Review **Open Access**

Yuan Yang, Zhen Qin[a], Wei Zeng[a], Ting Yang[a], Yubin Cao, Chaorong Mei* and Yu Kuang*

Toxicity assessment of nanoparticles in various systems and organs

DOI 10.1515/ntrev-2016-0047
Received March 28, 2016; accepted October 11, 2016; previously published online December 15, 2016

Abstract: In the past decades, much attention has been paid to toxicity assessment of nanoparticles prior to clinical and biological applications. While *in vitro* studies have been increasing constantly, *in vivo* studies of nanoparticles have not established a unified system until now. Predictive models and validated standard methods are imperative. This review summarizes the current progress in approaches assessing nanotoxicity in main systems, including the hepatic and renal, gastrointestinal, pulmonary, cardiovascular, nervous, and immune systems. Histopathological studies and specific functional examinations in each system are elucidated. Related injury mechanisms are also discussed.

Keywords: function; histopathology; *in vivo*; systematic assessment; nanoparticle; toxicity.

1 Introduction

Nanoparticles (NPs) are particles with the size of 1–100 nm. Current investigation progresses have rendered NP applications in biomedical, bioengineering, and optical fields. Much attention has been drawn to NPs' toxicology, which poses possible threat both medically and environmentally. From a biomedical perspective, NP toxicology reveals an interaction between the physicochemical characteristics of NPs and their biological effects. Assessments of NP toxicity need a set of design rules. During the past decades, *in vitro* toxic characterizations of NPs have been well summarized and compared. Marquis et al. [1] reviewed the analytical methods in respect to proliferation, necrosis, apoptosis, DNA damage, and oxidative stress, which are related to the main mechanisms of cytotoxicity. While *in vitro* studies have been performed extensively [2], the significance of nanotoxicity *in vivo* studies is being emphasized [3]. Relatively long-term, complicated, and animal-sacrificed *in vivo* studies come after *in vitro* assessments and are the inevitable progress before wide-range application. Although many reviews have summarized the methods in both *in vitro* and *in vivo* studies in certain nanostructures in different model systems (Table 1), systemic evaluation remains indefinite. Fischer [3] emphasized the importance of developing predictive models of NP toxicity assessment, whereas many researchers have focused on histological changes [25] and pharmacokinetic parameters like exposing [26], biodistribution [27, 28], biochemistry metabolism, and clearance. However, exploration of nanotoxicity remains superficial in sacrificed animals. Based on the *in vivo* results of NP pharmacokinetics, including homeostasis regulation, systemically evaluating the impact of NPs on the major systems, including the hepatic, renal, digestive, pulmonary, hematological, cardiovascular, nervous, immune systems, may provide profound insight into this field. This review summarizes the recent progress of toxic assessments of NPs from the perspective of single systems.

2 Systemic assessment

On a physiological basis, nanotoxicity can be tested from pathological changes, including morphologic changes (gross, microscopic, and ultrastructural changes) and functional damage. The mechanisms underlying NPs' physiological interactions range from cells to organs and were discussed in the latest review [29]. Additionally, in terms of

Correction note: Correction added after online publication December 15, 2016: Mistakenly this article was previously published online ahead of print containing wrong name for author Xidan Mei: The correct name is: Chaorong Mei.

[a]Zhen Qin, Wei Zeng, and Ting Yang contributed equally to this work
***Corresponding authors: Chaorong Mei,** Hospital of Chengdu Office of People's Government of Tibetan Autonomous Region, Chengdu, China, e-mail: xidanmei@163.com; and **Yu Kuang,** Department of Microbiology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China, e-mail: 1214791844@qq.com
Yuan Yang, Zhen Qin, Wei Zeng and Ting Yang: Department of Microbiology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China
Yubin Cao: West China School of Stomatology, Sichuan University, Chengdu, China

Table 1: Assessments of some NPs in medical use.

Categories	Application	Assessments		References
		In vitro	*In vivo*	
Fe_3O_4	Contrast agent (MRI) labeling and tracking	Cytotoxicity	Distribution	[4, 5]
Ag	Antimicrobial agent	Genotoxicity, cytotoxicity, cellular uptake	Pulmonary toxicity, hepatotoxicity, immunotoxicity	[6–8]
Au	Biolabel, biosensor, drug carriers	Cytotoxicity	Hepatotoxicity, spleen/lung toxicity	[9–11]
TiO_2	Biomedical ceramic implanted biomaterial sterilization	Cytotoxicity (lung, nervous, hematopoietic, etc.), genotoxicity, microvascular and mitochondrial dysfunction	Skin toxicity	[12, 13]
PEG	Drug carriers	Cytotoxicity	Immunotoxicity	[14, 15]
CNT	Building blocks	Toxicokinetics	Hepatotoxicity, pulmonary toxicity	[16–19]
PLGA	Drug carriers	Macrophage uptake, phototoxicity	Nephrotoxicity	[20–22]
SLN	Drug carriers	LD_{50}, cytotoxicity, tissue injury	Lung toxicity	[23, 24]

Fe_3O_4, Iron oxide; TiO_2, Titanium dioxide; PEG, Poly(ethylene glycol); CNT, Carbon nanotube; PLGA, Poly(D, llactideco-glycolide); SLN, solid lipid nanoparticles; LD_{50}, Median lethal dose.

in vivo studies, the selection of animal models is the initial step. Although many animal models have been built, a standard and predictive model is still underdetermined. Besides the most common experimental mice and rats, zebrafish [30, 31], rabbits [32], and *Caenorhabditis elegans* [33] were also used in nanotoxicology studies. As the explicitly known genome and developed application in toxicity investigation, mice and rats would be more appropriate models in nanotoxicology studies, which is addressed below (Figure 1).

In vivo assessments are divided into two main categories. One involves tissue structure changes [34, 35], apoptosis [36, 37], and inflammation infiltration in main organs (kidney, spleen, lung, brain, and heart). Another one targets certain systems, whose structural specificities are liable to concentrate NPs. For instance, hepatic sinusoid and kupffer cells are the fundamental structures for liver function in metabolism and detoxication, in which NPs are liable to be deposited, as well as in renal filtration

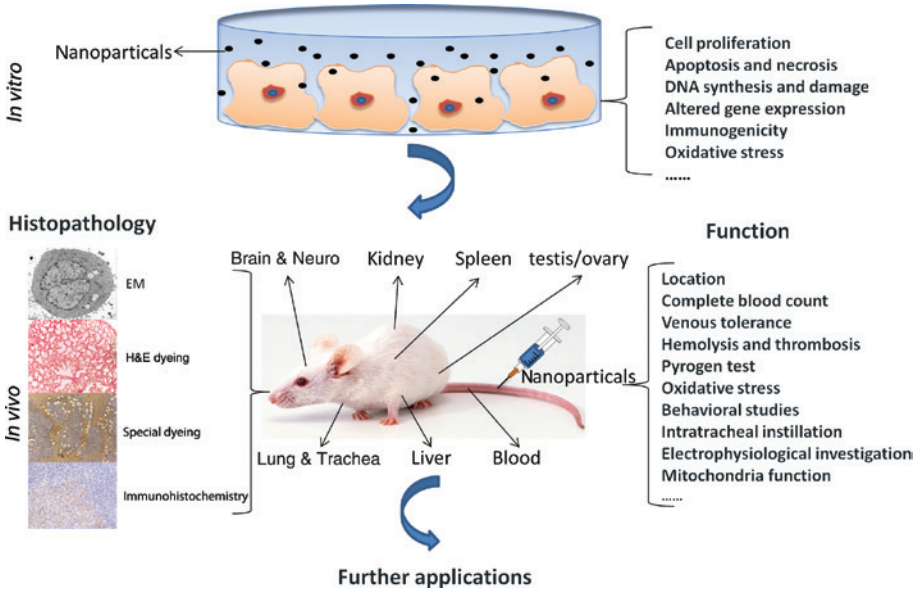

Figure 1: *In vitro* toxic characterization has been tested in respect to proliferation, necrosis, apoptosis, DNA damage, and oxidative stress. Mice and rats would be more appropriate models due to their explicit genome for toxicity tests of organs including hepatic and renal, gastrointestinal, pulmonary, hematological, cardiovascular, nervous, reproductive, and immune systems in terms of histopathological changes and functions.

membrane. Consequently, combined with the exposing ways (ingestion, injection, transdermal delivery, and inhalation) and applications, appropriate assessment models could be established, especially for the drug delivery of NPs [38].

2.1 Biodistribution

Any single physicochemical property of NPs can influence the distribution, which at a certain extent determines the material toxicity. Size [27, 39, 40], surface charge, constituent [41, 42], and chemistries of the coating could affect NP aggregation and excretion. Elucidating the biodistribution of NPs in organs is useful to guide the adjustment and modification of NPs.

The most common way of exploring the distribution is to remove the main organs [27] or tissues (lung, liver, kidney, heart, spleen, pancreas, brain, fat, and muscle) after animal sacrifice, then track the NPs according to their respective characteristics using physical detection methods. For some metal NPs, their intrinsic properties could be probed by specific instruments. Indeed, gold composite nanodevices in mouse tumor tissues could be explored by instrumental neutron activation analysis [27]. Si and Cd [34] concentrations could be determined by inductively coupled plasma optical emission spectrometry. For other varieties of NPs, some radiolabeled and fluorescent-labeled skills have been extensively applied. [^{3}H]-PLA [42] has served as a marker to show blood concentration and organ distributions of poly(ethylene glycol)-grafted nanocapsules quantitatively. Some fluorescent-labeled particles [43] in tissue homogenates were analyzed by plate reader. In some other materials [44], like quantum dots with heavy metal cores, it was more appropriate to apply multiplexing and multicolor imaging through single-particle Förster resonance energy transfer assays [45]. However, qualitative analysis with light microscope (LM) and electron microscope (EM) failed to show sufficient and significant evidence in current studies. Other imaging techniques, including typical imaging instruments like magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), single-photon emission computed tomography, optical imaging, and ultrasound, were introduced in the latest reviews [46, 47]. Furthermore, multiscale, real-time, and quantitative technologies were reported lately [48]. As some imaging contrast agents have potential unintentional toxicity, these tracing methods may aggravate the toxicity of NPs. Moreover, the combination of contrast agents may alter the characteristics and consequently change the biodistribution of NPs.

2.2 Hepatic and renal system

Hepatic sinusoid with Kupffer cells and renal glomerular basement membrane, as main metabolism and clearance organs, are fragile to toxic stimuli. Hepatic and renal toxicities are basic biosafety evaluation for drugs, as well as NPs. Oxidative stress can be determined by the index of superoxide dismutase (SOD) and glutathione peroxidase (GPx) [49]. Ultrastructural alterations of the liver and kidney are recommended primarily for morphologic changes [42]. Cationic nanobubbles [50] were visualized in histologic tests by section staining with haptoglobin and H&E. Afterward, tissue apoptosis was observed with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining.

2.2.1 Hepatic assessment

Immunohistochemistry was used to detect liver fibrosis and inflammation [50]. Serum enzymology analysis was used for hepatic function evaluation, mainly including alanine aminotransferase, aspartate aminotransferase [51], c-glutamyl transferase, and alkaline phosphatase [52], which indirectly and quantitatively reflect functional changes. Multiple automated hematology analyzer and automated chemistry analyzer are advantageous for the comprehensive analysis if conditions are permitted [53, 54].

2.2.2 Renal assessment

Histopathological study is useful to indicate renal glomerulus degeneration. The different pathologic changes, like glomerulosclerosis and collagenous tubulointerstitial matrix, can be confirmed by immunohistochemistry by detecting fibrotic and mesenchymal markers transforming growth factor-β1, interferon-6, type I collagen, fibronectin, and vimentin [55]. Cell proliferation can be assessed by measuring proliferating cell nuclear antigen [37]. Some kidney special dyeing, including PAS, PASM, and Masson, can be observed under LM [56]. The pathological diagnosis can be also determined by EM. From a functional aspect, kidney indices [57] are commonly measured. Gandhi et al. [58] tested renal variables including total proteins, albumin, and glomerular filtration rate. Urine protein, hematuria, urine albumin, and creatinine ratio [59] were also examined to evaluate the injury degree of the glomerular filtration membrane.

2.3 Gastrointestinal system

Compared with injection, oral administration of drugs or others is considered favorable due to convenience and compliance for patients. Drug bioavailability through oral administration, however, is limited because of physiological barriers of the gastrointestinal tract (GIT). With the improvement of nanocapsule transportation, some new drug delivery systems are gradually being developed, e.g. polymeric NP [60], which prevents biologicals from inactivation and degradation by acid and enzymatic barriers of the GIT. There are a majority of researches that report the benefits of NPs, such as the reversal of nonsteroidal anti-inflammatory drug-induced gastrointestinal injury [61] and radioprotection [62] from cancer radiotherapy. Cerium oxide NPs [63] were also reported to protect gastrointestinal epithelium from reactive oxygen damage. Nevertheless, the hazardous impact from NPs cannot be ignored. Exposure to TiO_2 NPs interfered in nutrient absorption of metal contents [64]. Histological assay should be the initial step in which GIT microvilli and epithelial atrophy [65, 66] are observed under EM, and the number of mast cells [64] in the stomach should be counted. Besides, functional tests of GIT were also necessary. Absorption function could be reflected indirectly by the evaluation of metal content and electrolyte. Acid-base unbalance like metabolic alkalosis occurred after the overstock of HCO_3^-, when certain mental NPs reacted drastically. A novel method quantitatively monitored the digestion of intramolecular-quenched protein under fluorescence spectroscopy in zebrafish [65] and showed digestive malfunction and developmental abnormalities.

2.4 Pulmonary system

Considering environmental issues, the initial researches of pulmonary toxicity were concentrated on inhalable particles [67, 68]. NP, as a new material with targeting and non-invasive characteristics in drug delivery system, is another category related to pulmonary impact. To detect pulmonary accumulation of NPs, MRI [69] was used to visualize antibody-conjugated superparamagnetic iron oxide (SPIO) NPs in a lipopolysaccharide-induced chronic obstructive pulmonary disease mice model. The most common method is intratracheal instillation for long-term [70, 71] and short-term [72] toxicity assessments, in which the bronchoalveolar lavage (BAL) fluid [73] is collected for biochemical analysis, including lactate dehydrogenase (LDH) assay. LDH index is useful to indicate pneumonocyte injury. As a crucial indicator of inflammatory reaction, the number of BAL-recovered neutrophils [74] is counted to indicate the extent of the inflammation. Although tissue studies are less commonly applied, pathology and apoptosis research was occasionally used in these systems. Additionally, lipid peroxidation (LPO) and glutathione production were used to reveal oxidative stress [75].

2.5 Cardiovascular system

For each of the NPs whose exposure is by way of intravenous injection, impact on the cardiovascular system is definite. The biocompatibility of hematology and serum drew lots of attention. Phlebitis is arranged at the first place of the common risk in clinical observation. Venous tolerance was explored in the auricular vein [76], where eye wetness, brachychronic breath, purple lump, blood clot, and edema were recorded. Typical symptoms of phlebitis [77] can be seen through pathological sections. Hemolysis and thrombosis are other common risks. While hemolysis was often tested *in vitro* [78], the vascular thrombosis model [79] of the carotid artery was applied by platelet aggregation and ATP release in rats. The routine study involving circulation was to count each variation of complete blood, including erythrocytes, total leukocytes, hemoglobin, and hematocrit [36].

As for cardiac injury, serum markers, like troponin-T, creatine kinase-MB, and myoglobin, should be analyzed [80]. Cardiac calcium concentration associated with contraction function and DNA damage also needs to be determined. Although energy-related function damage is concerned, ATP/ADP ratio and concentration of myoglobin in the cardiac cells were calculated less *in vivo*. Oxidative stress [81] biomarkers should be emphasized, including LPO, reactive oxygen species, and antioxidant enzymes like SOD, GPx, and catalase. Cardiac uptake of NPs was revealed through radioisotope [^{125}I] of iron oxide NPs [82]. An energetics study involving nanocarriers, which needs specific instrumentations, was performed by Vlasova et al. [83] by monitoring the cardiovascular parameters of arterial pressure and heart rate telemetrically, along with an *ex vivo* study on isolated aorta rings.

2.6 Nervous system

In the nervous system, assessments were focused on the drug delivery of solid lipid NPs (SLNs) in the brain. Especially, brain visualization was improved and drug permeability across the blood-brain barrier was assessed with

radiography techniques like PET and PET/CT system [84]. Magnetite-labeled NPs can act as a contrast agent in rat brain MRI [85]. Transportation was further assayed by non-invasive *in vivo* imaging and *ex vivo* optical imaging after injection via the carotid artery [86]. The uptake of drug into the brains was determined by the ratio of concentration in the brains (Cbr) to that in the plasma (Cpl) after intravenous injection. The impairment can be reflected by glutamate uptake dysfunction when NPs were translocated to the olfactory bulb [87]. Confocal fluorescence [86] studies were also applied to evaluate the uptake of brain endothelial cells. Meanwhile, acute toxicity was visualized by brain histology examination (LM & TEM) [88] and fluorescence imaging [85].

Nervous injury is mainly assayed by behavioral and electrophysiological studies. Behavioral studies were performed in a well-established animal model [89] with a series of clinical signs of toxicity, including tremors, convulsions, salivation, nausea, vomiting, diarrhea, lethargy, etc. Moreover, spatial learning ability and memory function associated with the hippocampus can be studied by examining the expression of related genes [90]. Electrophysiological investigation with electroencephalogram bands [91] is convenient and reliable. Peripheral electrical stimulus is one common method used in neurophysiology study, in which caudal sensory nerve action potentials [92] (caudal SNAPs) are recorded centrally from the tail. As the high oxidative metabolism in the brain, the function of brain mitochondria, which is easily isolated by differential centrifugation, is a good index to evaluate brain nerve injury [93, 94]. The respiratory chain [95] of the mitochondria provides opportunities for potential mechanism study.

2.7 Immune and reproductive system

The interactions between all new chemical and biological entities of NPs and the immune system need to be clarified prior to application in medicine and biology. Superficial modification of NPs determines their immunogenicity, which may cause immunotoxicity by interacting with the immune system [96]. Due to the complexity of the immune system, the toxicity assessment of NPs relies on overall changes of immune cytokines and molecules, immune organs, hematological system, etc. For instance, the generation and release of ILs and tumor necrosis factors are usually examined upon the use of NPs at high aspect ratio, some metal or certain cationic [97, 98]. Histological changes of major immune organs (e.g. spleen) are preferably observed by H&E dyeing [97, 99].

The toxicity assessment of the reproductive system is a relatively long-term study. Only a few researches [100–104] have focused on this kind of chronic study. However, it is a necessary step for the clinical approval of drug products. So far, various *in vivo* and *in vitro* models have been developed to study NP-associated toxicity in relative organs of this system (as reviewed in [105]). Different from other systems, the reproductive system is sexually divided into male female and male. Routinely for the male, the testicular tissue structure, the epididymal sperm parameters, the serous sexual hormones (e.g. testosterone), and the concentration of NPs in serum and testis need to be tested [106–108]. As for the female, sexual hormone (e.g. follicle stimulating hormone, luteinizing hormone, and estradiol) levels in serum need to be measured [105, 109]. Functions of major organs (ovary, uterus, and vaginal tract) also need to be evaluated [110]. The histopathological analysis is supposed to be done. Specifically, organs (testes, uteri, placenta, etc., of parental or offspring generation) with potential resorption of NPs are suggested to be inspected [111–114]. And last but not the least, reproductive index and offspring development need to be explored as some NPs were reported to penetrate the placenta-blood barrier in rats [115, 116]. Mostly, the teratogenicity of NPs on the fetus is of great concern [115, 117]. Also, the survival, growth, development, and reproduction of the offspring need to be mainly assessed upon prenatal exposure [101, 113, 118].

3 Summary

The application of NPs is increasing along with the development of nanotechnology, including drug delivery system [119], contrast agent of imaging, and engineering. Apart from the undesirable environmental NPs such as nanosilver nano-TiO_2 and carbon nanotubes [120], more attention needs to paid to medically applied nanoscale materials in systematical biosafety assessments. While *in vitro* studies indicate a promising direction, *in vivo* evaluation, as a closer step to the clinical application, more directly reflects the adaptation and injury of the organism. The present study elaborated the major systems and organ assessments from the aspects of general histopathology and specific function (Table 2). The ultrastructural changes and the relationships between tissues and NPs were clearly revealed through electric microscope. Original and specific pathologic changes were visualized by specific dyeing on paraffin sections. Functional injuries were detected by diverse biomarkers and high-tech

Table 2: Assessments of NP-related systems.

Systems	Related NPs	Assessment		References
		Histopathology	Function	
Hepatic	All	H&E dyeing, immunohistochemistry, TUNEL	Serum enzymology and hematology analysis	[50, 55]
Renal	All	Kidney special dyeing immunohistochemistry	Creatinine ratio	[56, 58, 60]
Gastrointestinal	Nanocapsules	GIT microvilli, epithelial atrophy (EM)	Blood metal content and electrolyte test	[67, 68]
Respiratory	SPIO	H&E dyeing, TUNEL	Intratracheal instillation	[70, 72–75]
Cardiovascular	Iron oxide	–	Venous tolerance, hemolysis and thrombosis, complete blood count	[36, 79, 80, 83]
Neuron	SLN	Brain histology, fluorescence imaging	Location, behavioral studies, electrophysiological investigation, oxidative stress, mitochondria function	[86, 87, 90, 91, 93, 95, 100]
Immune	All	H&E dyeing	Rabbit pyrogen test, LNPA, PFCA	[97, 120, 121]
Reproduction	All	H&E dyeing, TUNEL	Hematology, serum biochemical investigation, histopathological analysis, offspring development	[101, 111, 122–124]

SPIO, Superparamagnetic iron oxide; SLN, Solid lipid nanoparticles; LNPA, Lymph node proliferation assay; PFCA, Plaque-forming cell assay.

equipments. However, in further studies and applications, more attention should be paid to the correlations between internal environment affecting *in vivo* studies and some single factors controlled in the *in vitro* studies, although some investigations have indicated that no correlations existed between them [121, 125].

Acknowledgment: The authors gratefully acknowledge support from the Ability Improve Project (no. J1103604) of the China National Science Talents Cultivation Fund and Department of Microbiology and Experimental Technology Project (1083304121001) of Sichuan University for research support. The authors also thank the Departments of Histology, Embryology, and Neurobiology of Sichuan University for contribution of histological photos.

References

[1] Marquis BJ, Love SA, Braun KL, Haynes CL. Analytical methods to assess nanoparticle toxicity. *Analyst.* 2009, 134, 425–439.
[2] Love SA, Maurer-Jones MA, Thompson JW, Lin Y-S, Haynes CL. Assessing nanoparticle toxicity. *Annu. Rev. Anal. Chem.* 2012, 5, 181–205.
[3] Fischer HC, Chan WC. Nanotoxicity: the growing need for *in vivo* study. *Curr. Opin. Biotechnol.* 2007, 18, 565–571.
[4] Soenen SJ, De Cuyper M. Assessing cytotoxicity of (iron oxide – based) nanoparticles: an overview of different methods exemplified with cationic magnetoliposomes. *Contrast Media Mol. imaging.* 2009, 4, 207–219.
[5] Malvindi MA, De Matteis V, Galeone A, Brunetti V, Anyfantis GC, Athanassiou A, Cingolani R, Pompa PP. Toxicity assessment of silica coated iron oxide nanoparticles and biocompatibility improvement by surface engineering. *PLoS One.* 2014, 9, e85835.
[6] Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol. Adv.* 2009, 27, 76–83.
[7] Wijnhoven SW, Peijnenburg WJ, Herberts CA, Hagens WI, Oomen AG, Heugens EH, Roszek B, Bisschops J, Gosens I, Van De Meent D. Nano-silver–a review of available data and knowledge gaps in human and environmental risk assessment. *Nanotoxicology* 2009, 3, 109–138.
[8] Johnston HJ, Hutchison G, Christensen FM, Peters S, Hankin S, Stone V. A review of the in vivo and in vitro toxicity of silver and gold particulates: particle attributes and biological mechanisms responsible for the observed toxicity. *Crit. Rev. Toxicol.* 2010, 40, 328–346.
[9] Daraee H, Eatemadi A, Abbasi E, Fekri Aval S, Kouhi M, Akbarzadeh A. Application of gold nanoparticles in biomedical and drug delivery. *Artif. Cells Nanomed. Biotechnol.* 2016, 44, 410–422.
[10] Ambwani S, Kandpal D, Arora S, Ambwani TK. Cytotoxic effects of gold nanoparticles exposure employing in vitro animal cell culture system as part of nanobiosafety. In *2ND INTERNATIONAL CONFERENCE ON EMERGING TECHNOLOGIES: MICRO TO NANO 2015 (ETMN-2015): Contributory papers presented in 2nd International Conference on Emerging Technologies: Micro to Nano 2015*; AIP Publishing, 2016; pp 020091.
[11] Lin Z, Monteiro-Riviere NA, Kannan R, Riviere JE. A computational framework for interspecies pharmacokinetics, exposure and toxicity assessment of gold nanoparticles. *Nanomedicine* 2016, 11, 107–119.
[12] Iavicoli I, Leso V, Fontana L, Bergamaschi A. Toxicological effects of titanium dioxide nanoparticles: a review of in vitro mammalian studies. *Eur. Rev. Med. Pharmacol. Sci.* 2011, 15, 481–508.
[13] Yan J, Lin B, Hu C, Zhang H, Lin Z, Xi Z. The combined toxicological effects of titanium dioxide nanoparticles and bisphenol A on zebrafish embryos. *Nanoscale Res. Lett.* 2014, 9, 1–9.

[14] Rabanel J.-M, Hildgen P, Banquy X. Assessment of PEG on polymeric particles surface, a key step in drug carrier translation. *J. Control. Release.* 2014, 185, 71–87.
[15] Liao L, Zhang M, Liu H, Zhang X, Xie Z, Zhang Z, Gong T, Sun X. Subchronic toxicity and immunotoxicity of MeO-PEG-poly (D, L-lactic-co-glycolic acid)-PEG-OMe triblock copolymer nanoparticles delivered intravenously into rats. *Nanotechnology* 2014, 25, 245705.
[16] Lam C.-w, James JT, McCluskey R, Arepalli S, Hunter RL. A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. *Crit. Rev. Toxicol.* 2006, 36, 189–217.
[17] Du J, Wang S, You H, Zhao X. Understanding the toxicity of carbon nanotubes in the environment is crucial to the control of nanomaterials in producing and processing and the assessment of health risk for human: a review. *Environ. Toxicol. Pharmacol.* 2013, 36, 451–462.
[18] Aschberger K, Johnston HJ, Stone V, Aitken RJ, Hankin SM, Peters SA, Tran CL, Christensen FM. Review of carbon nanotubes toxicity and exposure – appraisal of human health risk assessment based on open literature. *Crit. Rev. Toxicol.* 2010, 40, 759–790.
[19] Johnston HJ, Hutchison GR, Christensen FM, Peters S, Hankin S, Aschberger K, Stone V. A critical review of the biological mechanisms underlying the in vivo and in vitro toxicity of carbon nanotubes: the contribution of physico-chemical characteristics. *Nanotoxicology* 2010, 4, 207–246.
[20] Zhang H, Xu J. Enhanced oral bioavailability of salmeterol by loaded PLGA microspheres: preparation, in vitro, and in vivo evaluation. *Drug Deliv.* 2016, 23, 248–253.
[21] Mody N, Tekade RK, Mehra NK, Chopdey P, Jain NK. Dendrimer, liposomes, carbon nanotubes and PLGA nanoparticles: one platform assessment of drug delivery potential. *AAPS PharmSciTech.* 2014, 15, 388–399.
[22] Asthana S, Jaiswal AK, Gupta PK, Dube A, Chourasia MK. Th-1 biased immunomodulation and synergistic antileishmanial activity of stable cationic lipid–polymer hybrid nanoparticle: biodistribution and toxicity assessment of encapsulated amphotericin B. Eur. J. Pharm. Biopharm. 2015, 89, 62–73.
[23] Paranjpe M, Finke J, Richter C, Gothsch T, Kwade A, Büttgenbach S, Müller-Goymann C. Physicochemical characterization of sildenafil-loaded solid lipid nanoparticle dispersions (SLN) for pulmonary application. *Int. J. Pharm.* 2014, 476, 41–49.
[24] Paranjpe M, Neuhaus V, Braun A, Mueller Goymann CC. Toxicity testing of sildenafil base loaded liposomes in in vitro and ex vivo models for pulmonary application. *Eur. J. Lipid Sci. Technol.* 2014, 116, 1129–1136.
[25] Kim SY, Lee YM, Baik DJ, Kang JS. Toxic characteristics of methoxy poly (ethylene glycol)/poly (ε-caprolactone) nanospheres, *in vitro* and *in vivo* studies in the normal mice. *Biomaterials.* 2003, 24, 55–63.
[26] Leite-Silva VR, Le Lamer M, Sanchez WY, Liu DC, Sanchez WH, Morrow I, Martin D, Silva HD, Prow TW, Grice JE. The effect of formulation on the penetration of coated and uncoated zinc oxide nanoparticles into the viable epidermis of human skin *in vivo*. *Eur. J. Pharm. Biopharm.* 2013, 84, 297–308.
[27] Balogh L, Nigavekar SS, Nair BM, Lesniak W, Zhang C, Sung LY, Kariapper MS, El-Jawahri A, Llanes M, Bolton B. Significant effect of size on the *in vivo* biodistribution of gold composite nanodevices in mouse tumor models. *Nanomed. Nanotechnol. Biol. Med.* 2007, 3, 281–296.
[28] Goel R, Shah N, Visaria R, Paciotti GF, Bischof JC. Biodistribution of TNF-α-coated gold nanoparticles in an in vivo model system. *Nanomedicine* 2009, 4, 401–410.
[29] Lane LA, Qian X, Smith AM, Nie S. Physical chemistry of nanomedicine: understanding the complex behaviors of nanoparticles in vivo. *Annu. Rev. Phys. Chem.* 2015, 66, 521–547.
[30] Vandhana S, Nithya M, Deepak A. Review on nano toxic effects in living organisms (mice and zebra fish). *Int. J. Innov. Res. Sci. Technol.* 2015, 1, 134–137.
[31] Fang Q, Shi X, Zhang L, Wang Q, Wang X, Guo Y, Zhou B. Effect of titanium dioxide nanoparticles on the bioavailability metabolism and toxicity of pentachlorophenol in zebrafish larvae. *J. Hazard. Mater.* 2015, 283, 897–904.
[32] Leonardi A, Bucolo C, Drago F, Salomone S, Pignatello R. Cationic solid lipid nanoparticles enhance ocular hypotensive effect of melatonin in rabbit. *Int. J. Pharm.* 2015, 478, 180–186.
[33] Zanni E, De Bellis G, Bracciale MP, Broggi A, Santarelli ML, Sarto MS, Palleschi C, Uccelletti D. Graphite nanoplatelets and *Caenorhabditis elegans*: insights from an in vivo model. *Nano Lett.* 2012, 12, 2740–2744.
[34] Guo M, Xu X, Yan X, Wang S, Gao S, Zhu S. *In vivo* biodistribution and synergistic toxicity of silica nanoparticles and cadmium chloride in mice. *J. Hazard. Mater.* 2013, 260, 780–788.
[35] Sayes CM, Marchione AA, Reed KL, Warheit DB. Comparative pulmonary toxicity assessments of C60 water suspensions in rats: few differences in fullerene toxicity in vivo in contrast to *in vitro* profiles. *Nano Lett.* 2007, 7, 2399–2406.
[36] Sarhan OMM, Hussein RM. Effects of intraperitoneally injected silver nanoparticles on histological structures and blood parameters in the albino rat. *Int. J. Nanomed.* 2014, 9, 1505.
[37] Coccini T, Barni S, Manzo L, Roda E. Apoptosis induction and histological changes in rat kidney following Cd-doped silica nanoparticle exposure: evidence of persisting effects. *Toxicol. Mech. Methods.* 2013, 23, 566–575.
[38] Gross E, Toste FD, Somorjai GA. Polymer-encapsulated metallic nanoparticles as a bridge between homogeneous and heterogeneous catalysis. *Catal. Lett.* 2015, 145, 126–138.
[39] De Jong WH, Hagens WI, Krystek P, Burger MC, Sips AJ, Geertsma RE. Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. *Biomaterials* 2008, 29, 1912–1919.
[40] Sonavane G, Tomoda K, Makino K. Biodistribution of colloidal gold nanoparticles after intravenous administration: effect of particle size. *Colloids Surf. B.* 2008, 66, 274–280.
[41] Hsieh C-M, Huang Y-W, Sheu M-T, Ho H-O. Biodistribution profiling of the chemical modified hyaluronic acid derivatives used for oral delivery system. *Int. J. Biol. Macromol.* 2014, 64, 45–52.
[42] Mosqueira VCF, Legrand P, Morgat J-L, Vert M, Mysiakine E, Gref R, Devissaguet J-P, Barratt G. Biodistribution of long-circulating PEG-grafted nanocapsules in mice: effects of PEG chain length and density. *Pharmaceut. Res.* 2001, 18, 1411–1419.
[43] Semete B, Booysen L, Lemmer Y, Kalombo L, Katata L, Verschoor J, Swai HS. *In vivo* evaluation of the biodistribution and safety of PLGA nanoparticles as drug delivery systems. *Nanomedicine* 2010, 6, 662–671.

[44] Li X, Yeh Y-C, Giri K, Mout R, Landis RF, Prakash Y, Rotello VM. Control of nanoparticle penetration into biofilms through surface design. *Chem. Comm.* 2015, 51, 282–285.
[45] Massey M, Wu M, Conroy EM, Algar WR. Mind your P's and Q's: the coming of age of semiconducting polymer dots and semiconductor quantum dots in biological applications. *Curr. Opin. Biotechnol.* 2015, 34, 30–40.
[46] Janib SM, Moses AS, MacKay JA. Imaging and drug delivery using theranostic nanoparticles. *Adv. Drug Deliver. Rev.* 2010, 62, 1052–1063.
[47] Park YI, Lee KT, Suh YD, Hyeon T. Upconverting nanoparticles: a versatile platform for wide-field two-photon microscopy and multi-modal *in vivo* imaging. *Chem. Soc. Rev.* 2015, 44, 1302–1317.
[48] Dobrucki LW, Pan D, Smith AM. Multiscale imaging of nanoparticle drug delivery. *Curr. Drug Targets.* 2015, 16, 560–570.
[49] Orazizadeh M, Fakhredini F, Mansouri E, Khorsandi L. Effect of glycyrrhizic acid on titanium dioxide nanoparticles-induced hepatotoxicity in rats. *Chem. Biol. Interact.* 2014, 220, 214–221.
[50] Pan T-L, Wang P-W, Al-Suwayeh S A, Huang Y-J, Fang J-Y. Toxicological effects of cationic nanobubbles on the liver and kidneys: biomarkers for predicting the risk. *Food Chem. Toxicol.* 2012, 50, 3892–3901.
[51] Yamagishi Y, Watari A, Hayata Y, Li X, Kondoh M, Tsutsumi Y, Yagi K. Hepatotoxicity of sub-nanosized platinum particles in mice. *Pharmazie* 2013, 68, 178–182.
[52] Cho W-S, Cho M, Jeong J, Choi M, Cho H-Y, Han BS, Kim SH, Kim HO, Lim YT, Chung BH. Acute toxicity and pharmacokinetics of 13 nm-sized PEG-coated gold nanoparticles. *Toxicol. Appl. Pharmacol.* 2009, 236, 16–24.
[53] Adamcakova-Dodd A, Stebounova LV, Kim JS, Vorrink SU, Ault AP, O'Shaughnessy PT, Grassian VH, Thorne PS. Toxicity assessment of zinc oxide nanoparticles using sub-acute and sub-chronic murine inhalation models. *Part. Fibre Toxicol.* 2014, 11, 1.
[54] Carneiro MLB, Peixoto RC, Joanitti GA, Oliveira RG, Telles LA, Miranda-Vilela AL, Bocca AL, Vianna LM, da Silva IC, de Souza AR. Antitumor effect and toxicity of free rhodium (II) citrate and rhodium (II) citrate-loaded maghemite nanoparticles in mice bearing breast cancer. *J. Nanobiotechnology.* 2013, 11, 1.
[55] Coccini T, Barni S, Mustarelli P, Locatelli C, Roda E. One-month persistence of inflammation and alteration of fibrotic marker and cytoskeletal proteins in rat kidney after Cd-doped silica nanoparticle instillation. *Toxicol. Lett.* 2015, 232, 449–457.
[56] Yanardağ R, Bolkent Ş, Özsoy-Saçan Ö, Karabulut-Bulan Ö. The effects of chard (*Beta vulgaris* L. var. cicla) extract on the kidney tissue serum urea and creatinine levels of diabetic rats. *Phytother. Res.* 2002, 16, 758–761.
[57] Gui S, Li B, Zhao X, Sheng L, Hong J, Yu X, Sang X, Sun Q, Ze Y, Wang L. Renal injury and Nrf2 modulation in mouse kidney following chronic exposure to TiO_2 nanoparticles. *J. Agric. Food Chem.* 2013, 61, 8959–8968.
[58] Gandhi S, Srinivasan B, Akarte AS. An experimental assessment of toxic potential of nanoparticle preparation of heavy metals in streptozotocin induced diabetes. *Exp. Toxicol. Pathol.* 2013, 65, 1127–1135.
[59] Petrica L, Vlad A, Gluhovschi G, Zamfir A, Popescu C, Gadalean F, Dumitrascu V, Vlad D, Popescu R, Velciov S. Glycated peptides are associated with proximal tubule dysfunction in type 2 diabetes mellitus. *Int. J. Clin. Exp. Med.* 2015, 8, 2516.
[60] Pridgen EM, Alexis F, Farokhzad OC. Polymeric nanoparticle technologies for oral drug delivery. *Clin. Gastroenterol. Hepatol.* 2014, 12, 1605–1610.
[61] Fricker G, Kromp T, Wendel A, Blume A, Zirkel J, Rebmann H, Setzer C, Quinkert R-O, Martin F, Müller-Goymann C. Phospholipids and lipid-based formulations in oral drug delivery. *Pharmaceut. Res.* 2010, 27, 1469–1486.
[62] Pamujula S, Kishore V, Rider B, Agrawal KC, Mandal TK. Radioprotection in mice following oral administration of WR-1065/PLGA nanoparticles. *Int. J. Radiat. Biol.* 2008, 84, 900–908.
[63] Colon J, Hsieh N, Ferguson A, Kupelian P, Seal S, Jenkins DW, Baker CH. Cerium oxide nanoparticles protect gastrointestinal epithelium from radiation-induced damage by reduction of reactive oxygen species and upregulation of superoxide dismutase 2. *Nanomedicine* 2010, 6, 698–705.
[64] Wang Y, Chen Z, Ba T, Pu J, Chen T, Song Y, Gu Y, Qian Q, Xu Y, Xiang K. Susceptibility of young and adult rats to the oral toxicity of titanium dioxide nanoparticles. *Small* 2013, 9, 1742–1752.
[65] Lin S, Wang X, Ji Z, Chang CH, Dong Y, Meng H, Liao Y-P, Wang M, Song T-B, Kohan S. Aspect ratio plays a role in the hazard potential of CeO_2 nanoparticles in mouse lung and zebrafish gastrointestinal tract. *ACS Nano.* 2014, 8, 4450–4464.
[66] Han X-Y, Du W-L, Huang Q-C, Xu Z-R, Wang Y-Z. Changes in small intestinal morphology and digestive enzyme activity with oral administration of copper-loaded chitosan nanoparticles in rats. *Biol. Trace. Elem. Res.* 2012, 145, 355–360.
[67] Wickramasinghe A, Karunaratne D, Sivakanesan R. PM 10-bound polycyclic aromatic hydrocarbons: biological indicators lung cancer risk of realistic receptors and 'source-exposure-effect relationship' under different source scenarios. *Chemosphere* 2012, 87, 1381–1387.
[68] Xia Z, Duan X, Tao S, Qiu W, Liu D, Wang Y, Wei S, Wang B, Jiang Q, Lu B. Pollution level inhalation exposure and lung cancer risk of ambient atmospheric polycyclic aromatic hydrocarbons (PAHs) in Taiyuan China. *Environ. Pollut.* 2013, 173, 150–156.
[69] Al Faraj A, Shaik AS, Afzal S, Al Sayed B, Halwani R. MR imaging and targeting of a specific alveolar macrophage subpopulation in LPS-induced COPD animal model using antibody-conjugated magnetic nanoparticles. *Int. J. Nanomed.* 2014, 9, 1491.
[70] Bermudez E, Mangum JB, Wong BA, Asgharian B, Hext PM, Warheit DB, Everitt JI. Pulmonary responses of mice rats and hamsters to subchronic inhalation of ultrafine titanium dioxide particles. *Toxicol. Sci.* 2004, 77, 347–357.
[71] Bermudez E, Mangum JB, Asgharian B, Wong BA, Reverdy EE, Janszen DB, Hext PM, Warheit DB, Everitt JI. Long-term pulmonary responses of three laboratory rodent species to subchronic inhalation of pigmentary titanium dioxide particles. *Toxicol. Sci.* 2002, 70, 86–97.
[72] Ma-Hock L, Burkhardt S, Strauss V, Gamer AO, Wiench K, van Ravenzwaay B, Landsiedel R. Development of a short-term inhalation test in the rat using nano-titanium dioxide as a model substance. *Inhal. Toxicol.* 2009, 21, 102–118.
[73] Paranjpe M, Müller-Goymann CC. Nanoparticle-mediated pulmonary drug delivery: a review. *Int. J. Mol. Sci.* 2014, 15, 5852–5873.
[74] Zhang DD, Hartsky MA, Warheit DB. Time course of quartz and TiO_2 particle-induced pulmonary inflammation and neu-

trophil apoptotic responses in rats. *Exp. Lung Res.* 2002, 28, 641–670.
[75] Braakhuis HM, Gosens I, Krystek P, Boere JA, Cassee FR, Fokkens PH, Post JA, van Loveren H, Park MV. Particle size dependent deposition and pulmonary inflammation after short-term inhalation of silver nanoparticles. *Part. Fibre Toxicol.* 2014, 11, 1.
[76] Zhang W, Wang G, See E, Shaw JP, Baguley BC, Liu J, Amirapu S, Wu Z. Post-insertion of poloxamer 188 strengthened liposomal membrane and reduced drug irritancy and *in vivo* precipitation superior to PEGylation. *J. Control. Release.* 2015, 203, 161–169.
[77] Laverman P, Dams ETM, Storm G, Hafmans TG, Croes HJ, Oyen WJ, Corstens FH, Boerman OC. Microscopic localization of PEG-liposomes in a rat model of focal infection. *J. Control. Release.* 2001, 75, 347–355.
[78] Fornaguera C, Calderó G, Mitjans M, Vinardell MP, Solans C, Vauthier C. Interactions of PLGA nanoparticles with blood components: protein adsorption coagulation activation of the complement system and hemolysis studies. *Nanoscale* 2015, 7, 6045–6058.
[79] Radomski A, Jurasz P, Alonso-Escolano D, Drews M, Morandi M, Malinski T, Radomski MW. Nanoparticle-induced platelet aggregation and vascular thrombosis. *Br. J. Pharmacol.* 2005, 146, 882–893.
[80] Baky N, Faddah L, Al-Rasheed N, Fatani A. Induction of inflammation DNA damage and apoptosis in rat heart after oral exposure to zinc oxide nanoparticles and the cardioprotective role of α-lipoic acid and vitamin E. *Drug Res.* 2013, 63, 228–236.
[81] Shafiee H, Mohammadi H, Rezayat SM, Hosseini A, Baeeri M, Hassani S, Mohammadirad A, Bayrami Z, Abdollahi M. Prevention of malathion-induced depletion of cardiac cells mitochondrial energy and free radical damage by a magnetic magnesium-carrying nanoparticle. *Toxicol. Mech. Method.* 2010, 20, 538–543.
[82] de Barros ALB, Chacko A-M, Mikitsh JL, Al Zaki A, Salavati A, Saboury B, Tsourkas A, Alavi A. Assessment of global cardiac uptake of radiolabeled iron oxide nanoparticles in apolipoprotein-E-deficient mice: implications for imaging cardiovascular inflammation. *Mol. Imaging Biol.* 2014, 16, 330–339.
[83] Vlasova MA, Tarasova OS, Riikonen J, Raula J, Lobach AS, Borzykh AA, Smirin BV, Kauppinen EI, Eletskii AV, Herzig K-H. Injected nanoparticles: the combination of experimental systems to assess cardiovascular adverse effects. *Eur. J. Pharm. Biopharm.* 2014, 87, 64–72.
[84] Frigell J, García I, Gómez-Vallejo V, Llop J, Penadés S. 68Ga-labeled gold glyconanoparticles for exploring blood-brain barrier permeability: preparation biodistribution studies and improved brain uptake via neuropeptide conjugation. *J. Am. Chem. Soc.* 2013, 136, 449–457.
[85] Vera NPM, Schmidt R, Langer K, Zlatev I, Wronski R, Auer E, Havas D, Windisch M, von Briesen H, Wagner S. Tracking of magnetite labeled nanoparticles in the rat brain using MRI. *PLoS One* 2014, 9, e92068.
[86] Liu D, Lin B, Shao W, Zhu Z, Ji T, Yang C. *In vitro* and *in vivo* studies on the transport of PEGylated silica nanoparticles across the blood-brain barrier. *ACS Appl. Mater. Interfaces* 2014, 6, 2131–2136.
[87] Win-Shwe T-T, Fujimaki H. Nanoparticles and neurotoxicity. *Int. J. Mol. Sci.* 2011, 12, 6267–6280.
[88] Blasi P, Schoubben A, Traina G, Manfroni G, Barberini L, Alberti PF, Cirotto C, Ricci M. Lipid nanoparticles for brain targeting III. Long-term stability and *in vivo* toxicity. *Int. J. Pharm.* 2013, 454, 316–323.
[89] Pradhan S, Patra P, Mitra S, Dey KK, Jain S, Sarkar S, Roy S, Palit P, Goswami A. Manganese nanoparticles: impact on non-modulated plant as a potent enhancer in nitrogen metabolism and toxicity study both *in vivo* and *in vitro*. *J. Agr. Food Chem.* 2014, 62, 8777–8785.
[90] Win-Shwe T-T, Yamamoto S, Fujitani Y, Hirano S, Fujimaki H. Spatial learning and memory function-related gene expression in the hippocampus of mouse exposed to nanoparticle-rich diesel exhaust. *Neurotoxicology* 2008, 29, 940–947.
[91] Horváth E, Oszlánczi G, Máté Z, Szabó A, Kozma G, Sápi A, Kónya Z, Paulik E, Nagymajtényi L, Papp A. Nervous system effects of dissolved and nanoparticulate cadmium in rats in subacute exposure. *J. Appl. Toxicol.* 2011, 31, 471–476.
[92] Negishi T, Koizumi F, Uchino H, Kuroda J, Kawaguchi T, Naito S, Matsumura Y. NK105 a paclitaxel-incorporating micellar nanoparticle is a more potent radiosensitising agent compared to free paclitaxel. *Br. J. Cancer.* 2006, 95, 601–606.
[93] Akopova O, Kolchinskaya L, Nosar V, Bouryi V, Mankovska I, Sagach V. Effect of potential-dependent potassium uptake on production of reactive oxygen species in rat brain mitochondria. *Biochemistry (Mosc).* 2014, 79, 44–53.
[94] Long TC, Saleh N, Tilton RD, Lowry GV, Veronesi B. Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity. *Environ. Sci. Technol.* 2006, 40, 4346–4352.
[95] Bhagavan HN, Chopra RK. Coenzyme: absorption tissue uptake metabolism and pharmacokinetics. *Free Radic. Res.* 2006, 40, 445–453.
[96] Dobrovolskaia MA, Shurin M, Shvedova AA. Current understanding of interactions between nanoparticles and the immune system. *Toxicol. Appl. Pharm.* 2016, 299, 78–89.
[97] Wang X, Tian J, Yong K-T, Zhu X, Lin MC-M, Jiang W, Li J, Huang Q, Lin G. Immunotoxicity assessment of CdSe/ZnS quantum dots in macrophages lymphocytes and BALB/c mice. *J. Nanobiotechnology.* 2016, 14, 1.
[98] Baron L, Gombault A, Fanny M, Villeret B, Savigny F, Guillou N, Panek C, Le Bert M, Lagente V, Rassendren F. The NLRP3 inflammasome is activated by nanoparticles through ATP ADP and adenosine. *Cell Death Dis.* 2015, 6, e1629.
[99] Silva AH, Locatelli C, Filippin-Monteiro FB, Martin P, Liptrott NJ, Zanetti-Ramos BG, Benetti LC, Nazari EM, Albuquerque CA, Pasa AA. Toxicity and inflammatory response in Swiss albino mice after intraperitoneal and oral administration of polyurethane nanoparticles. *Toxicol. Lett.* 2016, 246, 17–27.
[100] Wang Z, Chen Z, Zuo Q, Song F, Wu D, Cheng W, Fan W. Reproductive toxicity in adult male rats following intra-articular injection of cobalt–chromium nanoparticles. *J. Orthop. Sci.* 2013, 18, 1020–1026.
[101] Di Bona KR, Xu Y, Gray M, Fair D, Hayles H, Milad L, Montes A, Sherwood J, Bao Y, Rasco JF. Short-and long-term effects of prenatal exposure to iron oxide nanoparticles: influence of surface charge and dose on developmental and reproductive toxicity. *Int. J. Mol. Sci.* 2015, 16, 30251–30268.
[102] Hong J-S, Kim S, Lee SH, Jo E, Lee B, Yoon J, Eom I-C, Kim H-M, Kim P, Choi K. Combined repeated-dose toxicity study of silver

nanoparticles with the reproduction/developmental toxicity screening test. *Nanotoxicology* 2014, 8, 349–362.
[103] Kong L, Tang M, Zhang T, Wang D, Hu K, Lu W, Wei C, Liang G, Pu Y. Nickel nanoparticles exposure and reproductive toxicity in healthy adult rats. *Int. J. Mol. Sci.* 2014, 15, 21253–21269.
[104] Roychoudhury S, Nath S, Massanyi P, Stawarz R, Kacaniova M, Kolesarova A. Copper-induced changes in reproductive functions: *in vivo* and *in vitro* effects. *Physiol. Res.* 2016, 65, 11.
[105] Saunders M, Hutchison G, Carreira SC. Reproductive toxicity of nanomaterials. *Therapy* 2015, 40, 44.
[106] Li X, Yang X, Yuwen L, Yang W, Weng L, Teng Z, Wang L. Evaluation of toxic effects of CdTe quantum dots on the reproductive system in adult male mice. *Biomaterials* 2016, 96, 24–32.
[107] Layali E, Tahmasbpour E, Jorsaraei S. The effects of silver nanoparticles on oxidative stress and sperm parameters quality in male rats. *J. Babol Univ. Med. Sci.* 2016, 18, 48–55.
[108] Ren L, Zhang J, Zou Y, Zhang L, Wei J, Shi Z, Li Y, Guo C, Sun Z, Zhou X. Silica nanoparticles induce reversible damage of spermatogenic cells via RIPK1 signal pathways in C57 mice. *Int. J. Nanomed.* 2016, 11, 2251.
[109] Chattopadhyay A, Sarkar M, Sengupta R, Roychowdhury G, Biswas NM. Antitesticular effect of copper chloride in albino rats. *J. Toxicol. Sci.* 1999, 24, 393–397.
[110] Babaei H, Roshangar L, Sakhaee E, Abshenas J, Kheirandish R, Dehghani R. Ultrastructural and morphometrical changes of mice ovaries following experimentally induced copper poisoning. *Iran. Red Crescent Med. J.* 2012, 14, 558.
[111] Mozaffari Z, Parivar K, Roodbari NH, Irani S. Histopathological evaluation of the toxic effects of zinc oxide (ZnO) nanoparticles on testicular tissue of NMRI adult mice. *Adv. Stud. Biol.* 2015, 7, 275–291.
[112] Bai Y, Zhang Y, Zhang J, Mu Q, Zhang W, Butch ER, Snyder SE, Yan B. Repeated administrations of carbon nanotubes in male mice cause reversible testis damage without affecting fertility. *Nat. Nanotechnol.* 2010, 5, 683–689.
[113] Kubo-Irie M, Shinkai Y, Matsuzawa S, Uchida H, Suzuki K, Niki R, Oshio S, Takeda K. Prenatal exposure to rutile-type alumina-coated titanium dioxide nanoparticles impairs mouse spermatogenesis. *Fund. Toxicol. Sci.* 2016, 3, 67–74.
[114] Qi W, Bi J, Zhang X, Wang J, Wang J, Liu P, Li Z, Wu W. Damaging effects of multi-walled carbon nanotubes on pregnant mice with different pregnancy times. *Sci. Rep.* 2014, 4, 4352.
[115] Zhang W, Yang L, Kuang H, Yang P, Aguilar ZP, Wang A, Fu F, Xu H. Acute toxicity of quantum dots on late pregnancy mice: effects of nanoscale size and surface coating. *J. Hazard. Mater.* 2016, 318, 61–69.
[116] Shimizu M, Tainaka H, Oba T, Mizuo K, Umezawa M, Takeda K. Maternal exposure to nanoparticulate titanium dioxide during the prenatal period alters gene expression related to brain development in the mouse. *Part. Fibre Toxicol.* 2009, 6, 1.
[117] Lim J-H, Kim S-H, Lee I-C, Moon C, Kim S-H, Shin D-H, Kim H-C, Kim J-C. Evaluation of maternal toxicity in rats exposed to multi-wall carbon nanotubes during pregnancy. *Environ. Health Toxicol.* 2011, 26, e2011006.
[118] Huang X, Zhang F, Sun X, Choi K-Y, Niu G, Zhang G, Guo J, Lee S, Chen X. The genotype-dependent influence of functionalized multiwalled carbon nanotubes on fetal development. *Biomaterials* 2014, 35, 856–865.
[119] Gelperina S, Kisich K, Iseman MD, Heifets L. The potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis. *Am. J. Respir. Crit. Care Med.* 2005, 172, 1487–1490.
[120] Hendren CO, Lowry M, Grieger KD, Money ES, Johnston JM, Wiesner MR, Beaulieu SM. Modeling approaches for characterizing and evaluating environmental exposure to engineered nanomaterials in support of risk-based decision making. *Environ. Sci. Technol.* 2013, 47, 1190–1205.
[121] Nyk M, Kumar R, Ohulchanskyy TY, Bergey EJ, Prasad PN. High contrast *in vitro* and *in vivo* photoluminescence bioimaging using near infrared to near infrared up-conversion in Tm3+ and Yb3+ doped fluoride nanophosphors. *Nano Lett.* 2008, 8, 3834–3838.
[122] Watson C, Ge J, Cohen J, Pyrgiotakis G, Engelward BP, Demokritou P. High throughput screening platform for engineered nanoparticle-mediated genotoxicity using CometChip technology. *ACS Nano.* 2014, 8, 2118–2133.
[123] Nel A, Xia T, Meng H, Wang X, Lin S, Ji Z, Zhang H. Nanomaterial toxicity testing in the 21st century: use of a predictive toxicological approach and high-throughput screening. *Acc. Chem. Res.* 2012, 46, 607–621.
[124] Lai DY. Toward toxicity testing of nanomaterials in the 21st century: a paradigm for moving forward. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 2012, 4, 1–15.
[125] Das J, Das S, Paul A, Samadder A, Bhattacharyya SS, Khuda-Bukhsh AR. Assessment of drug delivery and anticancer potentials of nanoparticles-loaded siRNA targeting STAT3 in lung cancer *in vitro* and *in vivo*. *Toxicol. Lett.* 2014, 225, 454–466.

Bionotes

Yuan Yang
Department of Microbiology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China

Zhen Qin
Department of Microbiology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China

Zhen Qin received her MD from the West China School of Preclinical and Forensic Medicine, Sichuan University, where she is now a PhD candidate. Dr. Qin is studying mechanisms underlying the synergism between Influenza A virus and *Streptococcus pneumoniae* and recently became strongly interested in the application of nanomaterials in this field.

Wei Zeng
Department of Microbiology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China

Wei Zeng is a lecturer in West China School of Preclinical and Forensic Medicine, Sichuan University.

Ting Yang
Department of Microbiology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China

Ting Yang received her bachelor's degree from West China School of Preclinical and Forensic Medicine, Sichuan University.

Yubin Cao
West China School of Stomatology, Sichuan University, Chengdu, China

Yubin Cao received his bachelor's degree from West China School of Preclinical and Forensic Medicine, Sichuan University.

Chaorong Mei
Hospital of Chengdu Office of People's Government of Tibetan Autonomous Region, Chengdu, China
xidanmei@163@com

Chaorong Mei is a doctor from the Hospital of Chengdu Office of People's Government of Tibetan autonomous Region.

Yu Kuang
Department of Microbiology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China
1214791844@qq.com

Yu Kuang received her PhD from West China School of Preclinical and Forensic Medicine, Sichuan University, where she is working at now as a senior experimentalist of the pathogenic biology laboratory.