TOPICAL REVIEW — Magnetism, magnetic materials, and interdisciplinary research

Toxicity of superparamagnetic iron oxide nanoparticles: Research strategies and implications for nanomedicine*

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Superparamagnetic iron oxide nanoparticles (SPIONs) are one of the most versatile and safe nanoparticles in a wide variety of biomedical applications. In the past decades, considerable efforts have been made to investigate the potential adverse biological effects and safety issues associated with SPIONs, which is essential for the development of next-generation SPIONs and for continued progress in translational research. In this mini review, we summarize recent developments in toxicity studies on SPIONs, focusing on the relationship between the physicochemical properties of SPIONs and their induced toxic biological responses for a better toxicological understanding of SPIONs.

Keywords: superparamagnetic iron oxide nanoparticle, nanotoxicity, cytotoxicity, oxidative stress, reactive oxygen species

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1. Introduction

Nanotechnology is an emerging field with growing interest for its numerous applications ranging from information technologies to medicinal applications.[1] In 2008, the International Organization for Standardization (ISO) classified nanomaterials into three main groups, i.e., nanoparticles, nanoplates, and nanofibers.[2] A nanoparticle is defined as a material with all three external dimensions on a nanoscale (1 nm–100 nm). The dramatic increase in the use of nanoparticles in research, industry, and medicine has raised many questions about the potential toxicity.[3]

Among the most promising nanoparticles, superparamagnetic iron oxide nanoparticles (SPIONs) are the only magnetic nanoparticles that have been approved for clinical use to date.[4] SPIONs consist of magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3) cores coated with biocompatible organic/inorganic polymer. They show some unique properties such as superparamagnetism, high field irreversibility, high saturation field, and extra anisotropy contributions or shifted loops after field cooling.[5] Because of their versatile properties and biocompatibility, SPIONs have attracted a great deal of research interest and have been broadly used in bioscience and clinical research, including cell sorting;[6,7] tissue repair;[8–10] targeted drug delivery;[11–14] contrast agents for magnetic resonance imaging (MRI);[15,16] hyperthermia and magnetic field assisted radionuclide therapy.[17–21]

The increasing applications of SPIONs are accompanied with many risks and concerns on their toxicological properties and long-term influence on human health,[22–24] since the nanoscale properties can potentially induce cytotoxicity by impairing the functions of mitochondria, nucleus and DNA.[3,25–27] In the past decades, considerable efforts have been made to investigate the potential adverse biological effects and safety issues associated with SPIONs.[28–33] Those nanotoxicity studies in this area lead to the required information to make responsible regulatory decisions for future nanomedicines. The aim of this minireview is to summarize the current toxicity studies on SPIONs and explore the relationship between the physicochemical properties of SPIONs and their induced toxic biological responses.

2. Mechanism of toxicity

To date, much attention has been paid to the biocompatibility of SPIONs in the human body. Many studies have demonstrated that at doses of 100 μg/mL or higher, SPIONs with varying physicochemical characteristics may cause low toxicity or cytotoxicity (Fig. 1). As is well known, excessive reactive oxygen species (ROS), including free radicals such as the superoxide anion, hydroxyl radicals and the non-radical hydrogen peroxide, contribute to most intracellular and *in vivo* toxicities from SPIONs.[3,34,35] Because of their unique physicochemical properties, SPIONs present a large surface area for the generation of free radicals as a result of redox cycling at the particle surface. The ROS can also be generated

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from the leaching of iron ions from the surface degradation by enzymatic degradation. Subsequently, the ROS are transferred to the interior of the cell where they can produce oxidative stress by activating transcription factors for pro-inflammatory mediators.[36–39]

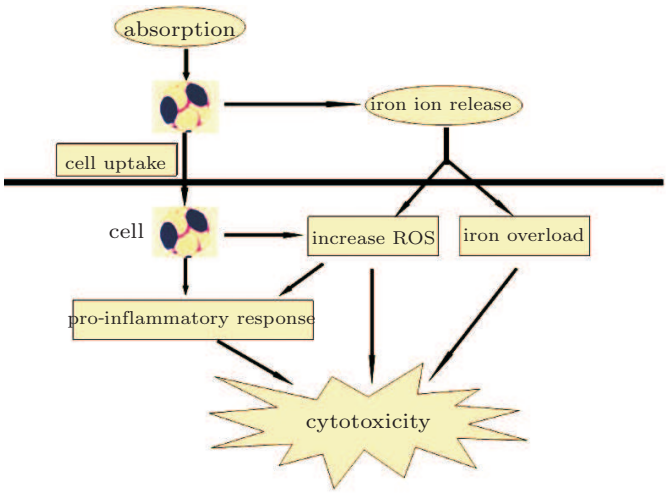


Fig. 1. Mechanism of cytotoxicity induced by SPIONs.

Furthermore, ROS can react with macromolecules and damage cells by peroxidizing lipids, changing proteins, disrupting DNA, interfering with signaling functions, and modulating gene transcription and finally causing cell death either by apoptosis or necrosis. It was found that alterations of intracellular signaling and pre-inflammatory response induced by SPIONs are correlated with the toxicity profiles of SPIONs. For example, cytosolic calcium is a key intracellular signaling molecule that controls a variety of cellular processes,[40] where ROS and oxidative stress resulting from SPIONs can modulate intracellular calcium signaling to activate the transcription factor NFκB and production of the pro-inflammatory cytokine TNFα.[41,42] and control inflammation.[43]

Another mechanism by which SPIONs can induce toxicity is *via* iron overload, which has toxic implications as excessive accumulation of the SPIONs. Since SPIONs are required to be magnetically targeted to a particular tissue/organ in biomedical application, high concentration of iron would be localized in the targeted tissue/organ.[44] Subsequently, high levels of free Fe ions in the exposed tissue can lead to an imbalance in its homeostasis and can cause aberrant cellular responses including cytotoxicity, oxidative stress, epigenetic events, inflammatory processes, and DNA damage,[26,45–47] which may initiate carcinogenesis or have a significant influence on future generations.[48] Furthermore, excess iron is associated with an increasing risk of cancer, particularly liver cancer. One of the important mechanisms is that iron, not only as a catalyst but also as a reactant, may contribute to free radical generation, which can promote the oxidation of proteins, peroxidation of membrane lipids, and modification of nucleic acids.[49–52] For example, Bhasin *et al*.[53] reported that spindle cell sarcoma and pleomorphic sarcoma were associated with iron-overload following intramuscular injections of SPIONs in rats.

3. *In vitro* cytotoxicity

The *in vitro* methods are extremely valuable for SPION safety assessments because they can produce specific and quantitative toxicity measurements rapidly and inexpensively without the use of animals. Nanoparticles often affect the metabolic activity of cells, membrane integrity of the cells, cell apoptosis, and proliferation. Therefore, the potential *in vitro* toxicity of SPIONs is initially determined as the viability of cells, cytotoxicity, oxidative stress, inflammatory reactions, and genotoxicity (Table 1).[54] Many assays have been widely used such as the lactate dehydrogenase (LDH) assay of cell membrane integrity, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for mitochondrial function, and immunochemistry biomarkers for apoptosis and necrosis (Fig. 2).

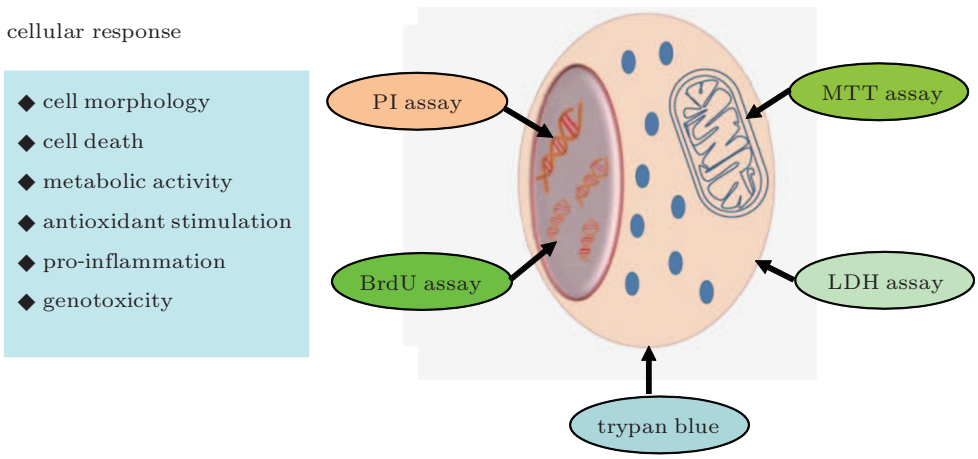


Fig. 2. *In vitro* nanotoxicity assays for SPIONs.

However, *in vitro* assays should be carried out with care since cells in culture do not experience the phase of pathogenic effects observed *in vivo*. And SPIONs can also interact with the assay components, interfere with the readout and sometimes contribute to erroneous results. For examples, in the classical dye-based assays such as MTT, the results would be disturbed by SPIONs due to the absorption of dye or dye products and the effect of ROS induced by SPIONs on the activity of mitochondrial enzymes. Additionally, some inherent issues such as dose, time and interaction between cells and matrix should be considered as well while analyzing the results, since they can also contribute to invalid data. Furthermore, because of the great difference between two-dimensional (2D) cells and three-dimensional (3D) tissues, one should be careful to apply the cell culture results to 3D tissues. The 2D cell culture may not accurately reflect the actual toxicity of SPIONs *in vivo* as it could not adequately represent the functions of 3D tissues, which have extensive cell-to-cell and cell-to-matrix interactions and different transport conditions.

The cytotoxicity of SPIONs was found to be highly dependent on a range of factors related to their physical properties, such as size, shape and surface coating. With the increase of particles' size, the area of surface increases, which becomes more reactive toward surrounding biological components and affects the biocompatibility of nanoparticles. A shape dependent nanotoxicity has been observed in a series of studies on different nanoparticles. The observed particle shapes include spherical shape, nanoworms, rod-shape, or magnetic beads, each of which has its own contact area with the cells and thus causes the difference in biocompatibility from others. For example, rod-shape SPIONs have been found to be endocytosed more slowly than spherical SPIONs.[55]

Table 1. *In vitro* cytotoxicity results of SPIONs.

Coating material	Nanoparticle size/nm	Concentration	Cell types	Exposure time	Toxicity assay	Results
Dextran	15	0.05 mg/mL	fibroblasts (human)	1 d–3 d	BrdU	result in cell death and reduced proliferation[59]
	100–150	0.1 mg/mL	macrophages (human)	7 d	MTS assay	Cells were viable (20%) after 7 days[95]
	30	1 mg/mL 10 mg/mL	malignant mesothelioma cells (human)	3 d	neutral red assay	1 mg/mL no toxic 10 mg/mL mildly toxic[96]
	20, 60	0.2–20 μM	primary peritoneal macrophages (rats and mice)	15 min–2 d	cytokine determination	result in an increased secretion of anti-inflammatory cytokines, and reduced production of pro-inflammatory cytokines[97]
		0, 1, 10, 100 μg Fe/mL	raw 264.7 (murine macrophage)	24-h	MTT, trypan blue	no significant difference between cells exposed to 0, 10, 10, or 100 mg Fe/mL SPIONs[98]
Polyvinyl alcohol	12.5	800 mM	L929 (mouse)	3 d	Crystal violet	confirmed the existence of gas vesicles inside cells[61]
	various shapes and size	0.2, 1, 5, and 20 mM	L929 (mouse)	3 h, 1 and 2 d	MTT	Toxicity was dependent on nanoparticle morphology and size[99]
	82	0.4, 0.8, and 1.6 mM	L929 (mouse)	1, 2 and 3 d	MTT	Toxicity was dependent on nanoparticle morphology and size[100]
Silica	50	4 mg/mL	A549 (human)		MTT	$IC_{50} = 4$ mg/mL[101]
	30–120	10 μg/mL	macrophages and dendritic cells (human)	2 d	MTT, trypan blue	dose- and size-dependent damage[102]
Tween 80	30	25 μg/mL–500 μg/mL	macrophages J774 (mouse)	1 h–6 h	MTT	dose- and time-dependent damage[103]
Protamine sulfate	80–150	50 μg/mL–250 μg/mL	MSC (human)	1 d–3 d	Comet	no mediated apoptosis in labeled MSCs.[104]
Uncoated	20–30	up to 80 μg/mL	A549 (human)	18 h	TB and ROS	no or low toxicity[105]
Uncoated	30, 47	up to 250 μg/mL	BRL 3A (rat)	1 d	LDH	no toxicity up to 100 μg/mL and significant toxic effects at 250 μg/mL[106]

The type of surface-coating materials of SPIONs and their breakdown products are important to determine their toxicity.[56] Uncoated SPIONs have very low dispersibility that can lead to precipitation and a high rate of agglomeration under physiological conditions. Proper coating can not only stabilize SPIONs, but also prevent the dissolution and

release of toxic ions. SPIONs are usually designed to be coated with an amphiphilic layer or bound to complex biological molecules such as antibodies, peptides, hormones or drugs.[57] The most common coatings are derivatives of dextran, polyethylene glycol (PEG), polyethylene oxide (PEO), poloxamers, and polyoxamines.[58] The cytotoxic potential of SPIONs with a range of surface coatings has been extensively investigated. For example, it was demonstrated that PEG-coated SPIONs produced negligible aggregation in cell culture media and reduced nonspecific uptake by macrophage cells.[21] However, Berry *et al.*[59] found that dextran-coated SPIONs could cause cell death and reduce proliferation similar to that caused by uncoated SPIONs. In the further study, significant membrane disruptions were observed in cells treated with dextran-coated SPIONs,[60] which may be attributed to the interactions among albumin, membrane fatty acids and phospholipids.

Cell culture medium is an important factor to influence the toxicity of SPIONs. Negatively charged uncoated SPIONs could bind to the serum proteins of cell culture medium and induce denaturation of proteins, which in turn can cause cytotoxicity.[61] Cell culture medium can also influence colloidal stability and cell interaction of SPIONs. Serum in the culture medium could induce agglomeration of the vinyl alcohol/vinyl amine copolymer-coated SPIONs and strongly inhibit cellular uptake of SPIONs.[62] Furthermore, proteins and other nutrients in cell culture medium may be adsorbed onto SPIONs and become unavailable for cellular activities, leading to the changes of cell growth and viability. Therefore, different medium recipes could influence the outcome of SPION cytotoxicity and optimal culture medium should be determined individually according to the type of SPIONs.[63,64]

The oxidation state of iron (Fe^{2+} or Fe^{3+}) in SPIONs is an additional key factor that determines the cytotoxicity of SPIONs.[48] Fe^{3+} ions are much more potent in inducing DNA damage than Fe^{2+}. It has been demonstrated that maghemite (Fe_2O_3) with an Fe^{2+}/Fe^{3+} ratio of 0.118 has a more significant genotoxicity than magnetite (Fe_3O_4) with an Fe^{2+}/Fe^{3+} ratio of 0.435. Much more effort is required to design and prepare SPIONs with good chemical stability.

4. *In vivo* toxicity of SPIONs

In vitro assays to investigate the toxicity of SPIONs are simpler, faster, and more cost-effective without ethical problems. However, little correlation between *in vivo* and *in vitro* toxicity of SPIONs has been demonstrated.[65] Researchers have found that some toxic responses of cells observed for SPIONs *in vitro*[66] were not exactly reproduced *in vivo*.[67] This may be attributed to the homeostasis maintained by the liver and kidneys, which could efficiently regulate any changes in pH, ionic strength and chemical composition of the blood plasma in the body.

Despite cost, time, and ethical considerations, *in vivo* tests in animal models are crucial for the study of SPION biological effect. *In vivo* assays have several priorities when studying the toxicity of SPIONs, such as determining the toxico-kinetics in the body (i.e., absorption, distribution, metabolism, and elimination) and evaluating the immunological, neurological, reproductive, cardiovascular and developmental toxicities to determine the chronic systemic toxicity of SPIONs.[23] Therefore, in order to understand their activity and potential toxicity it is necessary to conduct a systematic analysis of the pharmacokinetics of SPIONs, which can lead to improvements in the design of biocompatible SPIONs, a better understanding of SPION non-specificity toward tissues and cell types, and assessments of their basic distribution and clearance in the body.[68,69]

Generally, SPIONs are classified as biocompatible ones without severe toxic effects *in vivo* (Table 2).[70,71] However, the toxicity of SPIONs can be considered to be dose-dependent.[72] For example, intravenously injected Ferumoxtran-10 (dextran-coated SPIONs) at a dose of 2.6 mg Fe/kg in rats produced no changes in hemodynamic parameters whereas 13 mg Fe/kg dose caused a noticeable increase in aortic blood flow, but without any treatment-related cardiovascular or respiratory toxicity. The treatment-related clinical signs were observed only at a very high single dose (e.g., 126 mg Fe/kg, a dose 45 times higher than that used in human as MRI contrast agents). Additionally, repeated intravenous injection ($3 \sim 5$ times) at a dose of 17.9 mg Fe/kg in rats could lead to moderate changes in hematological parameters. Neurotoxicity study of ferumoxtran-10 showed some minor side effects on the central nervous system, including a lowered or increased spontaneous locomotor activity, rearing, exophthalmos, or mydriasis.[73] After injection of SPIONs, the serum iron level was noticeably increased. The chronic iron toxicity in humans may occur after administration of high doses of iron.

It is noteworthy to mention that the biological fate of SPIONs is strongly dependent on the composition and quantity of associated proteins at the surface of SPIONs,[74] which are determined by the physicochemical properties of SPIONs including surface morphology, surface charge density, coating material, nanoparticle size, and size distribution. Additionally, tissue/cell type is another crucial factor to influence the toxic response of SPIONs. For example, Hanini *et al.*[70] reported that SPIONs *in vivo* could induce toxicity in the liver, kidneys, and lungs while the brain and heart organs remained unaffected.

Table 2. *In vivo* toxicity results of SPIONs.

Toxicity study	Nanoparticle used	Concentration	Species	Administration route	Results
Distribution of SPIONs in different organs	SPION-albumin complexes	552 mg/mL	wistar rats	subcutaneous implant	relatively inert with slow, progressive local degradation over the six-month period and no distict structural alterations in the studied tissue[107]
Biocompatibility of SPIONs *in vivo*	γ–Fe_2O_3	0.8 mg/kg	wistar rats	intravenous injection; tail vein injection for clearance study	rapidly cleared through the urine; lead to toxicity in the liver, kidneys and lungs, the brain and heart remain unaffected[70]
Pulmonary toxicity and kinetic profile of TCL-SPIONs	TCL-SPIONs	0.6, 1.8, and 5.4 mg/kg for acute toxicity study, 1.8 mg/kg for kinetic study	BALB/c mice	intratracheally instillation	slightly induced pulmonary inflammation, distributed mainly in the lung and excreted in the urine *via* glomerular filtration[108]
Biodistribution, clearance, and biocompatibility of SPIOs	OA-pluronic coated iron oxide MNPs	10 mg Fe/kg.	sprague-Dawley rats	tail vein injection	did not cause long-term changes in the liver enzyme levels or induce oxidative stress[71]
Biocompatibility of magnetic fluid in two animal models	ferrofluid	0.5%–5% blood volume	sprague-Dawley rats; athymic nude mice	tail vein injection	no toxicity[109]
The selectivity of SPIONs accumulation in tumor of rats	G100 SPIONs suspensions	12 mg Fe/kg	male Fisher 344 rats	intravenous injection	accumulation of SPIONs in gliosarcomas is enhanced by magnetic targeting; quantified by MRI with no toxicity observation[110]
Systemic toxicity evaluation	Dox-TCL-SPIONs	12.5 mg Fe/kg, 0.64 mg Dox/kg	mice	intravenous injection	no toxicity in the major organs[111]
Safety evaluation of an undiluted direct injection of ferumoxides	ferumoxides	0.56 mg Fe/kg	patients with focal liver lesions	direct injection	direct injection of ferumoxides has safety and effectiveness profiles similar to those of slow infusion of the agent[112]

5. Blood compatibility

In terms of *in vivo* application of SPIONs, blood compatibility is an essential property. Should SPIONs be incompatible with bio-fluids such as blood, this could trigger coagulation and clot formation through adsorption of plasma proteins, platelet adhesion and activation of complement cascades. One of the primary screening tests on SPIONs toxicity is the haemolysis assay by using mammalian erythrocytes. The coagulation tendencies can be evaluated using widely available clinical assays including prothrombin time, activated clotting time, activated partial thromboplastin time and thrombin time,[23] which are useful in evaluating the intrinsic and extrinsic effects of SPIONs on the blood coagulation cascades. Intravenous injection (5 μM Fe/kg–40 μM Fe/kg) of Ferucarbotran, a clinically approved SPIONs coating with carboxydextran, was demonstrated to be safe except for a transient decrease in the specific clotting activity of blood coagulation Factor XI, which did not cause any clinically relevant adverse effects.

6. Biodistribution and elimination

The biodistribution of SPIONs used as an intravenous contrast agent for MRI is most widely studied.[58,75] After intravenous administration, SPIONs are distributed to various organs and tissues such as colon, lungs, bone marrow, liver, spleen, and the lymphatics.[76–78] The typical final biodistribution of SPIONs is 80%–90% in liver, 5%–8% in spleen and 1%–2% in bone marrow.[79,80] The physicochemical characteristics of nanoparticles such as size, surface morphology and surface charge, could influence their tissue distributions. After cellular uptake, SPIONs commonly reside in endosomes/lysosomes where they decompose into free iron, which is slowly released to the cytoplasm and eventually contributes to the total cellular iron pool.

The distribution of SPIONs is followed by rapid clearance from the systemic circulation, predominantly by action of the liver and spleen macrophages.[81] Generally, clearance and opsonization of SPIONs depend on their sizes and surface characteristics.[82,83] Differential opsonization accounts for variations in clearance rates and macrophage sequestration of SPIONs.[83] For example, 55% oleic acid/pluronic-coated SPIONs of injected dosage were accumulated in the liver of a rat. However, in the same animal model, 25% of injected dextran-coated SPIONs were eliminated *via* urine and feces.[84] Similarly, the distribution and elimination results obtained with ferumoxtran-10, a specific type of ultrasmall SPIONs coated with low molecular weight dextran, showed only

~ 20% of the iron ions injected were eliminated after 2 months through urine and feces in different animal models.[85] More attention should be paid to the mapping of the fate, kinetics, clearance and metabolism of SPIONs with different surface coatings, which would allow the development of predictive models of nanotoxicity (Fig. 3).[86]

SPIONs have many potential diverse applications and therefore there are a number of expected administration routes associated with the utilization of SPIONs. The distribution of SPIONs can be influenced by administration route. When being injected locally at the diseased site (e.g., tumor), SPIONs can undergo passive movement into the interstitial spaces around the administration site and gradually flow into lymphatic system. It was found that the inhaled SPIONs could cross the tight junctions/barriers such as the pulmonary epithelium, the blood-brain and blood-testis endothelium. Kwon *et al.*[87] reported that SPIONs administered by inhalation route to mice through the nose for 1 month, accumulated into the liver followed by testis, spleen, and brain. Intraperitoneally injected SPIONs were also distributed at high concentrations into the spleen and liver. Interestingly, SPIONs could cross the intact blood-brain barrier and were taken up by the neuronal cells.[28]

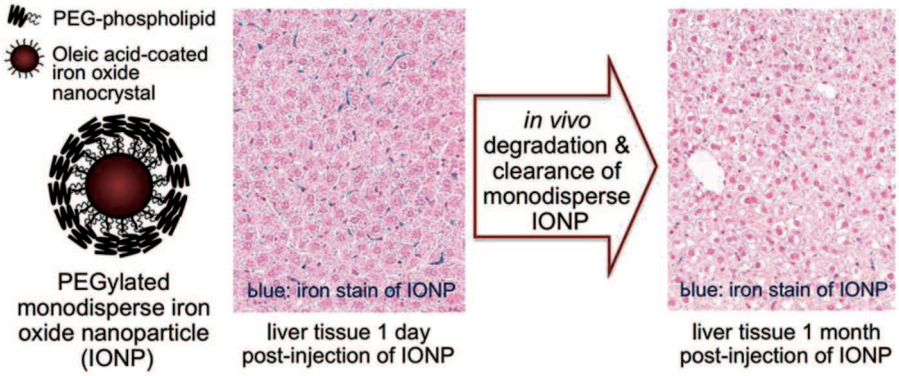


Fig. 3. Schematic overview of biodistribution, degradation, and clearance of PEGylated SPIONs in the liver. The SPIONs suitable for clinical translation require the optimization of pharmacokinetic/pharmacodynamic (PK/PD) to match residence time with the imaging needs. Reproduced with permission.[86] Copyright 2012, American Chemical Society.

7. In silico assays for nanotoxicity

An in silico method is a kind of fast and cost efficient approach to predict the nanotoxicity through integrated computational systems accounting for multiple variables associated with the biological interactions, which can supplement or replace some expensive and time-consuming assays, especially in the early design process of new types of SPIONs. By using computer-aided simulation, Dames *et al.*[88] observed that targeted aerosol delivery to the lung can be achieved with aerosol droplets comprising SPIONs in combination with a target-directed magnetic gradient field, which has been experienced in mice for the first time. Mathematical models were also developed by Sayes and Ivanov[89] to predict cellular membrane damage resulting from nanoparticles. Recently, Puzyn *et al.*[90] predicted the toxicity of 17 different metal oxide nanoparticles by using in silico assays. Such a method shows great potential for the future design of safe SPIONs.

However, in silico assay requires well-defined biological, toxicological, or pharmacological endpoints observed with similar nanoparticles, while for newly engineered nanoparticles, few toxicological or pharmacological data are available. Furthermore, as far as the accuracy is concerned, the results from in silico methods cannot be expected to exceed the data used to construct the model. Therefore, it is essential to validate the results of the mathematical model by using *in vivo* evaluation.

8. Surface engineering for SPIONs-based nanomedicine

Appropriate chemical design of both the core and the shell of SPION is extremely important for medical applications since naked SPIONs are quite unstable and even form bulk aggregates in biological fluids (i.e., blood). Generally, "green chemistry" avoiding the use of toxic chemicals is strongly recommended to prepare SPIONs suitable for preclinical and clinical applications and has attracted many efforts toward this goal. Owing to the progress in synthesis and surface modification, many new or improved methods have been developed to load a wide range of functionalities onto SPION surfaces, which can enhance its biological compatibility for SPION-based nanomedicine.[21]

Until now, a wide range of monomers, polymers, and inorganic biomaterials have been used for coating SPIONs, which provides a protective shell to stabilize SPION, avoid

agglomeration and prevent the dissolution and release of toxic ions. The coating materials on SPIONs are required to be biocompatible and biodegradable to avoid immune response and nonspecific adsorption of serum proteins after the intravenous administration. Biodegradable polymers such as dextran and carbohydrate derivatives are traditionally used as coating biomaterials of SPIONs to accomplish multiple purposes including enhancing the colloidal stability, increasing the blood circulation time and improving the biocompatibility of nanoparticles. Taking Feridex (FDA approved SPIONs) as an example, the surface coating of dextran can angle with the growing SPIO nanocrystals, protecting them from overgrowing and aggregating. Although the marketed or clinical-trial SPIONs (i.e., Combidex, Feridex and Resovist) are all coated with dextran or its derivatives, other hydrophilic polymers such as polyaspartic acid were demonstrated to be able to substitute dextran for SPION surface engineering. Additionally, polyethylene glycol (PEG), one of the most important hydrophilic polymers approved by FDA, is hemocompatible, non-antigenic and non-immunogenic with less side effects, which allows its extensive application for coating SPIONs.

Recently, high quality SPIO nanocrystals were synthesized in organic phase at higher temperatures for the better controlling of particle size and morphology.[91] However, these SPIO nanocrystals can be dispersed very well only in some organic solvents (i.e., chloroform, hexane, and tetrahydrofuran) with the coating of hydrophobic materials (i.e., oleic acid and oleylamine). Hence, it is quite necessary to transfer hydrophobic SPIO nanocrystals into a water phase with the help of amphiphilic biomaterials for certain biological application. To address such an issue, many surface engineering techniques have been developed, such as exchanging ligand and physical encapsulation, to increase the stability and biocompatibility of SPIONs. For example, we have tried to use ligands such as dopamine which has high affinity toward SPION surfaces. When being mixed, dopamine can cover the original coating oleic acid/oleylamine and lead to hydrophilicity.[21]

Polymeric micelles have emerged as good carriers for hydrophobic SPIO nanocrystals. They can solubilize SPIO nanocrystals in their inner cores and offer attractive characteristics such as a generally nanoscale size (10 nm–100 nm) and a propensity to evade scavenging by the mononuclear phagocyte system. Recently, Alkylated polyethylenimine (PEI), a typical amphiphilic block copolymer, was demonstrated to be used to form multiple SPIO nanocrystals containing micelles in aqua phase for cell labeling and MRI.[14,91] The polymer-encapsulated method is very flexible since it allows fir the preparation of SPIONs carrying a wide variety of stabilizers.

With the progress of surface engineering, there is a growing interest in developing SPIONs harboring various functions including cancer targeting, imaging, therapy, etc. Various kinds of antibodies, peptides and aptamers have been attached to SPIONs as targeting probes for specific biomarkers on target cells, providing a powerful route to the forming of multifunctional SPIONs for nanomedicine (Fig. 4).[21,92] In general, SPION surface engineered with targeting ligands has a large attractive force to bind the specific cells for targeted imaging/therapy. A typical experiment to test the SPIONs for their targeting effectiveness uses *in vitro* cultured cells that express unique biomarkers. Additionally, xenograft animal models are also widely used to evaluate SPIONs and explore basic pathophysiological mechanisms. However, it is noticeable that human tumors are often much more complex than the tumor xenografts in animal models, which could hinder the development of targeting SPIONs.

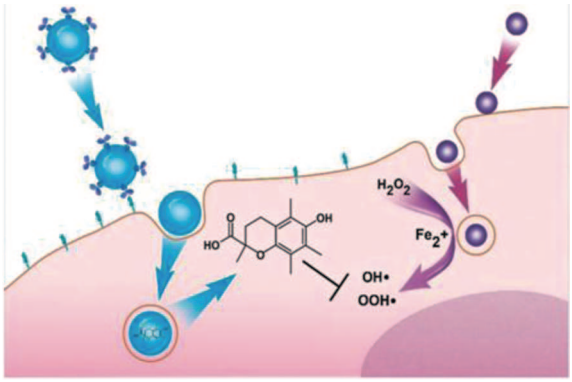


Fig. 4. Schematic diagram of the proposed protection mechanism of SPIONs coated with platelet endothelial cellular adhesion molecule-1antibodies (antiPECAM-1) and a targeted polymeric antioxidant (PTx). The antiPECAM-1/PTx SPIONs can bind to and internalize in endothelial cells and provide localized protection against the potential toxicity caused by SPIONs. Reproduced with permission.[92] Copyright 2013, Elsevier.

Surface charge plays a significant role in colloidal stability due to the behavior of the surface group in solution at a certain pH. Furthermore, the surface charge is one of the most important issues that affect the cell-nanoparticle interactions.[93] It can influence the cellular uptake efficiency of SPIONs, change organism/cellular responses to SPIONs and affect plasma protein binding hence the organ distribution and clearance of SPIONs (Fig. 5).[33,94] In general, surface functionalization with a positively charged group could enhance the uptake of SPIONs into cells. However, cationic surfaces with excess iron cations may induce hemolysis and platelet aggregation, which may be due to the affinity of cationic SPIONs to the negative phospholipid head groups or protein domains on cellular membranes.

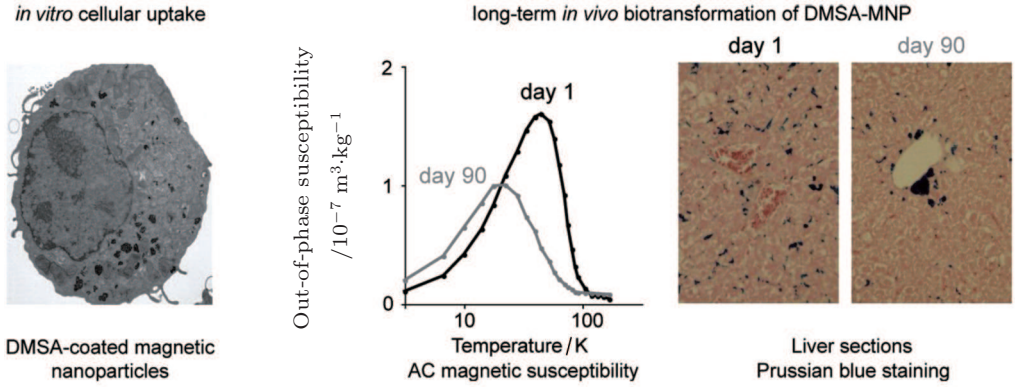


Fig. 5. Schematic overview of biocompatibility *in vitro*, and long-term biodistribution/clearance *in vivo* of SPIONs surface engineered with dimercaptosuccinic acid (DMSA). Negatively charged DMSA-SPIOs accumulating in liver tissues for extended periods of time could undergo a process of conversion from SPIOs to other non-SPIOs forms. The *in vivo* biotransformation confirms the promise of DMSA-coated SPIOs for clinical use. Reproduced with permission[94] Copyright 2013, Elsevier.

9. Conclusions and perspectives

Considering the wide preclinical and clinical applications of engineered SPIONs in the context of nanomedicine, it is crucial to understand the potential risks associated with exposure to SPIONs and the physiological effects produced by the surface coatings utilized for functionality. In this mini review, we first review the known mechanisms by which SPIONs can damage cells, including oxidative stress elicited by ROS. We provide a general discussion on the *in vitro* and *in vivo* toxicities of SPIONs while there are still a number of issues that need be clearly addressed prior to approving their clinical use. This field of nanotoxicity is important for the advancement of SPIONs in a wide range of applications. Studies in this field could lead to the required information to make responsible regulatory decisions for the development of next-generation SPIONs and for continued progress in translational research.

Some challenges need to be addressed including appropriate methods to assess the toxicity of novel SPIONs, such as generating gold standard and reference biomaterials for nanosafety testing, establishing *ex vivo* models for the specific routes of administration of nanomedicines, and developing in silico model approaches to predict the toxicological responses of SPIONs. As epigenetic changes may cause the reprogramming of gene expression long after the initial insult has been removed, epigenetic assessments should be tested early in the development of new SPIONs.

More work should be done on the design of functionalized SPIONs, which can not only be effectively and sufficiently internalized and are appropriately magnetisable, but also meet the demands of a particular application at the expense of no cellular toxicity. As for the *in vivo* pharmacokinetic studies on SPIONs, detection strategies must be capable of quantifying all of the major parts of SPIONs in tissues/organs since many multifunctional SPIONs are engineered with multiple components. Traditional radiolabeling of surface molecules coating on the SPIONs core is easily achieved, but the pharmacokinetic results might be misleading. In order to qualify the observed *in vivo* results, it is very important to understand how proteins interact with SPION surfaces, since this can potentially control the behavior of SPIONs *in vivo*. However, the studying of the toxicity aspects of SPIONs is lagged far behind their rapid development, and understanding the dynamic and complex interactions between SPIONs and biological systems is far from being complete. All of these factors give rise to conflicting results and slow down the development of this field.

In summary, the comprehensive characterization of SPIONs is often neglected prior to using them. It is crucial to encourage interdisciplinary research in the nanomedicine from the clinical, biological, engineering and toxicological point of view. Through the persistent efforts by multidisciplinary approaches, there is a great potential for further breakthrough developments in SPION designs for nanomedicine, which could solve the problem of "nanotoxicity" in the near future.

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