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Perturbation of Physiological Systems by Nanoparticles

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Abstract

Nanotechnology is having a tremendous impact on our society. However, societal concerns about human safety under nanoparticle exposure may derail the broad application of this promising technology. Nanoparticles may enter the human body *via* various routes, including respiratory pathways, the digestive tract, skin contact, intravenous injection, and implantation. After absorption, nanoparticles are carried to distal organs by the bloodstream and the lymphatic system. During this process, they interact with biological molecules and perturb physiological systems. Although some ingested or absorbed nanoparticles are eliminated, others remain in the body for a long time. The human body is composed of multiple systems that work together to maintain physiological homeostasis. The unexpected invasion of these systems by nanoparticles disturbs normal cell signaling, impairs cell and organ functions, and may even cause pathological disorders. This review examines the comprehensive health risks of exposure to nanoparticles by discussing how nanoparticles perturb various physiological systems as revealed by animal studies. The potential toxicity of nanoparticles to each physiological system and the implications of disrupting the balance among systems are emphasized.

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1. Introduction

The current era of nanotechnology is characterized by the wide application of various products based on nanomaterials or nanotechnology in almost all industrial sectors, in biomedicine, and in daily commodities. For example, nanomedicine potentially provides solutions for early diagnosis and personalized medicine for the treatment of complex diseases such as cancer and metabolic disorders[1]. Nanotechnologies also provide a potential solution to social challenges, such as energy shortages[2, 3] and environmental deterioration[4]. As of March 2011, there were 1317 nanotechnology-based consumer products on the market, including food containers, fabrics, sports equipment, antibiotic reagents, and electronic components[5].

Despite the advantages nanotechnology offers, the potential risk of intentional and unintended human exposure to nanoparticles is increasing as nanotechnology develops. Early studies on asbestos and antropogenic nanoparticles like diesel exhaust have shown that they can accumulate in human body, especially after daily exposure such as in the occupational settings. Due to their nanometer dimensions, both natural and synthetic nanoparticles behave similarly to small or biological molecules and cells. Nanoparticles can bind and perturb biological molecules in cells, such as DNA, lipids, and proteins. Long-term and short-term toxicities to humans and animals caused by nanoparticles have already become a serious concern. A large number of research reports have focused on the biological impact of nanoparticles on biomolecules, cells, organs, or isolated physiological systems. These publications are of great value in that they provide insights into crucial issues such as how nanoparticles perturb specific systems and whether they pose safety risks. However, no organ or system is an isolated target. Nanoparticles can translocate between organs, and signals can be transmitted across physiological boundaries. Therefore, it is extremely important to examine nanotoxicity at the whole-body level. Two studies in particular demonstrate that research that focuses on a single system may need to be re-considered. In one case, cytokines released by nanoparticle-stimulated cells on one side of a model membrane generated by layered cells caused DNA damage in cells on another side. Furthermore, the exposure of pregnant mice to cobalt chromium nanoparticles was shown to cause DNA damage in neonatal blood without an accumulation of nanoparticles in the neonate body[6, 7]. In another study,

inhaled multi-walled carbon nanotubes (MWCNTs) were shown to release signals from the lung that activate cyclooxygenase enzymes in the spleen and mediate immune suppression in mice[8]. These results suggest that nanoparticle exposure may have remote effects to distal organs by stimulating signals in the exposure portal of human body. The transmission of these signals to distal sites may cause toxicity. Therefore, the impact of nanoparticles may not be limited to a single physiological system.

The human body consists of multiple physiological systems that work together to maintain homeostasis. In the following, we summarize the current knowledge concerning the effects of nanoparticles on the 12 primary physiological systems. Our presentation includes *in vivo* toxicological evaluations and *in vitro* investigations using cells from various human organs. We focus primarily on synthetic nanoparticles since they are the major players in nanotechnology development and can be well characterized. A brief description of the absorption, distribution, metabolism, and excretion (ADME) properties of nanoparticles is first presented before nanotoxicity to various systems are discussed. With this review, we hope to provide a better understanding on whether nanoparticles can potentially compromise whole-body homeostasis and overall human health.

2. Absorption, distribution, metabolism, and excretion of nanoparticles

Increased applications of nanoparticle-based technology and products are increasing human exposure to nanomaterials. Intentional applications of nanoparticles to the human body in medicine result in exposures through implantation[9], intravenous injection, oral administration, inhalation, and even transdermal usage. Accidental exposures may occur *via* oral, respiratory, or dermal routes (Figure 1). After nanoparticles enter the bloodstream, they are distributed to various organs, where they are partially metabolized, excreted, or retained. The ADME processes of nanoparticles are complicated by their unique physiochemical properties. Wide variations in the chemical composition, size, shape, and surface properties of nanoparticles dictate the ADME behaviors of nanoparticles as well as their behaviors in cells. In this section, we will summarize the ADME process of nanoparticles.

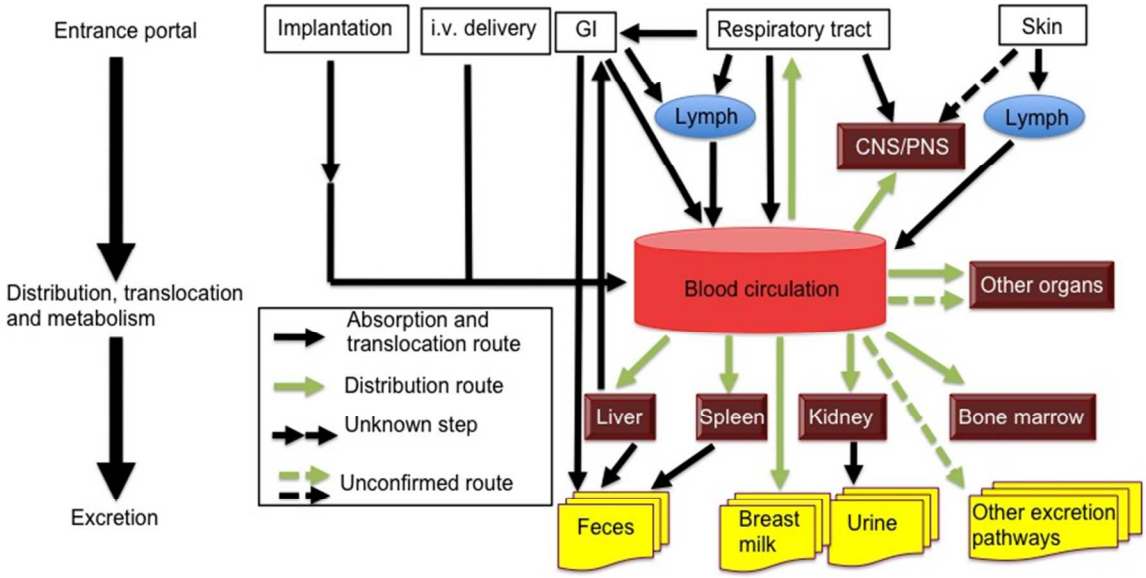

Figure 1. The biokinetics of nanoparticles. Although most of the absorption, translocation, and distribution routes of nanoparticles have been confirmed in recent years, a detailed understanding of these processes, including their rates, the retention time of nanoparticles, and the underlying mechanisms, is still lacking. Nanoparticles are primarily distributed to organs such as the liver, spleen, kidney, bone marrow, and CNS/PNS. Other organs and tissues that have been reported to accumulate nanoparticles include muscle, testis, placenta, and bone. Nanoparticles may exhibit different behaviors in different anatomical sites of the same physiological system. For example, nanoparticles in the

tracheobronchi and alveoli of the respiratory tract show different translocation and distribution properties. CNS, central nervous system. PNS, peripheral nervous system. i.v., intravenous, GI, gastrointestinal tract. Reproduced from Reference [10] with permission from Environmental Health Perspectives.

Various physiological barriers protect humans from exposure to foreign substances. For example, skin can prevent the absorption of hazardous substances from cosmetics. When the skin of experimental animals is exposed to nanoparticles, only a small number of nanoparticles are found in the circulation. However, smaller nanoparticles can penetrate skin and then undergo translocation to various organs[11].

After absorption, nanoparticles are carried by the bloodstream to various organs. Nanoparticles easily enter organs with high blood flow, such as the liver and spleen, and are effectively retained by the reticuloendothelial system (RES) of these organs[12, 13]. Small numbers of nanoparticles can also enter organs such as the brain[14, 15] and testis[16]. Delicate organs such as the brain and testis, as well as the fetus, are protected by dedicated protection barriers. Nanoparticles were also able to penetrate these barriers and pose potential risk[17]. The capability of nanoparticles to enter cells from various organs may be determined by their physicochemical properties. Besides the size[18, 19], shape[20], and aspect ratio[20], the aggregation status and surface chemistry also play important roles. The cell membrane consists of phospholipid bilayer and is negative charged. Therefore, the cationic nanoparticles are more readily to bind to cell surface and be internalized. For example, mesoporous silica nanoparticles coated with polyethyleneimine (cationic) shows an enhanced cell uptake compared with neutral or anionic nanoparticles[21]. However, since cationic nanoparticles may damage cell membrane and, after internalization, damage lysosomal compartment[22], they exhibit higher toxicity than neutral and anionic nanoparticles. Furthermore, functionalization of nanoparticles with ligand molecules recognizing specific cell membrane receptors may enhance their entrance into cells[23].

The inter-system translocation of nanoparticles is complicated. For example, after dermal or gastrointestinal exposure, ultrafine particles can enter the circulation *via* a lymph node-mediated process[24, 25]. After inhalation, there are at least four possible paths that nanoparticles can take: to lymph nodes, to the gastrointestinal tract, to the central

and/or peripheral nervous system, and to the blood circulation[10]. In *Caenorhabditis elegans,* ingested QDs were partly translocated to the reproductive system[26]. This translocation process is also partially controlled by the physicochemical properties such as surface charges[27].

Since the efficiency of absorbance and *in vivo* biodistribution of nanoparticles largely depend on their physicochemical properties, including shape, size, chemical composition, surface properties and the aggregation status, we list the main physicochemical properties of the nanoparticles, as well as the exposure routes, detection methods and main findings of the above studies in Table 1. Readers can refer to some recent review papers which have well summarized the relationship between nanoparticles' physicochemical properties and their *in vivo* behaviors and fate[28].

Table 1. Effects of physicochemical properties of nanoparticles on absorption and biodistribution.

Nanoparticle	Size(nm)	Surface chemistry	Administration route and exposure time	Animal model	Detection method	Main observations	Ref
TiO_2	10, 25 and 60	Hydrophobic or hydrophilic surface	Dorsal skin exposure, 60 days	Hairless mouse	TEM and atomic absorption spectrometry	Accumulation in spleen, lung, kidney and brain	11
TiO_2	80, 155	N/A	Intranasal instillation, 2, 10, 20 and 30 days	Mouse	Inducedly coupled plasma mass spectrometry (ICP-MS)	Accumulation in brain through olfactory bulb	15
MnO_2	30	N/A	Whole body inhalation, 12 days	Rat	Graphite furnace atomic absorption spectroscopy	Accumulation in CNS *via* olfactory bulb	14
Gold	2, 40	N/A	i.p. and i.v. injection, 1, 4 and 24 hours	Mouse	Autometallography	Macrophage uptake in liver, less in spleen, small intestine, lymph nodes	12
Gold	10-250	N/A	i.v. injection, 24 hours	Rat	ICP-MS	NPs of 10 nm entered testis and brain	17
CdTe(CdSe) core (shell) type II QDs	10 (naked); 18.8(coated)	Oligomeric phosphine	Intradermal injection, < 5 min	Mouse and pig	Near-infrared (NIR) excitation	Accumulation in sentinel lymph node	25
SWCNTs	1-3 ×100 (diameter × length)	N/A or coated by paclitaxel (PTX) - polyethylene	i.v. injection, 0.5, 2 and 4 hours	Mouse	Raman spectroscopy	Accumulation in liver and spleen, less in heart, lung, kidney,	13

		glycol (PEG)				stomach, intestine, muscle	
MWCNTs	20-30 × 0.5-2 μm	Carboxylated and aminated surface	i.v. injection, 0.17, 1 and 24 hours	Mouse	Cu^{64} label	Accumulation in testis	16
Polystyrene microspheres	50, 100, and 300	N/A	Gavage	Rat	Gel permeation chromatogrphy	Accumulation in liver and spleen *via* lymph node route	24

N/A, with no surface modification.

A large number of research in recent years have identified *in vivo* metabolism of nanoparticles[29]. The physiological conditions in bio-microenvironment (for example, acidic environment in stomach[18] and endosomes) and the degradation by enzymatic catalysis in cell lysosome may play primary roles. Endocytosis is the major route for the entrance of nanoparticle into cells. During this process, nanoparticles are enclosed by early or late endosomes, within which it is respectively slightly acidic (pH 6.2-6.5) or pronounced acidic (pH 4.5-5.5). Under such acidic microenvironment, some metallic nanoparticles, such as silver[30], quantum dots[31, 32], and iron oxide nanoparticles[33, 34], may undergo dissolution and release metallic ions. Non-metallic nanoparticles, like mesoporous SiO_2 nanoparticles[35] may be also partially dissolved in cells or *in vivo* by this way. Based on this understanding, manipulation of the suitable coatings can prolong nanoparticles' lifespan and reduce their toxicity[36].

Although *in vivo* evidence is still lacking, the degradation of nanoparticles by enzymatic catalysis is shown by some *in vitro* studies. For example, both SWCNTs and MWCNTs are prone to degradation by natural enzymes such as plant-derived horseradish peroxidase, and human neutrophil enzyme myeloperoxidase[37]. After the enzymatic degradation, the readiness of nanoparticles to induce toxicity may be reduced[37]. In the lung and the liver, large nanoparticles (with a cutoff size of ≈100 nm[38]) can be taken up by macrophages. Considering the large number of phase I and phase II enzymes in these cells (such as monooxygenase, transferases, esterases, and epoxide hydrolase), it is expected that the hepatic clearance of nanoparticles are associated with enzyme-catalyzed biodegradation[29].

Active and passive surface modifications may alter the metabolism profiles of

nanoparticles. For example, nanoparticles coated with polyethylene glycol may partially escape RES retention and may exhibit a prolonged circulation time in blood[39]**.** It was even shown that not only the type of modifying molecules, but the stereo isomers may play a role in determining the toxicity of nanoparticles. For example, a study found that D-Glutathione coated Cadmium Telluride (CdTe) QDs exhibited a lower cytotoxicity compared to its native counterpart, L-Glutathione[36]. *In vivo*, an important passive modification to nanoparticles is the formation of protein corona, layers of adsorbed protein molecules, on nanoparticle surface[40, 41]. Since the phagocytosis depends on the recognition of coating proteins by RES macrophages, the protein corona formation may help nanoparticle clearance[42]. However, it is not always the case, since protein corona may also simultaneously reduce nanoparticle aggregation making them smaller than 100 nm to escape phagocytosis[43].

Surface molecules on nanoparticles can also be modified *in vivo*. For example, some molecules on the nanoparticle surface may be cleaved off in endosomes, and nanoparticles may absorb various molecules. These properties are often used as a drug delivery strategy in nanomedicine applications[44]. Although nanoparticle metabolism plays a key role in determining nanotoxicity, our understanding in this area is still inadequate[37].

On the other hand, nanoparticles inside an organism may affect physiological systems and the metabolic process. For example, because of the high electron density on the surface[45], graphene oxide nanoparticles acted as an electron transfer mediator and catalyzed H_2O_2 decomposition to •OH in *Caenorhabditis elegans* (*C. elegans*)[46]. Involvement in physiological redox reactions has emerged as an important mechanism accounting for toxicity of nanoparticles[46, 47]. For this reason, special attentions should be paid to the health risks of nanoparticles under some pathophysiological conditions like diabetes and aging.

A noteworthy question is that the effects of both *in vitro* and *in vivo* metabolism of nanoparticles on their toxicity. Some nanoparticles, especially those metallic and metallic oxide nanoparticles, are prone to releasing ions inside or outside of organisms and cells[48, 49]. When the released ions are more toxic, it makes the evaluation of nanoparticle toxicity complicated. This is especially true for some quantum dots, most of which are made of

toxic heavy metals[49]. The release of ions is one way nanoparticles exert their toxicity and should be avoided by optimization of synthesis methods or chemical modifications.

Nanoparticles can be partially excreted (Figure 1). The excretion pathways may be related to the composition or physicochemical properties of the nanoparticles. For example, single-walled carbon nanotubes (SWCNTs) functionalized with polyethylene glycol are primarily cleared through feces and urine[39], whereas liposomes are eliminated *via* the hepatobiliary pathway[50]. Smaller nanoparticles, those less than 10 nm in diameter, can be eliminated by renal excretion[51], whereas nanoparticles larger than 80 nm in diameter are trapped by the liver and spleen and only slowly excreted in feces[50]. Nanoparticles degradable in macrophages may be cleared by the RES organs, while non-degradable particles may be deposited in organs for a long time [52, 53]. Other excretion pathways, including loss in saliva, sweat, and breast milk, are also possible[54].

Using lower organisms like *Caenorhabditis elegans* as study models, the ADME of nanoparticles are also found. CsSe@ZnS and CdTe quantum dots of 5-6 nm after ingestion exhibited a metabolic pathway different from food bacteria strains *Escherichia coli*. They were distributed to the intestinal and the reproductive system and in the digestive microenviroment, QDs were degraded because of the oxidation of the inner selenium[26].

The ADME properties of nanoparticles are quite diverse. Different nanoparticles are absorbed with different efficiencies and tend to be distributed in various organs. They can be dissolved, degraded, or stably trapped in the body. Nanoparticles are only partially excreted, and, in some organs, a fraction of the nanoparticles that enter the tissue may remain there for long periods. This persistence prolongs the perturbation of physiological systems and poses considerable risk to health.

After entering human body via different routes, both nanoparticles and small molecule drugs undergo ADME process. With a size intermediated between small molecules and bulk materials, nanoparticles exhibit many unique behaviors during these processes compared to small molecules. Understanding these differences will help us apply nanoparticles in a safer way. In the following Table 2, we compare the different properties of ADME between nanoparticles and small molecules.

Table 2. ADME property comparison between nanoparticles and small molecules.

		Nanoparticles	Small molecules
Absorption	Entrance portal	Oral, respiratory, dermal, injection, implantation… Cross barriers like skin, gut wall, alveolar membrane…	
Distribution	Distribution carrier	Blood circulation; Lymph circulation	
	Interaction with plasma	Protein corona on NPs surface	Protein-drug complex
	Passage through intercellular gap (e.g., tight junction, glomerular filtration)	Cutoff size loosely applies and many leak.	Cutoff size applies
	Passage through cell membrane	Endocytosis Membrane penetration and frustrated phagocytosis (for needle-like NPs)	Diffuse through membrane pore and lipid bilayers Carrier mediated transport; Endocytosis
Metabolism	Mediator	Peroxidase enzyme (e.g.,Myeloperoxidase); Physiological microenvironment (e.g., acidic environment in endosomes)	Phase I, II enzymes; Physiological microenvironment (e.g., gastric acid)
	Biological activity change	Help excretion; Decrease toxicity; Targeted drug delivery	Increase or decrease toxicity
	Site	Mainly in intracellular endosomes of macrophages in RES organs	Mainly in liver
Excretion	Major pathway	Urine and feces	Urine and feces
	Excretion efficiency	More difficult	Easier

3. Nanotoxicity to the respiratory and circulatory systems

Because dry nanoparticles easily become airborne, respiratory exposure is the most common route for nanoparticle intake. Within the respiratory system, there is an exchange of oxygen and carbon dioxide between the atmosphere and the body. The respiratory system comprises the airways, lungs, and respiratory muscles. In addition to providing gas exchange, the respiratory system has important roles in body defense and in the regulation of metabolic and endocrine functions. Thus, injury to the respiratory system often leads to disorders involving other bodily systems.

The circulatory system transports oxygen; nutrients such as glucose, amino acids, and fatty acids; biological messenger molecules such as hormones; and functional cells such as immune cells. It also facilitates the removal of wastes such as carbon dioxide and dead cell debris. The circulatory system consists of the heart, blood vessels, blood, lymph, and related structures. The close association of the circulatory and respiratory systems is best exemplified by the air exchange that occurs in the alveoli of the lungs. Within alveoli, oxygen diffuses into the blood through capillaries, and the oxygen-rich blood then travels back to the heart. Nanoparticles were reported to across alveolar epithelial and vascular endothelial cell layers and were translocated into the circulatory system after inhalation[55].

Respiratory toxicity. Inhaled nanoparticles are translocated to various organs from the lungs through the lymphatic vessels and blood[56, 57]. These translocations may result in toxicity to the whole body (Figure 2). Our current understanding of respiratory nanotoxicity can be classified into three categories: toxicity to respiratory organs, systemic toxicity after nanoparticles enter the circulation, and *in vitro* mechanisms.

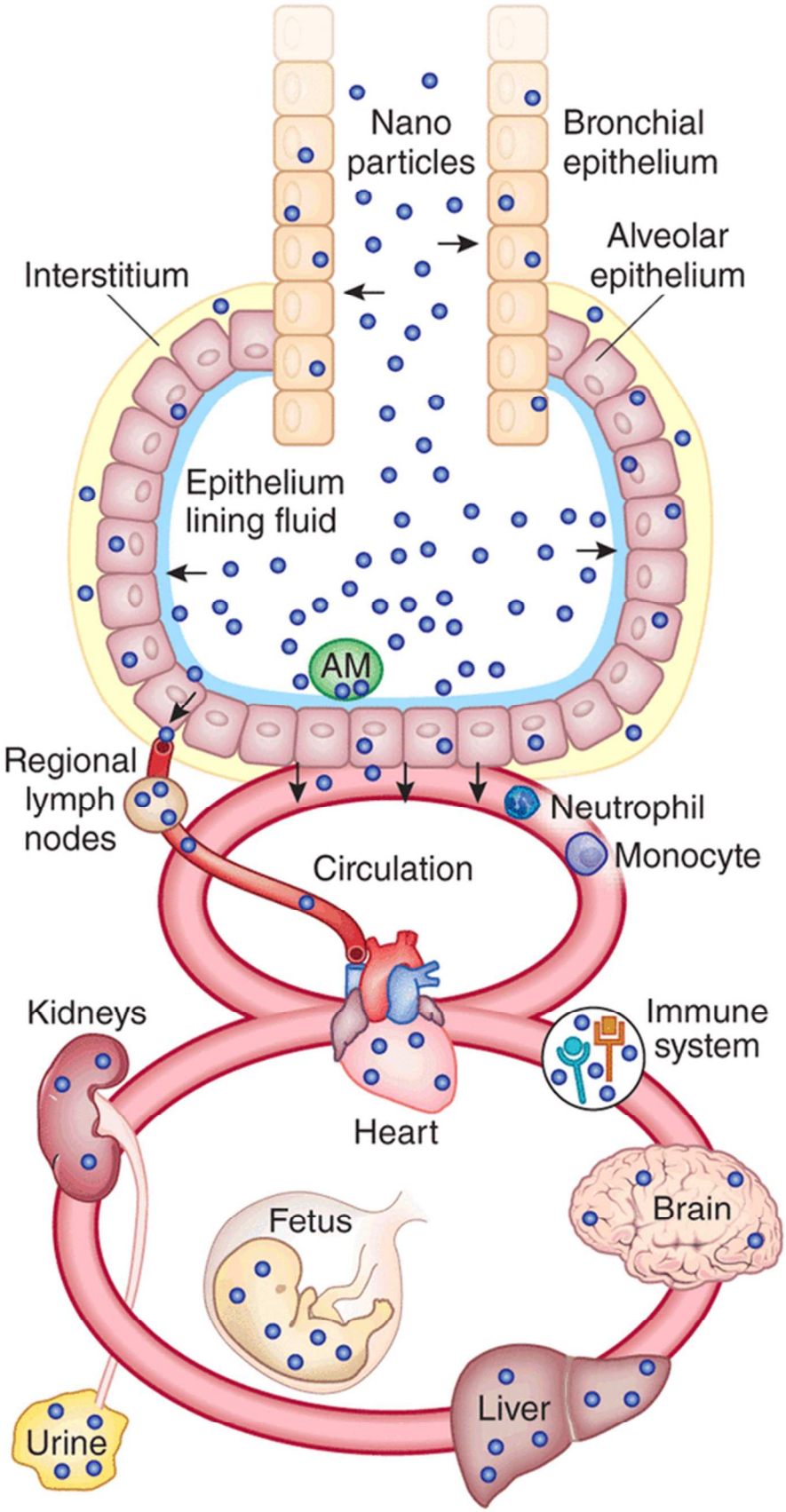
Nano particles
Bronchial epithelium
Alveolar epithelium
Interstitium
Epithelium lining fluid
AM
Regional lymph nodes
Neutrophil
Monocyte
Circulation
Kidneys
Immune system
Heart
Brain
Fetus
Liver
Urine

Figure 2. Schematic of nanoparticle translocation from the lung epithelium to regional lymph nodes and blood circulation. Nanoparticles circulating in blood may accumulate in various secondary organs of the body. This schematic highlights two important pathways of nanoparticle biodistribution from the lung: the pathway of lymphatic drainage toward regional lymph nodes and renal clearance from blood to urine. AM, alveolar macrophages. Reprinted with permission from Reference [57]. Copyright 2010, Nature Group.

Toxicity to the lung of animals. Because it is a reticuloendothelial system, the lung is one of the primary locations in which nanoparticles accumulate. The respiratory toxicity of nanoparticles has been investigated after exposure of animals to these particles by various dosing methods. One such dosing method is inhalation. After inhalation exposure of rats to MWCNTs (0.5 and 2.5 mg/m^3), the animals' lungs showed pronounced multifocal granulomatous inflammation[58]. Inhalation exposure to carbon nanofibers was also found to cause the formation of extrapulmonary fibers and inflammation in terminal bronchioles and alveolar ducts of rats[59]. Inhalation exposure of mice to TiO_2 nanoparticles caused lung inflammation[60]. After 15 days of inhalation exposure to rats, gold nanoparticles (30-100 and 5-8 nm in diameter) were found to have been translocated from the lung to organs such as the kidney, aorta, spleen, and heart. An analysis of biochemical indicators showed that damage occurred both to the lung and to these organs[61, 62]. In contrast, after 90 days of inhalation exposure to rats, smaller gold nanoparticles (4-5 nm) were not detected in any distal organs except the kidney, and lung inflammation was not significant[63].

Intratracheal instillation is a surrogate for inhalation exposure. Compared to inhalation exposure, intratracheal instillation typically delivers a higher dose to animals[64]. When administered by this route, various nanoparticles, including CNTs[65-67], carbon black[68], silver[69], TiO_2[60], iron oxide[70, 71], and CeO_2 nanoparticles[72], induced pulmonary inflammatory responses, granuloma formation[65-67, 73, 74], and fibrotic lung injury[65]. After intratracheal instillation, nanoparticles were taken up by both the alveolar macrophages and the alveolar epithelial cells. The entrance of nanoparticles in alveolar epithelium made it hard for them to be cleared from the lung[71]. TiO_2 nanorods generated hydroxyl radicals in the lungs of Sprague-Dawley rats and caused reversible pneumotoxicity with no significant alterations in pulmonary immune function[75]. ZnO nanoparticles induced

oxidative stress in the lungs of rats, as indicated by increased lipid peroxide, HO-1, and alpha-tocopherol levels[48]. Because ZnO nanoparticles can release zinc ion (Zn^{2+}) *in vivo*[48], effects from both forms of zinc have been investigated in alveolar type II epithelial cells[76]. The results show that ZnO nanoparticles induced less intracellular oxidative stress than dissolved zinc ions, suggesting that zinc ions are probably responsible for the induction of oxidative stress *in vivo*. Both carbon black[68] and TiO_2 nanoparticles[77] also induced genotoxicity (DNA strand breaks) in bronchoalveolar lavage cells and lung epithelial cells. Local immune suppression in the lung has been detected after single intratracheal instillation of iron oxide nanoparticles in mice[70].

Pharyngeal aspiration and intravenous injection have also been used for nanoparticle dosing. After pharyngeal aspiration, SWCNTs elicited acute pulmonary inflammation, progressive fibrosis, and granulomas in the lungs of mice[73]. Similarly, graphene nanoparticles extensively recruited inflammatory cells, including macrophages and granulocytes, into the lungs and pleural spaces of mice, leading to inflammation[78]. Following intravenous injection of a single dose of gold nanoparticles, the nanoparticles (20 nm) rapidly accumulated in the lungs; the number of nanoparticles in the lungs decreased with time without causing toxicity[79]. The respiratory toxicity and its relationship to properties of nanoparticles, exposure regiment, and testing animal models are summarized in Table 3.

Table 3.Respiratory toxicity of nanoparticles.

Nanoparticle	Characterization	Exposure regiment	Animal model	Main observations	Ref
MWCNTs	5–15 nm × 0.1–10 μm (diameter × length), 250–300 m^2/g. Containing 9.6% Al_2O_3, Fe and Co	Inhalation, 0.5 and 2.5 mg/m^3 for 90 days	Wistar rats	Multifocal granulomatous inflammation in the lungs	58
	5 nm × 5μm	Intratracheal instillation, 0.5, 2 and 5 mg/mouse for 60 days	Sprague–Dawley rats	Pulmonary inflammation and granuloma formation, Fibrotic lung injury	65
SWCNTs	N/A	Intratracheal instillation, 0.5 mg/kg for 3 and 14 days	ICR mice	Pulmonary inflammation and granuloma formation	66

	10-500 nm in diameter,aggregated, containing Fe, Ni, Co and Y	Intratracheal instillation, 0.1 or 0.5 mg/mouse for 7 and 90 days	B6C3F$_1$ mice	Pulmonary inflammation and granuloma formation	67
	1-4 nm in diameter, containing 0.23% Fe	Pharyngeal aspiration, 10-40 μg/mouse for 1-60 days	C57BL/6 mice	Pulmonary inflammation and granuloma formation	73
	1.4 nm ×1μm, Containing 30–40% amorphous carbon, 5% Ni and Co	Intratracheal instillation, 1 or 5 mg/kg, for 1-90 days	Crl:CD(SD)IGS BR rats	Pulmonary inflammation and granuloma formation	74
Carbon nanofibers	158 nm × 5.8 μm, < 0.5% metal	Inhalation, 0.54, 2.5, and 25 mg/m^3 for 90 days	Sprague Dawley rats	Formation of extrapulmonary fibers, Inflammation in bronchioles and alveolar ducts	59
Carbon black	14 nm, 300 m^2/g	Intratracheal instillation, single dose of 0.162 mg, analysis after 28 days	C57BL/6 mice	DNA strand breaks in bronchoalveolar lavage and lung epithelial cells	68
Graphene Nanoplatelets	5 μm by SEM, 100 m^2/g	Pharyngeal aspiration, 50 μg/mouse for 1 and 7 days	C57BL/6 mice	Inflammation in the lungs and pleural spaces	78
Gold	20 nm	Intravenous injection, 3 μg/mouse for 1-60 days	Wistar rats	Rapid accumulation in the lungs without toxicity	79
	30-100 and 5-8 nm	Inhalation, 2×10^6 NPs/cm^{3}for 5 and 15 days	Wistar rats	Down-regulation of muscle-related genes	61
	5-8 nm	Inhalation, 88 μg/m^3, for 90 days	Wistar-Kyoto rats	Uptake by alveolar macrophages and epithelial cells	62
	4-5 nm	Inhalation, 20 μg/m^3, for 90 days	Sprague Dawley rats	No translocation to distal organs, No pulmonary inflammation	63
Silver	240 nm	Intratracheal instillation, 125-500 μg /kg for 1-28 days	IRC mice	Pulmonary inflammation and granuloma formation	69
FeO	35 nm, 40 m^2/g	Intratracheal instillation, 0.5 mg/mouse for 1,2 and 6 days	BALB/c mice	Pulmonary inflammation and granuloma formation	70

Fe_2O_3	20 and 280 nm in diameter	Intratracheal instillation, 0.8 and 20 mg/kgbw for 30 days	Sprague Dawley rats	Pulmonary inflammation and granuloma formation	71
ZnO	20-50 nm in diameter	Intratracheal instillation, 0.2 mg/0.4 ml for 1, 24, 72hrs and 7 days	Wistar rats	Oxidative stress in the lungs	48
TiO_2	Aerodynamic size ≈ 100 nm	Inhalation, 0.8-28.5 mg/m^3,5 days, 4 hrs per day	C57BL/6J mice	Pulmonary inflammation	60
CeO_2	20 nm in diameter	Intratracheal instillation, 0.15-7 mg/kg for 1 and 28 days	Sprague Dawley rats		72

N/A, No data.

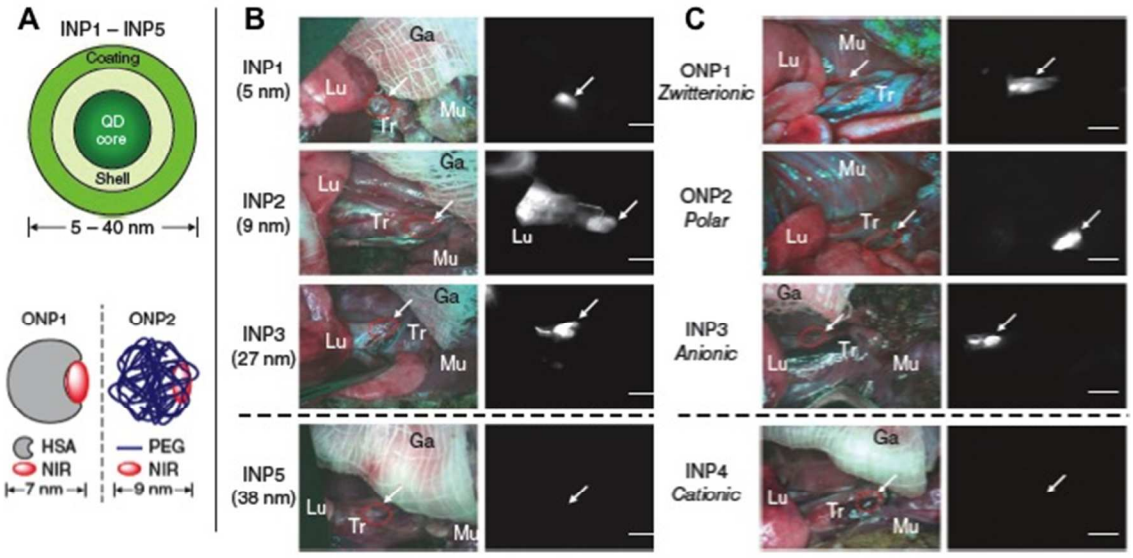

Figure 3. Size- and charge-dependent translocation of nanoparticles from lungs to lymph nodes. (A) Schematic structures of inorganic/organic hybrid nanoparticles (INPs, 800 nm emission) and organic nanoparticles (ONPs, 700 nm emission). Various charged organic ligands were used to coat the core/shell of INPs. (**B**) Size-dependent translocation of INPs from lungs to lymph nodes. Four INPs were administered to the lungs, and their translocation was observed 30 min after administration. Shown are representative (n = 3) images of color video (left) and NIR fluorescence (right). Ga, gauze; Lu, lung; Mu, muscle; Tr, trachea. The arrows and red dotted circles indicate lymph nodes. Scale bar, 500 μm. All NIR fluorescence images were obtained with identical exposure times and normalizations. (**C**) Charge-dependent translocation of nanoparticles from lungs to lymph nodes. ONP1 (zwitterionic), ONP2 (polar), INP3 (anionic), and INP4 (cationic) nanoparticles were administered to lungs, and their

translocation was observed 30 min after administration. Reproduced with permission from Reference [27]. Copyright 2010, Nature Group.

Systemic toxicity after inhalation by human. Nanotoxicity to humans has also been reported. After occupational exposure to polyacrylate nanoparticles for 5-13 months, subjects exhibited symptoms of intense itching, shortness of breath, and pleural effusions. Pathological examinations revealed nonspecific pulmonary inflammation, pulmonary fibrosis, and foreign-body granulomas of the pleura. Transmission electron microscopy revealed the presence of nanoparticles in the cytoplasm and karyoplasm of pulmonary epithelial and mesothelial cells as well as in the chest fluid of these subjects[80]. Although these symptoms are similar to those observed in animals studies, whether the polyacrylate nanoparticles alone or nanoparticles with adsorbed organic compounds together lead to the toxicity is unknown. In another case, a 38-year-old healthy male was operating a nickel metal arc process and exposed himself to nickel nanoparticles for about 90 minutes after removing mask. He died 13 days after inhalation of nickel nanoparticles with an estimated total dose of one gram[81]. The man had no history of respiratory diseases. However, necropsy found alveolar injuries throughout all lobes with alveolar hemorrhage and edema fluid and all damages could be characterized as adult respiratory distress syndrome (ARDS). Transmission electron microscope analysis found nickel nanoparticles (<25 nm) in the lung macrophages and in the urine and kidneys. These nanoparticles also caused lesions or necrosis to other organs such as brain, heart, kidney and spleen. Although the link between nanoparticle exposure and human pathology was not conclusively established in these cases, these alarming findings re-emphasize the urgency of nanotoxicity research.

Systemic toxicity after inhalation by animals. In addition to causing lung injury, nanoparticles can be translocated to other organs by blood flow and cause systemic toxicity. The translocation of nanoparticles from the lung to other organs may depend on the physicochemical properties of the nanoparticles. The translocation capability of organic and inorganic nanoparticles of different sizes and with different surface charges have been investigated after instillation[27]. The results of these studies showed that nanoparticles smaller than 6 nm freely migrated to the circulation 30 min after exposure in rats. Nanoparticles smaller than 34 nm were translocated to mediastinal lymph nodes,

whereas cationic nanoparticles tended to be retained in the lungs (Figure 3). Long fiber-like nanoparticles such as MWCNTs (>20 μm in length) were translocated from subpleural alveoli to the pleural space[82].

Another risk from inhalation of nanoparticles is to damage the function of innate immune machinery. *In vitro* research indicated that SWCNTs reduced the viability of the alveolar macrophages and impaired their phagocytic function[83]. After inhalation of MWCNTs, a reduction of T-cell proliferation under the stimulation of mitogen and an impaired immune response to sheep erythrocytes were found in mice. The activity of natural killer cells was also decreased while no tissue damage or lung inflammation were induced[84]. Mechanistic study revealed that MWCNTs stimulated the release of TGF-ß, an immunoregulatory factor from lung, and caused humoral immune suppression[8]. Since humoral immune functions form the first line of defensive against pathogen invasion, these findings indicates that the secondary infections caused by environmental nanoparticle exposure is a concern. Another molecular mechanism for systemic immune responses to pulmonary nanoparticle exposure may be the extracellularly secreted membrane vesicles named exosomes[85]. This will be discussed in the section of immune nanotoxicity.

Inhaled TiO_2 nanoparticles (21 nm) impaired the endothelium-dependent arteriolar dilation of the carotid artery in exposed rats. This response is similar to the type of pathophysiological microvascular consequences that occur in chronic diseases such as diabetes, hypertension, and heart failure[86]. In rats, inhaled Fe_2O_3 and ZnO nanoparticles were accumulated in the liver and lungs and resulted in alterations in biochemical markers in blood[87]. Gold nanoparticles had also been shown to cause systemic damage to distal organs[61, 62].

Airborne particle exposure is reported to be a trigger of acute cardiac events and cardiovascular disorders, especially in some susceptible populations. In a study of a spontaneously hypertensive rat model[88], twenty four hours after intratracheal instillation, SWCNTs induced the reconstruction of arterial vessel, perivascular myocytes degeneration, and peripheral vascular lesions in addition to local inflammatory responses and oxidative stress in lung tissues. These results suggested that individuals with existing cardiovascular diseases or carrying susceptible genes may be susceptible to the stimuli of

SWCNTs (or other nanoparticles). Age was found to be another susceptible factor[89]. Under the identical exposure conditions, SiO_2 nanoparticles induced more grave pulmonary damages in older mice.

In vitro studies related to respiratory nanotoxicity. *In vitro* investigations may reveal probable mechanisms underlying the respiratory toxicity of nanoparticles. Mercaptosuccinic acid capped CdTe QDs in human umbilical vein endothelial cells (HUVECs) impaired mitochondria and exerted endothelial toxicity through activation of mitochondrial death pathway[90]. In the type II pulmonary epithelial cell line A549, pristine MWCNTs damaged the cell membrane and induce intracellular oxidative stress, whereas hydroxyl MWCNTs crossed the cell membrane without causing membrane damage and induce apoptosis[91]. Studies using cells from the air-blood tissue barrier (A549 and U937 cells) showed that nanoparticles can decrease cell viability by inducing intracellular oxidative stress or by causing genotoxicity[92-96]. Some inflammation-related signaling pathways, including the NF-kappa B and MAP kinase pathways, were activated under such conditions and were believed to mediate these cytotoxicities[97]. Apoptosis in human bronchial epithelial cells (BEAS-2B) had been found to be mediated by the caspase family of proteins[98].

Circulation toxicity. Nanoparticles are transported to distal organs through the blood[99-102]. During this translocation process, nanoparticles alter the fluid dynamics of blood,[103] affect the vascular walls,[104] and adhere to the surfaces of blood vessels due to non-specific interactions from van der Waals, electrostatic and steric interactions[105]. This trend may be related to physical properties of nanoparticles like size and shape[106, 107]. For example, oblate-shaped nanoparticles showed a higher adhesion probability to the surface of blood vessels than spherical nanoparticles of the same volume[108] (Figure 4). In blood, the original properties of the circulating nanoparticles were changed by proteins and other molecules that form a protein corona on their surface[109]. This protein corona influences *in vivo* behaviors of nanoparticles such as cell uptake and biocompatibility[110, 111]. Protein adsorption also helps nanoparticle disperse better and cause a higher cellular nanoparticle accumulation[109].

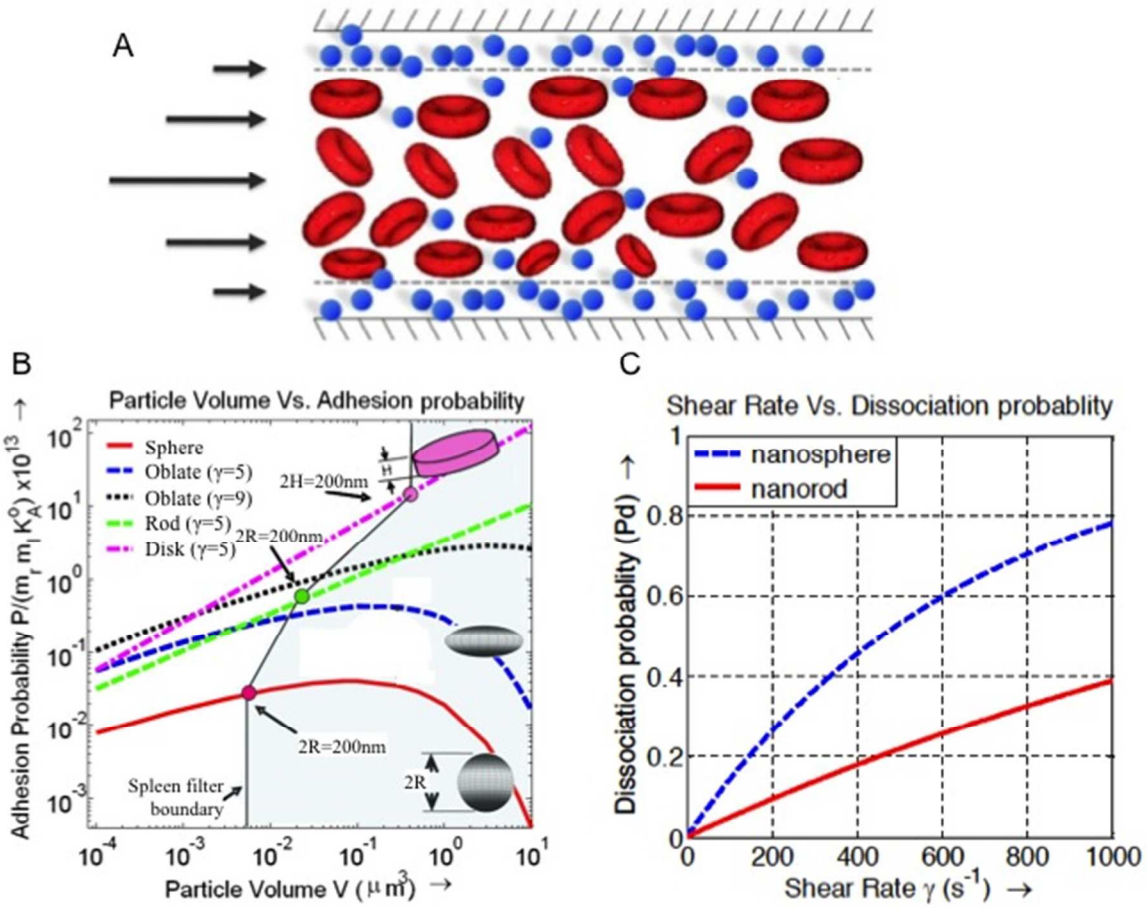

Figure 4. The transportation and distribution of nanoparticles in the blood are affected by their physical interactions with blood components. (A) When flowing through blood vessels, blood cells tend to concentrate near the center of the vessel because of the higher flow velocity in this region; this causes margination of nanoparticles toward and favors their adhesion to the vessel wall. (B) Adhesion probabilities of nanoparticles of various shapes are a function of particle volume. γ is the aspect ratio of nanoparticles. (C) For nanorods and nanospheres, particle dissociation probabilities are a function of the shear rate of the particles. The dissociation probability is normalized with shear rate zero. Reproduced with permission from Reference[112].

Nanoparticles are harmful to the circulatory system in several ways. After inhalation, various nanoparticles in the lungs of animal models stimulated the generation of oxidative stress and led to a release of pro-inflammatory mediators and coagulation factors, which were transmitted to circulation leading to cardiovascular lesions[113]. For example, in the ApoE$^{-/-}$ mouse model[114], nanoparticles such as CNTs, carbon black, and nickel hydroxide nanoparticles all accelerated plaque formation[115-117]. In low-density lipoprotein receptor knockout (LDLR/KO) mice, intratracheal exposure to carbon black

particles for 10 weeks exacerbated atherosclerotic lesions[118].

In blood, nanoparticles activate some coagulation pathways. For example, MWCNTs with different surface chemistries (pristine, carboxylated and amidated) damaged endothelial cell of blood vessel and triggered coagulation *in vivo*. *In vitro*, they exhibited obvious procoagulant activity as indicated by a study with activated partial thromboplastin time (aPTT) assays[119]. Mechanistic study showed that MWCNTs may stimulate thrombosis by activating both intrinsic and extrinsic pathways of coagulation via factor IX- and factor XII-dependent ways and the factor-independent way (Figure 5). Carbon nanoparticles (MWCNTs, SWCNTs, and mixtures thereof)[120] and injected silver nanoparticles[101] were also found to cause increased platelet aggregation, a key contributor to the formation of vascular thrombosis. The observed thrombus sizes were dose-dependent[121, 122]. The above studies demonstrate several of the adverse effects of nanoparticles on the cardiovascular system. These effects may cause significant risks, particularly in populations with at high risk for atherothrombosis.

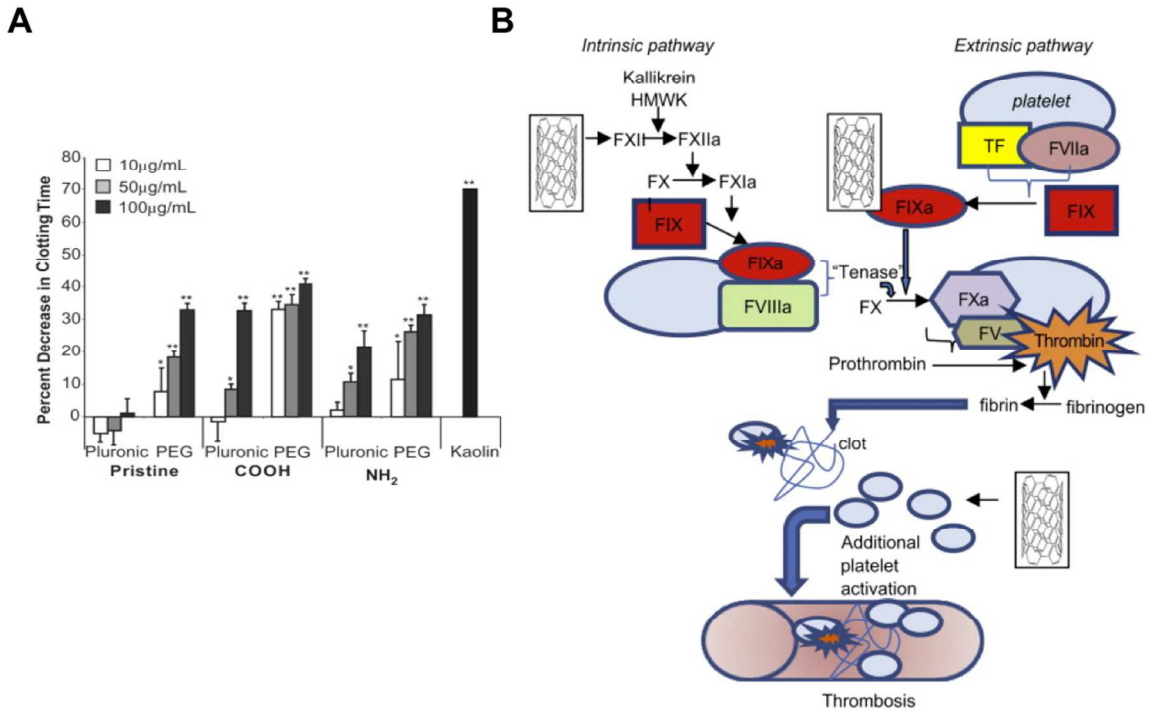

Figure 5. MWCNTs accelerate thrombopoiesis. (A) MWCNTs activate the intrinsic coagulation cascade. Activation of the intrinsic clotting cascade (ICC) was monitored by the activated partial thromboplastin time (aPTT) assay. Pristine, carboxylated, and amidated MWCNTs suspended in either pluronic F127 or distearoylphosphoethanolamine-(polyethylene glycol)-5000 were tested at the

indicated concentrations in pooled normal plasma (PNP). Kaolin, a negatively charged crystalline silicate, served as the positive control. The graph shows mean decreases in time to clot formation (±s.d.) normalized to the respective vehicle controls. □p < 0.02, □□p < 0.0005 relative to vehicle controls. (B) Model of MWCNT-mediated thrombosis. MWCNT can stimulate thrombosis through three independent mechanisms: activation of factor XII, binding of factor IXa, and activation of platelets. Nanotube association with FIXa appears to be more important than FXII in the nanotube-mediated activation of the intrinsic pathway. Reproduced with permission from Reference[119]. Copyright 2011, Elsevier.

Vascular endothelial cells form a dynamic interface between the circulatory system and nonvascular tissues. *In vitro* studies have shown that various nanoparticles damage the integrity and function of vascular endothelial cells. Exposure of HUVECs to water-soluble fullerene induced an accumulation of polyubiquitinated proteins and the release of lactate dehydrogenase, an indicator of cell lysis[123]. Iron oxide nanoparticles[124], CdTe quantum dots (QDs)[90], and SWCNTs[125] all decreased HUVEC viability and disrupted the cell cytoskeleton, leading to apoptotic cell death in a dose-dependent manner. Using freshly isolated human platelets as a study model, both silver nanoparticles[101] and amorphous SiO_2 nanoparticles[102] have been shown to lead to platelet aggregation. However, research on nanotoxicity to circulation is still rather inadequate at this time.

In summary, regardless of the exposure route, nanoparticles are retained by the lung reticuloendothelial system and the alveolar epithelium. Respiratory exposure also results in the transfer of nanoparticles to the blood circulation in alveoli, the nodal point of these two systems. Within the respiratory system, nanoparticles cause pulmonary inflammatory responses, granuloma formation, and fibrotic lung injury. They also cause systemic toxicity, primarily including compromised immune responses and systemic microvascular dysfunction. Within the circulatory system, nanoparticles alter the fluid dynamics of blood, generate intracellular oxidative stress, and induce inflammation that causatively leads to cardiovascular lesions, including platelet aggregation, thrombosis, and cardiovascular malfunction, in experimental animals (Figure 6). Populations with high atherothrombotic risk show a higher sensitivity to the adverse effects of nanoparticles on the cardiovascular system.

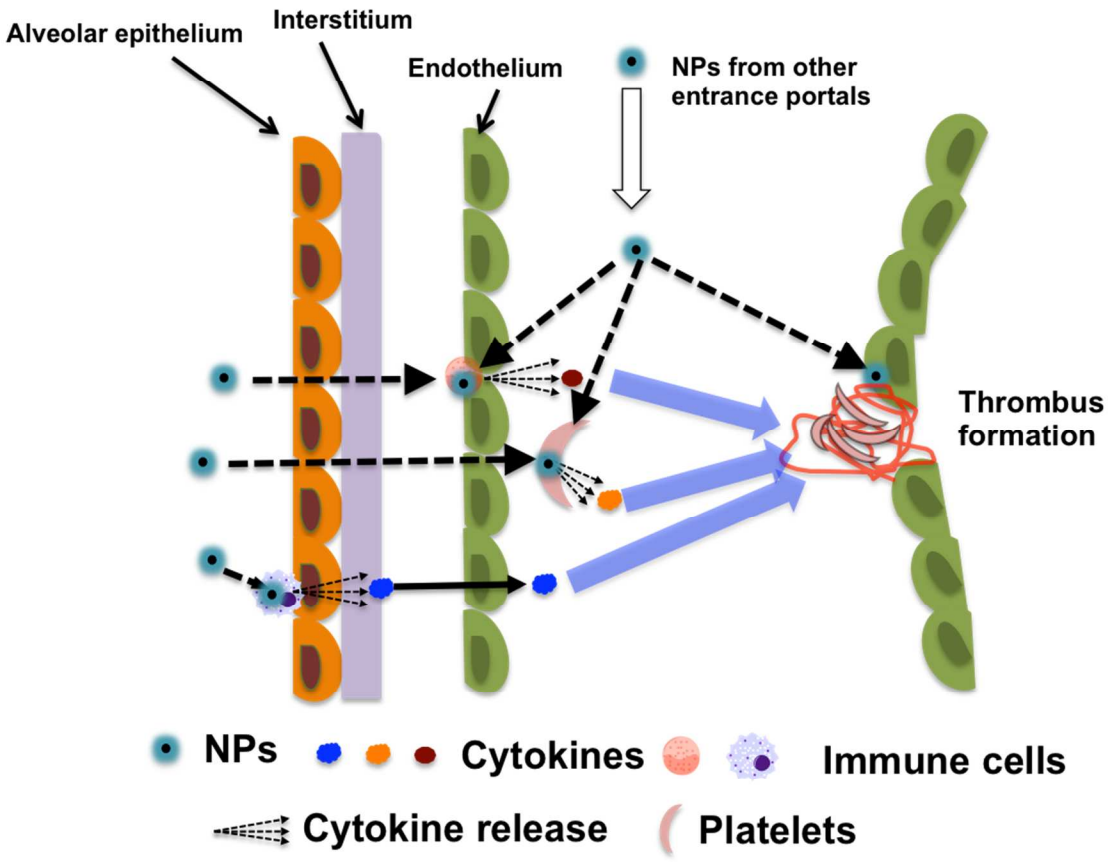

Figure 6. Nanoparticles-induced circulation toxicity after inhalation. After entrance *via* inhalation, nanoparticles induce circulation toxicity by three pathways. First, macrophages located in alveolar epithelium release cytokines after nanoparticle uptake. These cytokines migrate across endothelium of blood vessel and stimulate cardiovascular lesions (for example, thrombus formation). Second, nanoparticles migrate across interstitium and are taken up by macrophages located in the endothelium of blood vessel. This consequently induces release of cytokines to the blood and stimulates cardiovascular lesions. Third, some nanoparticles cross both interstitium and endothelium of blood vessel and are taken up by blood cells such as platelets. This may cause a release of cytokines to blood and stimulate cardiovascular lesions.

4. Nanotoxicity to the immune and hematopoietic systems

Hematopoiesis is the process by which hematopoietic stem cells develop into blood cells under the precise regulation of hematopoietic growth factors. In blood, there are three specific lineages of cells: erythroid cells, lymphocytes, and myelocytes. Erythroid cells function as oxygen carriers, and lymphocytes and myelocytes are responsive to immune defense signals. Impairment of the hematopoietic process may lead to disorders

in the functions of the immune and blood systems. Recent investigations have begun to indicate the adverse effects of nanoparticles on the immune system and on the process of hematopoiesis.

Immunotoxicity. The innate immune system and the acquired immune system together protect the body from invasion by pathogens. In each system, multiple biological molecules and cells have specific roles in recognizing, presenting, and combating invading pathogens. Although the thymus and spleen are specific immune organs, many immune cells circulating in various tissues throughout the body are not associated with any specific organ. Immunotoxicity refers to any permanent or reversible effect on components or functions of the immune system[126]; it includes immunosuppression, autoimmunity, hypersensitivity, and chronic inflammation. Nanoparticles from the ambient environment or biomedical applications will eventually enter the human circulatory system and be distributed to the entire body. In this process, the nanoparticles interact with the immune cells and immune organs. These interactions may lead to a loss of therapeutic efficiency due to clearance of nanoparticles by immune recognition[127] and/or damage to the body's defensive functions.

Although the issue of whether nanoparticles are immunogenic is still controversial, numerous studies suggest that nanoparticles function better as adjuvants than either alum or Freund's adjuvant, two traditionally used immune adjuvants[128]. Nanoparticles also appear to be immunosuppressors[129-131]. On one hand, this activity is desirable for the treatment of inflammatory disorders; on the other hand, it can potentially impair the ability of the body to perform surveillance against tumorigenesis and infections. Nanoparticles also induce the release of cytokines such as TNF, IL-12, and IFNγ[132]; activate Th1/Th2 response[133]; and stimulate acquired immunity[134], leading to inflammatory responses. These important immunological properties of nanoparticles have been previously reviewed[135]. We will only highlight some important recent findings below.

Immunological properties of nanoparticles. After acute respiratory exposure to a single dose of ambient nanoparticles (such as diesel-enriched nanoparticles) in a naïve mouse model, lung dendritic cells were activated. The activated dendritic cells selectively

provoked a Th2-biased immune activation of allogeneic CD4+ T cells[136]. Although engineered nanoparticles (carbon black and silver nanoparticles) were not shown to cause similar responses in the same investigation, the involvement of nanoparticles in the observed immunological effects cannot be excluded. Oral or peritoneal exposure of mice to polyethylene glycol-coated poly-DL-lactic-co-glycolic acid (PLGA) nanoparticles induced immune reactions. PLGA nanoparticles were taken up by macrophages in the peritoneal cavity, causing the production of anti-inflammatory cytokines and monocyte chemotactic protein-1 (MCP-1)[137]. Intratracheal instillation of four doses of iron oxide nanoparticles in mice reduced the number of lymph node cells that produced immunoglobulins against exotic immunogens[70].

The subcutaneous injection of MWCNTs into tumor-bearing mice has been shown to activate the complement system, stimulate cytokine production, and activate phagocytosis by macrophages[138]. These effects were beneficial to the host immune system's defensive function and inhibit the progression of tumor growth. In a rat allergic asthma model, intratracheal instillation of carbon nanoparticles with NO_2, a component in polluted air, decreased inflammatory reactivity and suppressed Th2-type cytokines in the lung and airway. This finding suggests that carbon nanoparticles inhibit the proinflammatory effects of NO_2 in the allergic asthma rat model[139].

Nanoparticles have also been investigated as immune adjuvants[140-142] and vaccine carriers[143, 144]. The size of the nanoparticle adjuvant or vaccine carrier has been shown to affect the immune response[145]. Nanoparticles enter and activate antigen-processing cells, thus inducing cellular immune responses. In contrast, micron-sized particles cannot be taken up efficiently by cells; hence, they only stimulate humoral immune responses. For example, a single immunization of rats with PLA nanoparticles (200–600 nm) stimulated a high level of IFN-gamma production, which upregulated MHC class I molecules and produced antibody isotypes favoring Th1-type immune responses. In comparison, immunization with larger particles (2–8 μm) promoted IL-4 secretion, upregulated MHC class II molecules, and favored Th2-type responses[146] (Figure 7). However, some investigations have reported contradictory results[147, 148]. An accurate description of the relationship between particle size and immune response has yet to be provided. A better understanding of the molecular mechanisms underlying nanoparticle-triggered immune

reactions is also needed.

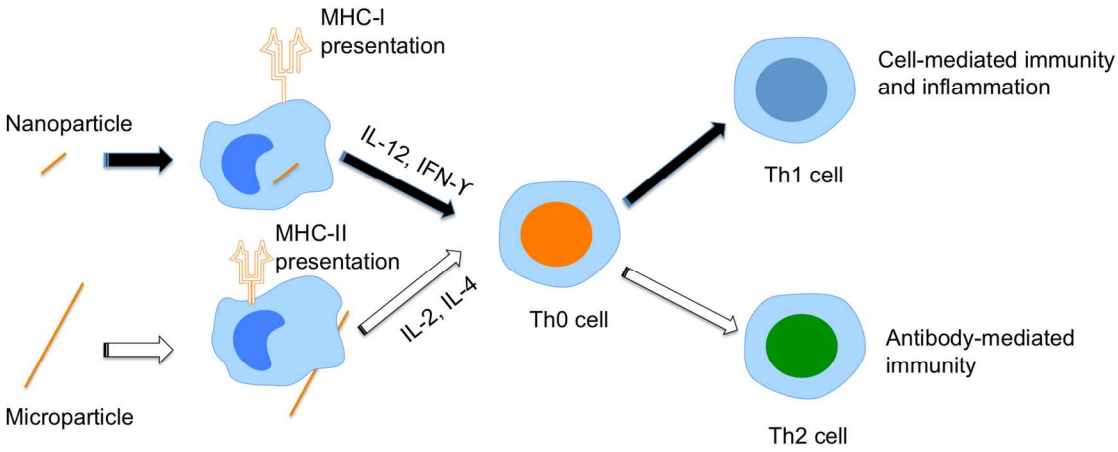

Figure 7. Nanoparticles trigger cell-mediated immunity while microparticles trigger antibody-mediated immunity. Because of their small size, nanoparticles can be phagocytosed by antigen presenting cells (APC) and induce antigen presentation via class I major histocompatibility complex (MHC) pathway. These events release cytokines such as IL-12 and IFN-□, which consequently determine the maturation of naïve T (Th0) cells towards Th1 cells and stimulate cell-mediated immune responses. In comparison, microparticles attach on the surface of APC cells and finally, stimulate the antibody-mediated immune responses.

Factors affecting immune responses to nanoparticles. Many factors affect the immune responses induced by nanoparticles. The physicochemical properties of nanoparticles, especially their surface chemistry, have been shown to be one of the important determinant factors. Surface modification of carboxylated MWCNT can change its binding target from primarily a mannose receptor to primarily a scavenger receptor, thus alleviating NF-κB activation and immunotoxicity[149]. The effects of other physicochemical factors of nanoparticles on specific immune responses await systemic investigation.

The immunological status of the tested animals affects the response of the immune system to nanoparticles. In healthy mice, inhalation of TiO_2 nanoparticles significantly elicited the formation of pulmonary neutrophilia and chemokine CXCL5 expression. However, in allergic mice, nanoparticles inhibited Th2-type inflammation, as indicated by a reduced infiltration of eosinophils and lymphocytes to the lungs and decreased expression of Th2 cytokines in the bronchoalveolar lavage. Exposure to nanoparticles

also decreased the number of mucus-producing goblet cells in the airway epithelium[150]. The suppression of inflammation by nanoparticles in allergic animal models has also been reported in other publications[151, 152].

Another crucial factor is the exposure route. When TiO_2 nanoparticles were delivered intraperitoneally to asthmatic mice, a Th2-dominant immune response was observed, as indicated by IgE production, an influx of inflammatory cells in the lung, and the presence of enhanced numbers of eosinophils[153]. This response differed from the results described above, which were obtained after inhalation. Additional investigation is needed to clarify the effects of nanoparticles on immune function to ensure their safe application in biomedicine and other areas.

Molecular mechanism and future challenges

Exposure to nanoparticles induces systemic immune responses.[62] Immune systems are stimulated by at least two pathways after pulmonary nanoparticle exposure. The first pathway involves the transmission of immunoregulatory factors released by macrophages in lung. TGF-ß is one of these factors. TFF-ß leads to the production and release of prostaglandin and IL-10 in spleen and causes T-cell dysfunction and a suppressed immune function[8]. The second involves the formation of exosomes, the secreted membrane vesicles bearing functional immune molecules. The exosomes also process immune signal transduction[154]. After pulmonary exposure of magnetic iron oxide nanoparticles, exosomes were formed in alveoli. They were quickly translocated into system circulation, stimulated the maturation of dendritic cells, and activated Th1-type immune response[85, 155]. This mechanism may account for the long-term inflammatory effects associated with nanoparticle exposure[156] and suggests that the control of immune signal transduction via exosomes may minimize the adverse immune responses caused by the inhaled nanoparticles.

An IL-1α mediated molecular mechanism has also been proposed. TiO_2 and SiO_2 nanoparticles, but not ZnO nanoparticles, induced inflammation in lung primarily by an IL-1 mediated pathway while knocking out either IL-1α or the receptor (IL-1R) in mice blocked pulmonary inflammation[157]. This pathway bears some similarities with that found for asbestos and micro-nanoparticles[158].

Many nanoparticles induce innate and acquired immune responses *via* toll-like receptors (TLRs). TLRs play a key role in recognizing pathogens, triggering innate immune defense responses, and activating adaptive immune functions. Nanoparticles such as poly (gamma-glutamic acid)[159], graphene[160], silver[161], and positively charged lipid nanoparticles[162] all impact immune systems *via* TLRs signaling. TLR4[159] and TLR2[161] seem to be nanoparticle-binding receptors. Poly (gamma-glutamic acid) nanoparticle-mediated immune reaction involves TLR4 and its adaptor protein MyD88 because immune cells from MyD88-knockout mice exhibit a lower level of inflammatory reactions than those from wild-type mice[159]. However, the total loss of expression of TLR4 by gene knockout does not completely suppress the induction of immune responses by nanoparticles, an observation that suggests the complexity of nanoparticles' interactions with the immune system and the possibility of other players in such responses[159].

In summary, nanoparticles exhibit the properties of immune adjuvants and immunosuppressors. In mice, they activate the complement system and decrease the population of lymph node cells that produce immunoglobulins against immunogens. Because they are efficiently taken up by antigen-processing cells, nanoparticles induce cellular immune responses rather than humoral immune responses. The physicochemical properties of nanoparticles, the immunological status of the test animals, and the exposure route all affect the response of the immune system to nanoparticles. Mechanistically, nanoparticles induce innate and acquired immune responses *via* cell surface receptors such as the mannose receptor, the scavenger receptor, and toll-like receptors (TLRs).

Although there have been several reports on the immunotoxicity of nanoparticles, the inconsistent results prevent reliable conclusions. A major challenge is the lack of universal and standardized guidelines for investigations of immunotoxicity[135]. This lack of standardization makes it difficult to compare research data from different groups and to draw firm conclusions. Other challenges in this area include a detailed understanding of effects from protein corona[109], the tendency of nanoparticles to agglomerate in biological media, their optical interference with assay systems, the chemical immunotoxicity of solvents, and contamination with endotoxins[163]. All of these problems

will need to be satisfactorily resolved to ensure the reliable assessment of immune nanotoxicity.

Hematopoietic toxicity. In adult humans and other adult mammals, the production and maturation of most blood cells occurs within the bone marrow, whereas lymphoid cells mature and are activated in the spleen, thymus, and lymph nodes. Under certain pathological conditions, the liver, thymus, and spleen may resume hematopoietic functions, causing pathological enlargement of these organs. Toxicity to the hematopoietic system may impair the production of blood cells by affecting the functions of hematopoietic stem cells or by damaging immune functioning, leading to severe diseases such as leucopenia, thrombocytopenia, neutropenia, and anemia.

Nanoparticles enter the bone marrow. The accessibility of nanoparticles to bone marrow is expected because bone marrow, like the liver and spleen, is one of the primary organs of the reticuloendothelial system (Figure 8). Of the various innate immune cells, lipid nanoparticles accumulated with higher efficiency in monocytes[164] and macrophages[165] than in lymphocytes, neutrophils, and dendritic cells. After oral administration or intravenous injection into mice, polystyrene nanoparticles[24] and iron oxide nanoparticles[166] were both detected in bone marrow. Taking advantage of this property, bone marrow-targeting drug delivery strategies that use nanoparticles with various surface chemistries have been developed [165, 167, 168].

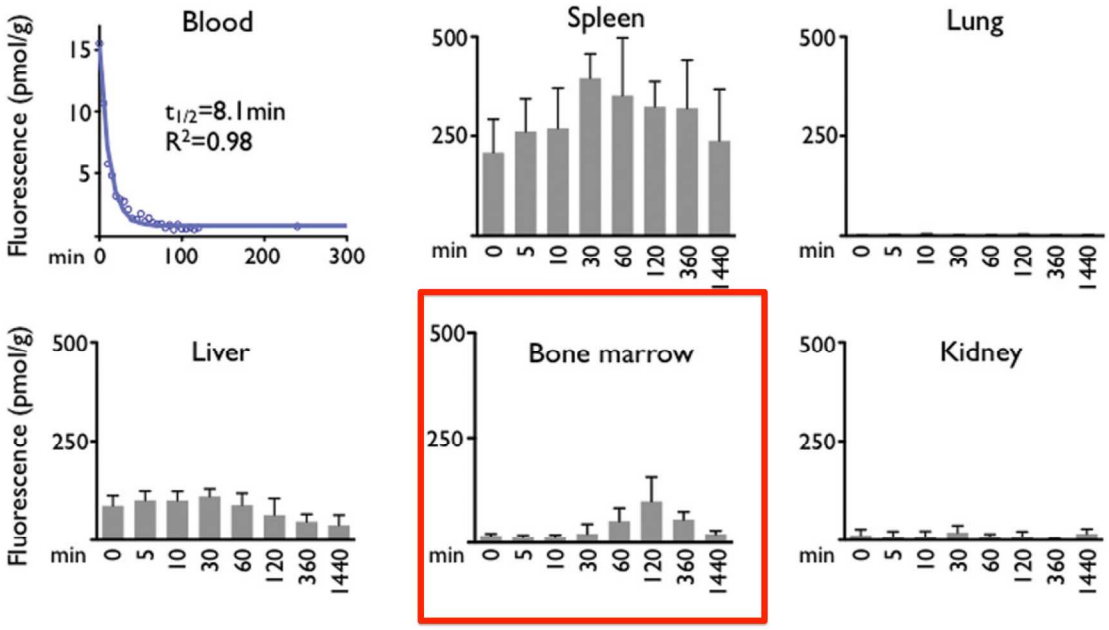

Figure 8. Accumulation of C12-200 lipid nanoparticles in bone marrow. Nanoparticles of 70-80 nm coated with siRNA molecules were injected into mice via tail vein and were rapidly cleared from the blood with a half-life of 8.1 min. The nanoparticles were found primarily distributed in the spleen, liver, and bone marrow, with less or no accumulation in the kidney or lung. Reproduced with permission from Reference [168]. Copyright 2011, Nature Group.

Nanoparticles that are harmless to the hematopoietic system. Some nanoparticles have been found to be relatively harmless to the hematopoietic system. Forty-eight hours after intravenous injection into rats, iron oxide nanoparticles were found in the bone marrow with no obvious acute toxicity[166]. In another research, human bone marrow stromal cells were labeled with superparamagnetic iron oxide nanoparticles; eight weeks after the labeled cells were transplanted into mice, there was no apparent adverse impact on the formation of hematopoietic supporting stroma[169]. Furthermore, nanoparticles did not affect phenotypic marker expression, colony formation, SDF-1-induced migration, or the differentiation into dendritic cells of hematopoietic stem cells[170]. These preliminary studies suggest that superparamagnetic iron oxide nanoparticles are relatively harmless to the hematopoietic system.

The effects of silver nanoparticles on the hematopoietic system have been investigated using a micronucleus assay. This assay uses erythrocytes in the bone marrow to detect damage to chromosomes and to the mitotic apparatus of mammalian cells. When damage

occurs, the frequency of micronucleated polychromatic erythrocytes in bone marrow increases. Twenty-eight days after the oral administration of silver nanoparticles to Sprague-Dawley rats, the animals showed no toxic effects in bone marrow, as indicated by a constant ratio of polychromatic erythrocytes/(polychromatic erythrocytes+normochromatic erythrocytes)[171].

Nanoparticles that adversely affect the hematopoietic system. Notwithstanding the above findings, some nanoparticles cause genotoxicity in bone marrow cells. Continuous oral exposure of rats to alumina nanoparticles (30/40 nm) at doses of 1000 and 2000 mg/kg for 30 or 48 hours resulted in the formation of micronuclei and chromosomal aberrations in the bone marrow cells, indicating genotoxicity of alumina nanoparticles to bone marrow[172]. When functionalized and pristine MWCNTs were intraperitoneally injected into Swiss-Webster mice at doses of 0.25, 0.5, and 0.75 mg/kg once a day for five consecutive days, a dose-dependent increase in micronucleus formation and chromosomal aberrations in bone marrow cells and DNA damage in leukocytes were observed[173]. The adverse effects of nanoparticles on hematopoiesis also depend on the route of administration. The administration of manufactured magnetic nanoparticles by inhalation (but not by intraperitoneal injection) for four weeks resulted in a decrease in the mean corpuscular volume and mean corpuscular hemoglobin content, two indicators of impaired erythrocyte function. Inhaled magnetic nanoparticles also decreased the production of platelets, increased the level of white blood cells in the bone marrow, and induced extramedullary hematopoiesis in the spleen[174].

Extramedullary hematopoiesis is indicative of pathological conditions such as anemia. Metallic nanoparticles (Fe_2O_3, Fe_3O_4, Sb_2O_3, gold, TiO_2, cobalt, and silver) affect primary cultures of hematopoietic progenitor cells from the bone marrow or immortalized cell lines in four ways: 1) nanoparticles with different chemical compositions affect the function of hematopoietic progenitor cells differently using colony formation capability as an indicator. For example, antimony oxide (Sb_2O_3) and cobalt nanoparticles show higher effect than other nanoparticles; 2) nanoparticles damage selectively to different hematopoietic progenitor cells, as indicated by their effects on the cells' morphology, proliferation, and differentiation; for example, cobalt nanoparticles preferentially affect erythroid and granulocytic–monocytic precursors, whereas Sb_2O_3 nanoparticles

selectively damage erythroid colony development; 3) hematopoietic progenitor cells show different sensitivities to nanoparticle exposure depending on the cells' biological status; for example, Sb_2O_3 nanoparticles adversely affect proliferating erythroid progenitors but not to differentiating erythroid progenitors; 4) findings on immortalized cell lines do not always agree with findings obtained in primary cells; for example, Sb_2O_3 nanoparticles damage erythroid progenitor cells but not affect human cell lines of hematopoietic origin such as K562, HL-60, CEM, CEM-R, Thp-1, Jurkat, and Molt-4[175].

In summary, nanoparticles enter the bone marrow, one of the primary organs of the reticuloendothelial system, and exhibit hematopoietic toxicity by causing genotoxicity to bone marrow cells. Nanoparticles compromise erythrocyte functions, reduce the production of platelets, increase the number of white blood cells, and induce extramedullary hematopoiesis in the spleen. Metallic nanoparticles of different chemical compositions show specific and variable toxicities to hematopoietic progenitor cells.

5. Nanotoxicity to reproduction and development

Reproductive and developmental toxicity refers to adverse effects on the human reproductive cycle at any stage, such as impairment of the capability to form healthy embryos in adult females by male or female factors[176]. Adverse effects manifested in the offspring at any point in the life span as a result of parental exposure are defined as developmental toxicity[177]. There is only a temporal difference between these two processes. Many environmental pollutants have adverse effects on the reproduction and development of animals and humans (Figure 9).

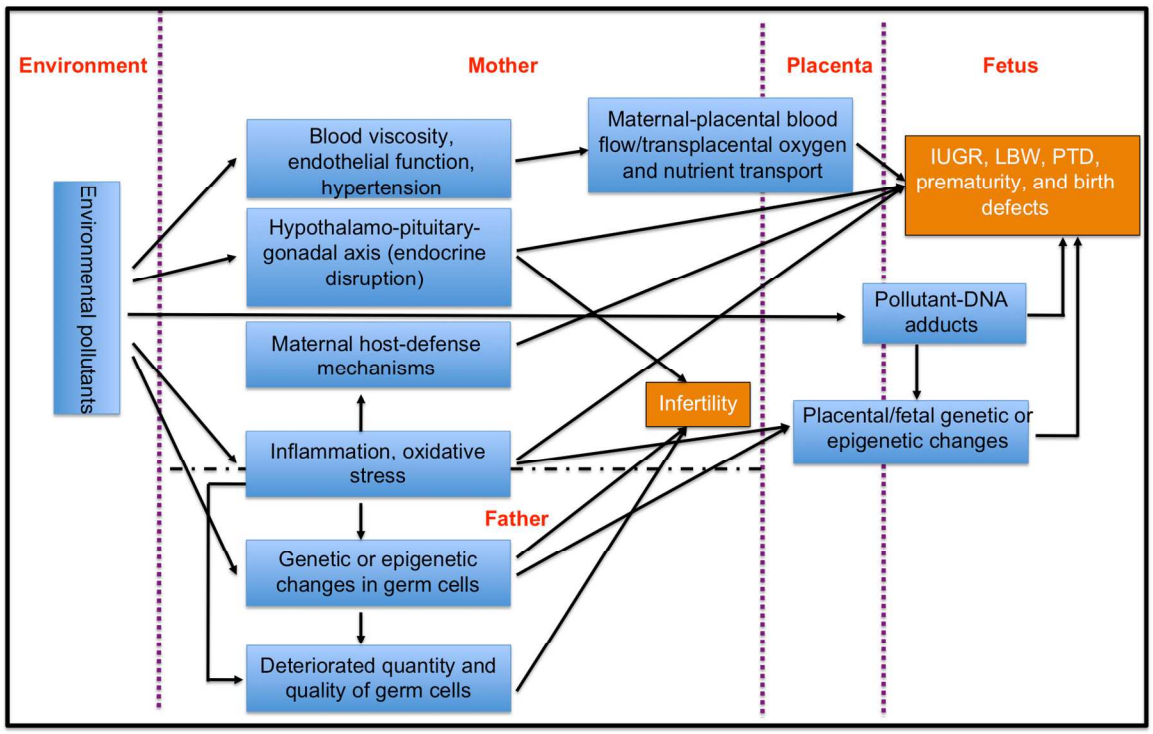

Figure 9. Impact of environment pollutants (including nanoparticles) on human reproduction, fertility, and development. LBW, low birth weight; IUGR, intrauterine growth retardation; PTD, preterm delivery. Reproduced with permission from Reference [178].

Reproductive nanotoxicity. In this section, we discuss the toxicity of nanomaterials to the male and female reproductive systems, including the adverse effects of nanoparticles on germ cells, on the physiological structure and function of the reproductive organs, and on fertility and their effects on the offspring.

Nanotoxicity to semen. Direct observation of nanotoxicity to the reproductive system comes from investigations of germ cells. Fresh semen from a healthy male was used to determine the effect of gold nanoparticles (9 nm) on human sperm activity[179]. At a concentration of 44 μg/mL, gold nanoparticles penetrated the heads and tails of sperm cells and caused 25% of sperm cells to become immotile. The effect of Fe_3O_4 nanoparticles coated with polyvinyl alcohol on bovine sperm cells has also been investigated[180]. Nanoparticles were taken up by sperm cells in a time-dependent manner; they bound to the acrosome in the sperm head and to mitochondria in the tail. There was no adverse effect on sperm motility or on the ability of the sperm to undergo the acrosome reaction (fertilize an egg), despite the fact that the acrosome and mitochondria

are involved in these actions.

Nanotoxicity to male reproduction. After pregnant Slc:ICR mice were subcutaneously injected with TiO_2 nanoparticles, nanoparticles are transferred to their offspring; the transferred nanoparticles accumulated in the Leydig cells, Sertoli cells, and spermatids in the testes of male pups, where they can be found on postnatal day 4 and at postnatal week 6. As a result, the seminiferous tubules were disorganized and disrupted. The mature sperm in the lumen of the tubules were also reduced in number. This treatment also led to a decrease in daily sperm production, epididymal sperm motility, and the number of Sertoli cells in male pups at postnatal week 6[181]. These data indicate that fetal exposure to nanoparticles harms the development of the male reproductive system. Similarly, after intratracheal administration to pregnant mice, carbon black nanoparticles altered testicular histology and reduced daily sperm production in male offspring[182, 183]. Adult male ICR mice exhibited vacuolation in seminiferous tubules, decreased daily sperm production, and increased levels of serum testosterone after intratracheal administration of carbon black[182]. Carbon black particles also impaired the function of Leydig cells, as indicated by the perturbation of testosterone levels after exposure. The adverse effects of carbon black on spermatogenesis depended on particle mass rather than the number of particles[182]. In another experimental paradigm, repeated intravenous injection of MWCNTs into male mice caused reversible testicular damage without affecting fertility[16]. After intravenous injections, MWCNTs were found to accumulate in the testes, and the oxidative stress level in the testes increased. MWCNT injection also decreased the thickness of the seminiferous epithelium in the testis at day 15, but both ROS level and the thinning of seminiferous epithelium were repaired by days 60 and 90. The quantity, quality, and integrity of the sperm and the levels of sex hormones were unaffected throughout the 90-day period. Fertility was not affected, as indicated by normal pregnancy and delivery success rates after the animals were mated with untreated female mice.

In vitro nanotoxicity to male reproduction. Nanoparticles have adverse effects on germ cell lines *in vitro*. Normal spermatogenesis is a complex biological process that is highly sensitive to environmental insults. Functional cells such as Leydig cells, Sertoli cells, spermatogonia, spermatids, and spermatozoa are involved in various stages of this

process. Chemicals and ultrafine particles perturb this process either by directly affecting the germ cells or indirectly by acting on the somatic cells in the testis. The effects of diesel exhaust particles, carbon black, and TiO_2 nanoparticles were investigated using the mouse Leydig cell line TM3, which is derived from testosterone-producing cells of the testis[184]. All of these nanoparticles were taken up by Leydig cells, and cell viability, cell proliferation, and gene expression were affected in a pattern that was unique to each nanoparticle. TiO_2 and diesel exhaust particles transiently suppressed the proliferation of Leydig cells, whereas the expression of heme oxygenase-1 (HO-1), a sensitive marker for oxidative stress, was markedly induced by treatment with diesel exhaust particles. Furthermore, carbon black and diesel exhaust particles slightly increased the expression of steroidogenic acute regulatory (StAR) protein, the factor that controlled mitochondrial cholesterol transfer.

The effects of silver (15-nm diameter), aluminum (30-nm diameter), and MoO_3 (30-nm diameter) nanoparticles on spermatogenesis were investigated using the C18-4 cell line, a cell line that was established from type A spermatogonia isolated from 6-day-old mouse testes[185]. Different nanoparticles caused various levels of toxicity to cells. Silver nanoparticles caused cell necrosis and apoptosis at a concentration of 10 μg/mL. The EC_{50} of silver nanoparticles for the reduction of cell viability was 7.75 μg/mL, whereas that of MoO_3 nanoparticles was 90 μg/mL. These results indicate that this cell line is more sensitive to the adverse effects of silver nanoparticles than of MoO_3 nanoparticles. The impact of silver nanoparticles on the proliferation of mouse spermatogonial stem cells, from which germ line cells in the adult testis develop, has also been investigated. Silver nanoparticles at concentrations higher than 10 μg/mL inhibited the proliferation of spermatogonial stem cells[186]. Silver nanoparticles also perturbed glial cell line-derived neurotrophic factor (GDNF)/Fyn kinase signaling, which was indispensable for spermatogonial stem cell proliferation.

Nanotoxicity to female reproduction. Research on nanotoxicity to female reproduction has focused on the effects of nanoparticle exposure on female reproductive organs. TiO_2 nanoparticles have been found to accumulate in the ovaries of mice and rats after *in vivo* or *in vitro* exposure[187-189]. In one study, intragastrically administered TiO_2 nanoparticles (5–6 nm) were shown to accumulate in mouse ovaries and increase the

expression of genes related to estradiol biosynthesis and progesterone metabolism, leading to decreased fertility[187]. However, after oral administration of TiO_2 nanoparticles, no abnormal pathological changes were found in mouse ovaries[188]. *In vitro*, TiO_2 nanoparticles in the ovaries of rats retarded follicle development and oocyte maturation[189].

Nanoparticle exposure can lead to the transplacental absorption of fetuses after implantation[190-192] and can cause toxicity to fetal development that may compromise fertility[193]. These effects will be discussed further in the section on developmental nanotoxicity.

In summary, although the direct exposure of human sperm to nanoparticles does not result in the impairment of semen function, the exposure of spermatogenic cells to nanoparticles leads to toxicity. The exposure of pregnant dams to nanoparticles can cause damage to the male reproductive systems of their pups, suggesting that the developing male reproductive system is especially vulnerable to nanoparticle toxicity. When given to male mice, nanoparticles cause reversible damage to the male reproductive organs, whereas fertility is minimally affected. Nanoparticle accumulation in the mouse ovary disturbs the normal balance of sex hormones. Nanoparticles also decrease fertility by causing toxicity to fetus development.

Developmental nanotoxicity. Developmental toxicity refers to any reversible or irreversible structural or functional alteration in a life form caused by pollutants. These alterations may interfere with physiological homeostasis, normal growth, differentiation, development, or behavior[177]. Because of their wide applications, nanoparticles may pose a threat to developmental safety both in animals and in humans.

Placental permeability. The placenta connects the developing fetus to the uterine wall. It provides nutrients, oxygen, and immunological protection and eliminates waste from the fetus *via* the mother's blood. In utero, the developing fetus is protected against the transmission of toxins from the mother by a placental barrier. This barrier may be compromised in certain diseases or by environmental pollutants. The transfer of nanoparticles from a pregnant mother to her offspring is often suggested as a possible mechanism of developmental toxicity. To investigate this issue, a human placental perfusion model, rodents, and zebrafish (*Danio rerio*) embryogenesis have been used.

Using an *ex vivo* human placental perfusion model, polystyrene nanoparticles were shown to cross the placental barrier but not to affect the viability of the placental explant. The effects were size-dependent, and transplacental activity was only evident for nanoparticles smaller than 240 nm[194]. In another study, polyethylene glycol-coated gold nanoparticles 10-30 nm in diameter were shown not to cross the placental barrier in perfused human placenta[195]. Therefore, placental permeability may depend on the chemical composition as well as the size of nanoparticles.

After intravenous injection into pregnant mice for two consecutive days, silica nanoparticles (70 nm) and TiO_2 nanoparticles (35 nm) accumulated in placental trophoblasts and were transferred to fetuses[192]. Exposure of mice to these nanoparticles by intravenous injection into the tail vein at gestational days 16 and 17 (vaginal plug = gestational day 1) led to 20% to 30% lower uterine weights, smaller fetuses, and a higher fetal resorption rate. Further studies showed that these effects may be caused by dysfunctions of the placenta. Neither fullerene (0.7 nm) nor silica nanoparticles (300 and 1000 nm) entered placental trophoblasts or induced the above consequences.Surface modifications on the silica nanoparticles with carboxyl or amine groups abolished such complications (Figure 10).

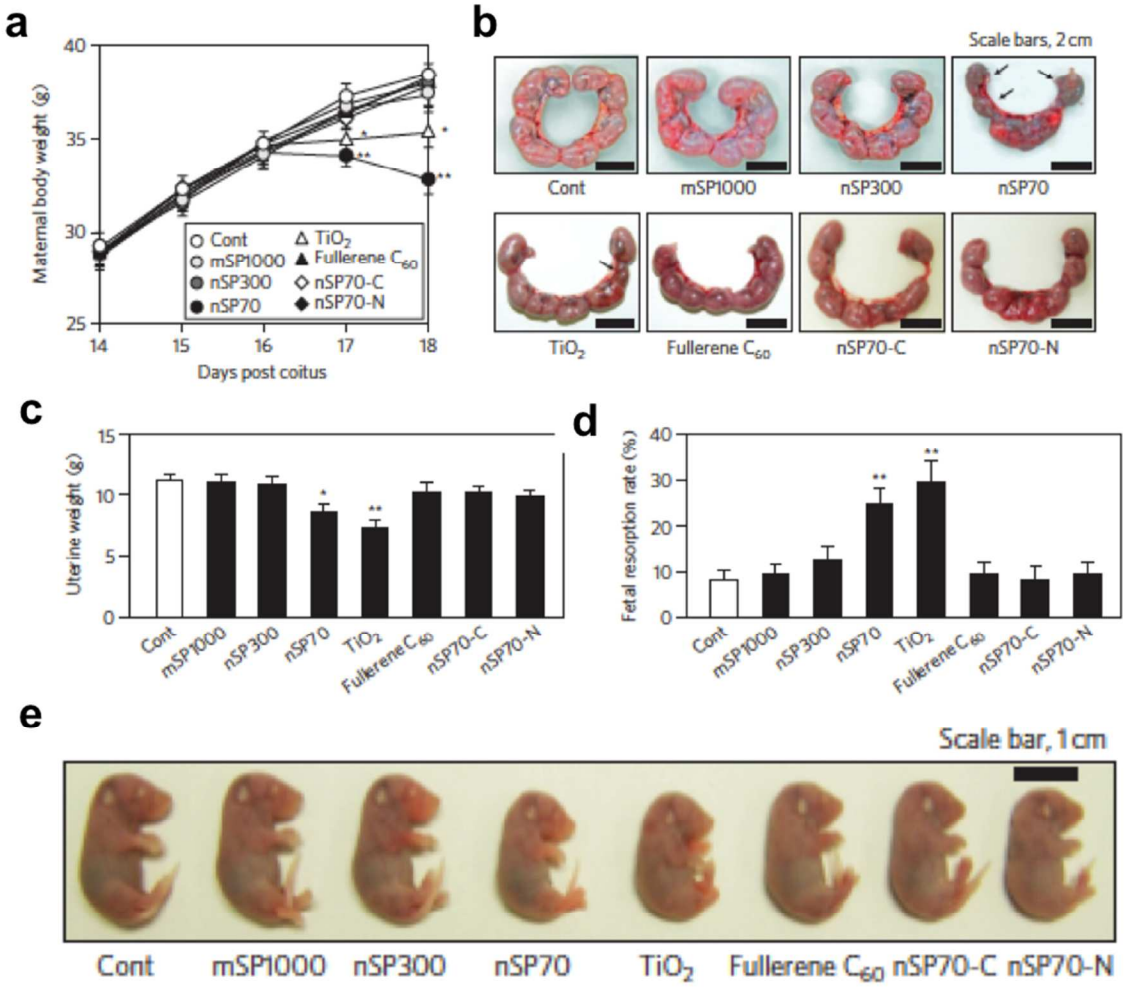

Figure 10. Complications of pregnancy in nSP70- and nano-TiO_2-treated mice. Pregnant mice were treated intravenously with 0.8 mg per mouse of silica particles with diameters of 70 nm (nSP70), 300 nm (nSP300), 1000 nm (mSP1000), nano-TiO_2, fullerene C_{60}, silica particles of 70 nm modified with COOH (nSP70-C) and NH_2 (nSP70-N), or PBS (control) on two consecutive days (GD16 and GD17). (a), Changes in maternal body weight. Maternal body weights were evaluated daily (**n**=11–24). (b)–(e), Pregnancy complications. Uteri from mice were excised at GD18 (b), and uterine weights (c) and fetal resorption rates (d) were evaluated (**n**=11–24). Fetuses (e) were excised from uteri. Reproduced with permission from Reference[192]. Copyright 2011, Nature Group.

In pregnant Sprague-Dawley rats, C60 nanoparticles were shown to penetrate the placenta and to be transferred to fetuses after absorption by the dams[191]. The transplacental passage of CdTe/CdS QDs of different sizes and with different surface cappings has also been reported[190]. Smaller QDs were more easily transferred than larger

QDs, and the QD transfer was dose-dependent. An inorganic silica shell or organic polyethylene glycol layer on QDs reduced this effect but did not eliminate it[190].

Some ^{198}Au-colloidal particles (5 and 30 nm) were transferred to the fetus one hour after intravenous injection to Wistar rats at gestational day 19[196]. Gold nanoparticles 5 nm in diameter exhibited a slightly higher transfer rate than gold nanoparticles 30 nm in diameter. The number of both types of gold nanoparticles in the fetal membrane and placenta was 100–300 times greater than in the fetus, suggesting that gold nanoparticles must cross both the chorioallantoic placenta and the yolk sac placenta to enter the fetus[196]. However, in another study, no ^{198}Au-colloidal nanoparticles (4–200 nm) were found in the amniotic fluid, fetal membranes, or fetus 15 min after a single injection of the nanoparticles into the iliac artery of pregnant Sprague-Dawley rats on gestational days 16–18. At that time point, nanoparticles had already entered the dam's circulation[197]. Similar negative results were also obtained in pregnant C57BL/6 mice 1, 4, and 24 hours after both intravenous and intraperitoneal injections of gold nanoparticles (2 and 40 nm)[12]. These contradictions may result from the use of inconsistent nanoparticle preparation or laboratory protocols. It is likely that nanoparticles can penetrate the placenta in variable amounts.

Placenta begins to develop after the implantation of the balstocyst into the maternal endometrium and matures at about week 12-13 after gestation in human. This process means at different time points of placental development, its protective capability to fetus from exotic toxicants may vary. This hypothesis is testified by a recent study. After exposure of pregnant mice to gold nanoparticles at different embryonic age (embryonic day (E) 5.5, 7.5, 11.5 and 13.5), nanoparticles exposed at the early embryonic age (E5.5 and E7.5) accumulated in fetus body, while at late embryonic age (E11.5 and E13.5), this accumulation dramatically decreased. This time window (between E7.5-E11.5) agrees well with the maturation of placenta barrier at E 10 in mouse[198]. This finding is important as it shows that the exposure of pregnant woman to nanoparticles at the early days of gestation should be especially concerned. Moreover, when the developmental toxicity of other nanoparticles is conducted, the gestation age of animal models should be selected with caution and explicitly indicated.

Toxicity to the fetus. After penetrating the placenta, nanoparticles can be transferred to

the fetus, where they may cause potential developmental toxicity. The developmental toxicity of C60 has been investigated in two studies. In one study, pregnant Slc mice were intraperitoneally injected with C60 nanoparticles on gestational day 10, and the embryos were examined 18 hours after injection[193]. At a dose of 50 mg/kg, C60 nanoparticles were distributed into the yolk sac and embryos, and half of the embryos were deformed in the head and tail regions. At a lower dose (25 mg/kg), abnormal embryos were observed at a lower frequency. At a dose of 137 mg/kg, all embryos died. The authors speculate that C60 nanoparticles severely disrupt the function of the yolk sac and embryonic morphogenesis. In another study, [^{14}C]C60 (0.3 mg/kg) was intravenously injected into pregnant (gestational day 15) and lactating Sprague-Dawley (lactation day 8) rats, and tissues of the dams were collected 24 and 48 hours later. In the pregnant rats 24 hours after injection, 3% of the injected radioactivity was found in the reproductive tract and 2% in the placenta. Radioactivity (0.87%) was also detected in the digestive systems of the fetuses, suggesting that C60 nanoparticles penetrated the placenta and were transferred to fetuses[191]. In lactating dams, radioactivity was detected in the reproductive tract (0.10–0.42%), mammary tissue (0.48–0.94%), and milk 24 hours after injection. It is noteworthy that C60 nanoparticles were also transferred to pups *via* lactation, as indicated by the increased radioactivity in the gastrointestinal tracts of pups 24 hours (0.28%) to 48 hours (0.43%) after injection.

The passage of nanoparticles from dams to fetuses causes damage to the fetus. When pregnant Slc:ICR mice were subcutaneously injected with TiO_2 nanoparticles, their male offspring showed lower body weights at postnatal day 4 and at postnatal week 6[181]. TiO_2 nanoparticles were transferred to the fetal brain and induced apoptosis in the mitral cells of the olfactory bulb[181]. The exposure of pregnant mice to TiO_2 nanoparticles disturbed the expression of genes associated with responses to oxidative stress, mitochondrial stress, and neurological functions in the brains of their pups from embryonic day 16. At later stages (after postnatal day 14), exposure to nanoparticles also altered the expression of genes related to cell apoptosis, inflammation, and neurotransmitter synthesis[199].

The transfer of nanoparticles to pups also adversely affects the offspring's reproductive systems[181] and general quality of life. The immune response of pregnant females is sensitive to air pollution, and this immune response increases the susceptibility

of the females' offspring to immune diseases such as asthma[200]. Exposure to TiO_2 nanoparticles and diesel exhaust particles is pro-inflammatory to pregnant mice and lead to allergy in neonates. After such exposure, increased airway hyperresponsiveness, increased numbers of eosinophils, and pulmonary inflammation are found in offspring[201].

In vitro studies. The developmental toxicity of cobalt ferrite and gold nanoparticles has been investigated *in vitro* using the embryonic stem cell line ES-D3[138]. Cobalt ferrite nanoparticles coated with silanes and gold nanoparticles coated with hyaluronic acid showed some toxicity. However, both of these nanoparticles are less embryotoxic than gold or cobalt ferrite salt[138].

The effects of QD on pre-implantation and post-implantation embryonic development have also been studied[202]. *In vitro* incubation with CdSe QD for 24 hours inhibited pre-implantation development of morulae into blastocysts. QDs also induced apoptosis in mouse blastocysts, leading to blastocyst death, and inhibit their proliferation. Post-implantation blastocyst development has been studied by transferring blastocysts to recipient mice. QD treatment reduced the number of blastocysts reaching later developmental stages and caused the resorption of post-implantation blastocysts and a reduction in fetal weight. These effects were significantly reduced by coating CdSe QD with a ZnS shell[202].

Developmental toxicity of nanoparticles in zebrafish models. Zebrafish is a widely used animal model for exploring human disease, development, and physiology[203]. Its genome has a high degree of homology to the human genome and similar responses to xeno-substances as mammals, such as oxidative stress and induction of foreign body metabolizing enzymes[204]. Exposure to TiO_2 nanoparticles (copper-loaded TiO_2 or pure TiO_2 nanoparticles)[205], TiO_2 nanoparticles under illumination[206], gold nanoparticles (0.8, 1.5 and 15 nm)[207], silver nanoparticles (3, 10, 50 and 100 nm, at concentrations of 100 and 250 μM)[208], copper nanoparticles (70 nm, >0.5 μg/mL)[209], zinc oxide (30 nm, 1-25 mg/mL)[210, 211] or iron oxide (30 nm, >10 μg/mL)[212] all decreased the hatch rate of embryos of fish and led to embryonic morphological malformations. Positively charged gold nanoparticles (1.3 nm) (coated with N,N,N trimethylammoniumethanethiol (TMAT)) also disrupted zebrafish's eye development and pigmentation by disturbing the expression

pattern of related genes like pax6, otx2, rx1 and sox10[213].

Toxicity of silver nanoparticles (40-180 nm) was related more to their surface physicochemical properties rather than their size (Table 4)[129]. Since silver ions were released from the surface of nanoparticles, they might contribute to the observed toxicity[208]. Both TiO_2 and silver nanoparticles also increased the levels of some biochemical indices (for example, glutathione, catalase activity, GST, GSR, and Sel N-1, a gene that is critical for zebrafish development) in embryos[205, 214].

Table 4. Toxicity of silver nanoparticles in zebrafish[215]. Copyright 2013, American Chemical Society.

NP	Stabiliser	Stabiliser Con.	Stabilizing Method	TEM diam (nm)	Shape	DLS diam (nm) in FSEW	zeta potential (mV) in FSEW	LC10, 24hpf	LC50, 24hpf	LC10, 48hpf	LC50, 48hpf
								ppm			
TSC-1	Trisodium citrate	1.25 mM	Electrostatic	46±5	colloid	94.2	-33.2	0.032	15.146	0.004	6.922
TSC-2				110±15	colloid	187.4	-38.6	0.023	18.395	0.003	2.427
PVP-1	Poly(vinylpyrrolidone)	0.1 wt%	Polymeric/ Steric	52±12	colloid	92.3	-3.62	0.004	0.464	0.002	0.061
PVP-2				140±12	colloid	248.3	-6.7	0.004	0.406	0.002	0.228
BIO-1	Gelatin	0.1 wt%	Biopolymeric/ Steric	48±6	colloid	108.2	3.36	0.137	57.41	0.001	5.891
BIO-2				155±17	colloid	260.8	3.69	0.043	43.497	0.005	3.043
Thiol-1	16-Mercaptohexacanoic Acid	10 mM	Thiol/ Electrostatic	53±2	colloid	102.3	-32.2	0.019	6.162	0.003	0.085
Thiol-2				108±9	colloid	144.7	-39.6	0.007	10.53	0.002	0.035
Si-1	Sigma-Aldrich, Cat No. 576832	N/A	N/A	78±24	colloid	297.2	-21.3	0.027	13.76	0.003	0.37
Si-2	Sigma-Aldrich, Cat No. 484059			204±38	colloid	312.1	-14.7	0.003	14.53	0.002	0.046
PI-1	PlasmaChem, PL-Ag150	N/A	N/A	140±23	colloid	212.7	-12.8	0.018	13.37	0.004	0.351
PI-2	PlasmaChem,PL-AgW200			14±4 (W); 181±16 (I)	rod	ND	-11.2	0.005	4.343	0.002	0.205
BBI-1	BBI Life Science, Cat No. EM. SC40	N/A	N/A	42±2	colloid	124.1	-18.5	0.023	5.28	0.003	3.455
BBI-2	BBI Life Science, Cat No. EM. SC80			77±8	colloid	184.6	-19.5	0.012	6.615	0.002	3.091

Diam, diameter. FSEW, filter sterilized egg water (FSEW) at pH 6.8−7.2. LC_{10} and LC_{50}, Lethal concentration for 10% or 50% of fish.

Effects of carbon nanotubes[216, 217], fullerenes[218] and chitosan nanoparticles[219] on zebrafish have also been investigated. Nanoparticles exhibited a dose–dependent

developmental toxicity at a level of microgram per milliliter (μg/mL, equal to ppm). Observed effects include a decreased hatch rate of embryos, embryo mortality, morphological malformations, and disturbed expression of related genes. Nanoparticle surface properties may play a role in the developmental toxicity. Some metal and metal oxide nanoparticles showed a nanoparticle-specific toxicity and ion release was only a minor contributor[220]. Considering the high degree of homology between the human genome and that of zebrafish, these studies may help our understandings of the developmental nanotoxicity.

6. Nanotoxicity to the digestive and urinary systems

Digestion and excretion are processes through which the body takes in energy and eliminates wastes. The physiological functions of the digestive system are to take in, transfer, and digest food; to absorb nutrients; and to excrete waste. The digestive system also provides immunological protection against infection and hypersensitivity. The urinary system is an important excretory system that filters soluble wastes in the blood and regulates electrolytes to maintain acid-base homeostasis in the body. These important systems are vulnerable to many environmental toxins. In this section, we will discuss the effects of nanoparticles on the digestive and urinary systems.

Digestive nanotoxicity. The digestive system consists of the digestive tract and the digestive glands. The digestive tract includes the oral cavity, the pharynx, the esophagus, and the gastrointestinal tract. The digestive glands include the salivary glands, the liver, and the pancreas. Gastric acid secreted by the stomach and various enzymes generated by the epithelial cells of the stomach and intestine assist digestion. The completion of digestive functions depends on the structural integrity of these organs and their coordinated regulation by the autonomic nervous system.

Oral uptake of nanoparticles is a concern because nanoparticles are widely used in the food industry as coloring agents, as food additives, and in food packaging materials[221]. Nanoparticles are also promising oral drug delivery carriers that can be used to increase the bioavailability of protein drugs[222, 223]. Furthermore, environmental pollution involving nanoparticles makes it likely that nanoparticles frequently enter the human digestive

system through drinking water[224]. Nanoparticles absorbed after administration by other routes may also enter the digestive tract. During oral uptake, nanoparticles have only transient contact with the oral cavity, pharynx, and esophagus. The stomach and intestines are the primary organs in which nanoparticles accumulate. Nanoparticles that escape gastrointestinal absorption are quickly eliminated in feces[225]. Therefore, investigations on the digestive toxicity of nanoparticles primarily concern their adverse effects on gastrointestinal functions.

Absorption of nanoparticles by the gastrointestinal tract. The gastrointestinal tract is composed of three layers: a layer of epithelial cells, its coating mucus, and a lamina propria underneath the epithelial cell layer. These three layers together prevent the entrance of harmful antigens and molecules into the blood. The mucus layer is the first defense layer and also serves as a lubricant for intestinal motility. Due to the protection offered by the mucus layer and the tight junctions between the epithelial cells, the rate of absorption of nanoparticles from the gastrointestinal tract is much lower than their rate of absorption when administered by other routes[226]. Under certain pathological conditions, however, the integrity or function of one or more of the layers of the gastrointestinal tract is compromised and the layers become permeable, causing disorders such as inflammatory bowel disease.

The absorption of nanoparticles in the gastrointestinal tract has been studied in rodent and other animal models. Despite their slow absorption, orally administered nanoparticles have been detected in distal organs. Silver nanoparticles orally administrated in rats, and TiO_2 nanoparticles in mouse were all accumulated in the wall of the ileum as well as in the liver, spleen, lung, and brain[188, 225]. After 28 days of oral exposure of Sprague-Dawley rats to silver nanoparticles, the particles were found to accumulate in various organs[171]. Polystyrene nanoparticles were also taken up through gastrointestinal mucosa after gavage feeding, and their uptake efficiency was size-dependent[24]. These findings demonstrate the potential for nanoparticle-based oral drug delivery[227, 228].

However, nanoparticles retained in the gastrointestinal tract may adversely affect its structure and function. For example, chitosan nanoparticles penetrated the mucus and epithelial cell layer and disrupted the tight junctions of the epithelial layer[223]. Ingested

silver nanoparticles were accumulated in the lamina propria of both the small and large intestine, where they disturbed the function of goblet cells and acted as an intestinal secretagogue[229].

Effects on gastrointestinal function. Oral administration of ZnO nanoparticles to mice at a very high dose (5 g/kg) stimulated acute responses including anorexia, vomiting, and diarrhea. Slight inflammation in the stomach and intestine was induced after 2 weeks. Histopathological observation and analysis of blood markers indicated renal damage and anemia[230]. Silver nanoparticles increased the amount of sialylated mucins in gastric mucus, similar to cases of active ulcerative colitis and small intestine carcinoma[229]. Establishing the relevance of these findings to the pathophysiology of the gastrointestinal tract requires further investigation. A lack of adverse effects after oral administration of nanoparticles has also been reported[171].

Nanoparticles may also exhibit positive effects on digestive functions. After intravenous injection of oxidized MWCNTs into mice with disordered gastric function, the MWCNTs were found to be distributed in the stomach and chyme, indicating that they were secreted from mucus cells. In these animals, exposure to MWCNTs decreased nitrogen monoxide content in the stomach to a normal level, increased acetylcholine content in the stomach, and enhanced gastric emptying and motility[231]. This result suggests a potential benefit of MWCNTs in gastric pharmaceutics. However, this finding also poses several questions, including whether this result can be reproduced in other animal models, what molecular mechanism underlies this effect, and what effect other nanoparticles may have. The answers to these questions will further facilitate our understanding of the interactions between nanoparticles and the digestive system.

In an *in vitro* model of the intestinal epithelium and an *in vivo* chicken model, acute oral exposure to polystyrene nanoparticles has been shown to disrupt iron transport in intestinal epithelial cells. In contrast, chronic exposure to these nanoparticles remodeled the intestinal villi and increased the surface area available for iron absorption. Evidently, the remodeling of intestinal villi was able to compensate for the lowered iron transport caused by nanoparticle exposure[232]. In addition to demonstrating a potential impact of nanoparticles on nutrient absorption by the gastrointestinal tract, this report emphasized

the complexity of nanoparticle interactions with the gastrointestinal tract. In lower invertebrate and vertebrate animal models, nanoparticles induce similar effects. In *Nereis diversicolor*; *Mytilus galloprovincialis; Porcellio scaber (Isopoda, Crustacea) and Mytilus edulis*; and zebrafish, nanoparticles of silver, CuO, TiO_2, and nickel accumulated in the digestive glands after dietary exposure, where they induced oxidative stress and destabilized digestive gland cell membranes[233-239]. Silver nanoparticles were also internalized into the animal's gut epithelium[236] and caused damage to its digestive functions[237].

In summary, after entering the digestive system, nanoparticles primarily interact with the stomach and intestines. Nanoparticles that escape gastrointestinal absorption are quickly eliminated in feces. In the gastrointestinal tract, nanoparticles damage intestinal structure and perturb its functions. Depending on their chemical nature, nanoparticles may pass through the tight junctions between intestinal epithelial cells and enter the circulation. The effects of nanoparticles on the gastrointestinal tract after administration by oral and other routes include enhanced gastric emptying and altered nutrient absorption. However, many of the effects on nanoparticles on the digestive system remain unexplored, such as how nanoparticles affect the immunological defense capability of the intestine and whether and how they affect the commensal bacteria that reside in the intestinal canal.

Urinary nanotoxicity. The urinary system consists of two kidneys, two ureters, the bladder, and the urethra. Nanotoxicity to the urinary system is a prioritized concern because nanoparticles readily accumulate in the kidney in addition to the reticuloendothelial system. The kidney is also an important organ for the elimination of nanoparticles[240, 241].

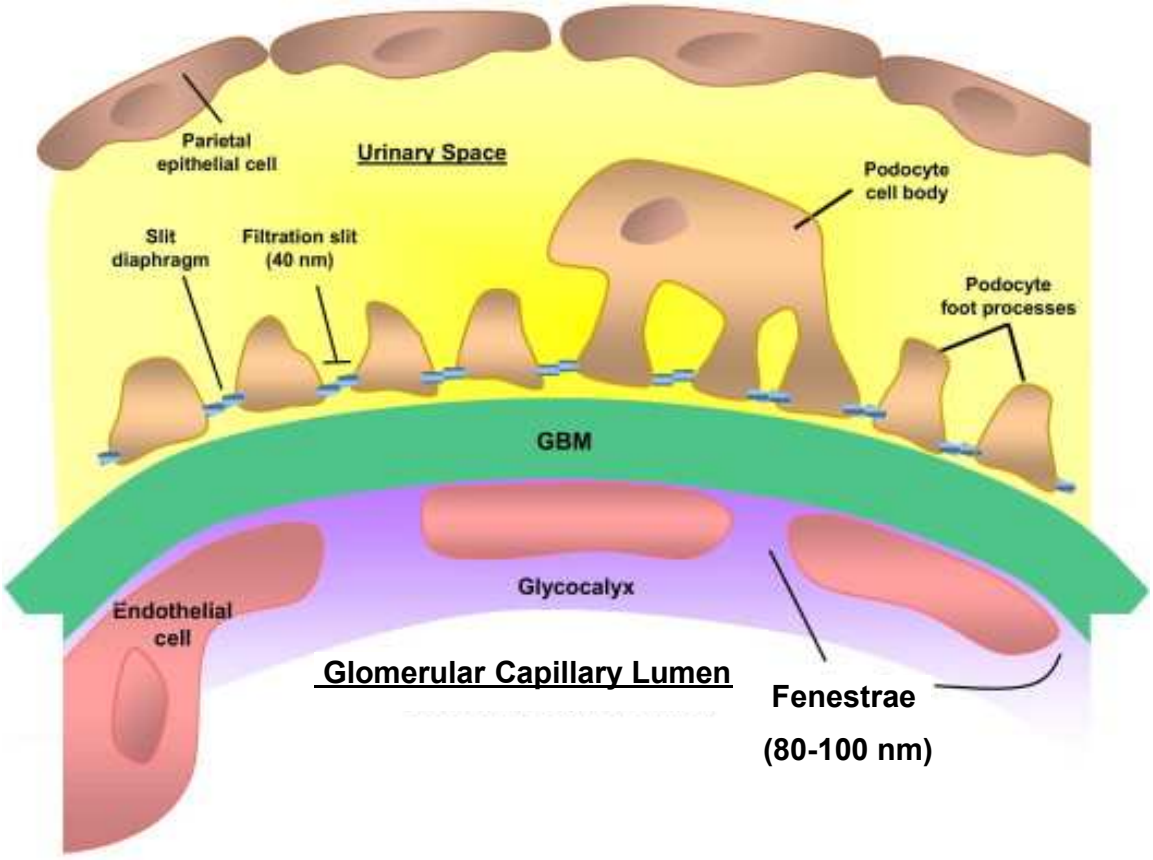

Figure 11. Structure of the glomerular capillary wall in kidney. The glomerular capillary wall consists of three layers: glomerular endothelial cells, glomerular basement membrane (GBM), and podocytes. The capillary wall acts as a glomerular filtration barrier, preventing the translocation of proteins and large molecules from the capillary lumen into the urinary space. The podocyte cell bodies lie with the urinary space, and the cells are attached to the GBM through foot processes. Adjacent foot processes are separated by a filtration slit of approximately 40 nm, bridged by the slit diaphragm. Disruption of the glomerular filtration barrier leads to proteinuria. Reprinted with permission from Reference [242]. Copyright 2011, Elsevier.

Size–dependent accumulation of nanoparticles in the kidney. The kidney is a natural blood filter, and its primary role is to remove wastes by producing urine. Structurally, the kidney comprises barriers with specific pore sizes (Figure 11). After entering the body *via* various portals, nanoparticles are absorbed into the blood and distributed to the kidney. Larger nanoparticles are primarily accumulated in the liver and spleen, whereas small nanoparticles (~ 5-10 nm)[51, 243] may pass these barriers and be rapidly excreted in urine. Larger nanoparticles that diffuse through the glomerular endothelial cell fenestrae (Figure

11) pores 80–100 nm wide are further barred by the glomerular basement membrane and the podocyte foot processes[244]. The glomerular basement membrane, together with the podocyte foot processes, imposes an apparent cutoff size of approximately 10 nm or molecular weight of 30-50 kDa. However, there are exceptions to this rule.

Probably due to their needle-like shape, some SWCNTs can penetrate the physical barriers present in the kidney and undergo excretion in urine. For example, within one minute after intravenous injection, SWCNTs (0.8-1.2 nm in diameters and 100-500 nm in length) were found in the bladders of mice[245]. After intravenous injection to mice, 50% of a preparation of SWCNTs were excreted in urine within 24 hours[246]. After intraperitoneal injection, SWCNTs were also detected in mice urine after 18 days[247]. Some spherical nanoparticles much larger than the suggested "cutoff size" can also be excreted in urine. For example, parenterally administrated magnetic nanoparticles approximately 100 nm in size were found in animals' bladders by magnetic resonance imaging, indicating that they were partially excreted in urine[248, 249]. Fluorescence-labeled silica nanoparticles 50 and 100 nm in size were also detected in urine as early as 12 hours after intravenous injection[250]. These findings suggest that some "larger" nanoparticles may bypass the glomerular filtration system of the kidney by unknown mechanisms. For needle-like nanoparticles, a mathematical model of rotational diffusivity was proposed to account for the observation.[162] However, this model did not explain behaviors of spherical nanoparticles. The material-dependent barrier-crossing capability of nanoparticles and nanoparticle-induced damages and malfunctions in the glomerular filtration barrier should be tested to understand more in this aspect.

Renal responses to nanotoxicity. The accumulation of nanoparticles in the kidney increases the kidney burden. However, studies have indicated that this organ is relatively insensitive to the adverse effects of nanoparticles. Daily intraperitoneal administration of naked gold nanoparticles (12.5 nm) of different doses (40, 200, and 400 μg/kg/day) to mice for eight consecutive days caused neither systemic toxicity nor changes in the levels of urea nitrogen or creatinine in blood[251]. Even at systemically toxic doses, nanoparticles may not exhibit toxicity to the kidney. Although intraperitoneal and intravenous injection of N-octyl-O-sulfate chitosan nanoparticles into mice led to systemic toxicity, as indicated by body weight loss and decreased food consumption, no associated

histopathological alterations were seen in the kidneys[252].

However, in other investigations in which very high doses were used, adverse effects of nanoparticles on the kidney have been reported. Intraperitoneal injection of TiO_2 at 1944 or 2592 mg/kg induced renal glomerular swelling and slight histopathological lesions in the kidneys of mice. Even at these high doses, TiO_2 nanoparticles did not affect blood urea nitrogen levels[253]. The kidney is one of the primary organs targeted by copper nanoparticles. A single oral gavage of copper nanoparticles (23.5 nm) at a dose of 232 mg/kg in mice caused dose-dependent pathological changes and grave injury to the kidneys and changed the blood urea nitrogen level. The toxicity to the urinary system was gender-dependent; male mice show more severe symptoms than female mice[254].

In summary, the glomerular filtration barrier in the kidneys prevents large nanoparticles (>10 nm) from being excreted in urine, with a few exceptions. Some needle-like nanoparticles bypass this barrier and enter the urine. Although the kidney is generally less sensitive to nanotoxicity than other organs, copper nanoparticles cause dramatic renal toxicity.

7. Nanotoxicity to the nervous and endocrine systems

The nervous and endocrine systems are essential for communication with the environment and the regulation of bodily functions. The nervous system uses networks of neurons that rapidly transmit signals between the brain and body; the endocrine system utilizes glands located throughout the body to regulate activities such as metabolism, digestion, blood pressure, and growth by secreting hormones. These two important systems converge at the hypothalamus, a part of the brain that controls the autonomic nervous system through neurons that project to the lateral medulla to regulate the behavior of major organs. Neurons in the hypothalamus also secrete a variety of hormones that impact the pituitary gland and regulate the body's endocrine network.

Nanotoxicity to the CNS. The human central nervous system (CNS) is protected by two physiological barriers: the blood–brain barrier (BBB) and the blood–cerebrospinal fluid barrier (BCSFB)[255, 256] (Figure 12). These barriers protect the microenvironment of the CNS from disturbance by hazardous xenobiotics. Although these barriers provide

crucial protection for the CNS, they make the delivery of CNS therapeutics difficult. Due to their small size, nanoparticles are able to penetrate these physiological barriers and reach the CNS. On one hand, this ability makes nanoparticles highly promising agents as potential therapeutic carriers for the treatment of CNS diseases[257]; on the other hand, it raises concerns regarding their possible unwanted toxic effects on the CNS.

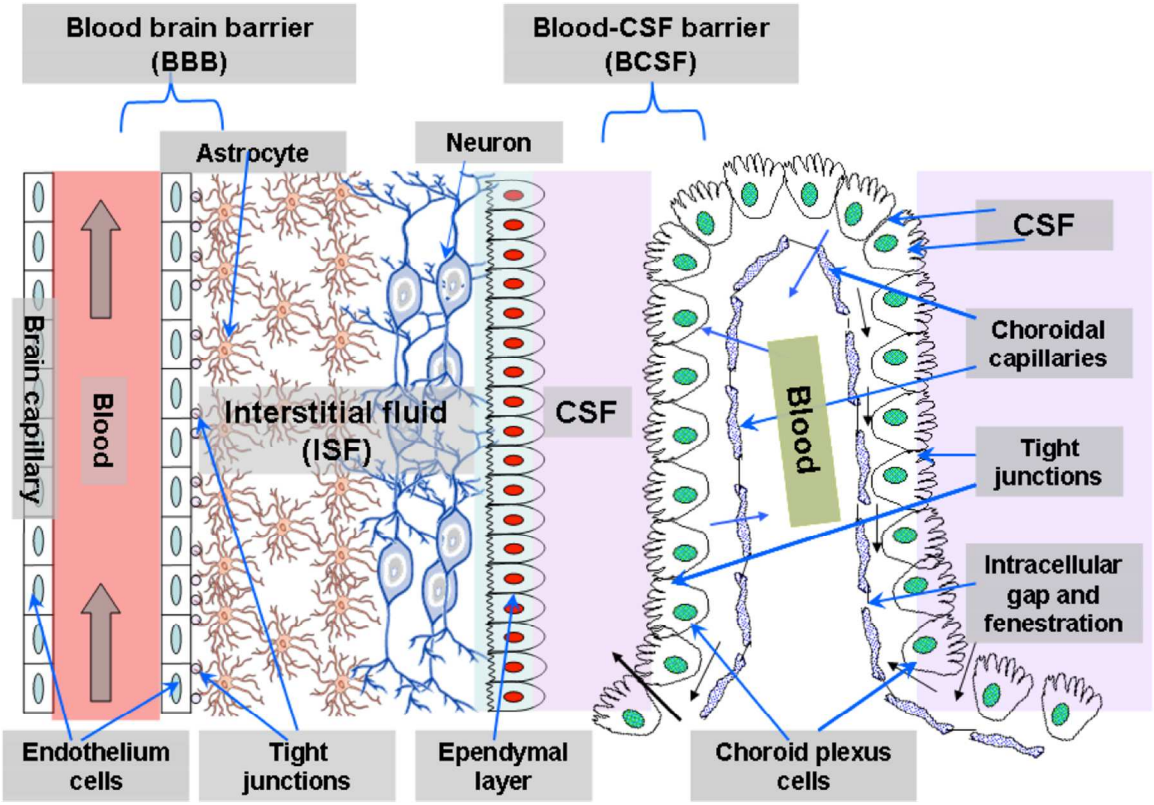

Figure 12. Overview of the two main barriers in the CNS. ISF: Interstitial fluid; CSF: Cerebrospinal fluid. Reprinted with permission from Reference [258].

Nanoparticle translocation into brain. The ability of nanoparticles to penetrate the BBB has been demonstrated in various experimental models, including an *in vitro* BBB model, rodent models, and other vertebrates like freshwater fish. There are at least three modes by which nanoparticles enter the CNS. First, nanoparticles can penetrate the BBB without damaging its integrity. CdSe/CdS/ZnS quantum rods coated with various biomolecules[259], iron oxide nanoparticles coated with a biocompatible chitosan copolymer[260], and magnetic nanoparticles coated with silica[261, 262] penetrate the BBB without affecting its functions. These nanoparticles may be ideal candidates for drug

delivery carriers for diagnosis and therapy of CNS diseases. Their ability to cross the BBB probably depends on their size. A study of the biodistribution of gold nanoparticles of various diameters after injection into the tail vein of rats demonstrated that whereas 0.3% of gold nanoparticles 10 nm in diameter were distributed in the brain after 24 hours, no gold nanoparticles with diameters of 50, 100, or 250 nm were detected[17].

Second, nanoparticles can penetrate the BBB by disrupting its integrity. This behavior has been shown by administering silver, aluminum, and copper nanoparticles to rats by various routes and testing the disruption of the BBB using Evans blue or radioiodine. The results show that leakage of Evans blue or radioiodine was found in different parts of the brain depending on nanoparticle, dose, and route of administration[263]. Compared to aluminum nanoparticles at equivalent doses, the disruptive effect was most prominent in animals treated with silver and copper nanoparticles[263]. Nanoparticles also enhance stress-induced BBB disruption. For example, silver and copper nanoparticles 50-60 nm in size exacerbated the BBB breakdown induced by hyperthermia and led to more severe cognitive dysfunction and brain pathology in rats[264]. Brain microvessel endothelial cells form the major component of the BBB. Therefore, most mechanistic investigations have focused on disturbance by nanoparticles of the functions of microvessel endothelial cells. CNS inflammation and functional abnormalities caused by nanoparticles may also be attributed to nanoparticle-induced perturbation of microvessel endothelial cells. In an *in vitro* BBB model involving primary rat microvessel endothelial cells, the application of silver nanoparticles increased BBB permeability and induced pro-inflammatory responses[265]. Treatment of human microvessel endothelial cells with Al_2O_3 nanoparticles reduced cell viability and downregulates the expression of tight junction proteins such as JAM-A, ZO-1, and ZO-2[266]. This treatment also caused fragmentation of the tight junction proteins claudin-5 and occludin *in vivo*.

The third route by which nanoparticles can translocate to the brain is along the olfactory nerve pathway, bypassing the BBB. Fe_2O_3 and TiO_2 nanoparticles administered by intranasal instillation[15, 267] and manganese oxide nanoparticles administered by respiratory exposure[14] can enter the brain by this route.

Behavioral disorders caused by nanoparticles. Behavior observation in mature

animals is a gold standard for evaluating perturbation of the CNS. After treatment with silver, copper, or aluminum nanoparticles by intraperitoneal injection, rats show mild to moderate deficits in cognitive and sensory-motor functions, as evidenced by poor performance in rotarod, grid walking, inclined plane angle, and footprint analysis tests. Moreover, more malfunctions are observed in animals when nanoparticles are administered by intravenous, intracarotid, or intracerebroventricular routes than when intraperitoneal administration is used. These results indicate that nanoparticles disrupt the protection of the brain by the BBB and induce brain damage, leading to sensory-motor dysfunction[268, 269]. In contrast, silica nanoparticles do not cause CNS disorders, probably due to their milder disruption of the BBB[270].

Nanoparticles are toxic to the CNS in two ways. First, the presence of nanoparticles in the cerebral compartment leads to oxidative stress and inflammation, causing damage to nerve cells in the brain. Because of the weak antioxidant capability and limited self-regenerative ability of neurons, the damage caused by nanoparticles in the CNS may be more severe than in other tissues. Second, the breakdown of the protective CNS barriers by nanoparticles[269] potentially enables the passage of biological molecules and toxic substances to the brain fluid microenvironment, leading to CNS toxicity.

Effects on neurotransmitters. Impaired CNS function is an important outcome of nanoparticle-induced neurotoxicity. Dopamine is a major neurotransmitter in neural systems. Its release from nerve cells provides signals that regulate movement and emotional responses. Both *in vitro* and i*n vivo* studies have demonstrated that exposure to some types of nanoparticles can deplete dopamine. Silver nanoparticles led to dopamine depletion in PC12 cells by perturbing intracellular redox balance, and manganese and copper nanoparticles affected the expression of enzymes involved in dopamine metabolism[271, 272]. PC12, a dopaminergic neuronal cell line, has been used as a cell model for *in vitro* neurotoxicity studies. Treatment of PC12 cells with SiO_2 nanoparticles caused a decrease in dopamine levels due to the downregulation of dopamine synthesis[270]. In rats, the depletion of dopamine caused by SiO_2 nanoparticles significantly reduced dopaminergic activity in the striatum[270]. These findings are especially alarming because dopamine depletion causes movement disorders characteristic of Parkinson's disease in mice[273]. Treatment with SiO_2 nanoparticles also affected the differentiation of PC12 cells

to neuron-like cells, as indicated by limited neurite extension following nerve growth factor induction[270]. Some metal or metal oxide nanoparticles may also cause neurotoxicity by releasing free ions. For example, after the exposure of *Cyprinus carpio* to CuO nanoparticles, released free Cu2+ ions inhibited the activity of cholinesterase, an important enzyme that hydrolyzes the neurotransmitter acetylcholine at cholinergic synapses[274].

Nanotoxicity to nerve cells. After penetrating the BBB, nanoparticles induce morphological alterations of nerve cells in the cerebral cortex, hippocampus, cerebellum, thalamus, hypothalamus, and brainstem and cause damage to myelinated fibers as well as the degeneration of nerve cells[275]. Silver and copper nanoparticles cause more neuronal changes than aluminum nanoparticles, and the hippocampus seems to be the most adversely affected organ by these nanoparticles in the brain[268, 276]. After intranasal instillation of SiO_2 nanoparticles in Sprague-Dawley rats, the nanoparticles traversed the olfactory bulb to the striatum, where they induced oxidative damage and inflammatory responses[270]. After female mice were exposed to TiO_2 nanoparticles via intranasally instillation for 30 days, size- and surface coating-dependent effects, such as the morphological changes of neurons and impaired production of monoamine neurotransmitters in the sub-brain regions were observed[274]. Mechanistically, TiO_2 nanoparticles caused a significant increase in lipid peroxidation and the levels of tumor necrosis factor alpha (TNF-α) and interleukin (IL-1ß) in the hippocampus[15].

In addition to tissue damage, nanoparticles cause perturbations of nerve cells. Brain cells include neurons and glial cells. Using PC12 cells as an *in vitro* neuron model, the length of MWCNTs was found to determine neuron's responses. Short MWCNTs (less than 2 μm) promoted the neuronal differentiation of PC12 cells, while longer MWCNTs (more than 30 μm) were retained in cells and caused damages [277]. SiO_2 nanoparticles at a dose of 200 μg/mL changed the morphology and decreased the viability of cultured PC12 cells. These effects were associated with the nanoparticle-induced disruption of cytoskeletal structures and an increase in cellular oxidative stress[270]. TiO_2 nanoparticles led to G2/M cell cycle arrest and cell apoptosis in PC12 cells by inducing oxidative stress[278]. Magnetic nanoparticles also induce a dose-dependent decrease in the viability of PC12 cells[279].

With their phagocytic capability, microglia constitute the first line of defense against invading pathogens or nanoparticles in the brain. They are also involved in the recruitment of immune cells from the periphery into affected areas[280]. Nanoparticles are significantly taken up by microglia in the brain, resulting in a decrease in their viability[281]. The uptake of TiO_2 nanoparticles by mouse microglial BV2 cells induced an increase in intracellular ROS and overexpression of genes involved in inflammation, cell cycle regulation, and apoptosis[282, 283]. In both studies, Fe_2O_3 nanoparticles were accumulated in different regions of mouse brain after intranasal exposure. They stimulated the release of reactive oxygen species from microglia and led to pathological alterations in CNS tissues[267, 284]. Like glial cells in CNS, astrocytes were specifically targeted and activated by TiO_2 nanoparticles[285].

Nanoparticle effects on the CNS may also involve other complicated interactions. Silver nanoparticles decreased the viability of human glioblastoma U251 cells by causing mitochondrial toxicity and DNA damage[286]. Nanoparticles have also been found to cause toxicity by influencing the electrophysiological properties of neurons. For example, CdSe QDs (2.38 nm) caused cell death in primary rat hippocampal neurons by increasing intracellular Ca^{2+} levels[287], and ZnO (20-80 nm) and CuO (60.6 nm) nanoparticles enhanced the excitability of neurons by disturbing K^+ currents[288, 289].

In summary, due to their small size, nanoparticles in circulation can enter the CNS. In the brain, nanoparticles damage both neurons and glial cells by inducing inflammation and cell apoptosis and by influencing the electrophysiological properties of neurons. Nanoparticles also affect nerve cell functions such as neurotransmitter release. As a consequence, nanoparticle exposure can lead to behavioral disorders in animals.

Endocrine nanotoxicity. The endocrine system works in conjunction with the nervous system to maintain physiological homeostasis. The endocrine system includes a series of glands, including the hypothalamus, pineal body, pituitary gland, thyroid and parathyroids, adrenals, testes, ovaries, and pancreas. Through the secretion of hormones, these glands regulate various important human functions ranging from metabolism to development to mood control. Dysregulation in hormone release or hormone function leads to various disorders. Compounds that alter the normal function of the endocrine

systems of animals and humans are called endocrine-disrupting chemicals (EDC). EDCs interfere with the synthesis, transport, metabolism, and elimination of hormones or disturb hormone receptor functions by directly binding to the receptors. Some pesticides and herbicides are EDCs. These compounds are found to increase hormone-dependent cancer risks (breast and prostate cancers)[290, 291], perturb immune system function[292], impair male fertility, and cause developmental toxicity[293]. The rapidly increasing applications of nanotechnology-based products have raised concerns regarding nanoparticles' potential toxicity to endocrine functions and questions regarding whether some nanoparticles might be similar to EDCs (Figure 13). The early research in this area has been primarily concentrated in the field of reproductive endocrine function.

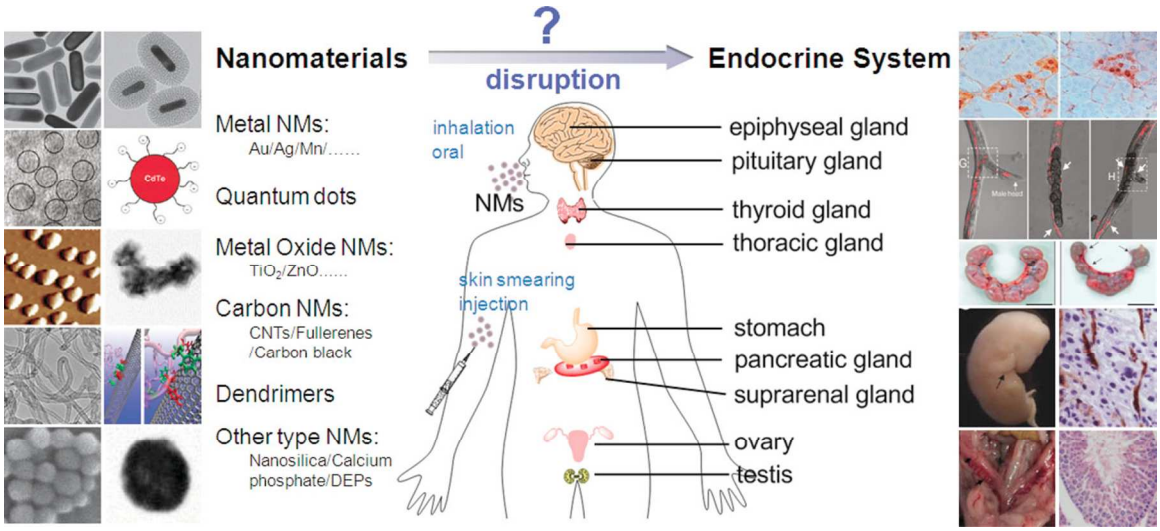

Figure 13. Nanoparticles enter the human body and pose a threat to human endocrine functions. Nanoparticles are shown on the left. On the middle and the right, various endocrine organs and possible adverse outcomes are indicated. Reproduced with permission from Reference [294]. Copyright 2013, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Effects of nanoparticles on the reproductive endocrine system. The function of the endocrine glands depends on the physiological conditions, and damage to these organs may affect endocrine functions. Important endocrine glands, for example, the hypothalamus, pineal and pituitary glands, and testes, are protected by the blood-brain or blood-testes barriers, whereas other glands have free access to nanoparticles in the blood. The biodistribution of nanoparticles in the endocrine glands and the related histopathological changes provide us with valuable insights. Radioactive copper (Cu^{64})-

labeled MWCNTs have been shown to accumulate in the testes of mice. The accumulation caused short-term damage to the testes, but this damage was repaired after 60 days[16]. In F344 rats, silver nanoparticles (56 nm) entered the adrenal gland, the thyroid gland, the ovaries, and the testes in a dose-dependent manner. However, at the applied doses, they did not cause apparent damage, as evidenced by organ indices or histopathology[295].

Nanoparticle exposure induces ROS and damage to reproductive organs, whereas it rarely alters sex hormone levels. After the intravenous injection of five doses of MWCNT into male mice, the levels of testosterone, luteinizing hormone, and follicle-stimulating hormone in serum were not changed[16]. The exposure of pregnant ICR mice to carbon black (14 nm) caused alterations in the male offspring, including a decrease in daily sperm production and damage to the seminiferous tubules, but did not decrease the level of serum testosterone[183]. In a 13-week evaluation of female zebrafish, exposure to TiO_2 nanoparticles (240-360 nm) decreased the number of eggs produced. A microarray analysis showed that although TiO_2 nanoparticles induce changes in the expression of thousands of genes in ovarian tissues, very few of these genes were the same as those whose expression is altered by EDCs[296]. These results indicate that TiO_2 nanoparticles may not perturb female reproductive functions at the hormonal level.

Despite the above findings, the issue of whether nanoparticles act as endocrine disruptors is still controversial because some investigations find they disturb hormone production. The addition of gold nanoparticles (10 nm) to cultures of rat ovarian granulosa cells increased the accumulation of estradiol-17 beta in the medium[297]. Carbon black (14 nm) increased the expression of steroidogenic acute regulatory protein, a factor needed for testosterone biosynthesis, in mouse testicular Leydig cells[184]. These *in vitro* outcomes have been confirmed by *in vivo* studies. The intratracheal administration of carbon black (14 and 56 nm) to male ICR mice increased testosterone levels[182]. The exposure of male F344 rats to nanoparticle-rich diesel exhaust (NR-DE) for one or two months impaired the function of Leydig and Sertoli cells, lowered plasma luteinizing hormone levels, and increased plasma immunoreactive inhibin concentrations[298]. NR-DE also increased the plasma concentrations of testosterone, progesterone, and corticosterone[298]. Furthermore, NR-DE stimulated the overexpression of proteins needed

for the biosynthesis of testosterone *via* a growth hormone receptor-mediated pathway[299]. However, comparative studies of NR-DE and filtered diesel exhaust suggested that adsorbed compounds, rather than the particles themselves, were responsible for these effects[300].

In vitro studies also suggest that nanoparticles do not impair effector cells' responses to hormones. Using renal epithelial cells, none of three carbon-based nanoparticles (fullerenes, SWCNTs, and MWCNTs) altered cellular responses to antidiuretic hormone, a hormone known to regulate both Na^+ and Cl^- in the principal cells[301].

In summary, nanoparticles' effects on reproduction have received much attention, whereas their effects on other endocrine functions have been less well studied. MWCNTs and carbon black accumulate in the testes of mice without affecting these organs' endocrine functions. Gold nanoparticles in rat ovarian granulosa cells increase the accumulation of estradiol-17 beta in the medium. Carbon black particles increase the expression of steroidogenic acute regulatory protein, a factor needed for testosterone biosynthesis, in mouse testicular Leydig cells.

8. Hepatoxicity caused by nanoparticles

The liver is a multi-functional organ in the human body. Its central role is to clear xenobiotic chemicals from the body by reducing their fat solubility and by changing their biological activity. This function is primarily carried out by a group of enzymes that are collectively termed cytochrome P450 (CYP). All blood coming from the stomach and the small intestine goes through the liver. In the liver, nutrients are broken down into forms that are easy for body tissues to use. The liver also aids the digestive system by secreting bile to help fat digestion. Furthermore, the liver is important for the storage and production of glucose, fatty acids, and iron. Thus, damage to the liver leads to a series of physiological consequences.

The liver is a major organ for nanoparticle accumulation because it is a reticuloendothelial system[302]. In the liver, both hepatocytes and Kupffer cells take up nanoparticles with a selectivity that is probably based on the surface properties of the nanoparticles[303] (Figure 14). Nanoparticles can be excreted from the liver *via* the biliary

pathway. For example, two months after intravenous injection, SWCNTs were partially cleared in feces[39]; surface charge on the particles accelerated their secretion rate[35]. Eleven days after exposure, approximately 5% of total hydroxylated SWCNTs administered intraperitoneally were excreted in feces[247].

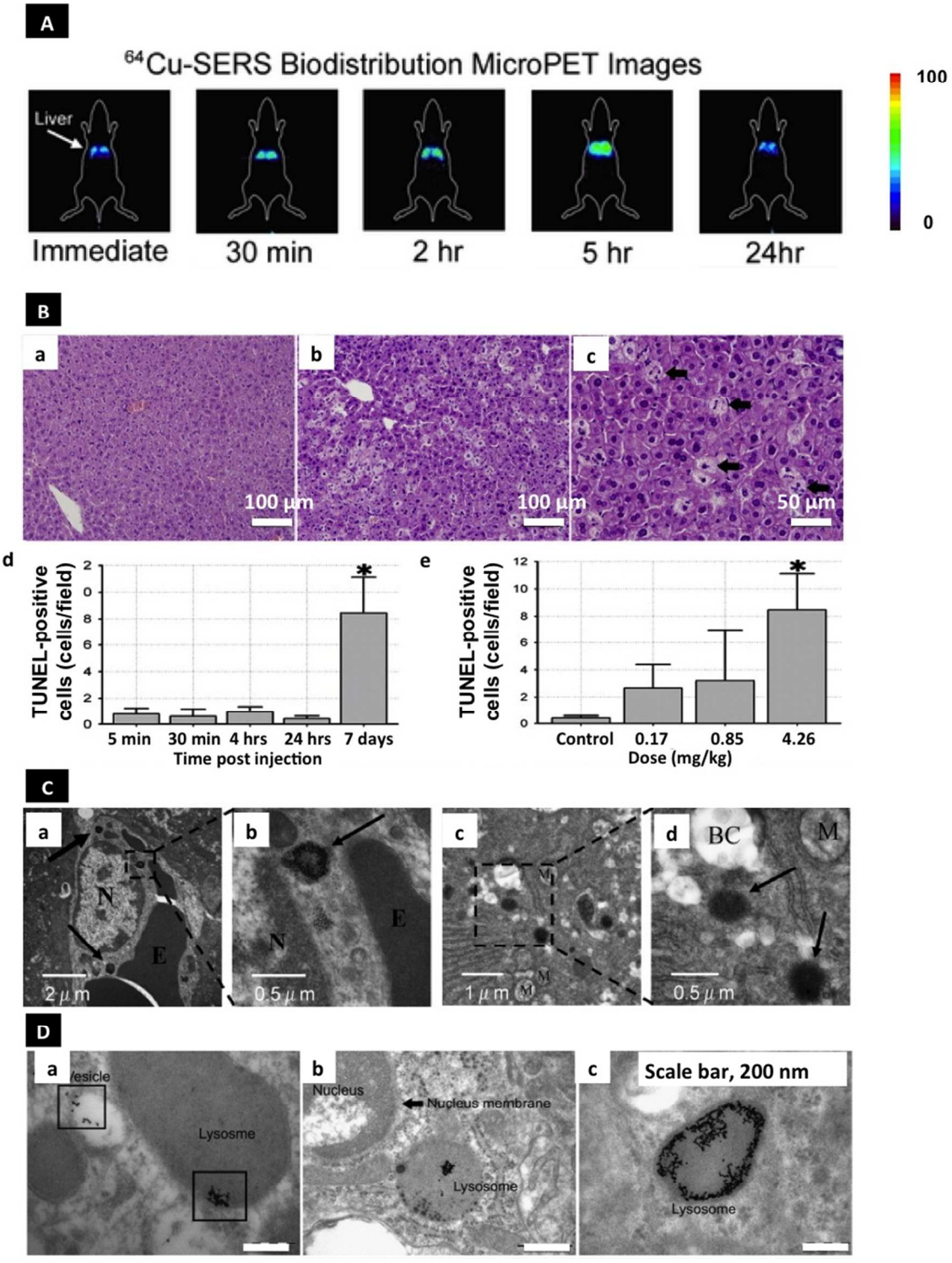

Figure 14. Nanoparticle uptake by Kupffer cells and hepatocytes in the liver. From upper to lower, the panels show the distribution of nanoparticles in the liver as observed at different levels. (A) Organ level distribution. At various times after the intravenous injection of gold surface-enhanced Raman scattering (SERS) nanoparticles radiolabeled with ^{64}Cu (100 μCi), mice were imaged using microPET. Nanoparticles primarily accumulate in the liver. (B) Tissue-level distribution and histopathology after hematoxylin and eosin staining of mouse liver following intravenous injection of 13-nm polyethylene glycol-coated gold nanoparticles *via* the tail vein. (a) and (b) show tissue sections prepared from control and treated mice 7 days after injection; (c) shows a higher magnification of liver tissue from a treated mouse. The arrows indicate apoptotic necrosis and acute inflammation. (d) Time course quantification of TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling)-positive cells. (e) Dose-dependent TUNEL-positive cell quantification. (C) Cellular-level distribution. Transmission electron microscopic images show nanoparticles (arrows) in endosomes of Kupffer cells (a, b) and hepatocytes (c, d) 45 min after the intravenous injection of iron oxide core HDL (high-density lipoprotein) nanoparticles into mice. N, nucleus; E, erythrocyte; M, mitochondria; BC, bile canaliculi. (D) Subcellular distribution. Thin-section transmission electron microscopy images of a mouse liver show the intracellular localization of polyethylene glycol-coated gold nanoparticles after intravenous injection. Reproduced with permission from References [304-306]. Copyright 2011, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. Copyright 2009 and 2011, Elsevier.

Although the liver possesses a self-protecting capability due to its antioxidant system and its various metabolizing enzymes, the long-term retention of nanoparticles increases the risk of hepatotoxicity[188, 307] (Figure 14). TiO_2, CNT, and SiO_2 nanoparticles induced hepatotoxicity, as indicated by abnormal serum levels of liver function indicators such as aspartate aminotransferase and alanine aminotransferase[188, 308]. This finding suggests that hepatocytes were injured by the retention of nanoparticles. Histopathologic examination confirmed these injures; bile duct hyperplasia, congestive dilation of the central veins, necrosis, fibrosis, and abnormal pigmentation can often be seen[295, 309]. Nanoparticles may also aggravate stress-induced apoptosis in liver, suggesting that they may be especially dangerous to patients with liver disease[310].

Only a few investigations have studied the effect of nanoparticles on liver functions. The cytochrome P450 family includes thousands of enzymes in animals (including human)[311], and most of those functioning to metabolize endogenous and exogenous toxic compounds principally locate in the liver. These enzymes account for approximately 75% of the metabolic reactions that take place in the human body and play important roles in

protecting the body from injuries caused by toxins. Orally administered silver nanoparticles did not have adverse effects on the activities of CYP1A, CYP2C, CYP2D, CYP2E1, or CYP3A in rats after exposure to a dose of 1.0 g/day for 14 continuous days. However, these nanoparticles inhibited the activity of CYP2C and CYP2D in rat liver microsomes at low doses (IC_{50} < 30 μg/mL)[312]. This pilot research only provides an indication of nanoparticles' effects on the metabolizing capability of the liver; it is possible that *in vivo,* the effects of silver nanoparticles on CYP enzymes are mitigated by additional protective mechanisms.

The effects of nanoparticles on other functions, such as the secretion of bile, the synthesis of glucose and fatty acids, and blood iron content, are largely unknown. Impairment of these functions may lead to symptoms such as fatigue, depression, and loss of appetite, all of which are often observed when animals are exposed to nanoparticles[313]. This finding suggests that nanoparticle exposure is a concern, especially for populations with liver disease, diabetes, or obesity.

In summary, both hepatocytes and Kupffer cells of the liver internalize nanoparticles. Nanoparticles cause hepatotoxicity and adversely affect the function of enzymes in the cytochrome P450 family. The effects of nanoparticles on other liver functions, such as the secretion of bile, the synthesis of glucose and fatty acids, and blood iron content, remain largely unknown.

9. Nanotoxicity to skin and bone

Skin and bone are important protective and supportive tissues of the human body. In this section, we will summarize the current understanding of potential nanotoxicity to these systems.

Nanotoxicity to skin. As the largest organ in the human body, skin interfaces with the environment and functions to defend and maintain normal physiological conditions. Structurally, skin consists of two primary layers: the epidermis and the dermis. The outermost sublayer of the epidermis is the stratum corneum (SC), which is composed primarily of keratinocytes. Drugs, toxins, or even nanoparticles may penetrate the skin barrier by targeting skin appendages (including hair, sweat glands, and hair follicles),

which are surrounded by networks of capillaries (Figure 15A).

Contact with nanoparticles in air, the use of nanoparticle-based clothing or nanoparticle-based skin products (for example, shampoos, lotions, and sunscreens), and transdermal therapeutic systems are the primary routes by which skin is exposed to nanoparticles. To determine whether nanoparticles can cause dermal toxicity, three key questions must be answered: 1) whether various nanoparticles can penetrate the dermal layers, 2) whether the contacting nanoparticles have adverse effects on dermal structure and function, and 3) whether skin-penetrating nanoparticles cause systemic toxicity. These questions are addressed below.

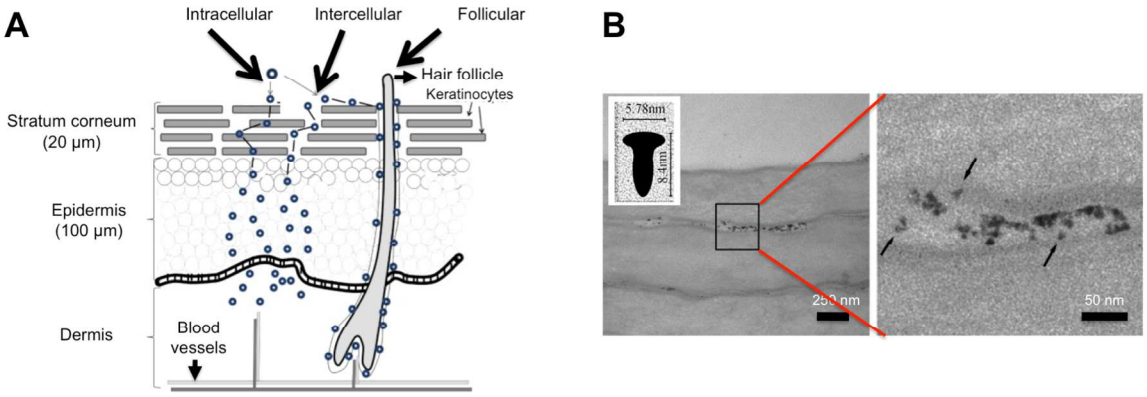

Figure 15. The probable pathways by which nanoparticles penetrate the dermal barrier. (A) A simplified schematic showing the structure of skin and three potential transdermal pathways for nanoparticles: intracellular, intercellular, and follicular routes. After passing through the stratum corneum, nanoparticles reach the epidermis and dermis. From the dermis, they may gain access to the blood circulation. (B) Nail-shaped quantum dots accumulated in the stratum corneum layer of porcine skin as observed by transmission electron microscopy. Reproduced with permission from References [314, 315]. Copyright 2008, American Chemical Society; Copyright 2008, 2012, Elsevier.

Transdermal passage of nanoparticles. The SC layer of the skin provides efficient protection against the intrusion of various nanoparticles. Because of this protective function, most nanoparticles administered to the skin are completely blocked by the intact SC layer. For example, although smaller SiO_2 nanoparticles (42 nm) can reach the SC layer of human skin explants, particles above 75 nm in size neither entered skin cells nor penetrated the SC layer[316]. QDs of different sizes were blocked by the SC layer of rat skin after exposure[317]. Even after mechanical flexing to facilitate penetration, QDs did not

pass the SC layer[315]. Human skin exhibits an even lower permeability than the skin of animals[318]. This property explains the observation that whereas neither intact nor abraded human skin was permeable to QDs[319], QDs penetrated deeply into abraded rat skin[320]. TiO_2 nanoparticles present in a mineral sunscreen only accumulated in the SC layer of human skin, as analyzed by a tape-stripping method[321]. Investigation by transmission electron microscopy showed that Cd/Se QDs primarily resided in the intercellular lipid bilayers of the SC[315] (Figure 15B). Very low penetration of gold nanoparticles of various sizes (10, 30, and 60 nm) has been observed in viable excised human skin after 24 hours[322].

The protective function of the SC layer against nanoparticles has been further confirmed by experiments with abraded skin. Ultraviolet radiation decreases the epidermal calcium gradient and disrupts SC lipids, thereby enhancing nanoparticle penetration into the skin. For example, commercial carboxyl QDs of 30 nm penetrated into the dermal layer of UV-irradiated SKH-1 mouse skin[323]. After the exposure of skin-abraded mice to polyethylene glycol coated Cd/Se QD (37 nm) for 24 and 48 hours, the QD were detected in the lymph nodes and liver, suggesting that they were absorbed into the circulation[324].

As noted above, some nanoparticles can reach the dermal layer. QDs (4.6 nm) coated with polyethylene glycol penetrated the epidermis and reached the dermis of porcine skin over a time window similar to that of occupational exposure (8 and 24 hours). Nanoparticles of smaller size and higher hydrophobicity appear to have greater penetrating ability[325]. The dermal penetration of other nanoparticles has also been reported. In an *in vitro* skin penetration assay using intact and damaged human skin, silver nanoparticles (25 nm) coated with polyvinylpyrrolidone were applied to skin for 24 h; the absorption of the silver nanoparticles by human skin was very low but detectable[326].

The transdermal penetration of nanoparticles after long-term exposure has also been investigated using animal models. In one study, minipigs were dosed daily for four weeks with sunscreen containing TiO_2 nanoparticles. Titanium was detected in the epidermis, but only negligible amounts were detected in the dermis of abdominal and neck skin[327].

After daily topical exposure of pigs' ears to TiO_2 nanoparticles (4 nm and 60 nm) for 30 days, nanoparticles were detected in the deep layer of the epidermis, whereas none were found in the dermis. After 60 days dermal exposure of hairless mice to TiO_2 nanoparticles, the particles were found to have penetrated the skin and to have been distributed into various organs *via* the circulation[11]. Although some of these results are contradictory, the differences may be due to the use of different types of nanoparticle preparations and different animal models. Overall, the research findings indicate that there is potential for damage to the skin due to exposure to nanoparticles.

How do nanoparticles penetrate the skin? Surface-modified fullerene nanoparticles migrated into deeper porcine skin layers through intercellular spaces[328]. The observed concentration gradient of nanoparticles from epidermis to dermis indicated that this penetration occurs by passive diffusion. The application of mechanical force to the skin may transiently increase the size of the intercellular spaces in the epidermis, accounting for the increased penetration of large nanoparticles after flexing[328]. Furthermore, pH may also play a role in dermal penetration by nanoparticles[329]. When a superficial incision was made in the back of a hairless mouse and Fe_3O_4 nanoparticles were applied at the incision site, the Fe_3O_4 nanoparticles can be shown by transmission electron microscopy to diffuse longitudinally *via* intracellular pathways to areas 30 μm from the application site[330]. Therefore, in addition to the intercellular and follicular pathways, translocation *via* intracellular pathways may also contribute to the transdermal penetration of nanoparticles[323, 331] (Figure 15).

Dermal nanotoxicity. Nanoparticles can be taken up by various eukaryotic cells, including dermal fibroblasts[332] and keratinocytes[333, 334]. SiO_2 nanoparticles[316] topically applied to human skin explants and QDs[323] similarly applied to mice primarily accumulated in the SC. The particles were mainly taken up by activated Langerhans cells, a dendritic cell type present in the epidermis, and relatively less by keratinocytes. In comparison with cultured keratinocytes[315, 334-338] and Langerhans cells[339], nanoparticle uptake by cells in skin tissue is much lower due to the protection provided by the SC layer.

Nanoparticles in skin cells cause cytotoxicity characterized by induction of ROS[340]

and the release of inflammatory factors[341, 342]. This damage may lead to dermal irritation[343] and skin sensitization[344]. The exposure of skin cells to silver nanoparticles and SWCNTs caused ultrastructural and morphological changes in the cells[345] and increased the expression of IL-6[315, 334], the cytokine responsible for mediating dermal irritation. The potential of various nanoparticles, including silver, TiO_2, ZnO_2 nanoparticles, and MWCNTs, to cause dermal irritation and skin sensitization has been investigated using porcine skin, a human skin equivalent model, and in rabbits and mice. The results of these *ex vivo* and *in vivo* studies indicate that these nanoparticles did not cause phototoxicity, acute cutaneous irritation, or skin sensitization[334, 346-349]; in fact, the results suggest a relatively low toxicity of nanoparticles to normal dermal functions. However, TiO_2 nanoparticles have been shown to cause collagen loss in the skin of exposed animals, which might lead to skin aging[11, 350].

The incidence of atopic dermatitis in industrialized countries has increased three-fold over the past three decades, and chronic exposure to urban fine particles is speculated to be a risk factor[351]. Although nanoparticle-induced atopic dermatitis has not been reported, some nanoparticles, including TiO_2, polystyrene, and amorphous SiO_2 nanoparticles, exacerbate atopic dermatitis by acting as allergens after intradermal injection[352-354]. This activity may further enhance the excessive induction of total IgE and cause a stronger systemic Th2 response[354].

Phototoxicity describes an inflammatory skin reaction that results from the topical application of chemical substances and subsequent exposure to light, particularly ultraviolet A radiation (320–400 nm)[355]. Because some nanoparticles are used in cosmetics, their potential phototoxicity is a matter of concern. Nanoparticles such as TiO_2 exhibit photocatalytic activity. Nitration of tyrosine residues is a post-translational modification of proteins that can occur under oxidative and nitrative stress. In one study, TiO_2 nanoparticles led to tyrosine nitration of bovine serum albumin in a UV-irradiated reaction mixture. These nanoparticles also initiated protein tyrosine nitration in mouse skin homogenates. This activity may lead to skin aging and the development of chronic cutaneous diseases[356]. However, TiO_2 and polystyrene nanoparticles were not phototoxic to skin and did not cause erythema or edema in animal models[346]. This conclusion was also supported by results from other test models, such as Balb/c 3T3 mouse fibroblasts

and a human skin equivalent model[346]. In contrast, in cultured cells irradiated with ultraviolet and visible light, soluble fullerene (nano-C60$(OH)_{22–26}$) and iron-doped $LiNbO_3$ nanoparticles caused phototoxicity by the induction of free radicals[357, 358]. These results suggest that the induction of phototoxicity by nanoparticles may depend on their chemical composition.

Systemic toxicity after dermal adsorption. Once the integrity of the SC layer is damaged, nanoparticles can penetrate the epidermis and enter the richly vascularized dermis, from which systemic absorption may occur. To date, only a few investigations have reported the biodistribution of nanoparticles after absorption in the dermis. In most cases, absorption and biodistribution only occur in compromised skin or after long-term exposure. For example, after four weeks of exposure of minipigs to TiO_2 nanoparticles in sunscreen, a small number of the TiO_2 nanoparticles migrated to the dermis, but they did not enter the circulatory system[327]. After exposure of hairless mice to TiO_2 with hydrophobic or hydrophilic surfaces of different sizes (10, 25, and 60 nm) for 60 days, the nanoparticles penetrated the skin, entered the circulation, and accumulated in the spleen, heart, and liver. Pathological lesions caused by oxidative stress were found in the skin and liver. The collagen content of the skin was notably decreased, potentially leading to skin aging[11] (Figure 16).

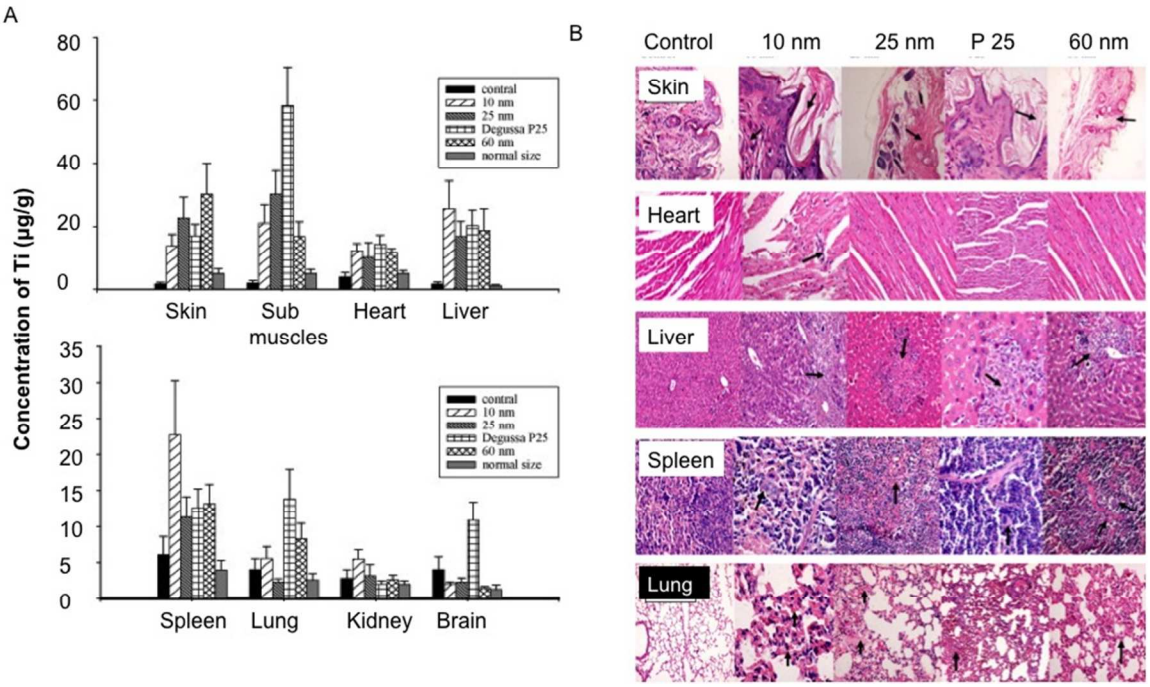

Figure 16. Nanoparticles accumulate in distal organs after skin penetration. (A) The content of titanium in various tissues of hairless mice after dermal exposure to TiO_2 nanoparticles for 60 days. (B) Histopathological evaluation of the organs of hairless mice after dermal exposure to TiO_2 nanoparticles of various sizes for 60 days. Samples were stained with hematoxylin and eosin (H&E) and observed at 100×. The arrows indicate pathological changes in various tissues. Small TiO_2 nanoparticles (10 nm) are anatase crystal, while larger ones (25 and 60 nm) are rutile. Degussa P25 represents commercial TiO_2 nanoparticles of 21 nm containing 25% rutile and 75% anatase forms. 10 and 60 nm TiO_2 nanoparticles either have a hydrophobic surface (10 and 60 nm) or a hydrophilic surface (25 nm and Degussa P25). All nanoparticles have a purity ≥ 99.5%. Reproduced with permission from Reference [11]. Copyright 2009, Elsevier.

In summary, after short-term exposure, nanoparticles primarily accumulate in the SC layer of the epidermis of healthy skin. Long-term exposure or exposure of damaged skin increases the risk of percutaneous absorption of nanoparticles. The transdermal properties of nanoparticles depend on the composition and physicochemical properties of the nanoparticles and on the skin itself. Dermal nanotoxicity caused by nanoparticles can result in accelerated skin aging, dermal irritation, skin sensitization, atopic dermatitis, and dermal phototoxicity. Nanoparticles may also enter the circulation after dermal exposure and cause lesions in distal organs.

Nanotoxicity to bones. Bone is composed of osseous tissue, marrow, endosteum, and periosteum and is often closely associated with nerves, blood vessels, and cartilage. It is a specialized dense connective tissue that provides mechanical support for the whole body, protection for internal organs, and attachment for muscles. The marrow inside bones is an organ that produces blood cells and stores minerals in the body. Osseous tissue includes an organic matrix such as collagen and complex minerals including calcium, phosphate, carbonate, citrate, hydroxyl, and sodium. Concerns regarding nanotoxicity to bone tissue have been raised due to the increased exposure of humans to nanoparticles and to the application of nanomedicine to the treatment of bone diseases such as osteoporosis[359, 360], bone fractures[361, 362], and other bone disorders[361] and in dentistry[363, 364]. In these applications, nanoparticles directly replace natural bone tissue or are remineralized and resorbed into bones to facilitate bone growth or recovery. Nanoparticles are also used as reinforcing agents to enhance the mechanical strength of other polymer scaffolds[365] (Figure 17).

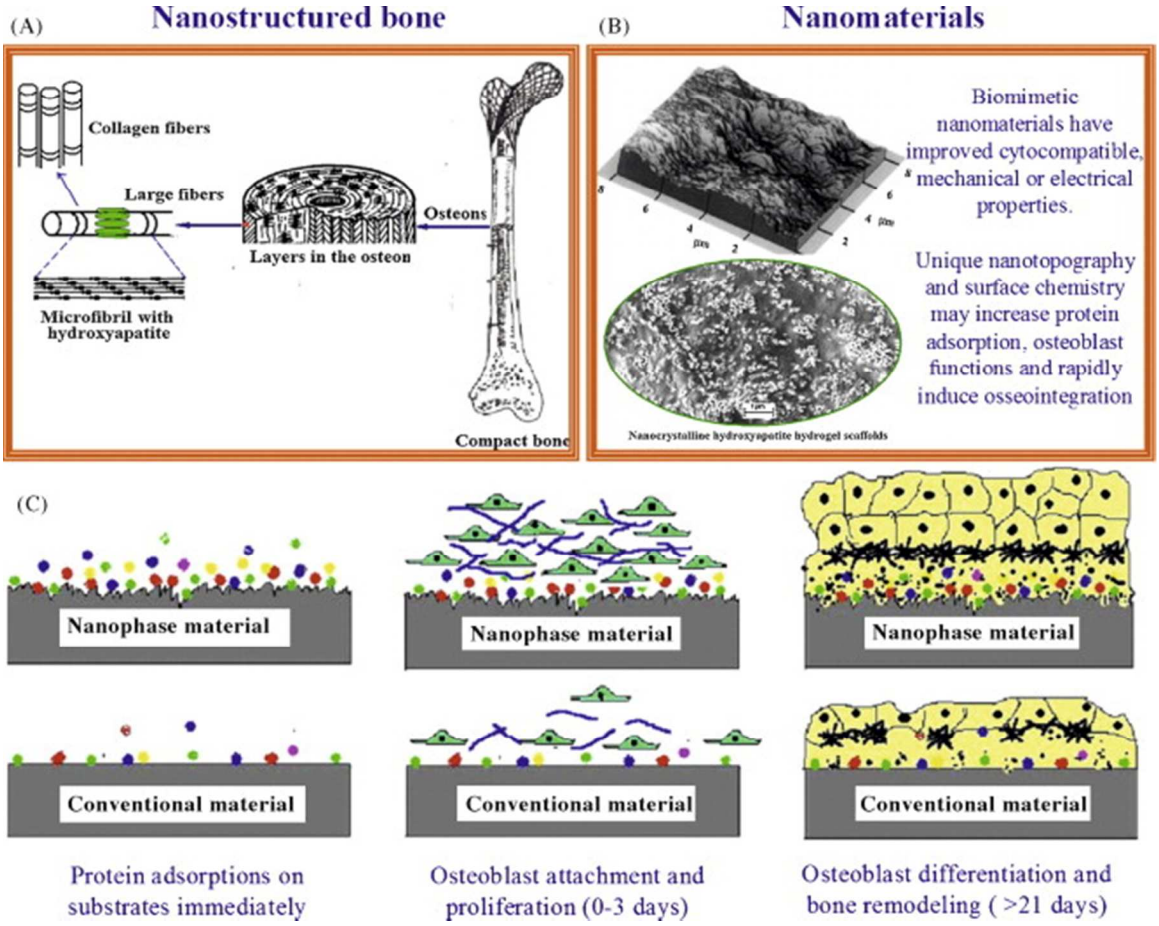

Figure 17. The biomimetic advantages of nanomaterials. (A) The nanostructured hierarchal self-assembly of bone. (B) Nanophase titanium (top, atomic force microscopy image) and nanocrystalline hydroxyapatite/helical rosette nanotubes hydrogel scaffold (bottom, scanning electron microscopy image). (C) Schematic illustration of the mechanism by which nanomaterials may be superior to conventional materials for bone regeneration. The bioactive surfaces of nanomaterials mimic those of natural bones, thus promoting protein adsorption and more efficiently stimulating new bone formation than conventional materials. Reprinted with permission from Reference [366]. Copyright 2009, Elsevier.

The effects of nanoparticles on bone have been investigated both *in vivo* and *in vitro* in recent years. Findings representative of this work are summarized below.

In vivo evaluation. The translocation and accumulation of nanoparticles in various organs are key factors that determine whether nanoparticles cause systemic toxicity and damage to secondary organs. Oral exposure of CD-ICR mice to ZnO nanoparticles (1~5 g/kg) caused zinc accumulation in bones as well as in other organs. However, no impairment of bone structure or function was observed[367]. Inhaled iridium nanoparticles

(2–4 nm) and carbon nanoparticles (5–10 nm) were retained in soft tissue and bone. Smaller nanoparticles exhibit higher levels of translocation and accumulation[368].

MWCNTs implanted into mouse muscle can specifically inhibit osteoclastic bone resorption *in vivo* by inhibiting osteoclast differentiation[369]. Studies of the molecular mechanism of this effect have shown that the incorporation of MWCNTs into osteoclast precursor cells suppressed the nuclear translocation of NFATc1, a transcription factor essential for osteoclastogenesis. In this way, MWCNTs specifically inhibited osteoclast differentiation[369]. Although this effect may benefit their application in bone tissue engineering, it also raises the concern that MWCNTs may perturb *in vivo* homeostasis between bone formation and resorption. Nanoparticles have also been found to cause genotoxicity in bone marrow cells after exposure in animals[172, 173]. This finding is discussed in the section on hematopoietic toxicity.

In vitro evaluation. Bone defects caused by trauma or due to pathological or physiological bone resorption stimulate a process called bone regeneration. In bone regeneration, bone marrow stromal cells in the non-hematopoietic compartment of the bone marrow differentiate into mature osteoblasts. These osteoblasts play important roles in maintaining calcium homeostasis in the bone matrix by producing matrix components and minerals as well as by mineralizing the matrix[370]. Efforts have been made to develop nanobiomaterials for bone regeneration. For example, due to their ability to sustain osteoblast proliferation and bone-forming function, MWCNTs and SWCNTs are often used as osteoblastic cell growth substrates[371]. SWCNTs on thin-film substrates induce the release of endogenous factors that stimulate the synthesis of extracellular matrix and increase cell survival[365]. In addition, osteoblasts display increased cell adhesion to the rough surface of nanoparticles compared to their adhesion to conventional metal surfaces, suggesting that nanomaterials may enhance the bone-forming function of the cells[372, 373]. SWCNTs, hydroxyapatite, TiO_2, and silver nanoparticles have been evaluated for their effects on cell calcification and mineralization in MC3T3-E1 bone cells[374]. All tested nanoparticles enhance mineralization, as indicated by an increased number and larger area of mineral nests in MC3T3-E1 bone cells. MicroRNA expression analysis after exposure of the cells to silver nanoparticles shows that these effects may result from the altered expression of genes associated with bone formation, such as Runx2, Dlx3, and

Msx2. These results indicate a potential benefit of nanoparticles in bone tissue engineering.

Osteoclasts are specialized cells responsible for bone resorption and their function is important to maintain bone volume and calcium homeostasis. *In vitro* study indicated that MWCNTs were incorporated into precursor cells of osteoclasts and inhibited their differentiation into mature osteoclasts under the protection of specific cytokines, such as receptor activator of nuclear factor-κ B ligand and macrophage colony stimulating factor. In comparison, carbon black did not show such effects[369]. This effect led to the *in vivo* inhibition of osteoclastic bone resorption.

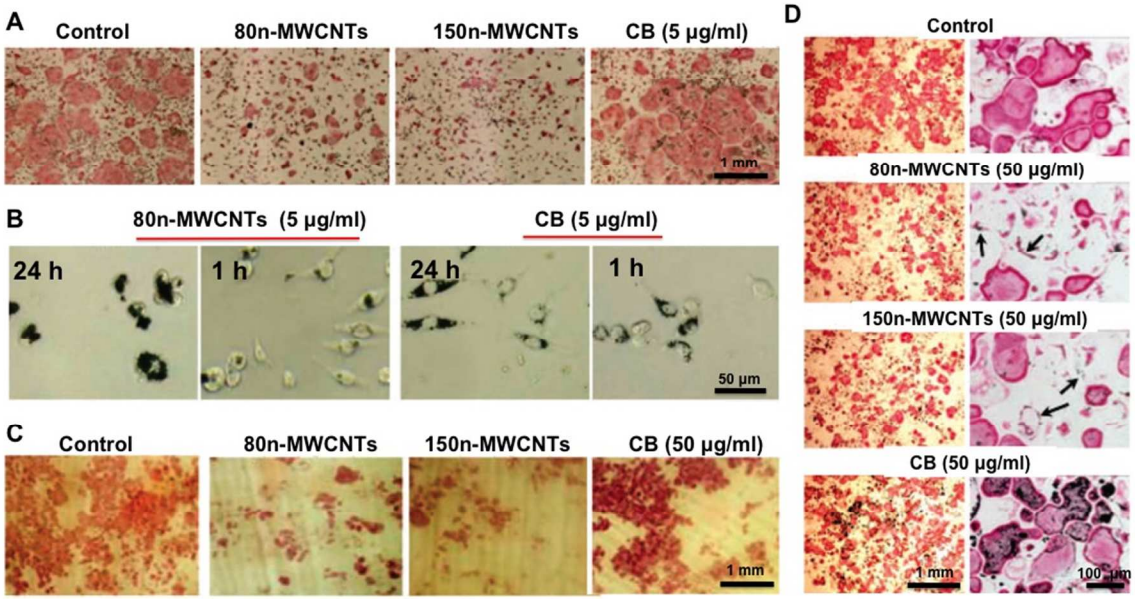

Figure 18. MWCNTs inhibit osteoclast differentiation and function. (A) MWCNTs incorporated into BMMØ (precursor cells of osteoclasts) inhibited the differentiation of BMMØ into osteoclasts. BMMØ were treated with or without 80n-MWCNTs, 150n-MWCNTs, or CB for 24 h followed by induction of osteoclast differentiation by the addition of the differentiation factors RANKL (100 ng/mL) and M-CSF (25 ng/mL). The red cells are TRAP (tartrate-resistant acid phosphatase)-positive cells. TRAP is a marker enzyme present in osteoclasts. Carbon black (CB) 80 nm in size was used as a control. (B) The cellular incorporation of MWCNTs accounts for their inhibition of differentiation. The phase-contrast microscopy images show that BMMØ cells incorporated MWCNTs after incubation for 24 hours but not after incubation for 1 h. Treatment of MWCNTs for 1 hour did not inhibit osteoclast differentiation of BMMØ. (C) MWCNTs inhibit the pit-forming activity and survival of osteoclasts. Osteoclast preparations were cultured for 36 hours on dentine slices with or without 80n-MWCNTs, 150n-MWCNTs, or CB. The dentine slices were then recovered and stained with

Mayer's hematoxylin to visualize the resorption pits. The red region represents the resorption pits formed on the dentine slices. (D) MWCNTs suppressed the RANKL-supported survival of purified osteoclasts. Arrows indicate MWCNTs in areas where MWCNTs-containing osteoclasts died. There was no effect of CB on RANKL-supported osteoclast survival. Reproduced with permission from Reference [369]. Copyright 2009, American Chemical Society.

Although nanoparticles may be helpful in bone tissue engineering, some nanoparticles with promising potential applications in biomedicine have been found to be detrimental to the formation of bone tissue. Superparamagnetic iron oxide nanoparticles used for stem cell tracking and magnetic resonance imaging impair the chondrogenic and osteogenic differentiation of human mesenchymal stem cells by altering the intracellular cytokine production profile of the cells[375], and TiO_2 nanoparticles induce cytotoxicity in murine MC3T3-E1 preosteoblasts[376].

In summary, bone formation is a well-orchestrated process that involves many cell types and signaling pathways. Recent investigation has shown that nanoparticles are promising for use in bone tissue engineering. However, nanoparticles may disturb cell signaling networks in bone tissue, leading to pathological consequences. Nanoparticles can also impair chondrogenic and osteogenic differentiation.

10. Hereditary and carcinogenic toxicity of nanoparticles

In addition to their potential toxicity to physiological systems and organs, nanoparticles may cause hereditary or carcinogenic toxicity. Hereditary toxicity refers to any damage to cellular DNA; it includes changes in the structure or the number of genes resulting from the direct or indirect perturbation of DNA or non-DNA targets. When such damage occurs in germ cells such as spermatocytes or oocytes, it becomes heritable. DNA damage to somatic cells may result in carcinogenicity. In this section, we will discuss both types of toxicity.

Recent findings repeatedly suggest that nanoparticles may have the potential to initiate or promote cancers (Figure 19). First, nanoparticles may enter cell nuclei and directly damage hereditary materials. Second, inflammation caused by nanoparticles can lead to the production of oxidants and mitogens that cause secondary DNA damage[377]. Third, nanoparticles with high aspect ratios exhibit an asbestos-like shape, and asbestos is

known to cause cancer in the lung and in pleural and peritoneal mesothelium. Fourth, our group and other groups have found that nanoparticles disturb cellular signaling pathways[378-380]. These perturbations may contribute to carcinogenesis. Fifth, nanoparticles can enter mitochondria, where they induce cellular oxidative stress and cause damage to DNA. They also disrupt DNA repair machinery[286].

Both *in vivo* and *in vitro* assays are used to evaluate the potential hereditary toxicity of nanoparticles. A bacterial reverse-mutation bioassay, the Ames test, has been used to evaluate the *in vitro* hereditary toxicity of chemicals. A number of mammalian cell-based hereditary toxicity assays, such as the mammalian chromosome aberration test, the mammalian cell gene mutation test, the micronucleus test, and the Comet assay, have also been developed[381, 382].

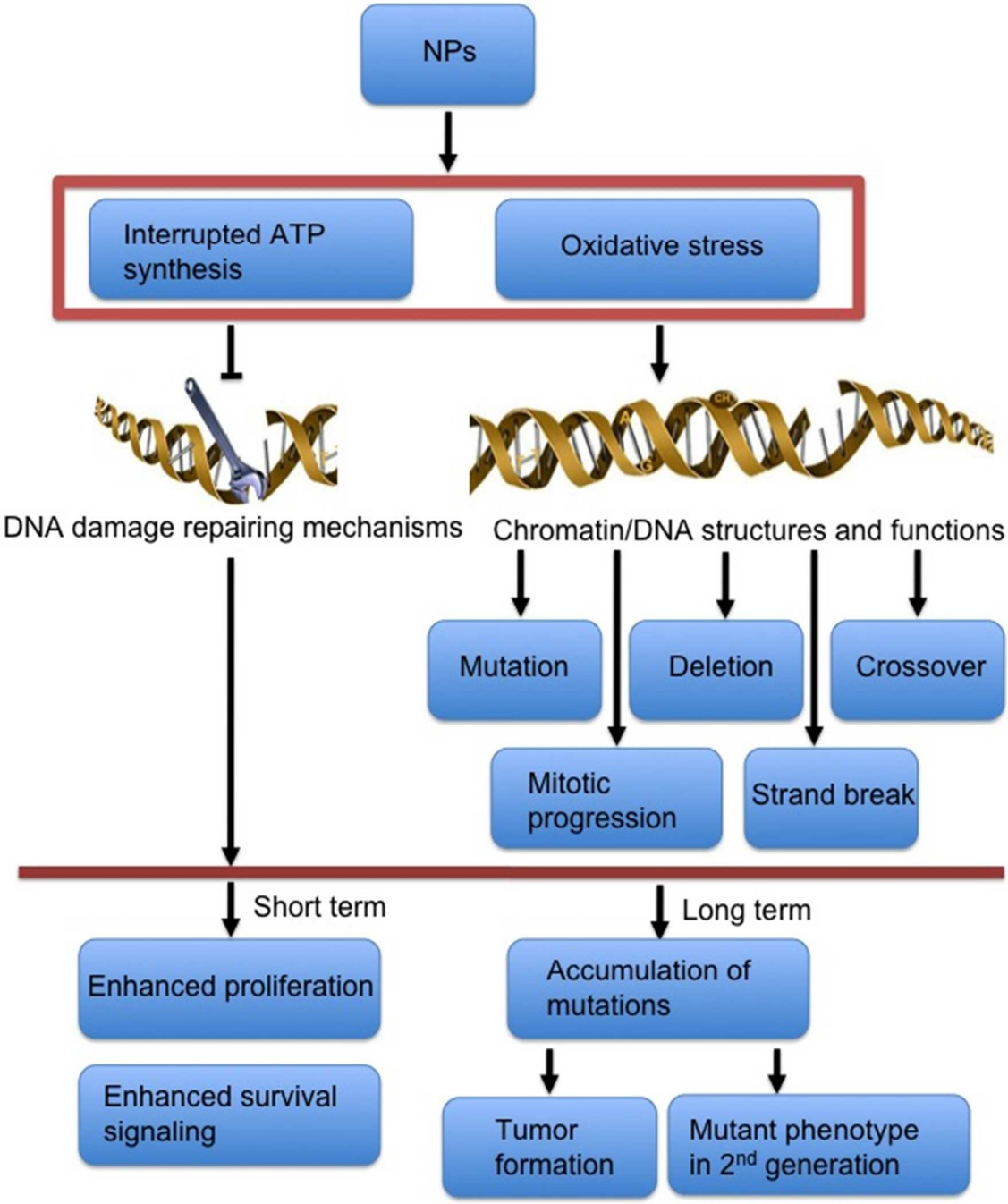

Figure 19. Nanoparticles may cause damage to hereditary materials by inhibiting cellular ATP synthesis and inducing oxidative stress. These perturbations either damage chromatin/DNA structures and functions or interfere with cellular DNA repair mechanisms. As a short-term consequence of these perturbations, cells exhibit enhanced proliferation and survival signaling that is characteristic of tumor growth. In the long term, these effects may lead to tumorigenesis or to a mutant phenotype in the second generation, depending on the cell type.

In vivo hereditary and carcinogenic toxicity. The hereditary toxicity of TiO_2 nanoparticles has been investigated because of its broad applications in various areas such as industry, catalysis, cosmetics, and tissue engineering. Animal experiments have provided evidence that TiO_2 nanoparticles induce hereditary toxicity in animal cells. The oral administration of TiO_2 nanoparticles to mice for five days at a dose of 500 mg/kg induced DNA strand breaks and chromosomal damage in blood cells, bone marrow cells, and liver cells. The oral administration in drinking water of TiO_2 nanoparticles (300 μg/mL) to pregnant dams for 10 days from 8.5 to 18.5 days post-coitum caused increased DNA deletions in the offspring, as assayed in fetal retinal pigment epithelium[383]. By analyzing inflammatory responses after treatment, the authors conclude that TiO_2 nanoparticles caused hereditary toxicity *in vivo via* a mechanism associated with inflammation and/or oxidative stress. The exposure of rats to TiO_2 nanoparticles (100 mg/kg) by intratracheal instillation for 15 months increased mutations in the hypoxanthine–guanine phosphoribosyl transferase (HPRT) gene in type 2 alveolar epithelial cells[384]. This gene encodes an enzyme critical for DNA synthesis. In comparison, another nanoparticle, carbon black, exhibited a higher level of perturbation of the same gene[77]. However, the exposure of cells to these nanoparticles *in vitro* did not induce similar effects. An *in vitro* co-culture study suggested that the production of oxidants by neutrophils exposed to nanoparticles may be associated with an increased frequency of mutations[77]. This study demonstrates the complexity of nanoparticle effects *in vivo* and the limitation of *in vitro* experiments for studies of hereditary toxicity.

The results of some studies on the carcinogenicity of TiO_2 nanoparticles are even more alarming. In rats, 129 weeks after intratracheal instillation of TiO2 nanoparticles, the particles induced tumor formation in the lungs of the animals[385]. The implantation of TiO_2 nanoparticles in rats converted poorly tumorigenic and nonmetastatic QR-32 fibrosarcoma cells to tumorigenic cells[386]. As with other nanoparticles, the excretion of TiO_2 nanoparticles after intravenous and subcutaneous injection in mice is very slow. Even after 26 weeks, these nanoparticles were still detectable in major organs, including the liver and spleen[387]. This type of long-term accumulation may cause genotoxicity or carcinogenicity.

Although whether MWCNTs alone cause cancer in mice was not suggested, in one

recent study, MWCNTs were found to be a potent promoter of carcinogenicity[388]. In that study, 7 days after treatment with methylcholanthrene, a known carcinogen, inhalation of MWCNTs in B6C3F1 mice by an occupational exposure dose (5 mg/m^3, 5 hours/day, 5 days/week) dramatically increased both the incidence of carcinogenicity in mice and the number of tumors per animal. Three days after a single intratracheal administration of 0.5 or 2 mg MWCNT (11 nm in diameter and 700 nm in length) to Wistar rats, micronuclei can be detected in lung epithelial cells[389]. In ApoE$^{-/-}$ mice, the potential hereditary toxicity of fullerenes C60 and SWCNTs have been investigated by the Comet assay using bronchoalveolar lavage cells. C60 (agglomerates of about 150 nm by DLS measurement) was less likely to induce DNA damage than SWCNTs[390], and both CNTs and C60 were less genotoxic than carbon black (agglomerates of about 1.2 μm by DLS measurement)[391]. Consistent with this result, C60 did not show clastogenic ability after two doses of 88 mg/kg administered by gavage to mice[392].

For some time, researchers have considered the question of whether the similarity in the shape of CNTs and asbestos fibers confers similar toxicity on the two agents and whether MWCNT exposure can cause mesothelioma, a cancer of the lining of the lungs. In one study that addressed this question, long MWCNTs (20 μm in length) were injected into the peritoneal (abdominal) cavities of mice at a dose of 1.5 mg/kg. Seven days after exposure, asbestos-like and length-dependent inflammation and the formation of granulomas were observed[393] (Figure 20). Although MWCNTs were not detected at the mesothelial lining, this research warrants additional investigation of the possible carcinogenicity of MWCNTs.

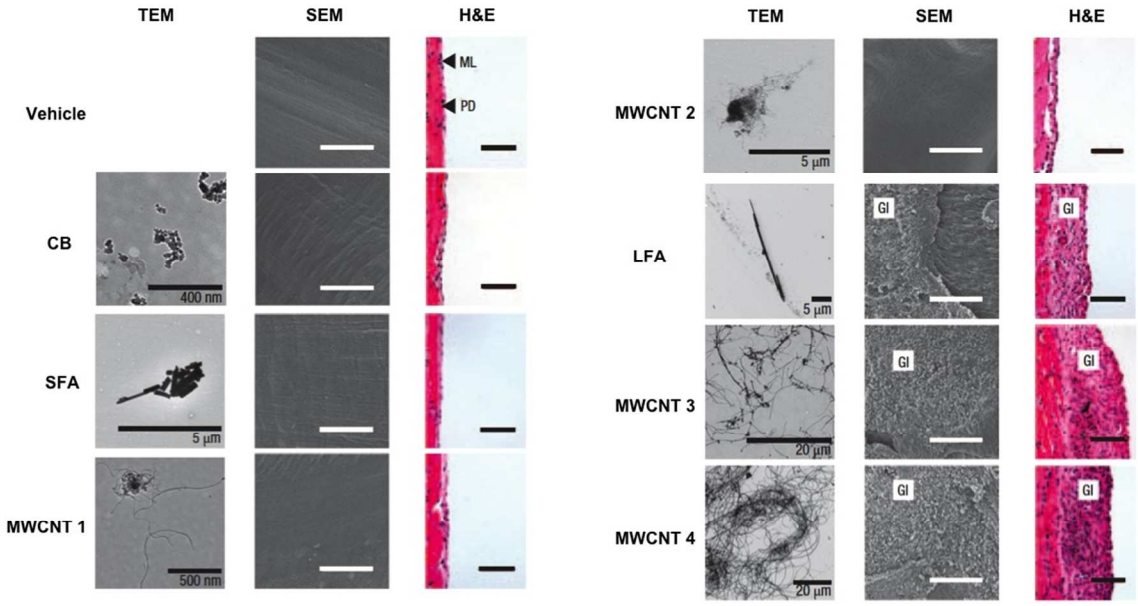

Figure 20. Long-fiber particles (LFA) and MWCNT3 and 4, but not short ones (carbon black (CB), short-fiber amosite (SFA), and MWCNT1, 2) cause granulomas on the peritoneal side in mice. In each panel, the first column shows transmission electron microscopy (TEM) images of the particles. The middle and right columns show scanning electron microscopy (SEM) images and hematoxylin and eosin (H&E)-stained sections of diaphragm tissue, showing the presence of granulomatous inflammation (GI) in mice exposed to LFA or MWCNT3 or 4. The muscular portion of the peritoneal diaphragm (PD) and the mesothelial layer (ML) are aligned to show granulomatous inflammation at the peritoneal aspect of the diaphragm surface. Scale bars in scanning electron microscopy images: 200 µm. Scale bars in H&E images: 50 µm. Long- (short-) fiber amosite is an amphibole form of asbestos and is used as a positive control. CB is used as a non-fiber-shaped control. Reproduced with permission from Reference [393]. Copyright 2008, Nature Group.

Hereditary and carcinogenic toxicity of nanoparticles is also studied with *Drosophila melanogaster* as a model. In *Drosophila melanogaster*, gold nanoparticles (15 nm, 3µg/g per day for 25 days) caused mutant phenotypes in offspring, including deformations of the wings, eyes, and thorax[394]. These findings indicate that animal fetuses are more sensitive to nanoparticle exposure than adults. Investigation in *Drosophila melanogaster* showed that silver nanoparticles (40 nm) at concentrations ranging from 0.1–10 mM increased the formation of mutant clones in larval cells by inducing somatic recombination[109]. However, in Sprague-Dawley rats, different conclusions have been reached. Exposure of Sprague-Dawley rats to silver nanoparticles (60 nm) up to 1000 mg/kg for 28 days by oral treatment did not lead to differences in the number of

micronucleated polychromatic erythrocytes or in the ratio of polychromatic erythrocytes to total erythrocytes compared to controls[171]. Both parameters are indicators for chromosome damage. Because of the differences in animal models, nanoparticle preparation, and assay methods in these two studies, it is difficult to reach firm conclusions from these results.

In vitro hereditary and carcinogenic toxicity. *In vitro* studies indicated that after short- and long-term exposure to TiO_2 nanoparticles, human cells exhibited changes similar to those seen in malignant transformation. These changes included enhanced proliferation, prolonged survival, and genetic instability that facilitate the accumulation of mutations. Long-term (~12 weeks) exposure of NIH3T3 and human fibroblast HFW cells to TiO_2 nanoparticles (15 nm) at a dose of 10 μg/ml enhanced cell anchorage-independent growth, micronuclei formation, and multinucleation[395]. These alterations are all associated with tumorigenesis[396, 397]. TiO_2 nanoparticles affected cellular mitotic progression at anaphase and telophase, leading to the appearance of aberrant multipolar spindles and altered chromatin alignment/segregation due to altered signaling of polo-like kinase 1 (PLK1). Similar observations have been made in human lymphoblastoid cell line[398]. TiO_2 nanoparticles may also cause DNA damage by the ataxia telangiectasia mutant (ATM) protein and Chk2 mediated mechanisms[399], although this is still controversial[400]. All of these *in vitro* results indicate that TiO_2 nanoparticles are probably genotoxic. Because of these and similar results obtained using experimental animals and cell culture, TiO_2 nanoparticles have been classified as possibly carcinogenic to humans (class 2B) by the International Agency for Research on Cancer[341].

Gold nanoparticles are often reported to be non-genotoxic[390, 401, 402]. However, hereditary toxicity of gold nanoparticles has also been reported. In MRC-5 lung fibroblast cells, treatment with gold nanoparticles (20 nm) at a dose of 1 nM disturbed the expression of heterogeneous nuclear ribonucleoprotein C1/C2 and secernin-1, proteins associated with DNA repair[403]. In human cell models, silver nanoparticles (5-260 nm) induced DNA damage[286, 404, 405] and increased the frequency of formation of micronuclei[405, 406]. Nanoparticle-induced oxidative stress and the interruption of ATP synthesis in cells may contribute to the observed hereditary toxicity of the

nanoparticles[405].

At doses that do not reduce cell viability, SWCNTs[407], MWCNTs[389, 408], and mixtures of the two[409] all caused DNA damage that can be detected by the Comet assay. MWCNTs also increased the ratio of multinucleated cells in Chinese hamster lung cells[410]. However, except after treatment with MWCNTs at high concentrations (36 μg/mL), no DNA damage was observed by micronucleus assay. SWCNTs induced micronucleus formation and double-strand DNA damage in lymphocytes and fibroblasts, respectively. In comparison, MWCNTs induced lymphocyte micronuclei and anaphase bridges between nuclei in bi-nucleated cells but did not induce double-strand DNA damage. This finding indicates that MWCNTs may act simultaneously as clastogenic and aneugenic agents[411]. This was also confirmed by analyses of the expression of base excision repair protein 8-oxoguanine-DNA glycosylase 1, double-strand break repair protein Rad 51, and other DNA damage indices, including the phosphorylation of H2AX histone at serine 139 and the SUMO modification of XRCC4[408]. A study of mutagenesis using an endogenous molecular marker shows that MWCNTs increased mutation frequency by 2-fold compared to untreated cells[408]. In contrast, C60 at concentrations of up to 200 μg/mL caused no chromosomal aberrations *in vitro*[392]. Despite the fact that toxicity has been reported *in vitro*, SWCNTs showed no obvious genotoxicity in rats after oral exposure[412]. We summarize the characterization of nanoparticles and the cell models used in all of these studies in Table 5.

Table 5. Characterization of nanoparticles and cell models used in *in vitro* hereditary and carcinogenic toxicity studies.

Nanoparticle	Size (nm)	Dosing regiment	Cell model	Assay methods	Major observations	Ref
TiO_2	2-30	0.5 ng/mL - 50 μg/mL, 12 weeks	NIH3T3 and human fibroblast HFW cells	Colony formation assay, Anchorage independent growth assay	Increased cell survival and growth, Increased multinucleated cell and micronucleus numbers, G2/M phase arrest,	395

					Aberrant multipolar spindles and chromatin alignment/segregation.	
TiO_2	6.5	0-130 µg/mL, 6, 24 and 48 hours	Human lymphoblastoid cell WIL2-NS	CBMN[1] assay, HPRT[2] mutagenicity assay, Comet assay	2.5-fold increase in frequency of micronucleated and binucleated cells, 5-fold increases in olive tail moment, 2.5-fold increases in mutation frequency	398
TiO_2	15	1-100 µg/mL, 24 hours	Human dermal fibroblasts	Immuno-cytochemistry analysis, Immunofluorescence microscopy	Increased phosphorylation of H2AX, ATM[3], and Chk2, Inhibited DNA synthesis rate and replicon initiation frequency	399
TiO_2	28	5 and 15 µg/mL, 12 hours	Human lung epithelial A549 cells	Comet Assay, Immunofluorescence Staining	No effects	400
Cobalt	20				Induced ROS[4], DNA damage, Increased phosphorylation of ATM	
Gold	20	1nM, 72 hours	Human fetal lung fibroblast MRC-5 cells	Two dimensional gel electrophoresis, Comet assay, Florescence in situ hybridization assay	Induced DNA strand and chromosomal breaks	403
Silver	6-20	25-400 µg/mL, 24-72 hours	Human lung fibroblast IMR-90 cells, Human glioblastoma U251cells	SCGE[5] assay, CBMN assay	DNA damage and G2/M phase arrest	286
Silver	40-60	50 and 100 µg/mL, 3 hours	Human peripheral blood cells	Comet assay	Induced DNA damage	404
Silver	43-260	0.01-10 µg/mL, 24 hours	Human normal bronchial epithelial BEAS-2B cells	Comet assay, Micronucleus assay	Induced DNA breakage and micronucleus formation	405
Silver	5	10-30 µg/mL, 28 hours	Human lympho-blastoid	Ames test, Micronucleus assay	Increased 3.17-fold in micronucleus	406

			TK6 cells		frequency	
SWCNTs	0.4-1.2	12- 96 μg/cm^2, 24 hours	Lung fibroblast V79 cells	Comet assay, Micronucleus assay, Ames test	Induced DNA damage and micronucleus formation	407
SWCNTs	2 nm x 4-15 μm	1 mg/mL, 72 hours	Human blood samples	Micronucleus analysis	No effects	412
MWCNTs	10-30 nm x 1-2 μm					
MWCNTs	50	5 and 100 μg/mL, 2 and 4 hours	Mouse embryonic stem cells	Western blot, Adenine phosphoribosyltransferase molecular marker	Increased expression of base excision repair protein OGG1, double strand break repair protein Rad 51, Increased and phosphorylation of H2AX histone, and SUMO modification of XRCC4	408
MWCNTs	11 × 700	10-150 μg/mL, 6 or 24 hours	Human and rat epithelial cells	Micronucleus assay	2-fold increase in micronucleus frequency	389
MWCNTs	90 nm × 5.0μm	12.5-400 μg/mL, 7 days	Chinese hamster lung cells	Chromosome aberration assay, Micronucleus assay, HPRT mutagenicity assay	No structural chromosome aberration, No induction of micronuclei, No HPRT mutagenicity	410
MWCNTs	20–40 nm× 1–5 μm	0.5–30 μg/mL, 24 hours	Human dermal fibroblasts HDMEC, Human whole blood cells	Micronucleus assay, Enumeration of γH2AX foci	Increased double strand breaks, Induced lymphocyte micronuclei and anaphase bridges among nuclei	411
Pristine and amide SWCNTs	N/A				Induced micronuclei formation, Decreased proliferation potential	
CNT mixture (~50% SWCNTs, ~40% other CNTs)	1.1 nm × 0.5-100 μm	3.8-380 μg/mL, 24-72 hours	Human bronchial epithelial BEAS 2B cells	Comet assay, Micronucleus assay	Increase micronucleated cells	409
Pristine and oxidized nanodiamon	4-5	5 and 100 μg/mL, 24 hours	Mouse embryonic stem cells	Western blot, Scanning Electron Microscopy	Increased expression of DNA repair	413

ds					proteins	
Fullerene	35-140	100-200 μg/mL, 24 hours	Chinese hamster lung cells	Ames assay, Chromosomal aberration test	No mutagenic response, No increase in chromosomal aberrations	[392]

[1]CBMN, cytokinesis block micronucleus assay
[2]HPRT, hypoxanthine-guanine phosphoribosyltransferase gene mutation assay
[3]ATM, ataxia telangiectasia mutant
[4]ROS, reactive oxygen species
[5]SCGE, single cell gel electrophoresis assay
N/A, No data

The genotoxic potential of other nanoparticles, such as QD[414], nanodiamond[413], aluminum oxide[172], cobalt[400], cobalt chrome alloy[415], and maghemite nanoparticles[416], has also been investigated. However, comparison of these results is difficult because of the great differences in the experimental protocols used. DNA damage assessed by classic assays is positive in most studies conducted to date[417]. Inefficient DNA repair may be the primary risk for nanoparticle-induced hereditary and carcinogenic toxicity. DNA damage is the consequence of nanoparticles' 'double hits' on DNA. On one hand, intracellular oxidative stress induced by nanoparticles directly damages DNA molecules; on the other hand, nanoparticles also interfere with cellular DNA repair mechanisms, for instance, by inhibiting cellular ATP synthesis[385].

In summary, nanoparticles cause damage to the hereditary material primarily by oxidative stress- and inflammation-mediated mechanisms. Although few types of nanoparticle-induced DNA damage found *in vitro* have been confirmed *in vivo*, nanoparticles have been shown to cause chromosomal DNA damage in some vulnerable cell types such as blood cells and bone marrow cells *in vivo*. In addition, chronic inflammation, which can occur as a consequence of nanoparticle exposure, may convert nontumorigenic cells to tumorigenic cells or even induce tumor formation in animals.

11. Concluding remarks

From various exposure routes, nanoparticles tend to be absorbed with different efficiencies and distributed in various organs. They may be partially dissolved, degraded, excreted or stably trapped in organs. Nanoparticles can be retained by the lung causing pulmonary inflammatory responses, granuloma formation, and fibrotic injury or be transferred to the blood circulation in alveoli causing systemic toxicity. Nanoparticles may alter the fluid dynamics of blood, generate intracellular oxidative stress, and induce inflammation that causatively leads to platelet aggregation, thrombosis, and cardiovascular malfunction. Nanoparticles exhibit the properties of immune adjuvant and immunosuppressor. They activate the complement system and decrease the population of lymph node cells that produce immunoglobulins against immunogens. Since they are efficiently taken up by antigen-processing cells, nanoparticles induce cellular immune responses rather than humoral immune responses. Induction of innate and acquired immune responses by nanoparticles is probably through the activation or perturbation of cell surface receptors. When nanoparticles enter the bone marrow, they may compromise erythrocyte functions, reduce the production of platelets, increase the number of white blood cells, and induce extramedullary hematopoiesis in the spleen. The exposure of pregnant dams to nanoparticles causes damage to the male reproductive systems of their pups. When male mice are exposed to nanoparticles such as carbon nanotubes, reversible damages to the male reproductive organs are observed whereas fertility is minimally affected. Nanoparticle accumulation in the mouse ovary disturbs the normal balance of sex hormones. After entering the digestive system, some nanoparticles escape gastrointestinal absorption and are quickly eliminated in feces. Remaining nanoparticles may enhance gastric emptying and alter nutrient absorption. Nanoparticles may also damage intestinal structure and pass through the tight junctions between intestinal epithelial cells entering the circulation. However, effects of nanoparticles on the immunological defense capability of the intestine and the commensal bacteria that reside in the intestinal canal remain unexplored. Since the glomerular filtration barrier in the kidneys prevents large nanoparticles (>10 nm) from being excreted in urine, smaller hydrophilic nanoparticles and some needle-like nanoparticles may bypass this barrier and enter the urine. Although the kidney is generally less sensitive to nanotoxicity compared

to other organs, copper nanoparticles are known to cause renal toxicity. Due to their nanometer size, nanoparticles in circulation may also enter the CNS. They damage neurons and glial cells by inducing inflammation and cell apoptosis and perturb the electrophysiological properties of neurons. Nanoparticles also affect nerve cell functions such as neurotransmitter release. Liver is one of the major nanoparticle accumulation organs. Kupffer cells and, to a lesser degree, hepatocytes internalize nanoparticles that perturb the function of liver enzymes such as those in cytochrome P450 family. Other effects of nanoparticles on liver functions, such as the secretion of bile, the synthesis of glucose and fatty acids, and blood iron content, remain largely unknown. After a short-term exposure, nanoparticles primarily accumulate in the stratum corneum layer of the epidermis in healthy skin. Long-term exposure or exposure of damaged skin increases the risk of percutaneous absorption of nanoparticles. Nanoparticle-induced dermal toxicity results in an accelerated skin aging, dermal irritation, skin sensitization, and atopic dermatitis in addition to entering the circulation to mediate systemic toxicity. Recent investigations have shown that nanoparticles are promising in bone tissue engineering. However, nanoparticles may disturb cell signaling networks such as impairing chondrogenic and osteogenic differentiation in bone tissues leading to pathological consequences. The impact of nanoparticles on hereditary material is primarily through generation of oxidative stress and inflammation. Nanoparticles have been shown to cause chromosomal DNA damage in more vulnerable blood cells and bone marrow cells *in vivo*. Furthermore, nanoparticle-mediated chronic inflammation may convert non-tumorigenic cells to tumorigenic cells or even induce tumor formation in animals. A working model indicating possible nanotoxicity to physiological systems is proposed in figure 21.

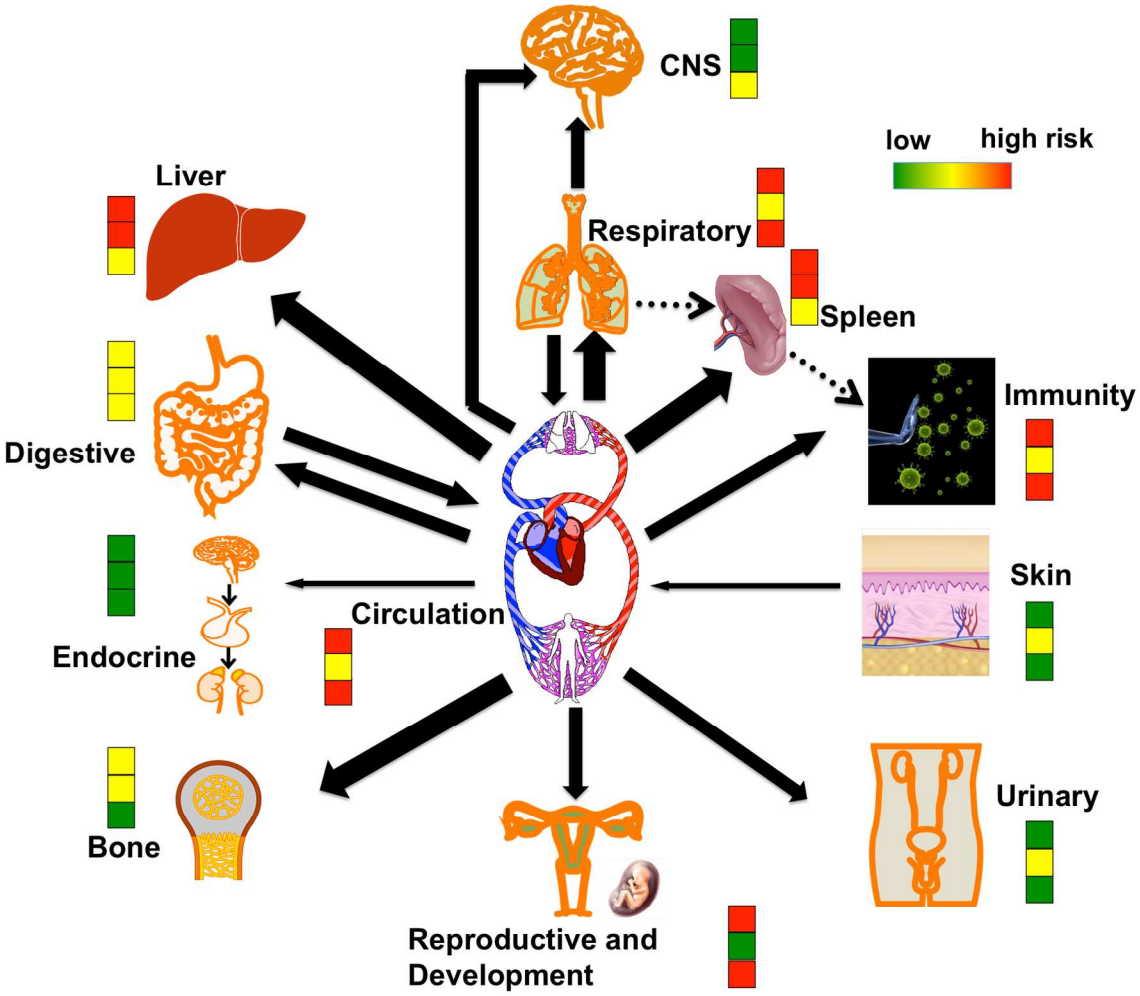

Figure 21. A working model indicating possible nanotoxicity to physiological systems. In the three-frame bar, three frames (from upper to lower) represent the probability of nanoparticle accumulation in organs or systems, self-repair capability (including the inclination of nanoparticle degradation to facilitate the excretion) of the system, and the observed toxicity from available literatures. Green, yellow and red (as shown in scale bar) indicate the levels of toxicity from low to high. These scales are only based on available data and are not conclusive because of differences in dose, nanoparticle preparation, and animal models. Arrows show the direction of nanoparticle translocation. The width of lines indicates readiness of nanoparticle's translocation. Dash lines show the reported cross-system effects.

Investigations have also shown that nanoparticles can have significant effects across systems and organs due to the close association between organs and systems. The impact of nanoparticles on one organ may be transmitted to distant organs due to the extensive intra- and inter-system communications that occur in the body. Nanoparticles can

penetrate various biological barriers, and nanotoxicity can also be transferred across generations. Therefore, assessments of the impact of exposure to nanoparticles at the level of the whole body and across generations are needed to provide a comprehensive understanding of nanotoxicity.

Perturbation or toxicity of nanoparticles to physiological systems is largely determined by the basic interactions between a nanoparticle and a biomolecule or cell. Such interactions are heavily affected by nanoparticle's size, shape, chemical composition, and surface properties. This makes it possible for us to modify nanoparticles and modulate their biological activities. However, nanoparticle's property is not the only factor that determines its toxicity to physiological systems. Different physiological systems have quite different capabilities to counter nanotoxicity. These capabilities include nanoparticle-degrading capability, antioxidant capability and cellular self-repair capability in addition to biological protection barriers such as the blood-brain barrier and the blood-testis barrier. Therefore, physiological systems also play a deciding role in the toxicity of nanoparticles (Figure 22). Another crucial factor is how nanoparticles are absorbed, translocated, distributed, metabolized, and excreted. Very complex interactions between nanoparticles and systems mentioned above jointly determine the eventual perturbations.

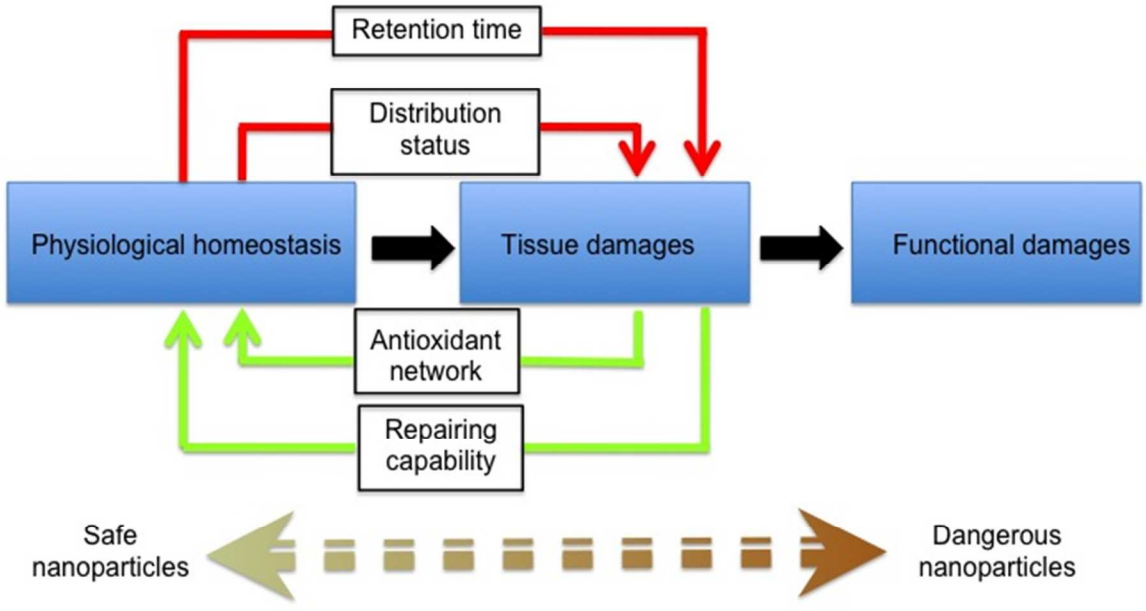

Figure 22. A simplified description of nanotoxicity to physiological systems. Safer nanoparticles with higher biological inertness are more biocompatible with physiological systems than dangerous nanoparticles that are more biologically active. Physiological systems with higher antioxidant or repair capabilities exhibit higher tolerance to nanotoxicity. The accumulation and retention of the particles are determined both by the characteristics of the nanoparticles themselves and by the characteristics of their target physiological systems.

12. Acknowledgments

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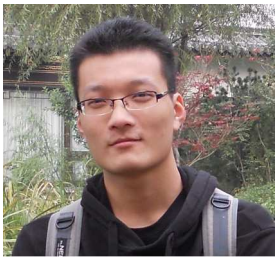

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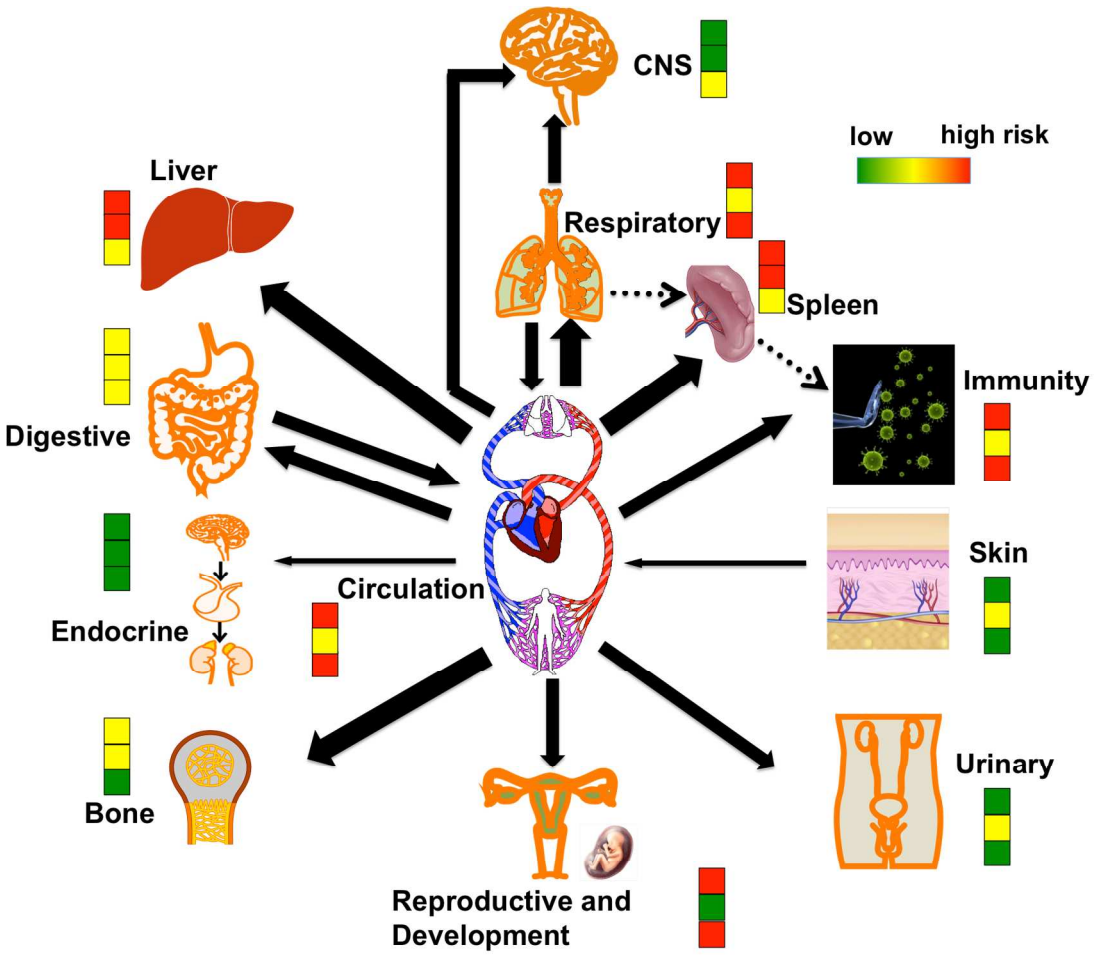

Nanoparticle translocation and potential toxicity at the physiological system level.