e72. Ahmed J, Gubrud M. Anticipating military nanotechnology. Technol Soc Magazine IEEE 2004;23:33e40. Burch WM. Passage of inhaled particles into the blood circulation in humans. Circulation 2002;106:e141e2. Takenaka S, Karg E, Roth C, et al. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ Health Perspect 2001;109(Suppl

4):547e51. Oberdorster G, Sharp Z, Atudorei V, et al. Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol 2004;16:437e45. De Lorenzo AJD. The olfactory neuron and the bloodebrain barrier. In: Ciba Foundation symposium e internal secretions of the pancreas (colloquia on endocrinology). John Wiley & Sons; 2008. pp. 151e76. Tjalve H, Henriksson J, Tallkvist J, et al. Uptake of manganese and cadmium from the nasal mucosa into the central nervous system via olfactory pathways in rats. Pharmacol Toxicol 1996;79:347e56. Tallkvist J, Henriksson J, d'Argy R, et al. Transport and subcellular distribution of nickel in the olfactory system of pikes and rats. Toxicol Sci 1998;43:196e203. Tjalve H, Henriksson J. Uptake of metals in the brain via olfactory pathways. Neurotoxicology 1999;20:181e95. Henriksson J, Tjalve H. Manganese taken up into the CNS via the olfactory pathway in rats affects astrocytes. Toxicol Sci 2000;55:392e8. Persson E, Henriksson J, Tjalve H. Uptake of cobalt from the nasal mucosa into the brain via olfactory pathways in rats. Toxicol Lett 2003;145:19e27. Elder A, Gelein R, Silva V, et al. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect 2006;114:1172e8. Zhang Y, Chen W, Zhang J, et al. In vitro and in vivo toxicity of CdTe nanoparticles. J Nanosci Nanotechnol 2007;7:497e503. Popovsky M. Nanotechnology and environmental insurance. Columbia J Environ Law 2011;36:125. Brunner TJ, Wick P, Manser P, et al. In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. Environ Sci Technol 2006;40:4374e81. Sager TM, Kommineni C, Castranova V. Pulmonary response to intratracheal instillation of ultrafine versus fine titanium dioxide: role of particle surface area. Part Fibre Toxicol 2008;5:17. ILSI Risk Science Institute. The relevance of the rat lung response to particle overload for human risk assessment: a workshop consensus report. Inhal Toxicol 2000;12:1e17. National Institute for Occupational Safety and Health. NIOSH pocket guide to chemical hazards and other databases. Publication No. 2002-140. Cincinnati: US Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute of Occupational Safety and Health, DHHS (NIOSH); 2002.
[31] National Institute for Occupational Safety and Health. Occupational exposure to titanium dioxide. Publication No. 2011-160 Cincinnati. US Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute of Occupational Safety and Health, DHHS (NIOSH); 2011. [32] World Health Organization. Titanium dioxide. EHC 24. Geneva: WHO; 1982. [33] Nordberg GF, Fowler BA, Nordberg M, et al. Titanium. In: Handbook of the toxicology of metals. 3rd ed. Amsterdam: Elsevier; 2007. [34] Rahman Q, Lohani M, Dopp E, et al. Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts. Environ Health Perspect 2002;110:797e800. [35] Hedenborg M. Titanium dioxide induced chemiluminescence of human polymorphonuclear leukocytes. Int Arch Occup Environ Health 1988;61:1e6. [36] Duan Y, Liu J, Ma L, et al. Toxicological characteristics of nanoparticulate anatase titanium dioxide in mice. Biomaterials 2010;31:894e9. [37] Chen J, Dong X, Zhao J, et al. In vivo acute toxicity of titanium dioxide nanoparticles to mice after intraperitioneal injection. J Appl Toxicol 2009;29:330e7. [38] Liang G, Pu Y, Yin L, et al. Influence of different

sizes of titanium dioxide nanoparticles on hepatic and renal functions in rats with correlation to oxidative stress. J Toxicol Environ Health A 2009;72:740e5. [39] Wang J, Chen C, Liu Y, et al. Potential neurological lesion after nasal instillation of TiO2 nanoparticles in the anatase and rutile crystal phases. Toxicol Lett 2008;183:72e80. [40] Nel A, Xia T, Madler L, et al. Toxic potential of materials at the nanolevel. Science 2006;311:622e7. [41] Cho WS, Duffin R, Poland CA, et al. Metal oxide nanoparticles induce unique inflammatory footprints in the lung: important implications for nanoparticle testing. Environ Health Perspect 2010;118:1699e706. [42] Li Y, Li J, Yin J, et al. Systematic influence induced by 3 nm titanium dioxide following intratracheal instillation of mice. J Nanosci Nanotechnol 2010;10:8544e9. [43] Wang JX, Li YF, Zhou GQ, et al. Influence of intranasal instilled titanium dioxide nanoparticles on monoaminergic neurotransmitters of female mice at different exposure time. Zhonghua Yu Fang Yi Xue Za Zhi 2007;41:91e5 [in Chinese]. [44] Wang J, Liu Y, Jiao F, et al. Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO2 nanoparticles. Toxicology 2008;254:82e90. [45] Ma L, Liu J, Li N, et al. Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO2 delivered to the abdominal cavity. Biomaterials 2010;31:99e105. [46] Hu R, Gong X, Duan Y, et al. Neurotoxicological effects and the impairment of spatial recognition memory in mice caused by exposure to TiO2 nanoparticles. Biomaterials 2010;31:8043e50. [47] Jeon YM, Park SK, Lee MY. Toxicoproteomic identification of TiO2 nanoparticle-induced protein expression changes in mouse brain. Animal Cells Syst 2011;15:107e14. [48] Long TC, Saleh N, Tilton RD, et al. Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity. Environ Sci Technol 2006;40:4346e52. [49] Long TC, Tajuba J, Sama P, et al. Nanosize titanium dioxide stimulates reactive oxygen species in brain microglia and damages neurons in vitro. Environ Health Perspect 2007;115:1631e7.

[50] Liu S, Xu L, Zhang T, et al. Oxidative stress and apoptosis induced by nanosized titanium dioxide in PC12 cells. Toxicology 2010;267:172e7. [51] Shin JA, Lee EJ, Seo SM, et al. Nanosized titanium dioxide enhanced inflammatory responses in the septic brain of mouse. Neuroscience 2010;165:445e54. [52] Yamashita K, Yoshioka Y, Higashisaka K, et al. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. Nat Nanotechnol 2011;6:321e8. [53] Takeda K, Suzuki K, Ishihara A, et al. Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve systems. J Health Sci 2009;55:95e102. [54] Shimizu M, Tainaka H, Oba T, et al. Maternal exposure to nanoparticulate titanium dioxide during the prenatal period alters gene expression related to brain development in the mouse. Part Fibre Toxicol 2009;6:20. [55] Takahashi Y, Mizuo K, Shinkai Y, et al. Prenatal exposure to titanium dioxide nanoparticles increases dopamine levels in the prefrontal cortex and neostriatum of mice. J Toxicol Sci 2010;35:749e56. [56] Brayner R, Ferrari-Iliou R, Brivois N, et al. Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Lett 2006;6:866e70. [57] Roselli M, Finamore A, Garaguso I, et al. Zinc oxide

protects cultured enterocytes from the damage induced by Escherichia coli. J Nutr 2003;133:4077e82. [58] Deng X, Luan Q, Chen W, et al. Nanosized zinc oxide particles induce neural stem cell apoptosis. Nanotechnology 2009;20:115101. [59] Zhao J, Xu L, Zhang T, et al. Influences of nanoparticle zinc oxide on acutely isolated rat hippocampal CA3 pyramidal neurons. Neurotoxicology 2009;30:220e30. [60] Han D, Tian Y, Zhang T, et al. Nano-zinc oxide damages spatial cognition capability via over-enhanced long-term potentiation in hippocampus of Wistar rats. Int J Nanomed 2011;6:1453e61. [61] Darroudi M, Sabouri Z, Oskuee RK, et al. Neuronal toxicity of biopolymer-template synthesized ZnO nanoparticles. Nanomed J 2014;1:88e93. [62] Yamamoto O, Komatsu M, Sawai J, et al. Effect of lattice constant of zinc oxide on antibacterial characteristics. J Mater Sci Mater Med 2004;15:847e51. [63] Ghule K, Ghule AV, Chen B-J, et al. Preparation and characterization of ZnO nanoparticles coated paper and its antibacterial activity study. Green Chem 2006;8:1034e41. [64] Tam K, Djuriic A, Chan C, et al. Antibacterial activity of s ZnO nanorods prepared by a hydrothermal method. Thin Solid Films 2008;516:6167e74. [65] Zhang L, Jiang Y, Ding Y, et al. Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids). J Nanopart Res 2007;9:479e89. [66] Reddy KM, Feris K, Bell J, et al. Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. Appl Phys Lett 2007;90:2139021e3. [67] Heinlaan M, Ivask A, Blinova I, et al. Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria Vibrio fischeri and crustaceans Daphnia magna and Thamnocephalus platyurus. Chemosphere 2008;71:1308e16. [68] Franklin NM, Rogers NJ, Apte SC, et al. Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and ZnCl2 to a freshwater microalga (Pseudokirchneriella subcapitata): the importance of particle solubility. Environ Sci Technol 2007;41:8484e90. [69] Zhu X, Zhu L, Duan Z, et al. Comparative toxicity of several metal oxide nanoparticle aqueous suspensions to zebrafish

(Danio rerio) early developmental stage. J Environ Sci Health A Tox Hazard Subst Environ Eng 2008;43:278e84. Wang J, Wang B, Wang T-C, et al. Acute toxicological impact of nano- and submicro-scaled zinc oxide powder on healthy adult mice. J Nanopart Res 2008;10:263e76. Finley JW, Davis CD. Manganese deficiency and toxicity: are high or low dietary amounts of manganese cause for concern? Biofactors 1999;10:15e24. Hazardous Substances Data Bank. Manganese compounds. National Library of Medicine; 2006. http://toxnet.nlm.nih. gov/cgi-bin/sis/search/r?dbsþhsdb:@termþ@rnþ@ relþ7439-96-5. National Institute for Occupational Safety and Health. NIOSH pocket guide to chemical hazards. Publication No. 2005-149. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health; 2007. US Centers for Disease Control (ATSDR). Toxicological profile for manganese; 2000. http://www.atsdr.cdc.gov/ toxprofiles/tp.asp?id¼102&tid¼23 [accessed 03.03.11]. Dobson AW, Erikson KM, Aschner M. Manganese neurotoxicity. Ann N Y Acad Sci 2004;1012:115e28. Fechter LD, Johnson DL, Lynch R. The relationship of particle size to olfactory nerve uptake of a non-soluble form of

manganese into brain. Neurotoxicology 2002;23:177e83. Brenneman KA, Wong BA, Buccellato MA, et al. Direct olfactory transport of inhaled manganese ((54)MnCl(2)) to the rat brain: toxicokinetic investigations in a unilateral nasal occlusion model. Toxicol Appl Pharmacol 2000;169:238e48. Rao DB, Wong BA, McManus BE, et al. Inhaled iron, unlike manganese, is not transported to the rat brain via the olfactory pathway. Toxicol Appl Pharmacol 2003;193:116e26. Dorman DC, Struve MF, James RA, et al. Influence of particle solubility on the delivery of inhaled manganese to the rat brain: manganese sulfate and manganese tetroxide pharmacokinetics following repeated (14-day) exposure. Toxicol Appl Pharmacol 2001;170:79e87. Vitarella D, Wong BA, Moss OR, et al. Pharmacokinetics of inhaled manganese phosphate in male Sprague-Dawley rats following subacute (14-day) exposure. Toxicol Appl Pharmacol 2000;163:279e85. Gulson B, Mizon K, Taylor A, et al. Changes in manganese and lead in the environment and young children associated with the introduction of methylcyclopentadienyl manganese tricarbonyl in gasoline e preliminary results. Environ Res 2006;100:100e14. Cohen DD, Gulson BL, Davis JM, et al. Fine-particle Mn and other metals linked to the introduction of MMT into gasoline in Sydney, Australia: results of a natural experiment. Atmos Environ 2005;39:6885e96. Stefanescu D, Khoshnan A, Patterson P, et al. Neurotoxicity of manganese oxide nanomaterials. J Nanopart Res 2009;11:1957e69. Hussain SM, Javorina AK, Schrand AM, et al. The interaction of manganese nanoparticles with PC-12 cells induces dopamine depletion. Toxicol Sci 2006;92:456e63. ´ ´ ´ ¨ Oszlanczi G, Vezer T, Sarkozi L, et al. Functional neurotoxicity of Mn-containing nanoparticles in rats. Ecotoxicol Environ Saf 2010;73:2004e9. Singh SP, Kumari M, Kumari SI, et al. Genotoxicity of nanoand micron-sized manganese oxide in rats after acute oral treatment. Mutat Res 2013;754:39e50. Diana MS, Ali K, Paul HP, et al. Neurotoxicity of manganese oxide nanomaterials. J Nanopart Res 2009;11:1957e69. Ahamed M, Alsalhi MS, Siddiqui MK. Silver nanoparticle applications and human health. Clin Chim Acta 2010;411:1841e8.

[89] Lee HY, Park HK, Lee YM, et al. A practical procedure for producing silver nanocoated fabric and its antibacterial evaluation for biomedical applications. Chem Commun (Camb); 2007:2959e61. [90] Jain P, Pradeep T. Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. Biotechnol Bioeng 2005;90:59e63. [91] Zhang YY, Sun J. A study on the bio-safety for nano-silver as anti-bacterial materials. Zhongguo Yi Liao Qi Xie Za Zhi 2007;31:36e8. 16 [in Chinese]. [92] Wijnhoven SWP, Peijnenburg WJGM, Herberts CA, et al. Nano-silver e a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology 2009;3:109e38. [93] Kim YS, Kim JS, Cho HS, et al. Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in SpragueeDawley rats. Inhal Toxicol 2008;20:575e83. [94] Arora S, Jain J, Rajwade JM, et al. Cellular responses induced by silver nanoparticles: in vitro studies. Toxicol Lett 2008;179:93e100. [95] Arora S, Jain J, Rajwade JM, et al. Interactions of silver nanoparticles with primary mouse fibroblasts and liver cells. Toxicol Appl Pharmacol 2009;236:310e8. [96] Soto K, Garza KM, Murr LE. Cytotoxic effects of aggregated nanomaterials. Acta Biomater 2007;3:351e8. [97] Powers CM,

Badireddy AR, Ryde IT, et al. Silver nanoparticles compromise neurodevelopment in PC12 cells: critical contributions of silver ion, particle size, coating, and composition. Environ Health Perspect 2011;119:37e44. [98] Tang J, Xiong L, Zhou G, et al. Silver nanoparticles crossing through and distribution in the bloodebrain barrier in vitro. J Nanosci Nanotechnol 2010;10:6313e7. [99] Trickler WJ, Lantz SM, Murdock RC, et al. Silver nanoparticle induced bloodebrain barrier inflammation and increased permeability in primary rat brain microvessel endothelial cells. Toxicol Sci 2010;118:160e70. [100] Tang J, Xiong L, Wang S, et al. Distribution, translocation and accumulation of silver nanoparticles in rats. J Nanosci Nanotechnol 2009;9:4924e32. [101] Liu Y, Guan W, Ren G, et al. The possible mechanism of silver nanoparticle impact on hippocampal synaptic plasticity and spatial cognition in rats. Toxicol Lett 2012;209:227e31. [102] Zhang Y, Ferguson SA, Watanabe F, et al. Silver nanoparticles decrease body weight and locomotor activity in adult male rats. Small 2013;9:1715e20. [103] Yin N, Liu Q, Liu J, et al. Silver nanoparticle exposure attenuates the viability of rat cerebellum granule cells through apoptosis coupled to oxidative stress. Small 2013;9:1831e41. [104] Hood E. Nanotechnology: looking as we leap. Environ Health Perspect 2004;112:A740e9. [105] Kong SD, Lee J, Ramachandran S, et al. Magnetic targeting of nanoparticles across the intact bloodebrain barrier. J Control Release 2012;164:49e57. [106] Zhou R, Acton PD, Ferrari VA. Imaging stem cells implanted in infarcted myocardium. J Am Coll Cardiol 2006;48:2094e106. [107] Cromer Berman SM, Walczak P, Bulte JW. Tracking stem cells using magnetic nanoparticles. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2011;3:343e55. [108] Singh N, Jenkins GJ, Asadi R, et al. Potential toxicity of superparamagnetic iron oxide nanoparticles (SPION). Nano Rev 2010;1:5358. http://dx.doi.org/10.3402/nano.v1i0.5358. [109] Calderon-Garciduenas L, Reed W, Maronpot RR, et al. Brain inflammation and Alzheimer's-like pathology in individuals exposed to severe air pollution. Toxicol Pathol 2004;32:650e8. Peters A, Veronesi B, Calderon-Garciduenas L, et al. Translocation and potential neurological effects of fine and ultrafine particles a critical update. Part Fibre Toxicol 2006;3:13. Calderon-Garciduenas L, Solt AC, Henriquez-Roldan C, et al. Long-term air pollution exposure is associated with neuroinflammation, an altered innate immune response, disruption of the blood-brain barrier, ultrafine particulate deposition, and accumulation of amyloid beta-42 and alpha-synuclein in children and young adults. Toxicol Pathol 2008;36:289e310. Lundqvist M, Stigler J, Cedervall T, et al. The evolution of the protein corona around nanoparticles: a test study. ACS Nano 2011;5:7503e9. Mahmoudi M, Lynch I, Ejtehadi MR, et al. Proteinenanoparticle interactions: opportunities and challenges. Chem Rev 2011;111:5610e37. Pisanic II TR, Blackwell JD, Shubayev VI, et al. Nanotoxicity of iron oxide nanoparticle internalization in growing neurons. Biomaterials 2007;28:2572e81. Wang B, Feng W, Zhu M, et al. Neurotoxicity of low-dose repeatedly intranasal instillation of nano- and submicronsized ferric oxide particles in mice. J Nanopart Res 2009;11:41e53. Wu J, Ding T, Sun J. Neurotoxic potential of iron oxide nanoparticles in the rat brain striatum and hippocampus. Neurotoxicology 2013;34:243e53. Miller RM, Kiser GL, Kaysser-Kranich TM, et al. Robust dysregulation of gene expression in

substantia nigra and striatum in Parkinson's disease. Neurobiol Dis 2006;21:305e13. Coffey ET, Smiciene G, Hongisto V, et al. c-Jun N-terminal protein kinase (JNK) 2/3 is specifically activated by stress, mediating c-Jun activation, in the presence of constitutive JNK1 activity in cerebellar neurons. J Neurosci 2002;22:4335e45. Wang B, Feng WY, Wang M, et al. Transport of intranasally instilled fine Fe2O3 particles into the brain: microdistribution, chemical states, and histopathological observation. Biol Trace Elem Res 2007;118:233e43. Wang B, Wang Y, Feng W, et al. Trace metal disturbance in mice brain after intranasal exposure of nano- and submicron-sized Fe2O3 particles. Chem Anal 2008;53:927e42. Kim NH, Park SJ, Jin JK, et al. Increased ferric iron content and iron-induced oxidative stress in the brains of scrapieinfected mice. Brain Res 2000;884:98e103. Gaasch JA, Lockman PR, Geldenhuys WJ, et al. Brain iron toxicity: differential responses of astrocytes, neurons, and endothelial cells. Neurochem Res 2007;32:1196e208. Castellani RJ, Moreira PI, Liu G, et al. Iron: the redox-active center of oxidative stress in Alzheimer disease. Neurochem Res 2007;32:1640e5. Wang Y, Wang B, Zhu MT, et al. Microglial activation, recruitment and phagocytosis as linked phenomena in ferric oxide nanoparticle exposure. Toxicol Lett 2011;205:26e37. Evans GW. Copper homeostasis in the mammalian system. Physiol Rev 1973;53:535e70. Sternlieb I. Copper and the liver. Gastroenterology 1980;78:1615e28. Nalbandyan RM. Copper in brain. Neurochem Res 1983;8:1211e32. Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol 1990;186:1e85.

[129] Galhardi CM, Diniz YS, Faine LA, et al. Toxicity of copper intake: lipid profile, oxidative stress and susceptibility to renal dysfunction. Food Chem Toxicol 2004;42:2053e60. [130] Aruoja V, Dubourguier HC, Kasemets K, et al. Toxicity of nanoparticles of CuO, ZnO and TiO2 to microalgae Pseudokirchneriella subcapitata. Sci Total Environ 2009;407:1461e8. [131] Sharma HS, Ali SF, Tian ZR, et al. Chronic treatment with nanoparticles exacerbate hyperthermia induced bloodebrain barrier breakdown, cognitive dysfunction and brain pathology in the rat. Neuroprotective effects of nanowired-antioxidant compound H-290/51. J Nanosci Nanotechnol 2009;9:5073e90. [132] Li F, Zhou X, Zhu J, et al. High content image analysis for human H4 neuroglioma cells exposed to CuO nanoparticles. BMC Biotechnol 2007;7:66. [133] Prabhu BM, Ali SF, Murdock RC, et al. Copper nanoparticles exert size and concentration dependent toxicity on somatosensory neurons of rat. Nanotoxicology 2010;4:150e60. [134] Xu LJ, Zhao JX, Zhang T, et al. In vitro study on influence of nano particles of CuO on CA1 pyramidal neurons of rat hippocampus potassium currents. Environ Toxicol 2009;24:211e7. [135] Trickler WJ, Lantz SM, Schrand AM, et al. Effects of copper nanoparticles on rat cerebral microvessel endothelial cells. Nanomedicine (Lond) 2012;7:835e46. [136] Karlsson HL, Cronholm P, Gustafsson J, et al. Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. Chem Res Toxicol 2008;21:1726e32. [137] Liu Z, Liu S, Ren G, et al. Nano-CuO inhibited voltage-gated sodium current of hippocampal CA1 neurons via reactive oxygen species but independent from G-proteins pathway. J Appl

Toxicol 2011;31:439e45. [138] Xiao AY, Wei L, Xia S, et al. Ionic mechanism of ouabain-induced concurrent apoptosis and necrosis in individual cultured cortical neurons. J Neurosci 2002;22:1350e62. [139] Janvier NC, McMorn SO, Harrison SM, et al. The role of Naþ-Ca2þ exchange current in electrical restitution in ferret ventricular cells. J Physiol 1997;504:301e14. [140] Sitte HH, Farhan H, Javitch JA. Sodium-dependent neurotransmitter transporters: oligomerization as a determinant of transporter function and trafficking. Mol Interv 2004;4:38e47. [141] Armstrong CM. Sodium channels and gating currents. Physiol Rev 1981;61:644e83. [142] Yang N, George Jr AL, Horn R. Molecular basis of charge movement in voltage-gated sodium channels. Neuron 1996;16:113e22. [143] Chen J, Zhu J, Chob H-H, et al. Differential cytotoxicity of metal oxide nanoparticles. J Exptl Nanosci 2008;3:321e8. [144] Perreault F, Pedroso Melegari S, Henning da Costa C, et al. Genotoxic effects of copper oxide nanoparticles in Neuro 2A cell cultures. Sci Total Environ 2012;441:117e24. [145] Darlington TK, Neigh AM, Spencer MT, et al. Nanoparticle characteristics affecting environmental fate and transport through soil. Environ Toxicol Chem 2009;28:1191e9. [146] Zhang QL, Li MQ, Ji JW, et al. In vivo toxicity of nanoalumina on mice neurobehavioral profiles and the potential mechanisms. Int J Immunopathol Pharmacol 2011;24(1 Suppl.): 23Se9S. [147] Li Y, Yu S, Wu Q, et al. Chronic Al2O3-nanoparticle exposure causes neurotoxic effects on locomotion behaviors by inducing severe ROS production and -disruption of ROS defense mechanisms in nematode Caenorhabditis elegans. J Hazard Mater; 2012:219e30. Huang N, Yan Y, Xu Y, et al. Alumina nanoparticles alter rhythmic activities of local interneurons in the antennal lobe of Drosophila. Nanotoxicology 2013;7:212e20. Chen L, Yokel RA, Hennig B, et al. Manufactured aluminum oxide nanoparticles decrease expression of tight junction proteins in brain vasculature. J Neuroimmune Pharmacol 2008;3:286e95. Zhang Q, Xu L, Wang J, et al. Lysosomes involved in the cellular toxicity of nano-alumina: combined effects of particle size and chemical composition. J Biol Regul Homeost Agents 2013;27:365e75. Li XB, Zheng H, Zhang ZR, et al. Glia activation induced by peripheral administration of aluminum oxide nanoparticles in rat brains. Nanomedicine 2009;5:473e9.

[152] Wu J, Wang C, Sun J, et al. Neurotoxicity of silica nanoparticles: brain localization and dopaminergic neurons damage pathways. ACS Nano 2011;5:4476e89. [153] Kim Y-J, Yang SI. Neurotoxic effects by silica TM nanoparticle is independent of differentiation of SH-SY5Y cells. Mol Cell Toxicol 2011;7:381e8. [154] Zhang Y, Ali SF, Dervishi E, et al. Cytotoxicity effects of graphene and single-wall carbon nanotubes in neural phaeochromocytoma-derived PC12 cells. ACS Nano 2010;4:3181e6. [155] Zhang Y, Xu Y, Li Z, et al. Mechanistic toxicity evaluation of uncoated and PEGylated single-walled carbon nanotubes in neuronal PC12 cells. ACS Nano 2011;5:7020e33. [156] Tin-Tin-Win-Shwe, Yamamoto S, Ahmed S, et al. Brain cytokine and chemokine mRNA expression in mice induced by intranasal instillation with ultrafine carbon black. Toxicol Lett 2006;163:153e60.

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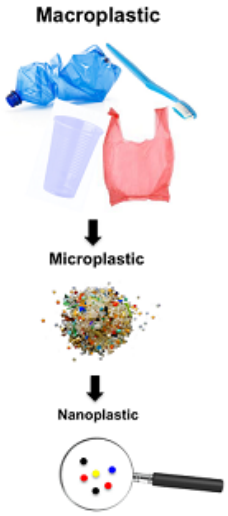
NanoPlastic

Polystyrene degrades into nanoplastics.
The formation of nanoplastic particles increase over time.
Results suggest a continuous process of plastic surface erosion.



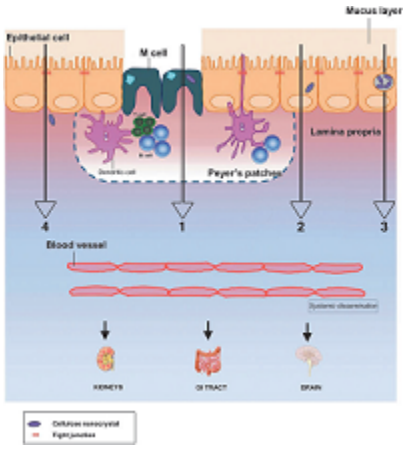
The amount of nano- and microplastic in the aquatic environment rises due to the industrial production of plastic and the degradation of plastic into smaller particles. Concerns have been raised about their incorporation into food webs. Little is known about the fate and effects of nanoplastic, **especially for the freshwater environment**. In this study, effects of **nano-polystyrene (nano-PS)** on the growth and photosynthesis of the green alga *Scenedesmus obliquus* and the growth, mortality, neonate production, **and malformations of the zooplankter** *Daphnia magna* were assessed. **Nano-PS reduced population growth and reduced chlorophyll concentrations in the algae**. Exposed *Daphnia* showed a **reduced body size and severe alterations in reproduction. Numbers and body size of neonates were lower, while the number of neonate malformations among neonates rose to 68% of the individuals**. These effects of **nano-PS were observed between 0.22 and 103 mg nano-PS/L. Malformations occurred from 30 mg of nano-PS/L onward. Such plastic concentrations are much higher than presently reported for marine waters as well as freshwater, but may eventually occur in sediment pore waters**. As far as we know, these results are the first to show **that direct life history shifts in algae and *Daphnia* populations may occur as a result of exposure to nanoplastic**.

Comment [O]: **The release of plastic materials into the environment is recognised as an important pollution related issue (Sutherland et al., 2010 and UNEP, 2011). Once in the environment plastics undergo abiotic and biotic weathering processes that cause their degradation and fragmentation into increasingly smaller particles, commonly termed microplastics (MPs; often defined**

Comment [O]: **During the first half of the project we successfully developed new molecular-models for two common hydrophobic polymers, namely polypropylene (PP) and polyethylene (PE). *The sum of propylene glycol and glycol ethers was associated with increased***

Distribution and effects of plastic pollution that results from insufficient resource efficiency in a world that consumes 100 million tonnes of plastic annually. Plastics are of concern both for their chemical toxicity associated with the toxic additives and monomers often found in plastic products, and the adverse ecological and toxicological effects caused by the solid materials themselves

Wastewater treatment plants have been identified as a potential source of microplastics, as many plastic particulates can be found both in sewage sludge and the treated effluents- It is expected particularly in areas where biosolids are applied to agricultural lands that there will be elevated microplastic levels since these materials are extremely slow to mineralise.



Microplastics might be a vector for hazardous substances because they can sorb persistent, bioaccumulating and or toxic chemicals (e.g. POPs, endocrine disrupters). Thus, plastic particles may facilitate the entrance of these substances into the food chain, potentially threatening human health both chemically and by particle toxicity.

Glycol ethers. **Glycol ethers, a chemical class with > 80 compounds, are used in a broad array of cleaning applications because of their combined hydrophilic and lipophilic nature**. They are often used in paints, varnishes, and cosmetics and have been detected in a variety of household products (Kwon et al. 2008; Plaisance et al. 2008). Biomonitoring methods are currently being developed, so large-scale studies are limited**. In human studies, exposure to glycol ethers has been associated with low sperm mobility** (Cherry et al. 2008), **hematological effects** (Starek et al. 2008), **and asthma and allergies** (Choi et al. 2010).

In the present study, we analyzed all samples for 2-butoxyethanol and 2,2-methoxyethoxyethanol, and in a later second sampling round, we analyzed 14 additional samples for six other glycol ethers. **We detected glycol ethers in 3 conventional cleaners, face lotion, polish/wax, sunscreen, and in alternative shaving cream, pillow protector, and sunscreen samples**. Of the 5 conventional samples with detectable 2-butoxyethanol, only the carpet cleaner was labeled as containing 2-butoxyethanol. **When analyzed and detected, other glycol ethers were not listed on labels**. Although we detected phenoxyethanol in conventional and alternative sunscreen samples, we did not detect this chemical in some conventional and alternative samples comprising products labeled as containing this compound; levels may have been < LOD.

Comment [O]: ***Impact of Endocrine Disrupting NPs on Reproductive Health***
Hormones play a key role in influencing the development of the reproductive system and subsequently in controlling its activities once developed. For this reason, most of the research carried out on EDCs in the last two decades has focused its attention on reproductive

Comment [O]: **Limit Of Detection**

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How fasting helps fight fatty liver disease

Scientists at Helmholtz Zentrum München have new information on what happens at the molecular level when we go hungry. Working with the Deutsches Zentrum für Diabetesforschung (German Center for Diabetes Research -- DZD) and the Deutsches Krebsforschungszentrum (German Cancer Research Center -- DKFZ) **they were able to show that upon deprivation of food a certain protein is produced that adjusts the metabolism in the liver**. The results are published in the Open Access Journal 'EMBO Molecular Medicine'. The growing number of overweight people has long been one of modern society's pressing issues. In particular the resulting metabolic diseases such as type 2 diabetes and corresponding secondary conditions can have serious consequences for health. **A reduced intake of calories, such as in the framework of an intermittent fasting diet, can help to whip the metabolism back into shape** -- but why does this happen?-- This is the question that Prof. Dr. Stephan Herzig, Director of the Institute for Diabetes and Cancer (IDC) at the Helmholtz Zentrum München, and Dr. Adam J. Rose, head of the 'Protein metabolism in health and disease' research group at the DKFZ in Heidelberg, wanted to answer. "Once we understand how fasting influences our metabolism we can attempt to bring about this effect therapeutically," Herzig states

Stress molecule reduces the absorption of fatty acids in the liver

In the current study, the scientists looked for liver cell genetic activity differences that were caused by fasting. With the help of so-called transcript arrays, they were able to show that especially the gene for the protein GADD45β was often read differently depending on the diet: **the greater the hunger, the more frequently the cells produced the molecule, whose name stands for 'Growth Arrest and DNA Damage-inducible'**. As the name says, the molecule was previously **associated with the repair of damage to the genetic information and the cell cycle, rather than with metabolic biology**.- **Subsequent simulation tests showed that GADD45β is responsible for controlling the absorption of fatty acids in the liver**. Mice who lacked the corresponding gene were more likely to develop fatty liver disease. **However when the protein was restored, the fat content of the liver normalized and also sugar metabolism improved**. The scientists were able to **confirm the result also in humans: a low GADD45β level was accompanied by increased fat accumulation in the liver and an elevated blood sugar level-The stress on the liver cells caused by fasting consequently appears to stimulate GADD45β production, which then adjusts the metabolism to the low food intake**," Herzig summarizes. The researchers now want to use the new findings for therapeutic intervention in the fat and sugar metabolism so that the positive effects of food deprivation might be translated for treatment-The stress on the liver cells caused

Comment [O]: **GADD45β='Growth Arrest and DNA Damage-inducible'**

by fasting consequently appears to stimulate GADD45β production, which then adjusts the metabolism to the low food intake," Herzig summarizes. The researchers now want to use the new findings for therapeutic intervention in the fat and sugar metabolism so that the positive effects of food deprivation might be translated for treatment **Story Source-**The above post is reprinted from materials provided by **Helmholtz Zentrum Muenchen - German Research Centre for Environmental Health**. -**Journal Reference**-J. Fuhrmeister, A. Zota, T. P. Sijmonsma, O. Seibert, S. C ng r, K. Schmidt, N. Vallon, R. M. de Guia, K. Niopek, M. Berriel Diaz, A. Maida, M. Blu her, J. G. Okun, S. Herzig, A. J. Rose. **Fasting-induced liver GADD45 restrains hepatic fatty acid uptake and improves metabolic health**. *EMBO Molecular Medicine*, 2016; DOI: 10.15252/emmm.201505801 Helmholtz Zentrum Muenchen - German Research Centre for Environmental Health. "How fasting helps fight fatty liver disease." ScienceDaily. ScienceDaily, 9 May 2016. <www.sciencedaily.com/releases/2016/05/160509085347.htm>.

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Application of dental nanomaterials- potential toxicity to the central nervous system

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Received 26 December 2014

Accepted for publication 16 March 2015

Published 14 May 2015 Volume 2015:10(1) Pages 3547—3565

DOI https://dx.doi.org/10.2147/IJN.S79892

Checked for plagiarism Yes

Review by Single-blind

Peer reviewer comments 5

Editor who approved publication: Dr Lei Yang

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Abstract: **Nanomaterials are defined as materials with one or more external dimensions with a size of 1–100 nm**. Such materials possess typical nanostructure-dependent properties **(eg, chemical, biological, optical, mechanical, and magnetic)**, which may differ greatly from the properties of their bulk counterparts. **In recent years, nanomaterials have been widely used in the production of dental materials, particularly in light polymerization composite resins and bonding systems, coating materials for dental implants, bioceramics, endodontic sealers, and mouthwashes**. However, the dental applications of nanomaterials yield not only a significant improvement in clinical treatments **but also growing concerns regarding their biosecurity**. The brain is well protected by the blood–brain barrier (BBB), which separates the blood from the cerebral parenchyma. **However, in recent years, many studies have found that nanoparticles (NPs), including nanocarriers, can transport through the BBB and locate in the central nervous system (CNS).** Because the CNS may be a potential target organ of the nanomaterials, it is essential to determine the neurotoxic effects of NPs. In this review, possible dental nanomaterials and their pathways into the CNS are discussed, **as well as related neurotoxicity effects underlying the in vitro and in vivo studies**. Finally, we analyze the limitations of the current testing methods on the toxicological effects of nanomaterials. This review contributes to a better understanding of the nano-related risks to the CNS as well as the further development of safety assessment systems.

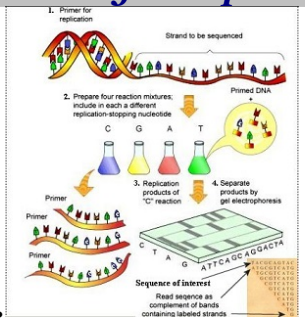
Keywords: dental, nanomaterials, central nervous system, toxicity, testing methods, risk assessment

A Letter to the Editor has been received and published for this article.

Introduction

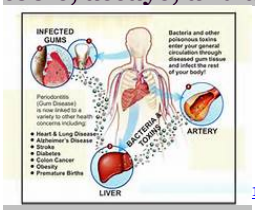
Nanomaterials are defined as materials composed of unbound particles or particles in an aggregate or agglomerate state with one or more external dimensions with a size ranging from 1 nm to 100 nm.[1] Such materials possess typical nanostructure-dependent properties **(eg, chemical, biological, optical, mechanical, and magnetic),** which distinguish them from their bulk counterparts. Because of their new and unique properties, nanomaterials are becoming ubiquitous in various products, such as sunscreens, cosmetics, medical supplies, clothing, and building materials. The global demand for nanomaterials and nano-enabled devices is expected to approach US$1 trillion by 2015.[2] The overwhelming increase in the amount of nanotechnology-related products has had major impacts on both society and the environment.--The benefits of nanomaterials to modern medicine have been particularly tremendous. **In recent years, nanomaterials have been widely used in the production of dental materials**, **including light**

polymerization composite resins[3,4] and bonding systems, coating materials for dental implants,[5] bioceramics, endodontic sealers,[6] and mouthwashes.[7] However, in addition to yielding significant improvements in clinical treatments, the applications of dental nanomaterials **have also created growing concerns regarding their biosecurity**. Because **the nanomaterials are similar in size to DNA molecules, proteins, viruses, and biological molecules, some of their biological effects may lie in the interaction mechanisms between living things and the environment**, which has not yet been distinctly understood. In fact, nanoparticles (NPs) **are a type of mesoscopic system that possesses a special surface effect, a small size effect, and a macroscopic quantum tunneling effect**. When reduced to the nanoscale, many benign materials may exhibit appreciable cellular toxicity. For example, TiO_2, a common substrate material for dental implants, was previously classified as being biologically inert in humans and animals and has been used as a negative control particle in a variety of toxicological studies. **Nevertheless, several possible adverse effects of TiO_2 NPs on human health have been recently discovered**.[8,9] Additionally, in vitro data have also demonstrated **the cellular toxicity of zinc oxide nanomaterials (nano-ZnO), which have been developed for numerous anti-infection applications.[10]---Indeed, nanomaterials are not inherently benign; they can affect biological behaviors at different levels, including the cellular, subcellular, and protein levels**. After exposure, some **nanomaterials readily travel throughout the body, deposit in target organs, penetrate cell membranes, lodge in the mitochondria, and trigger injurious responses**. In recent years, many studies have demonstrated that **nanomaterials can accumulate in the heart, liver, spleen, lungs, and kidneys of animals**.[11,12] The brain is different from other organs, as the blood–brain barrier (BBB) can prevent the majority of substances from entering the brain. **However, existing research has shown that nanomaterials have relatively easily crossed the BBB into the brain, and the crossing of the BBB by nanomaterials was attributed to their small sizes and high surface activities**. Furthermore, **these nanomaterials may even translocate into the brain by the olfactory and sensory nerves**.[13,14] All of these findings have suggested that the ***central nervous system (CNS) could be damaged and a range of pathogenic effects may be experienced upon exposure to nanomaterials***.



Researchers have conducted many in vivo and in vitro studies to explore the interactions **between the nanomaterials and biological macromolecules, cells, organs, and tissues, and the**

majority of these studies have found that the effects of the biological toxicities of the nanomaterials may be induced by the mechanisms of oxidative stress and inflammatory reactions.[15,16] However, one problem that has arisen is whether the traditional methods and techniques utilized in the analysis of the toxicities of the nanomaterials are accurate and reliable. Further questions have arisen regarding whether **the unique physicochemical properties of the nanomaterials have introduced new mechanisms of injury and whether these new mechanisms will lead to new pathologies**. Even if the nanomaterials do not introduce new pathologies, **there could be new, novel mechanisms of injury that require special tools, assays, and approaches to assess their toxicities.**



[17] ---This review is mainly intended to provide a detailed introduction of the applications of dental nanomaterials along with **their potential neurotoxic effects**. Possible dental **nanomaterials and their pathways into the CNS are stated first, and the neurotoxic effects and related mechanisms behind the in vitro and in vivo studies are further discussed.** Finally, we highlight the limitations of the current investigative methods and provide some suggestions on some aspects of future researches. We hope this review will contribute to a better understanding of the nano-related risks to the CNS and the further development of safety assessment systems.

Possible commercial dental nanomaterials

Alongside the industrialization process of nanotechnology, dental nanomaterials have been widely utilized, and the opportunities for people to come into contact with nanomaterials have improved greatly. Nanotechnology-based materials have led to great improvements in clinical treatments and have driven the innovation of numerous conventional dental materials. Major applications of nanomaterials in the dental field are described in this section, and a summary of these examples is provided in **Composite resins and bonding systems** Schematic of the blood–brain barrier and the associated components of the neurovascular unit. **Note:** Reprinted from *Adv Drug Deliv Rev*, 64(7), Chen Y, Liu L. Modern methods for delivery of drugs across the blood–brain barrier. 640–665., Copyright (2012), with permission from Elsevier.[46]Neurotoxicity on the in vitro BBB model

in recent years, nanomaterials have been reported to be able to overcome the BBB and to produce biologic effects on the CNSII.[92,93] In many situations, the microvascular endothelial cells of the human brain are used as an in vitro BBB model, such as hCMEC/D3 cells,[94] **human brain microvascular endothelial cells,[95] and human cerebral endothelial cells**.[96] Rat is another common experimental animal due to its availability of

Comment [O]: **Prosthodontics is an important branch of the oral medicine. With the improvement of people's living standards and the promotion of oral health knowledge, prosthodontics increasingly received widespread attention. Prosthodontics is mainly for dental defects, treatment after tooth loss, such as lays, crowns, and dentures, also including the use of artificial prostheses for periodontal disease, temporomandibular joint disease, and maxillofacial tissue defects**

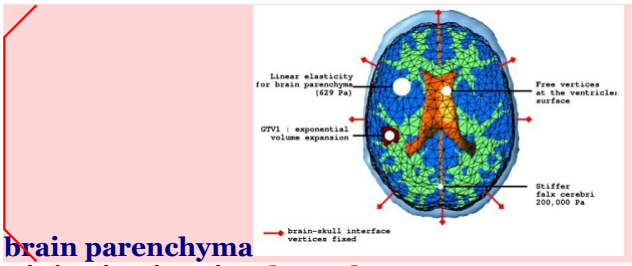
Comment [O]: **Nanomaterials can be divided into four categories of nanopowder, nanofiber, nanomembrane, and nanoblock**

resources and pathological models.[97,98] In addition to vascular endothelial cells, astrocytes play a key role in the induction and maintenance of the integrity of the BBB.[99] Thus, the two types of coculture models that have been widely utilized are as follows[100,101]: **(1) brain microvascular endothelial cells + astrocytes and (2) peripheral vascular endothelial cells + astrocytes. Other studies have also utilized "endothelial cells + microglia"[102] and "endothelial cells + pericytes" cocultured systems.**[97] At present, cell-based BBB models are the most extensively used because they are easy to obtain and maintain and they are highly effective for the screening studies of drugs and nanocarrier systems. In a recent review by Wong et al[103] **the authors summarized different types of BBB models, including isolated brain capillaries, cell-based/free models, and dynamic in vitro models** Despite these attempts to mimic in vivo conditions, each of the in vitro BBB models possesses their own advantages and disadvantages, and none of them are completely ideal. More effective in vitro BBB models must be developed for the evaluation of the deliveries of therapeutic agents in further investigations.

Figure 4 In vitro cell culture models for the studies on drug and NP transport through the BBB.
Note: Reprinted from *Adv Drug Deliv Rev*, 64(7), Wong HL, Wu XY, Bendayan R. Nanotechnological advances for the delivery of CNS therapeutics. 686–700., Copyright (2012), with permission from Elsevier.[103]
Abbreviations: NP, nanoparticle; BBB, blood–brain barrier.Different in vitro studies have focused on evaluating various aspects, such as pharmacology, transport, migration, and the metabolic activity of the BBB. Research has also been focused on DNA damage, the morphological and functional changes of the mitochondria, endoplasmic reticulum, lysosomes, and other organelles, and the transportation mode of internalization, transcytosis and exocytosis. ***NPs have been demonstrated to be taken up by mammalian cells by such mechanisms as pinocytosis, endocytosis dependent on caveolae and lipid raft composition, and phagocytosis.***[104] The intracellular sites of the localization of NPs vary depending on the cell type and applied method**. NPs have entered the endothelial cell monolayer and have accumulated along the endo-lysosomal pathway, which affected the normal morphology and function of the BBB itself.** For example, Brun et al[98] observed an **accumulation of TiO_2 NPs in the endothelial cells of the brain by using an in vitro cell-based rat BBB model**. ***An intense inflammatory response associated with a modulation of the endothelial cell functioning of the brain was also observed. Therefore, an impaired transport capacity resulting from the dysfunction of the endothelial cells of the BBB might constitute the first step in the neurodegeneration process***.--Although numerous studies have considered the effects of nanomaterials to the BBB itself, a distinct lack of knowledge exists with respect to the biological effects of NP accumulation within the BBB of the neighboring cells in the CNS, particularly over the long term. Wiley et al[105] **observed that transferrin-containing gold NPs reached**

Comment [O]: **How the test were done to determine the effect or impact of nano materials**

Comment [O]: **Brain Damage~ see other links on site in regard to 10 different nanometals that can cause brain lobotomy at different parts of the brain—see "Neurotoxicity of nanoscale materials in the June script of the show 2016 " to get a more indepth report on how this causes compromise and debilitation to everyone**



and accumulated in the brain parenchyma following an intravenous injection in mice through a receptor-mediated transcytosis pathway. Raghnaill et al[106] **also reported an accumulation over time, but there was no degradation of 100 nm PS COOH NPs within the lysosomes of the BBB model in vitro**. Thus, possible **long-term toxicity must be considered, and this toxicity may result from the accumulation of known "toxic" and "nontoxic" NPs**.

Neurotoxicity on glial cells or neuroglia

Among all of the neuroglial cells, astrocytes and microglia have received the most attention. The **astrocyte** is thought to induce the barrier phenotype of cerebrovascular endothelial cells during development through the release of soluble factors, such as vascular endothelial growth factor. **Recently, many studies have attempted to determine the specifics of the NP–astrocyte interactions. For instance, the ZnO NP–astrocyte interaction was reported to induce an oxidative stress that could trigger cell apoptosis by activating the JNK signaling pathway in cultured primary astrocytes.**[107] A similar finding was observed in the interactions between **superparamagnetic iron oxide NPs and astrocytes**.[108] Mixed glial cultures have often been established from the cerebral cortices of neonatal Sprague-Dawley rats and purified astrocytes. Another common cell line is human glial cells (U87 astrocytes).[109] As the sentinels of the CNS, microglia are the first cells to respond to a disruption of the brain homeostasis and the entry of foreign particles or infectious agents. **Once activated, microglia can generate ROS and reactive nitrogen species (RNS) and even elicit an inflammatory response**. In most cases, the macrophage cell line was adopted to evaluate its activation and inflammatory reaction. **In a neurotoxicity study of Si NPs performed by Choi et al[77] even low levels of NPs were capable of increasing ROS and RNS production and inducing cytokine release. These changes had an adverse effect on the microglial function and surrounding neurons. This result was consistent with other toxicity studies that have been conducted more recently**.[110,111] **It was also determined that exposure to Fe_2O_3 NPs did not cause a significant release of inflammatory factors even though cell phagocytosis and a generation of ROS and NO were observed.**[112] This finding indicated that **microglial activation may also act as an alarm and defense system in the processes of the exogenous NPs invading and accumulating in the brain**.

Comment [O]: **The brain parenchyma is the functional tissue in the brain. It's comprised of two types of cells that are used specifically for cognition and controlling the rest of the body. The remaining brain tissue is known as *stroma*, which is the supportive or structural tissue. Damage or trauma to the brain parenchyma often results in a loss of cognitive ability or even death.**

Comment [O]: **This is saying that they do not break down and are accumulating in the brain**

Comment [O]: **astrocyte [as´tro-sīt] a neuroglial cell of ectodermal origin, characterized by fibrous or protoplasmic processes; collectively called ASTROGLIA or MACROGLIA.**

Neurotoxicity on neurons

Neuronal cell lines commonly used for in vitro studies include the following: 1) rat PC12 neuronal cells,[113,114] which have been derived from a **pheochromocytoma of the rat adrenal medulla**; PC12 cell lines are commonly **used for the neurobiological and neurochemical assessment of NP-induced neurotoxicity; and 2) a human SHSY5Y neuroblastoma cell line**,[115,116] which is perceived as an appropriate cell model for the assessment of neurotoxicity because it possesses many biochemical and functional properties of neurons.[117] Additionally, primary culture cell lines have also been used in the **evaluation of the neurotoxicity of NPs; these lines include human cortical neuronal cells (HCN-1A), rat dopaminergic neurons (N27), rat primary neuronal cells,[118] embryonic rat striatum or cerebellar granule cells,[119] and hippocampal CA1 and CA3 neurons**.[120,121] It has now been confirmed that **some nanomaterials can exploit the endocytotic pathways both to cross the BBB endothelium in vivo and to enter the neurons or glial cells in vitro.[122]** For instance, Vilella et al[66] discovered that **there was an uptake of NPs in hippocampal neurons that were prepared from rats at embryonic day 18 or 19. Aside from intracellular accumulation, there was also evidence that different metal oxide NPs affect the membrane potentials of neurons and increase the neuronal firing rate by changing the responses of the potassium channels**.[90] **This finding was consistent with a toxicity study of nano-CuO on CA1 hippocampal neurons performed** by Xu et al.[123] Furthermore, this **toxic effect may have a physiological impact on animal behavior, which was demonstrated in rats by testing their spatial cognition capabilities.**[81] Recently, the impact of nanomaterials on the CNS, particularly the hippocampal neuronal cells, has been illustrated in a comprehensive review by Yang et al.[124]

Studies on cell-to-cell communication

The CNS is composed of a dense network of neurons and glial cells that are highly interconnected. Therefore, cell-to-cell communication is an important factor in maintaining a functional organization. In recent years, **tunneling nanotubes (TNTs) were reported as a new principle of cell-to-cell communication. As a form of membrane continuity, TNTs may be efficient communication tunnels that facilitate information and material exchange. Such communication may even occur over a relatively long distance**.[125] **Considering that NPs can be transported intra- and intercellularly within vesicles after internalization by the vesicle, this cell-to-cell transport may be mediated by TNT-like structures in glial and neuronal cells in vitro.** Furthermore, the transport was dependent on F-actin and was increased by the induction of TNT-like structures.[126] Nevertheless, the influence of nanomaterials on cell-to-cell communication in the CNS remains unclear; thus, more in-depth studies are warranted.

Comment [O]: **Pheochromocytoma is a relatively rare tumor of the adrenal glands or of similar specialized cells outside of the adrenal glands**

Comment [O]: **Telling you here it creates a tunnel or a connection to get the particles from point A to Point B and can increase the signals of communication**

Other problems and future research prospects

Alongside the rapid advances in the development of nanotechnology-based materials, it has become **imperative to elucidate the toxicity of NPs.** However, the **safety evaluation systems of nanomaterials lag far behind their emerging development and applications.** Although researchers have obtained some important information, the risks of NP exposure are not understood sufficiently well to enable the development of a science-based risk assessment. Because investigation into the possible harmful effects of NPs has only been conducted for a few years, it is not surprising that many studies suffer from shortcomings. Therefore, better testing and evaluation systems are urgently needed.

Limitations of the testing methods

Cytotoxicity in vitro is typically estimated with colorimetric tests.[127] However, Monteiro-Riviere et al[128] determined that MTT and neutral red assays, two classical dye-based assays, may produce invalid results in the testing of cell viability when applied with nanomaterials due to their interactions and/or adsorption of the dye/dye products. **Furthermore, carbon nanomaterials can interact with assay markers to cause variable results in classical toxicological studies.** This finding is consistent with the results of Griffiths et al.[129] For these reasons, such interactions in cytotoxicity assays must be considered. Another challenge of the testing methods lies in the accurate detection of nanomaterials in biological objects**. At present, flow cytometry, induced coupled plasma mass spectroscopy, confocal microscopy, the radioactive tracer technique, and transmission electron microscopy in combination with energy-dispersive X-ray spectroscopy are commonly used to study the cellular uptake of NPs**.[130,131] However, there is not a single method that is satisfactory in obtaining precise information for all types of nanomaterials. **Therefore, a combination of the utilization of different testing methods is suggested to provide more accurate results**. Furthermore, different assays should be employed according to the certain types of NPs, as well as in addition to imaging techniques.[132]

Limitations of the experimental models

Under in vivo conditions, nanomaterials could yield different effects compared with in vitro experiments.[133] Although the observations from in vivo studies are **more representative of the situations in living organisms**, in some cases, these studies may provide inaccurate results. The challenges are largely related to the experimental models (animals), which are difficult to control and could be affected by various unpredictable factors. Additionally, other considerations, such as **dosimetry, the optimization of the dispersion of NPs, the evaluation of the interactions between the nanomaterials and cells, and their biodistributions, create more challenges for in vivo toxicity assessments.**[134] Compared with animal studies, in vitro studies are less

Comment [O]: **nanocomposites composed of nanomaterials and traditional metals, ceramics, resin, or other matrix materials have been widely used in prosthodontics because their properties, such as modulus elasticity,**

Comment [O]: **Dental materials of dentures can be divided into mainly three categories: resin, ceramic, and metal. They are important to fabricate dental prosthesis, which directly contacts with the oral mucosa and is under long-term**

Comment [O]: **]. Nanomaterials can be divided into four categories of nanopowder, nanofiber, nanomembrane, and nanoblock, in which development of nanopowder is longest, and its technology is most**

expensive, ethically ambiguous, and most importantly, easier to control and reproduce. The first step toward understanding **how NPs will react in the body often involves cell culture studies. An increasing number of in vitro cytotoxicity studies of different nanomaterials using various cell lines, incubation times, and colorimetric assays have been published**. However, many problems still exist in the studies performed under in vitro conditions. First, the appropriate selections **of a set of sensitive cell lines** and in vitro assays measuring the different cytotoxicity endpoints are essential to ensure the accurate identification of nanomaterial cytotoxicity.[135] However, for a certain NP, the selection of the most appropriate cell line is still difficult. To some extent, more sensitive cell models are required to determine the cytotoxicity of a certain type of NP. **One example is with the use of nano-ZnO. The majority of the toxicity studies specific to ZnO NPs have relied on the use of immortalized cell lines, which display altered sensitivities to foreign materials/chemicals due to their changes in metabolic processes and significant genetic instabilities. Nevertheless, the toxicity of ZnO NPs on normal primary human cells and their potential immunomodulatory effects are often neglected**. Furthermore, the cytotoxic response varies with different types of cell lines and nanomaterials, making it difficult to develop predictive models because of the lack of detailed and systematic investigations.[136] Finally, the toxic effects of NPs on rat cell lines (a common in vitro model) may not be able to accurately reflect the effects in humans.---In vitro investigations will not be able to completely determine the in vivo situations until further in vivo analyses have been performed to confirm their findings.[133] In a recent review by Donaldson et al[137] the authors stated that cells in culture did not experience the range of pathogenic changes that might occur under in vivo conditions, which were partially related to the issues of translocation, toxicokinetics, and coordinated tissue responses. Some other studies have also cast doubt on the results obtained from in vitro models, especially in models in submersed conditions when NPs were suspended in media that could impact the dispersion and dissolution.[15,138]

Until now, the understanding of nanomaterial neurotoxicology has been extremely limited. In-depth studies are warranted, particularly when considering the recent emphasis on the use of nanocarriers for drug delivery in the brain.[139] Here, we have provided some suggestions on the research prospects that require further detailed investigations.

As indicated by Laurent et al the effects of the **protein corona on NP–cell interactions are often ignored at the nano–bio interface**.[140] Because in vitro biological studies typically use low amounts (10% dilution or less, depending on cell types) of animal-derived serum, which is present in in vivo studies, NP coronas are likely to form at different protein-to-NP ratios between the in vitro and in vivo studies.[141] In this sense, in vitro models that evaluate the NPs for brain-related diseases are supposed to use the corona-coated NPs to reflect the real in vivo situations,[142] as the protein corona may cover the designed functional groups and significantly reduce the ability of NPs to cross through the

Comment [O]: **In this section they are basically soft pedaling the danger here ~ irregardless of the soft cell line use if there is going to be a reaction where the cells terminate-mutate or become assimilated through a mutation**

Comment [O]: **Usually the cell lines of the rats are more durable and are more adaptable then the human cell line and because there is no model to accurately predict the effect which is really very unscientific to make such a statement the**

Comment [O]: **biological integration is usually unsatisfactory, and some patients are prone to allergies, causing skin, mucous membrane inflammation [22, 30, 31]. Satisfactory biological integration of the implant surfaces with the surrounding host tissues is one of the most important elements for long-term success of dental implants**

cell barriers.[143,144] Another consideration with respect to the protein corona arises from the evaluation of its structural evolution over time. NPs will interact with tissues and cells in living organisms, including passing through cellular membranes and being transported to the final subcellular locations. Therefore, the detailed changes of the nanomaterial corona at these stages and their implications require further study. Additionally, as described in the limitations of the in vitro models, more appropriate cell lines should be developed. Takhar and Mahant[145] recently suggested the possibility of using transgenic cell lines carrying human genes, which may be more predictive to situations involving humans than the traditional rat cells.

With regard to animal studies, the effects of the life stages should be considered. First, fetal life and early childhood are vulnerable periods. These life stages are of great importance for the rapid growth of whole organism, cell differentiation, and organogenesis, and in the case of the brain, are involved in critical processes in neurodevelopment. If toxic exposures occur at these stages, they could alter the trajectory of the development of the brain, which may have minor effects in the early years and profound implications later in life.[146] Currently, growing evidence from animal research has confirmed that the CNS is highly vulnerable to chemical injury during development.[147] Therefore, particular attention should be given to determine the influence of nanomaterial exposure at these developmental stages.

Aging may also represent an important factor in the susceptibility of NP-induced neurotoxicity. **Aged brains have demonstrated an increase in cytokine and microglial activation and are more vulnerable to environmental insults, particularly in pro-inflammatory stimuli**,[148,149] **including various NPs**. **In recent years, it has been predicted that many neurodegenerative diseases can result from the cumulative exposure throughout a lifetim**e.[150] This finding was consistent with the observations in another toxicity research conducted by Qin et al.[151] **In this animal study, chronic neuroinflammation in response to a single intraperitoneal injection of lipopolysaccharide, a potent inflammatory stimulus, in young adult mice only culminated in dopaminergic neurotoxicity in aged animals**. **Other associated factors, such as the sex and genetic background, should also be investigated**.[152,153] **Recently, it was reported that the differential expression of the enzyme paraoxonase 2 (PON2) between male and female brains may be responsible for a number of sex differences with regard to neurotoxicity**.[152] **Gene–nanomaterial interactions also played an important role in NP-induced neurotoxicity, as genetic polymorphisms may modulate individual susceptibilities to nanomaterials**. Given the prominent role of oxidative stress, genetically based differences in antioxidant enzymes may predispose certain individuals to significant air pollution neurotoxicities.[153]

Comment [O]: **based on a gene code a person could have a more susceptible break down on regard to nano inducments and as the nanao accumulates as a result of the genetics of the person could cause more then usual sympotoms**

A continuous exposure may result in the significant accumulation of NPs in a secondary target organ. Therefore, it is important to obtain data on the retention characteristics of NPs in both primary and secondary target organs, as well as NP elimination pathways. **No data on NP elimination in the CNS are available yet.** It is conceivable that the CSF, via its connections to the nasal lymphatic system and to the circulation of blood, could be an excretory pathway for the brain, and this topic should be investigated in future studies. Indeed, from his review on CSF barriers, Segal[154] concluded that the CSF may act as not only a compartment for the distribution of substances to different brain regions but also an elimination route for waste products into the blood circulation because the brain has no lymphatics. However, this is a single study and need to be complemented by more systematic research on nanomaterial elimination.

Comment [O]: **Primarily because the nano particles will translocate and polymorph with other things in the body and can get trapped and accumulate in every major organ and skeletal tissue**

Summary

Nanomaterials have made major contributions to modern dentistry in various areas, including composite resin and bonding systems, coating materials for dental implants, and dental restorations. The wide applications of these dental nanomaterials have created more exposure opportunities to these NPs in both dental staff and patients. Because the CNS may be a potential target organ of nanomaterials, it is essential to determine the neurotoxic effects of NPs. Although the impact of NPs on the CNS has received considerable attention in recent years, the data and findings obtained from the in vivo and in vitro studies are still limited. The limitations of the present testing methods and the experimental models also make it difficult to establish a science-based evaluation system. Better testing and evaluation systems are urgently needed. In conclusion, more efforts are required to ensure the safe use of nanomaterials.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31070857, 50973045, 51172283, 81400557), and the Project on the Integration of Industry, Education and Research of Guangdong Province, People's Republic of China (2012B091000147).

Disclosure

The authors declare no conflicts of interest in relation to this paper.

References

1.Bleeker EA, de Jong WH, Geertsma RE, et al. Considerations on the EU definition of a nanomaterial: science to support policy making. *Regul Toxicol Pharm*. 2013;65(1):119–125.2.Juanola-Feliu E. The nanotechnology revolution in Barcelona: innovation and creativity by universities. *Manage Int*. 2009;13:111–123.3.Kasraei S, Sami L, Hendi S, Alikhani MY, Rezaei-Soufi L, Khamverdi Z. Antibacterial properties of composite resins incorporating silver and zinc oxide

nanoparticles on Streptococcus mutans and Lactobacillus. *Restor Dent Endod*. 2014;39(2):109–114.4.Niu LN, Fang M, Jiao K, et al. Tetrapod-like zinc oxide whisker enhancement of resin composite. *J Dent Res*. 2010;89(7):746–750.5.Memarzadeh K, Sharili AS, Huang J, Rawlinson SC, Allaker RP. Nanoparticulate zinc oxide as a coating material for orthopedic and dental implants. *J Biomed Mater Res A*. 2015;103(3):981–989.6.Javidi M, Zarei M, Naghavi N, Mortazavi M, Nejat AH. Zinc oxide nano-particles as sealer in endodontics and its sealing ability. *Contemp Clin Dent*. 2014;5(1):20–24.7.Frohlich E, Roblegg E. Models for oral uptake of nanoparticles in consumer products. *Toxicology*. 2012;291(1–3):10–17.8.Keelan JA. Nanotoxicology: nanoparticles versus the placenta. *Nat Nanotechnol*. 2011;6(5):263–264.9.Shimizu M, Tainaka H, Oba T, Mizuo K, Umezawa M, Takeda K. Maternal exposure to nanoparticulate titanium dioxide during the prenatal period alters gene expression related to brain development in the mouse. *Part Fibre Toxicol*. 2009;6:20.10.Lu S, Duffin R, Poland C, et al. Efficacy of simple short-term in vitro assays for predicting the potential of metal oxide nanoparticles to cause pulmonary inflammation. *Environ Health Perspect*. 2009;117(2):241–247.11.Lee CM, Jeong HJ, Yun KN, et al. Optical imaging to trace near infrared fluorescent zinc oxide nanoparticles following oral exposure. *Int J Nanomedicine*. 2012;7:3203–3209.12.Wang Y, Chen Z, Ba T, et al. Susceptibility of young and adult rats to the oral toxicity of titanium dioxide nanoparticles. *Small*. 2013;9(9–10):1742–1752.13.Stern ST, McNeil SE. Nanotechnology safety concerns revisited. *Toxicol Sci*. 2008;101(1):4–21.14.Medina C, Santos-Martinez MJ, Radomski A, Corrigan OI, Radomski MW. Nanoparticles: pharmacological and toxicological significance. *Br J Pharmacol*. 2007;150(5):552–558.15.Adamcakova-Dodd A, Stebounova LV, Kim JS, et al. Toxicity assessment of zinc oxide nanoparticles using sub-acute and sub-chronic murine inhalation models. *Part Fibre Toxicol*. 2014;11:15.16.Ma L, Liu J, Li N, et al. Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO_2 delivered to the abdominal cavity. *Biomaterials*. 2010;31(1):99–105.17.Nel A, Xia T, Madler L, Li N. Toxic potential of materials at the nanolevel. *Science*. 2006;311(5761):622–627.18.Balos S, Pilic B, Petronijevic B, Markovic D, Mirkovic S, Sarcev I. Improving mechanical properties of flowable dental composite resin by adding silica nanoparticles. *Vojnosanit Pregl*. 2013;70(5):477–483.19.Wang R, Bao S, Liu F, et al. Wear behavior of light-cured resin composites with bimodal silica nanostructures as fillers. *Mat Sci Eng C Mater*. 2013;33(8):4759–4766.20.Xu HH, Weir MD, Sun L, et al. Strong nanocomposites with Ca, PO(4), and F release for caries inhibition. *J Dent Res*. 2010;89(1):19–28.21.Xia Y, Zhang F, Xie H, Gu N. Nanoparticle-reinforced resin-based dental composites. *J Dent*. 2008;36(6):450–455.22.Wang W, Sun X, Huang L, et al. Structure-property relationships in hybrid dental nanocomposite resins containing monofunctional and multifunctional polyhedral oligomeric silsesquioxanes. *Int J Nanomedicine*. 2014;9:841–852.23.Melo MA, Cheng L, Zhang K, Weir MD, Rodrigues LK, Xu HH. Novel dental adhesives containing nanoparticles of silver and amorphous calcium phosphate. *J Biomed Mater Res B Appl Biomater*. 2013;29(2):199–210.24.Wagner A, Belli R, Stotzel C, Hilpert A, Muller FA, Lohbauer U. Biomimetically- and hydrothermally-grown HAp nanoparticles as reinforcing fillers for dental adhesives. *J Adhes Dent*.

2013;15(5):413–422.25.Habekost LV, Camacho GB, Lima GS, Ogliari FA, Cubas GB, Moraes RR. Nanoparticle loading level and properties of experimental hybrid resin luting agents. *J Prosthodont*. 2012;21(7):540–545.26.Jallot E, Nedelec JM, Grimault AS, et al. STEM and EDXS characterisation of physico-chemical reactions at the periphery of sol-gel derived Zn-substituted hydroxyapatites during interactions with biological fluids. *Colloid Surf B*. 2005;42(3–4):205–210.27.Krisanapiboon A, Buranapanitkit B, Oungbho K. Biocompatability of hydroxyapatite composite as a local drug delivery system. *J Orthop Surg (Hong Kong)*. 2006;14(3):315–318.28.Qi X, Li H, Qiao B, et al. Development and characterization of an injectable cement of nano calcium-deficient hydroxyapatite/multi(amino acid) copolymer/calcium sulfate hemihydrate for bone repair. *Int J Nanomedicine*. 2013;8:4441–4452.29.Huber FX, Belyaev O, Hillmeier J, et al. First histological observations on the incorporation of a novel nanocrystalline hydroxyapatite paste OSTIM in human cancellous bone. *BMC Musculoskelet Disord*. 2006;7:50.30.Yang C, Lee JS, Jung UW, Seo YK, Park JK, Choi SH. Periodontal regeneration with nano-hyroxyapatite-coated silk scaffolds in dogs. *J Periodontal Implant Sci*. 2013;43(6):315–322.31.An SH, Matsumoto T, Miyajima H, Nakahira A, Kim KH, Imazato S. Porous zirconia/hydroxyapatite scaffolds for bone reconstruction. *Dent Mater*. 2012;28(12):1221–1231.32.De Aza AH, Chevalier J, Fantozzi G, Schehl M, Torrecillas R. Crack growth resistance of alumina, zirconia and zirconia toughened alumina ceramics for joint prostheses. *Biomaterials*. 2002;23(3):937–945.33.Uno M, Kurachi M, Wakamatsu N, Doi Y. Effects of adding silver nanoparticles on the toughening of dental porcelain. *J Prosthet Dent*. 2013;109(4):241–247.34.Han Y, Kiat-amnuay S, Powers JM, Zhao Y. Effect of nano-oxide concentration on the mechanical properties of a maxillofacial silicone elastomer. *J Prosthet Dent*. 2008;100(6):465–473.35.Acosta-Torres LS, Mendieta I, Nunez-Anita RE, Cajero-Juarez M, Castano VM. Cytocompatible antifungal acrylic resin containing silver nanoparticles for dentures. *Int J Nanomedicine*. 2012;7:4777–4786.36.Karlsson M, Palsgard E, Wilshaw PR, Di Silvio L. Initial in vitro interaction of osteoblasts with nano-porous alumina. *Biomaterials*. 2003;24(18):3039–3046.37.Pardun K, Treccani L, Volkmann E, et al. Characterization of wet powder-sprayed zirconia/calcium phosphate coating for dental implants. *Clin Implant Dent Relat Res*. 2015;17(1):186–198.38.Uezono M, Takakuda K, Kikuchi M, Suzuki S, Moriyama K. Hydroxyapatite/collagen nanocomposite-coated titanium rod for achieving rapid osseointegration onto bone surface. *J Biomed Mater Res B Appl Biomater*. 2013;101(6):1031–1038.39.Mendonca G, Mendonca DB, Aragao FJ, Cooper LF. Advancing dental implant surface technology – from micron- to nanotopography. *Biomaterials*. 2008;29(28):3822–3835.40.Lebold T, Jung C, Michaelis J, Brauchle C. Nanostructured silica materials as drug-delivery systems for doxorubicin: single molecule and cellular studies. *Nano Lett*. 2009;9(8):2877–2883.41.Mu L, Feng SS. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol): PLGA nanoparticles containing vitamin E TPGS. *J Control Release*. 2003;86(1):33–48.42.Melancon MP, Lu W, Zhong M, et al. Targeted multifunctional gold-based nanoshells for magnetic resonance-guided laser ablation of head and neck cancer. *Biomaterials*. 2011;32(30):7600–7608.43.Erdogan S. Liposomal nanocarriers for tumor imaging. *J Biomed*

Nanotechnol. 2009;5(2):141–150.44.Weir A, Westerhoff P, Fabricius L, Hristovski K, von Goetz N. Titanium dioxide nanoparticles in food and personal care products. *Environ Sci Technol*. 2012;46(4):2242–2250.45.Geraets L, Oomen AG, Schroeter JD, Coleman VA, Cassee FR. Tissue distribution of inhaled micro- and nano-sized cerium oxide particles in rats: results from a 28-day exposure study. *Toxicol Sci*. 2012;127(2):463–473.46.Chen Y, Liu L. Modern methods for delivery of drugs across the blood–brain barrier. *Adv Drug Deliv Rev*. 2012;64(7):640–665.47.Patel T, Zhou J, Piepmeier JM, Saltzman WM. Polymeric nanoparticles for drug delivery to the central nervous system. *Adv Drug Deliv Rev*. 2012;64(7):701–705.48.Beduneau A, Saulnier P, Benoit JP. Active targeting of brain tumors using nanocarriers. *Biomaterials*. 2007;28(33):4947–4967.49.Spector R. Nature and consequences of mammalian brain and CSF efflux transporters: four decades of progress. *J Neurochem*. 2010;112(1):13–23.50.Kao YY, Cheng TJ, Yang DM, Wang CT, Chiung YM, Liu PS. Demonstration of an olfactory bulb-brain translocation pathway for ZnO nanoparticles in rodent cells in vitro and in vivo. *J Mol Neurosci*. 2012;48(2):464–471.51.Hwang SR, Kim K. Nano-enabled delivery systems across the blood–brain barrier. *Arch Pharm Res*. 2014;37(1):24–30.52.Caraglia M, De Rosa G, Salzano G, et al. Nanotech revolution for the anti-cancer drug delivery through blood–brain-barrier. *Curr Cancer Drug Tar*. 2012;12(3):186–196.53.Barbu E, Molnar E, Tsibouklis J, Gorecki DC. The potential for nanoparticle-based drug delivery to the brain: overcoming the blood–brain barrier. *Expert Opin Drug Deliv*. 2009;6(6):553–565.54.Jain KK. Nanobiotechnology-based strategies for crossing the blood–brain barrier. *Nanomedicine (Lond)*. 2012;7(8):1225–1233.55.Watson RE, Desesso JM, Hurtt ME, Cappon GD. Postnatal growth and morphological development of the brain: a species comparison. *Birth Defects Res B Dev Reprod Toxicol*. 2006;77(5):471–484.56.Yamashita K, Yoshioka Y, Higashisaka K, et al. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. *Nat Nanotechnol*. 2011;6(5):321–328.57.Okada Y, Tachibana K, Yanagita S, Takeda K. Prenatal exposure to zinc oxide particles alters monoaminergic neurotransmitter levels in the brain of mouse offspring. *J Toxicol Sci*. 2013;38(3):363–370.58.Yu LE, Yung LYL, Ong CN, et al. Translocation and effects of gold nanoparticles after inhalation exposure in rats. *Nanotoxicology*. 2007;1(3):235–242.59.Biddlestone-Thorpe L, Marchi N, Guo K, et al. Nanomaterial-mediated CNS delivery of diagnostic and therapeutic agents. *Adv Drug Deliv Rev*. 2012;64(7):605–613.60.Wang B, Feng WY, Zhu MT, et al. Neurotoxicity of low-dose repeatedly intranasal instillation of nano- and submicron-sized ferric oxide particles in mice. *J Nanopart Res*. 2009;11(1):41–53.61.Shah L, Yadav S, Amiji M. Nanotechnology for CNS delivery of bio-therapeutic agents. *Drug Deliv Transl Res*. 2013;3(4):336–351.62.De Lorenzo AJ. *The olfactory neuron and the blood–brain barrier*. London: CIBA Foundation Symposium Series J&A Churchill; 1970.63.Thorne RG, Pronk GJ, Padmanabhan V, Frey WH 2nd. Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. *Neuroscience*. 2004;127(2):481–496.64.Dhuria SV, Hanson LR, Frey WH 2nd. Intranasal delivery to the central nervous system: mechanisms and experimental considerations. *J Pharm Sci*. 2010;99(4):1654–

1673.65.Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect*. 2005;113(7):823–839.66.Vilella A, Tosi G, Grabrucker AM, et al. Insight on the fate of CNS-targeted nanoparticles. Part I: Rab5-dependent cell-specific uptake and distribution. *J Control Release*. 2014;174:195–201.67.Amara S, Ben-Slama I, Mrad I, et al. Acute exposure to zinc oxide nanoparticles does not affect the cognitive capacity and neurotransmitters levels in adult rats. *Nanotoxicology*. 2014;8(suppl 1):208–215.68.Zhang L, Bai R, Li B, et al. Rutile TiO_2 particles exert size and surface coating dependent retention and lesions on the murine brain. *Toxicol Lett*. 2011;207(1):73–81.69.Kumar V, Kumari A, Guleria P, Yadav SK. Evaluating the toxicity of selected types of nanochemicals. *Rev Environ Contam T*. 2012;215:39–121.70.Li Y, Li J, Yin J, et al. Systematic influence induced by 3 nm titanium dioxide following intratracheal instillation of mice. *J Nanosci Nanotechno*. 2010;10(12):8544–8549.71.Ze Y, Hu R, Wang X, et al. Neurotoxicity and gene-expressed profile in brain-injured mice caused by exposure to titanium dioxide nanoparticles. *J Biomed Mater Res A*. 2014;102(2):470–478.72.Kwon JT, Seo GB, Jo, et al. Aluminum nanoparticles induce ERK and p38MAPK activation in rat brain. *Toxicol Res*. 2013;29(3):181–185.73.Marano F, Hussain S, Rodrigues-Lima F, Baeza-Squiban A, Boland S. Nanoparticles: molecular targets and cell signalling. *Arch Toxicol*. 2011;85(7):733–741.74.Shrivastava R, Raza S, Yadav A, Kushwaha P, Flora SJS. Effects of sub-acute exposure to TiO_2, ZnO and Al_2O_3 nanoparticles on oxidative stress and histological changes in mouse liver and brain. *Drug Chem Toxicol*. 2014;37(3):336–347.75.Hu R, Zheng L, Zhang T, et al. Molecular mechanism of hippocampal apoptosis of mice following exposure to titanium dioxide nanoparticles. *J Hazard Mater*. 2011;191(1–3):32–40.76.Win-Shwe TT, Fujimaki H. Nanoparticles and neurotoxicity. *Int J Mol Sci*. 2011;12(9):6267–6280.77.Choi J, Zheng QD, Katz HE, Guilarte TR. Silica-based nanoparticle uptake and cellular response by primary microglia. *Environ Health Perspect*. 2010;118(5):589–595.78.Tobe EH. Mitochondrial dysfunction, oxidative stress, and major depressive disorder. *Neuropsych Dis Treat*. 2013;9:567–573.79.Zhang Y, Yu CG, Huang GY, Wang CL, Wen LP. Nano rare-earth oxides induced size-dependent vacuolization: an independent pathway from autophagy. *Int J Nanomedicine*. 2010;5:601–609.80.Chen L, Miao Y, Chen L, et al. The role of low levels of fullerene C60 nanocrystals on enhanced learning and memory of rats through persistent CaMKII activation. *Biomaterials*. 2014;35(34):9269–9279.81.Han DD, Tian YT, Zhang T, Ren GG, Yang Z. Nano-zinc oxide damages spatial cognition capability via over-enhanced long-term potentiation in hippocampus of Wistar rats. *Int J Nanomedicine*. 2011;6:1453–1461.82.Li T, Shi TT, Li XB, Zeng SL, Yin LH, Pu YP. Effects of Nano-MnO_2 on dopaminergic neurons and the spatial learning capability of rats. *Int J Env Res Pub He*. 2014;11(8):7918–7930.83.Kim EM, Palmer P, Howard V, et al. Effect of Intracerebroventricular injection of TiO_2 nanoparticles on complex behaviour in the rat. *J Nanosci Nanotechno*. 2013;13(12):8325–8330.84.Cui Y, Chen X, Zhou Z, et al. Prenatal exposure to nanoparticulate titanium dioxide enhances depressive-like behaviors in adult rats. *Chemosphere*. 2014;96:99–104.85.Sorce S, Krause KH. NOX enzymes in the central nervous system: from signaling to disease. *Antioxid Redox Sign*.

2009;11(10):2481–2504.86.Massaad CA, Klann E. Reactive oxygen species in the regulation of synaptic plasticity and memory. *Antioxid Redox Sign*. 2011;14(10):2013–2054.87.Schappi MG, Jaquet V, Belli DC, Krause KH. Hyperinflammation in chronic granulomatous disease and anti-inflammatory role of the phagocyte NADPH oxidase. *Semin Immunopathol*. 2008;30(3):255–271.88.Thomas MP, Chartrand K, Reynolds A, Vitvitsky V, Banerjee R, Gendelman HE. Ion channel blockade attenuates aggregated alpha synuclein induction of microglial reactive oxygen species: relevance for the pathogenesis of Parkinson's disease. *J Neurochem*. 2007;100(2):503–519.89.Hu R, Gong X, Duan Y, et al. Neurotoxicological effects and the impairment of spatial recognition memory in mice caused by exposure to TiO_2 nanoparticles. *Biomaterials*. 2010;31(31):8043–8050.90.Krol S. Challenges in drug delivery to the brain: nature is against us. *J Control Release*. 2012;164(2):145–155.91.Armulik A, Genové G, Mäe M, et al. Pericytes regulate the blood–brain barrier. *Nature*. 2010;468(7323):557–U231.92.Wohlfart S, Gelperina S, Kreuter J. Transport of drugs across the blood–brain barrier by nanoparticles. *J Control Release*. 2012;161(2):264–273.93.Powers CM, Bale AS, Kraft AD, et al. Developmental neurotoxicity of engineered nanomaterials: identifying research needs to support human health risk assessment. *Toxicol Sci*. 2013;134(2):225–242.94.Ye D, Raghnaill MN, Bramini M, et al. Nanoparticle accumulation and transcytosis in brain endothelial cell layers. *Nanoscale*. 2013;5(22):11153–11165.95.Weiss CK, Kohnle MV, Landfester K, et al. The first step into the brain: uptake of NIO-PBCA nanoparticles by endothelial cells in vitro and in vivo, and direct evidence for their blood–brain barrier permeation. *Chem Med Chem*. 2008;3(9):1395–1403.96.Halamoda Kenzaoui B, Chapuis Bernasconi C, Guney-Ayra S, Juillerat-Jeanneret L. Induction of oxidative stress, lysosome activation and autophagy by nanoparticles in human brain-derived endothelial cells. *Biochem J*. 2012;441(3):813–821.97.Hanada S, Fujioka K, Inoue Y, Kanaya F, Manome Y, Yamamoto K. Cell-based in vitro blood–brain barrier model can rapidly evaluate nanoparticles' brain permeability in association with particle size and surface modification. *Int J Mol Sci*. 2014;15(2):1812–1825.98.Brun E, Carriere M, Mabondzo A. In vitro evidence of dysregulation of blood–brain barrier function after acute and repeated/long-term exposure to TiO_2 nanoparticles. *Biomaterials*. 2012;33(3):886–896.99.Ogunshola OO. In vitro modeling of the blood–brain barrier: simplicity versus complexity. *Curr Pharm Design*. 2011;17(26):2755–2761.100.Pilakka-Kanthikeel S, Atluri VSR, Sagar V, Saxena SK, Nair M. Targeted brain derived neurotropic factors (BDNF) delivery across the blood–brain barrier for neuro-protection using magnetic nano carriers: an in-vitro study. *PLoS One*. 2013;8(4):e62241.101.Gromnicova R, Davies HA, Sreekanthreddy P, et al. Glucose-coated gold nanoparticles transfer across human brain endothelium and enter astrocytes in vitro. *PLoS One*. 2013;8(12):e81043.102.Sumi N, Nishioku T, Takata F, et al. Lipopolysaccharide-activated microglia induce dysfunction of the blood–brain barrier in rat Microvascular endothelial cells co-cultured with microglia. *Cell Mol Neurobiol*. 2010;30(2):247–253.103.Wong HL, Wu XY, Bendayan R. Nanotechnological advances for the delivery of CNS therapeutics. *Adv Drug Deliv Rev*. 2012;64(7):686–700.104.AshaRani PV, Hande MP, Valiyaveettil S. Anti-

proliferative activity of silver nanoparticles. *BMC Cell Biol*. 2009;10:65.105.Wiley DT, Webster P, Gale A, Davis ME. Transcytosis and brain uptake of transferrin-containing nanoparticles by tuning avidity to transferrin receptor. *P Natl Acad Sci U S A*. 2013;110(21):8662–8667.106.Raghnaill MN, Bramini M, Ye D, et al. Paracrine signalling of inflammatory cytokines from an in vitro blood brain barrier model upon exposure to polymeric nanoparticles. *Analyst*. 2014;139(5):923–930.107.Wang J, Deng X, Zhang F, Chen D, Ding W. ZnO nanoparticle-induced oxidative stress triggers apoptosis by activating JNK signaling pathway in cultured primary astrocytes. *Nanoscale Res Lett*. 2014;9(1):117.108.Pickard MR, Jenkins SI, Koller CJ, Furness DN, Chari DM. Magnetic nanoparticle labeling of astrocytes derived for neural transplantation. *Tissue Eng Part C Methods*. 2011;17(1):89–99.109.Lai JC, Lai MB, Jandhyam S, et al. Exposure to titanium dioxide and other metallic oxide nanoparticles induces cytotoxicity on human neural cells and fibroblasts. *Int J Nanomedicine*. 2008;3(4):533–545.110.Xue Y, Wu J, Sun J. Four types of inorganic nanoparticles stimulate the inflammatory reaction in brain microglia and damage neurons in vitro. *Toxicol Lett*. 2012;214(2):91–98.111.Huerta-García E, Pérez-Arizti JA, Márquez-Ramírez SG, et al. Titanium dioxide nanoparticles induce strong oxidative stress and mitochondrial damage in glial cells. *Free Radic Biol Med*. 2014;73:84–94.112.Wang Y, Wang B, Zhu MT, et al. Microglial activation, recruitment and phagocytosis as linked phenomena in ferric oxide nanoparticle exposure. *Toxicol Lett*. 2011;205(1):26–37.113.Wu J, Sun JA, Xue Y. Involvement of JNK and P53 activation in G2/M cell cycle arrest and apoptosis induced by titanium dioxide nanoparticles in neuron cells. *Toxicol Lett*. 2010;199(3):269–276.114.Liu SC, Xu LJ, Zhang T, Ren GG, Yang Z. Oxidative stress and apoptosis induced by nanosized titanium dioxide in PC12 cells. *Toxicology*. 2010;267(1–3):172–177.115.Valdiglesias V, Costa C, Kiliç G, et al. Neuronal cytotoxicity and genotoxicity induced by zinc oxide nanoparticles. *Environ Int*. 2013;55:92–100.116.Valdiglesias V, Costa C, Sharma V, et al. Comparative study on effects of two different types of titanium dioxide nanoparticles on human neuronal cells. *Food Chem Toxicol*. 2013;57:352–361.117.Xie HR, Hu LS, Li GY. SH-SY5Y human neuroblastoma cell line: in vitro cell model of dopaminergic neurons in Parkinson's disease. *Chin Med J (Engl)*. 2010;123(8):1086–1092.118.Chiang HM, Xia Q, Zou X, et al. Nanoscale ZnO induces cytotoxicity and DNA damage in human cell lines and rat primary neuronal cells. *J Nanosci Nanotechnol*. 2012;12(3):2126–2135.119.Zieminska E, Stafiej A, Struzynska L. The role of the glutamatergic NMDA receptor in nanosilver-evoked neurotoxicity in primary cultures of cerebellar granule cells. *Toxicology*. 2014;315:38–48.120.Zhao J, Xu L, Zhang T, Ren G, Yang Z. Influences of nanoparticle zinc oxide on acutely isolated rat hippocampal CA3 pyramidal neurons. *Neurotoxicology*. 2009;30(2):220–230.121.Liu Z, Ren G, Zhang T, Yang Z. Action potential changes associated with the inhibitory effects on voltage-gated sodium current of hippocampal CA1 neurons by silver nanoparticles. *Toxicology*. 2009;264(3):179–184.122.Grabrucker AM, Garner CC, Boeckers TM, et al. Development of novel Zn^{2+} loaded nanoparticles designed for cell-type targeted drug release in CNS neurons: in vitro evidences. *PLoS One*. 2011;6(3):e17851.123.Xu LJ, Zhao JX, Zhang T, Ren GG, Yang Z. In vitro study on influence of nano particles of CuO on

CA1 pyramidal neurons of rat hippocampus potassium currents. *Environ Toxicol*. 2009;24(3):211–217.124.Yang Z, Liu ZW, Allaker RP, et al. A review of nanoparticle functionality and toxicity on the central nervous system. *J R Soc Interface*. 2010;7(suppl 4):S411–S422.125.Wang Y, Cui J, Sun X, Zhang Y. Tunneling-nanotube development in astrocytes depends on p53 activation. *Cell Death Differ*. 2011;18(4):732–742.126.Tosi G, Vilella A, Chhabra R, et al. Insight on the fate of CNS-targeted nanoparticles. Part II: intercellular neuronal cell-to-cell transport. *J Control Release*. 2014;177:96–107.127.Park EJ, Yi J, Kim Y, Choi K, Park K. Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism. *Toxicol In Vitro*. 2010;24(3):872–878.128.Monteiro-Riviere NA, Inman AO, Zhang LW. Limitations and relative utility of screening assays to assess engineered nanoparticle toxicity in a human cell line. *Toxicol Appl Pharmacol*. 2009;234(2):222–235.129.Griffiths SM, Singh N, Jenkins GJ, et al. Dextran coated ultrafine superparamagnetic iron oxide nanoparticles: compatibility with common fluorometric and colorimetric dyes. *Anal Chem*. 2011;83(10):3778–3785.130.Kettiger H, Schipanski A, Wick P, Huwyler J. Engineered nanomaterial uptake and tissue distribution: from cell to organism. *Int J Nanomedicine*. 2013;8:3255–3269.131.Raspopov RV, Gmoshinskii IV, Popov KI, Krasnoiarova OV, Khotimchenko SA. [Methods of nanoparticles control in food and biological objects. Report 1. Use of microscopic and chromatography investigation methods]. *Vopr Pitan*. 2012;81(2):4–11. [Russian].132.Arora S, Rajwade JM, Paknikar KM. Nanotoxicology and in vitro studies: the need of the hour. *Toxicol Appl Pharmacol*. 2012;258(2):151–165.133.Clift MJ, Gehr P, Rothen-Rutishauser B. Nanotoxicology: a perspective and discussion of whether or not in vitro testing is a valid alternative. *Arch Toxicol*. 2011;85(7):723–731.134.Dhawan A, Sharma V. Toxicity assessment of nanomaterials: methods and challenges. *Anal Bioanal Chem*. 2010;398(2):589–605.135.Kroll A, Dierker C, Rommel C, et al. Cytotoxicity screening of 23 engineered nanomaterials using a test matrix of ten cell lines and three different assays. *Part Fibre Toxicol*. 2011;8:9.136.Hanley C, Thurber A, Hanna C, Punnoose A, Zhang JH, Wingett DG. The Influences of cell type and ZnO nanoparticle Size on immune cell cytotoxicity and cytokine induction. *Nanoscale Res Lett*. 2009;4(12):1409–1420.137.Donaldson K, Borm PJ, Castranova V, Gulumian M. The limits of testing particle-mediated oxidative stress in vitro in predicting diverse pathologies; relevance for testing of nanoparticles. *Part Fibre Toxicol*. 2009;6:13.138.Kim JS, Peters TM, O'Shaughnessy PT, Adamcakova-Dodd A, Thorne PS. Validation of an in vitro exposure system for toxicity assessment of air-delivered nanomaterials. *Toxicol In Vitro*. 2013;27(1):164–173.139.Costantino L, Boraschi D. Is there a clinical future for polymeric nanoparticles as brain-targeting drug delivery agents? *Drug Discov Today*. 2012;17(7–8):367–378.140.Laurent S, Burtea C, Thirifays C, Hafeli UO, Mahmoudi M. Crucial ignored parameters on nanotoxicology: the importance of toxicity assay modifications and "cell vision". *PLoS One*. 2012;7(1):306–314.141.Lesniak A, Campbell A, Monopoli MP, Lynch I, Salvati A, Dawson KA. Serum heat inactivation affects protein corona composition and nanoparticle uptake. *Biomaterials*. 2010;31(36):9511–9518.142.Mahmoudi M, Monopoli MP, Rezaei M, et al. The protein corona mediates the impact of nanomaterials and

slows amyloid beta fibrillation. *Chembiochem*. 2013;14(5):568–572.143.Mirshafiee V, Mahmoudi M, Lou KY, Cheng JJ, Kraft ML. Protein corona significantly reduces active targeting yield. *Chem Commun*. 2013;49(25):2557–2559.144.Salvati A, Pitek AS, Monopoli MP, et al. Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. *Nat Nanotechnol*. 2013;8(2):137–143.145.Takhar P, Mahant S. In vitro methods for nanotoxicity assessment: advantages and applications. *Arch Appl Sci Res*. 2011;3(2):389–403.146.Block ML, Elder A, Auten RL, et al. The outdoor air pollution and brain health workshop. *Neurotoxicology*. 2012;33(5):972–984.147.Rodier PM. Environmental causes of central nervous system maldevelopment. *Pediatrics*. 2004;113(4):1076–1083.148.Sparkman NL, Johnson RW. Neuroinflammation associated with aging sensitizes the brain to the effects of infection or stress. *Neuroimmunomodulat*. 2008;15(4–6):323–330.149.von Bernhardi R, Tichauer JE, Eugenin J. Aging-dependent changes of microglial cells and their relevance for neurodegenerative disorders. *J Neurochem*. 2010;112(5):1099–1114.150.Carvey PM, Punati A, Newman MB. Progressive dopamine neuron loss in Parkinson's disease: the multiple hit hypothesis. *Cell Transplant*. 2006;15(3):239–250.151.Qin LY, Wu XF, Block ML, et al. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia*. 2007;55(5):453–462.152.Giordano G, Tait L, Furlong CE, Cole TB, Kavanagh TJ, Costa LG. Gender differences in brain susceptibility to oxidative stress are mediated by levels of paraoxonase-2 expression. *Free Radical Bio Med*. 2013;58:98–108.153.Costa LG, de Laat R, Dao K, Pellacani C, Cole TB, Furlong CE. Paraoxonase-2 (PON2) in brain and its potential role in neuroprotection. *Neurotoxicology*. 2014;43:3–9.154.Segal MB. The choroid plexuses and the barriers between the blood and the cerebrospinal fluid. *Cell Mol Neurobiol*. 2000;20(2):183–196.

The matrices of traditional composite resins have generally been comprising various types of inorganic fillers. Applications of nano-sized fillings in the resin matrices have overcome some of the mechanical limitations and have significantly improved their clinical performance. Commonly used nanomaterials include nano-ZnO,[3,4] nano-silica,[18,19] nano-calcium phosphate and calcium fluoride (nano-$Ca_3(PO_4)_2$ and CaF_2, respectively),[20] and nano-TiO_2.[21] In addition to composite resins, the utilization of nanomaterials in dental adhesives has also effectively improved their bonding strengths and mechanical properties. For instance, polyhedral oligomeric silsesquioxanes hybrid nano-composites have polymerized with silicon-based nanomaterials to form a novel type of bonding material that possessed a large mechanical strength and good thermostability.[22] Furthermore, the antibacterial properties of the bonding agents could be greatly improved by the inclusion of nano-sized silver and calcium phosphate.[23] Other possible additions have included nano-hydroxyapatite (nano-HAp)[24] and nano-silica.[25]

Root filling materials

Root canal filling materials are supposed to effectively kill the bacteria in the periodical lesions, densely seal the apical zones, and promote healing. However, the brittleness of the root canal often increases after treatment due to the large size of the traditional HAp. The mismatch of the elastic modulus between the root dentin and fillings has also resulted in percolation. In contrast, nano-HAp represents a unique advantage in this aspect because its structure is similar to natural inorganic bone. Nano-HAp was able to induce osteogenesis[26] and further improve the bacteriostatic and antibacterial effects of the root fillings.[27] Considering its good bioactivity, nano-HAp was also used as an optimum replacement material in the repair of bone defects.[28,29] For example, Yang et al[30] demonstrated that nano-HAp-coated silk scaffolds effectively guided the regeneration of periodontal and bone tissue. Similarly, porous ZrO_2/HAp composite scaffolds were also reported to possess excellent mechanical properties and cellular/tissue compatibilities.[31]

Bioceramics and associated dental prosthesis

Nanostructured bioceramics, which are constructed by a plasma-coating or chemical deposition process, generally possess enhanced mechanical properties, such as a better resistance to crack propagation[32] and an increased fracture toughness and Vickers hardness.[33] Additionally, the utilization of nano-sized Ti-, Zn-, and Ce-oxide has greatly improved the mechanical properties of a maxillofacial silicone elastomer.[34] Nano-sized silver may be an effective addition to denture-based materials to improve their antifungal properties and biocompatibility.[35] Thus, the applications of nanomaterials have the potential to effectively improve the comprehensive properties, including the mechanical, chemical, and biological properties, of different types of conventional dental materials.

Coating materials for dental implants

Good osseointegration at the implant–bone interface is essential for the success of dental implantation, but, unfortunately, this still remains a significant challenge. In recent years, a number of studies have reported the increased success rates of implants through the applications of a nano-coating on the surface, a nano-ceramic, and artificial nano-bone materials. For example, nano-porous alumina,[36] nano-zirconia/nano-$Ca_3(PO_4)_2$,[37] nano-ZnO,[5] and nano-HAp[38] have been utilized to increase the surface bioactivities of dental implants to achieve superior osseointegration. The advantages of the nanoscale modifications of dental implant surfaces have been presented in a recent review by Mendonca et al.[39]

Target delivery and imaging in tumor chemotherapy

A key problem in the use of chemotherapy for oral malignant tumors is how to improve the local concentrations of the drug while reducing the systemic side effects. To solve this problem, novel NP-based drug delivery strategies have been

studied where the NPs are the drug carriers that can transport the anticancer drugs to the tumor sites, which further increases the therapeutic efficacy. For example, Lebold et al[40] applied mesoporous thin silica films with nanoscale pores as drug carriers that were incorporated with doxorubicin, a widely used anticancer drug. The mesoporous silica nanomaterials demonstrated a sustained and controlled release of the anticancer drugs. Mu and Feng[41] discussed the advantages of manufacturing polymeric NPs (vitamin E d-alpha-tocopheryl polyethylene glycol 1000 succinate) for the controlled release of paclitaxel and other anticancer drugs. Another application of targeted therapy with novel NPs involves tumor imaging. Superparamagnetic iron oxide with special surface modifications has been utilized to guide the laser ablation of maxillofacial cancer because these manufactured NPs are magnetic resonance-active and can be selectively heated up for simultaneous imaging.[42] Similarly, liposomal nanocarriers also possess special advantages in their use for tumor radiography and imaging due to their good encapsulation of drugs and gadolinium.[43]

Aside from the aforementioned therapeutic uses, many nanomaterials, such as nano-TiO_2 and nano-ZnO, have been utilized in everyday dental items, including toothpastes and mouthwashes.[7,44] Considering the various applications of dental nanomaterials listed above, we should admit the outstanding contributions of nanomaterials to modern medicine. In the meantime, however, the risks of nanomaterials to human health have also significantly increased accompanied with more exposure opportunities.

Possible pathways for entering the CNS

Based on the principles of toxicology, nearly all substances are potentially toxic to humans, and the key lies in the dose and method of exposure. The people who most likely come into contact with dental nanomaterials are the production, research, and development staff, as well as the dental staff and patients. In clinical situations, most of the dental nanomaterials were directly applied in the oral cavity or maxillofacial region, allowing the nanomaterials to easily enter into the bloodstream (or lymph fluid) via absorption through oral mucosa or through the digestive tract after swallowing. In addition, opportunities for exposure to nanomaterials may also occur with the utilization of dental tools. At present, tungsten carbide (WC) nanowires, which are a new form of nano-WC, have been applied in the production of carbide micro-drills, including in dental drills and burrs. Thus, dental staff and patients may face abrasive NPs directly during a grinding or polishing process, especially considering that many dental prosthetic materials also contain nano-metals (eg, Co, Cr, Au, Ag, Ti), resins (Si), and ceramics (eg, Zr, Al, Li, Mg, Fe). Once these NPs are absorbed into our bloodstreams, they can be distributed to different organs, including the liver, spleen, kidneys, heart, lungs, and brain. Compared with the other organs, these substances are still required to cross the BBB or blood–cerebrospinal fluid (CSF) barriers to reach the brain. In addition to the systemic pathways, nanomaterials can directly translocate to the brain via nerves.[45] The possible pathways of dental nanomaterials entering the CNS are described below.

BBB pathway

The BBB is mainly composed of the cerebrovascular endothelium, which is sealed with tight junctions (TJs). Additional structures, such as pericytes, astrocyte end-feet, and a discontinuous basal membrane, are supportive cells to the BBB. All of these structures associated with the surrounding neurons constitute a complex and functional "neurovascular unit"[46] (Figure 1). The unique structural characteristics of the BBB are the intracellular TJs and the absence of Weibel–Palade bodies, which are significantly different from other vascular endothelial cells and can prevent most of the substances from entering into the CNS. In addition to these physical barriers, the BBB also possesses some metabolic barriers to the delivery of therapeutic agents.[47] First, the endothelia of the BBB are deficient in pinocytic vesicles, and thus, they can only allow for the low pinocytosis of certain substrates. Second, a series of intra- and extracellular enzymes that are expressed by the cellular components will limit the transport of a substance through the BBB. The complex interactions between the drugs and these enzyme systems often lead to therapeutic failure. Finally, efflux systems (such as P-glycoprotein) of the endothelial cells also play an important role in the elimination of harmful endogenous and exogenous molecules. Another associated structure that serves to prevent potentially harmful substances from entering the brain is known as the blood–CSF barrier.[48] This barrier is formed by choroid plexus epithelial cells, which possess similar TJs but a smaller surface area compared with the BBB endothelia. The blood–CSF barrier helps to prevent macromolecules from penetrating into the CSF, and this function is further reinforced by the active transport systems, which actively remove therapeutic organic acids from the CSF.[49]