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Metabolism of Nanomaterials *in Vivo*: Blood Circulation and Organ Clearance

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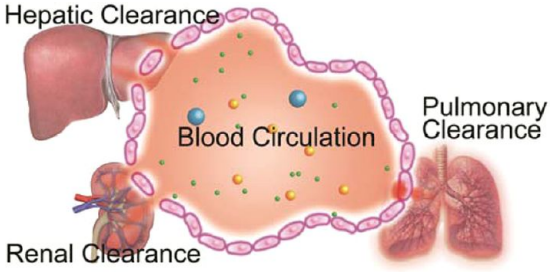

Before researchers apply nanomaterials (NMs) in biomedicine, they need to understand the blood circulation and clearance profile of these materials *in vivo*. These qualities determine the balance between nanomaterial-induced activity and unwanted toxicity. NMs have heterogeneous characteristics: they combine the bulk properties of solids with the mobility of molecules, and their highly active contact interfaces exhibit diverse functionalities. Any new and unexpected circulation features and clearance patterns are of great concern in toxicological studies and pharmaceutical screens. A number of studies have reported that NMs can enter the bloodstream directly during their application or indirectly via inhalation, ingestion, and dermal exposure. Due to the small size of NMs, the blood can then transport them throughout the circulation and to many organs where they can be stored.

In this Account, we discuss the blood circulation and organ clearance patterns of NMs in the lung, liver, and kidney. The circulation of NMs in bloodstream is critical for delivery of inhalable NMs to extrapulmonary organs, the delivery of injectable NMs, the dynamics of tissue redistribution, and the overall targeting of drug carriers to specific cells and organs. The lung, liver, and kidney are the major distribution sites and target organs for NMs exposure, and the clearance patterns of NMs in these organs are critical for understanding the *in vivo* fate of NMs.

Current studies suggest that multiple factors control the circulation and organ clearance of NMs. The size, shape, surface charge, surface functional groups, and aspect ratio of NMs as well as tissue microstructures strongly influence the circulation of NMs in bloodstream, their site-specific extravasation, and their clearance profiles within organs. Therefore structure design and surface modification can improve biocompatibility, regulate the *in vivo* metabolism, and reduce the toxicity of NMs.

The biophysicochemical interactions occurring between NMs and between NMs and the biological milieu after the introduction of NMs into living systems may further influence the blood circulation and clearance profiles of NMs. These interactions can alter properties such as agglomeration, phase transformations, dissolution, degradation, protein adsorption, and surface reactivity. The physicochemical properties of NMs change dynamically *in vivo* thereby making the metabolism of NMs complex and difficult to predict. The development of *in situ*, real-time, and quantitative techniques, *in vitro* assays, and the adaptation of physiologically-based pharmacokinetic (PBPK) and quantitative structure–activity relationship (QNSAR) modeling for NMs will streamline future *in vivo* studies.

1. Introduction

With the rapid development of nanotechnology, many innovative nanomaterials (NMs) have been chemically synthesized and are widely used in a multitude of fields. It is well-known that NMs combine the properties of solid and the mobile ability of molecules, thereby exhibiting many new and unexpected metabolism kinetic features *in vivo*.[1,2] With respect to occupational and biomedical applications, NMs can enter the bloodstream following inhalation, ingestion, injection, or dermal exposure. Once in the bloodstream, NMs rapidly circulate within the body and are taken up in organs and tissues. In particular, they tend to accumulate in the reticuloendothelial system (RES). Rapid clearance from the bloodstream with subsequent

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overaccumulation in nontarget organs is regarded as the main cause of side effects of NMs and is also a major challenge in the delivery of NMs to other sites of interest.[3,4]

According to the basic principle for safety, biomedical agents should be effectively cleared from the body and have little accumulation within the organs. So far, most *in vivo* studies indicate that NMs show nonspecific uptake in the RES, have high tendency to accumulate within the organs over an extended time, and are slowly degraded or excreted from the body. For instance, a study in mice showed that gold nanoparticles (40 nm) administered intravenously or intraperitoneally remained in the liver even after 6 months.[5] Such substantial NMs storage in tissues and organs makes it difficult for them to undergo further biodegradation or excretion, which may lead to unwanted toxicity.[6] The efficient clearance of NMs determines the balance between NM-induced activity and toxicity and is also a prerequisite for *in vivo* application of NMs, particularly when used as drug delivery systems or clinical therapeutics.

In sharp contrast to molecules and bulk materials, NMs *in vivo* show a gradual clearance process and unique clearance profile. NMs have relatively large surface-to-volume ratios and relatively more clusters of molecules or atoms on the nanosurface. These properties limit their mobility in various *in vivo* compartments. The mobility of NMs shows dynamically changing properties *in vivo*, depending on the particle physicochemical characteristics, for example, size, shape, aspect ratio, surface charge, and surface functionality.[1] When NMs are exposed to biological systems, the large number of chemically active sites on the nanosurface may initiate complex biophysicochemical reactions. As a result, the agglomeration, dissolution, adsorption, and biochemical activities of NMs are altered, and consequently influence their *in vivo* behaviors.

In this Account, we will discuss the physicochemical principles that determine the circulation and clearance features of NMs *in vivo*. Additionally, the novel metabolic and excretion profiles of NMs, which arise due to the interactions of NMs with tissue microstructure and specific biomicroenvironments, will be addressed. Finally, we propose some directions for better elucidation of *in vivo* NM circulation and clearance from technical, experimental, and theoretical perspectives.

2. Blood Circulation

NM circulation in the bloodstream is critical for inhalation-based delivery to extrapulmonary organs, injectable delivery, tissue redistribution dynamics, and the overall targeting of drug carriers to specific cells and organs. NMs in the bloodstream could be uptaken by vascular endothelial cells or alternatively, could extravasate into interstitium via transcytosis or paracellular pathways.[2,3] The patent junctions of lymph vessels facilitate the further redistribution of interstitialized NMs via lymphatics and lymphatic nanodrug delivery.[7] The immunoresponse to NMs has become a focus of more recent studies.[8] So far, uptake in the RES organs (i.e., the liver and spleen) is observed as the main destination of NMs circulating within the bloodstream.[6,9−11] Rapid clearance from the circulation with a consequent overaccumulation in the liver and spleen is regarded as the main cause of NM side effects and insufficient delivery of NMs to target sites.[4] Thus, the factors that control the blood circulation and the site-specific extravasation of NMs are of great concern in toxicological studies and pharmaceutical screening.

2.1. Effects of the Physio-anatomical Features of Vasculature. The blood circulation of NMs and their exchange between tissue vasculature and interstitium is a multifaceted process. One decisive factor is the vascular physio-anatomy. As shown in Figure 1, in some tissues, the extravasation of NMs is quite efficient due to the openings in discontinuous or fenestrated capillaries. Fenestrated capillaries are also found around tumors due to rapid construction of new vascular structures. These structures possess enhanced permeability, a phenomenon referred to as enhanced permeation retention effect.[3] Extravasation of NMs is restricted in some important organs, for example, brain, by tight junctions formed between the endothelial cells, but penetration of NMs across blood−brain barrier was observed in both *in vitro* and *in vivo* studies.[12,13]

2.2. Effects of the Physicochemical Characteristics of NMs. The blood circulation and site-specific extravasation of NMs is also dependent on physicochemical characteristics of NMs, which have been investigated extensively to understand the mechanisms of nanotoxicology and to develop nanomedicines. Small NMs might be removed from the blood by renal clearance (<5 nm) or rapid liver uptake (10−20 nm),[14] whereas large NMs are filtered in the sinusoidal spleen (>200 nm),[4] or are recognized and cleared by RES.[3] Therefore, NMs between 20 and 200 nm can remain in the circulation for an extended period of time.[4]

Additionally, recent results indicate that particle shape and surface properties may play important roles in biological half-life, biodistribution, and cellular internalization.[2] Internalization kinetics of cylindrical nanoparticles with high aspect ratios have been shown to be significantly faster than those with low aspect ratios.[15] The design of nonspherical or flexible nanoparticles can dramatically extend circulation time *in vivo*. Furthermore, it is established that nanoparticles

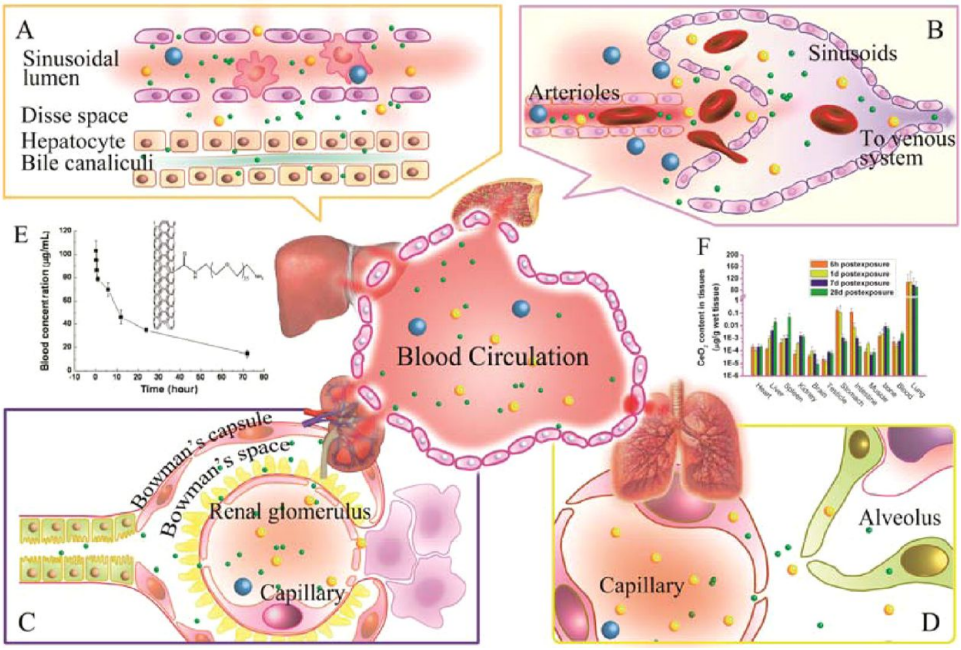

FIGURE 1. The tissue-specific extravasation of NMs. (A) The hepatic sinusoidal endothelial cells possess open fenestraes sized 100−200 nm that facilitate the NM diffusion. Smaller NMs (10−20 nm) are removed from blood via rapid liver uptake, whereas larger NMs (≥200 nm) are effectively cleared by Kupffer cells. (B) In sinusoidal spleen (as in rat and human), blood flows through the discontinuous capillary into splenic venous system. Nondeformable entities sized above 200 nm may be cleared from blood by splenic filtration. (C) The capillary fenestraes in the glomeruli have size between 10 and 100 nm, but the basal lamina can block the penetration of particles larger than 5 nm. (D) The endothelia of lung, muscle, and bone capillaries are generally characterized by a continuous morphology that allows only small particles sized below 3 nm to cross the interendothelial cell slits. (E) The blood concentration of PEG−SWCNTs versus time following intravenous administration to mice.[8] (F) The translocation to the secondary target organs and the intertissue redistribution of nanoceria via blood circulation.[9]

with neutral or negatively charged surfaces have a reduced plasma protein adsorption and low rate of nonspecific cellular uptake, while positively charged NMs are expected to have a high nonspecific internalization rate and short blood circulation half-life.[16] Blood circulation patterns of NMs may also be further influenced by surface ligands[3] and surface chirality.[17]

2.3. Effects of the Interactions between NMs and Biomicroenvironment. NM surface properties highly influence blood circulation and biodistribution. However, such properties are altered upon nano−bio interfacial reactions between the NMs and their surrounding bioenvironment. The newly formed nano−bio interface comprises dynamically physicochemical, kinetic, and thermodynamic exchanges between the nanosurfaces and the biological components (biological fluids, membranes, cell components, proteins, DNA, etc.).[18] Protein adsorption to the NM surface is one result of the interfacial reaction, leading to a protein corona that gives the NMs their biological identity.[19]

A prominent consequence of coating with proteins is the opsonization of NMs, which allows RES macrophages to easily recognize and remove these NMs. Surface modification of NMs with chemical and biological agents, such as poly(ethylene glycol) (PEG) can create a hydrophilic protective layer around the NMs, which sterically hinders absorption of opsonin proteins, thereby blocking the opsonization process.[3]

It is noteworthy that formation of protein corona does not always speed up the clearance of NMs from blood circulation. For instance, a particle hiding behind a corona of bystander proteins might have a reduced nonspecific affinity to the cell surface than a particle with a naked surface.[19] Proteins that are not recognized by any receptors of the cell or are bound to the NMs without presenting a relevant receptor-binding sequence, will provide the particle stealth with respect to the cells. Ge et al.[20] investigated the interactions of single-wall carbon nanotubes (SWCNTs) with four serum proteins and found that comparatively, bovine fibrinogen could reconstitute the most compact form and the most protein layers on the SWCNT surface, which effectively reduced the cytotoxicity of SWCNTs. This result suggests that spontaneous and rapid coating with proteins would influence NM engulfment and elimination by immune cells in the bloodstream, as well as NM clearance and delivery to the

intended target sites. Some researches have demonstrated that protein corona assisted in the transcellular passage of NMs through the blood–brain barrier.[12,13]

3. Organ Clearance of Nanomaterials

Physicochemical characteristics of NMs, such as size, shape, surface charge, surface functional group, and aspect ratio as well as the specific biomicroenvironment in the target organ strongly influence NM clearance profiles. Numerous *in vivo* studies suggest that the lung, liver, and kidney are the major distribution sites and target organs for NM exposure. Therefore, the physicochemical-based features of NM clearance in these organs are of great concern.

3.1. Pulmonary Clearance of Nanomaterials. The lung is in direct contact with the environment, and it is likely to be the first port of entry for the inhaled NMs into the body. Results on direct effects of exposure to ambient and model airborne ultrafine particles have been reported from human, rodent, and *in vitro* cell culture studies[21] and are the basis for understanding of the fate of inhaled NMs.[22] Figure 2 shows the possible clearance, storage, and extrapulmonary transport profile of NMs in lung.

Airway and alveolar macrophages (AMs) are at the forefront of lung defense. Phagocytic uptake, in concert with mucociliary transport, is the main mechanism for foreign intruder removal. However, our results and those of others,[10,11,22] demonstrate that AMs-mediated clearance is size-selective and phagocytosis of NMs smaller than 100 nm is quite inefficient. It should be noted that sometimes the "size" refers not to the dimensions of primary particles, but to those of agglomerates that cells see. NMs tend to agglomerate due to interparticle forces at the electrical double layer.[10,11] Ions in the aqueous airways where NMs meet AMs will compress the electrical double layer on the NM surface, leading to further agglomeration.[10] Thus, agglomerates with remarkably enlarged size and varied shape are always found in alveoli during the initial stage of exposure, especially when NMs are administered either at high aerosol concentrations or as suspensions (intratracheal/intranasal instillation). These agglomerates are large enough to be efficiently eliminated by phagocytic uptake followed by mucociliary movement.

Phagocytic uptake of NMs or agglomerates may be mediated by material characteristics, including size and geometry; "softness" versus stiffness, and surface adsorption/coating.[16,22] In some interesting work, coating with pulmonary surfactant-associated proteins enhanced the opsonin-dependent uptake of NMs.[23] Therefore, phagocytic uptake may be enhanced after the formation of a protein corona.

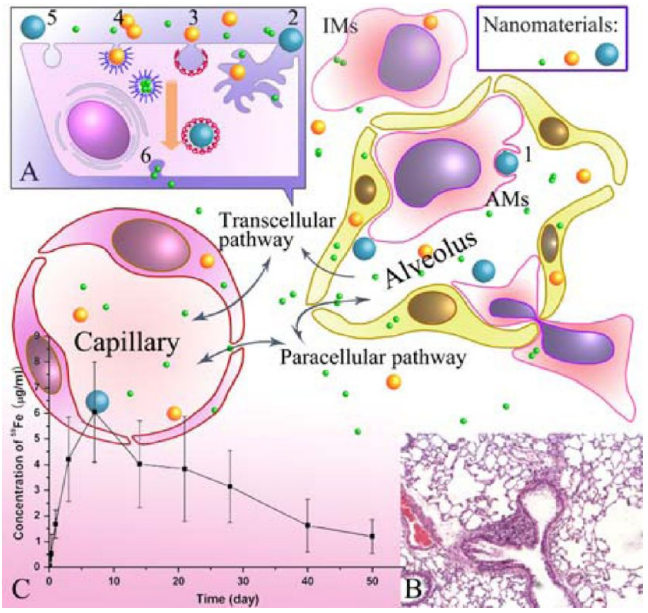

FIGURE 2. The pulmonary clearance of NMs *in vivo*. Phagocytosis (1) with subsequent clearance up the airway tree via mucociliary transport is inefficient for clearance of NMs. Other internalization mechanisms (A) include macropinocytosis (2), clathrin-mediated endocytosis (3), caveolae-mediated endocytosis (4), clathrin- and caveolae-independent endocytosis (5). NMs in alveolus could be translocated into the interstitium via a paracellular or transcellular pathway, which is the cellular uptake of NMs with the subsequent exocytosis within secretory vesicles (6). NMs may deposit in interstitium for an extremely long time due to their resistance to phagocytic uptake, which would lead to lung emphysema and prosign of lung fibrosis (B).[25] Some of the interstitialized NMs could eventually penetrate into the blood circulation (C).[10] IMs: interstitial macrophages; AMs: alveolar macrophages.

The protein corona may, on the other hand, reduce interparticle agglomeration. Agglomeration is usually quite loose; thus, the protein corona could easily overcome the agglomeration tendency by reducing surface energy and increasing hydrophilicity and steric stability. Under such circumstances, well-dispersed NMs with size below 100 nm could easily escape phagocytosis.[22] In addition, albumin adsorption significantly decreases the cellular association and uptake of NMs by AMs.[23] Given that a leak of serum albumin into alveoli is a common symptom of pulmonary exposure to NMs,[24] coating with albumin might sometimes prevent the AM-mediated clearance. Our previous research demonstrated a rapid agglomeration of nanoceria after its delivery into airways, and the agglomerates were redispersed by the presence of serum albumin.[10] Correspondingly, *in vivo* results showed an efficient clearance of nanoceria during the initial stage of intratracheal-instillation exposure, followed with a pronounced reduction in AM-mediated clearance between 8 and 28 days postexposure.[10]

Other mechanisms like macropinocytosis or pinocytosis, that is, clathrin- or aveolae-mediated endocytosis,[25] are possible but so far seem not to induce NM accumulation in macrophages and lead to macrophage-associated removal of NMs.[22] Intracellular particle dissolution is an additional clearance pathway; its rate depends on particle physicochemical characteristics such as size, density, surface area, and chemical composition.[26]

Because of the small size, NMs are likely to translocate beyond the epithelial barrier into the interstitium.[22] NMs might accumulate in interstitium for a long time due to their resistance to phagocytic uptake. The durability or biopersistence of NMs might finally cause pulmonary inflammation, fibrosis, and cancer.[26,27] Some of the interstitialized NMs could be further transported into the systemic circulation and induce impairment in extrapulmonary organs.[21]

3.2. Hepatic Clearance of Nanomaterials. Liver is the main organ of metabolic clearance of most drugs and xenobiotics. Evidence has shown that NMs are preferentially deposited in liver under systemic exposure, resulting in prolonged retention within the organ and in some instances significant hepatotoxicity.[28−30] Hepatic lobules, the structural unit of liver, consist of parenchymal cells (i.e., hepatocytes) and nonparenchymal cells, such as Kupffer cells (KCs), sinusoidal endothelial cells, stellate, and intrahepatic lymphocytes. These cells participate in the hepatic clearance pathways of NMs.

Phagocytic KCs and hepatocytes (the dominant cells in liver) represent two major pathways for hepatic clearance of NMs (Figure 3). The size of NMs plays an important role in modulating target cell type as well as the degradation pathway in liver. Generally, NMs larger than ∼200 nm are effectively cleared by KCs because slow blood flow in liver sinusoids allows enough time for phagocytosis and macropinocytosis of NMs. The endothelium of hepatic sinusoid is discontinuous with fenestrations approximately 100−200 nm in diameter.[31] Thus, NMs smaller than the fenestrations can cross the endothelium into the Disse space, then enter the lymphatic circulation or be taken up by hepatocytes.[32,33] Recent work showed that polystyrene NMs (20 nm) were internalized by hepatocytes and were observed within bile canaliculi, indicating a possible elimination via bile.[33]

Protein adsorption on the NM surface significantly affects their specific binding or uptake by liver cells. KC-mediated clearance is the main mechanism for NMs opsonized with plasma protein because KCs possess numerous receptors for selective endocytosis of these NMs. Our previous work on CdSe@S QDs (21 nm) showed that QDs could bind to

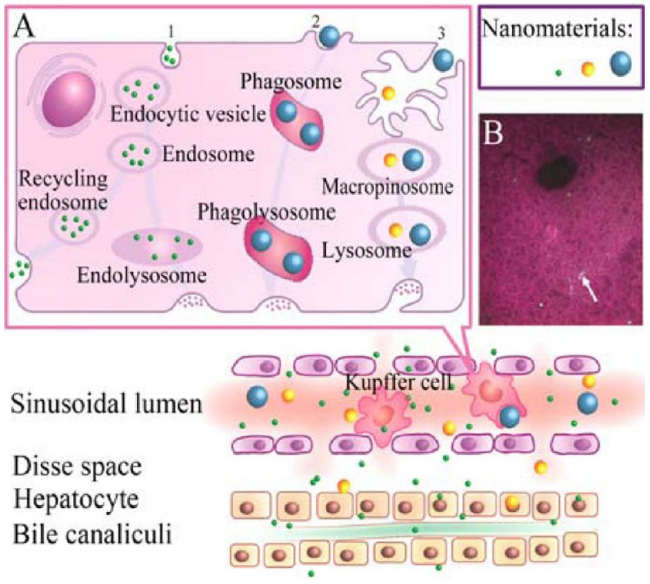

FIGURE 3. Hepatic clearance of NMs *in vivo*. Larger NMs can be taken up and cleared by Kupffer cells; smaller NMs can also across the endothelium into the Disse space and then be returned to lymphatic circulation or be taken up by hepatocytes and subsequently involved in a billary excretion pathway. (A) Kupffer cell mediated clearance pathways of NMs: (1) NMs via caveolae- or clathrin-mediated endocytosis have three different fates, accumulation in the endocytic vesicle, transport to endosome compartment and then be clearance by an endolysosomal pathway, or transport to a recycling endosome and release into blood circulation as intact NMs;[23] (2) NMs via phagocytosis can be internalized into phagosome and then be cleared via phagolysosomal pathway; (3) NMs can be taken up into macropinosomes via macropinocytosis and then be degraded by fusion of macropinosomes with lysosomes. (B) Fluorescence images of QDs in the liver. Arrows show the aggregated QDs accumulated in the liver lobules.[34]

albumin, transferrin, and γ-globulin in plasma and aggregate up to several hundred nanometers. The most protein bound QDs were metabolized in liver and excreted via feces within five days. However, 8.6% of the injected dose in the binding state still remained in hepatic tissue and was difficult to eliminate.[34] It is widely accepted that NMs modified via PEGylation can lessen their opsonization by plasma proteins and reduce the uptake by KCs. The blood circulation of PEG-SWCNTs was found to prolong up to 1 day and near-completely be cleared in the liver at around 2 months.[35]

The biophysicochemical interaction between NMs and biological components (biological fluids, phagosome, endosome, lysosome, proteins, enzymes, etc.) determines the specific clearance pathway of NMs. Lysosomal-associated degradation is a dominant pathway for KC clearance of NMs because of the higher lysosomal enzyme activities. When NMs are trapped in the KC lysosome, the acidic microenvironment of KCs and the large number of acid hydrolase enzymes may cause dissolution or enzymatic degradation

of NMs. Ours and other studies[11,36] demonstrated that pristine or dextran-coated magnetic iron oxide nanoparticles could be entrapped in lysosomal vesicles and dissolved or degraded by lysosomal α-glucosidase. Importantly, SWCNTs have been found to be biodegraded by enzymatic catalysis, including horseradish peroxidase, myeloperoxidase, and heme oxygenase-1.[37,38] It is known that there are a large number of phase I and phase II enzymes in liver, for example, monooxygenase, transferases, esterases, and epoxide hydrolase, that are expressed. Thus, the hepatic clearance of NMs could be associated with enzyme-catalyzed biodegradation, although *in vivo* evidence is still lacking at this time. However, for those NMs that are difficult to break down by intracellular processes, such as inert gold nanoparticles, NMs remain within the cells and deposit in the liver for a long time.[5] In therapeutic or toxicological studies, when NM doses overwhelm the hepatic biodegradation capacity, the excess NMs will accumulate in the organ over a long period of time as well.

3.3. Renal Clearance of Nanomaterials. Compared with the liver, the kidney is minimally involved in intracellular catabolism. Renal excretion represents a preferable clearance pathway for NMs from body. However, our previous work in mice orally exposed to copper and zinc oxide nanoparticles showed resultant morphological and pathological damage in the renal glomerulus and renal tubules.[29,30] The understanding of renal clearance of NMs is fundamental for the toxicity assessment and the *in vivo* application of NMs.

Renal clearance involves glomerular filtration, tubular secretion, and tubular reabsorption. Glomerular filtration is the first step in renal clearance of NMs and directly affects renal clearance capability. In this section, we will mainly focus on the unique patterns of glomerular filtration of NMs and the subsequent effects on renal clearance of NMs (Figure 4). In doing so, it is pertinent to discuss the anatomy and physiology of the glomerulus.

The glomerulus is a specialized vascular bed consisting of three distinct but closely interacting layers: a fenestrated endothelium (with diameter of 80−100 nm), the glomerular basement membrane (GBM, with the average thickness of 200−400 nm depending on species), and podocytes that form a special extracellular structure called the slit diaphragm (with the slit width of ∼30−40 nm and physiologic pore size of 4−5 nm in the slit diaphragm between podocytes).[39] The GBM pore size, the slit diaphragm, and fixed negatively charged components of endothelium such as proteoglycans render the glomerular barrier highly size- and charge-selective.[40,41] Renal clearance studies in mice

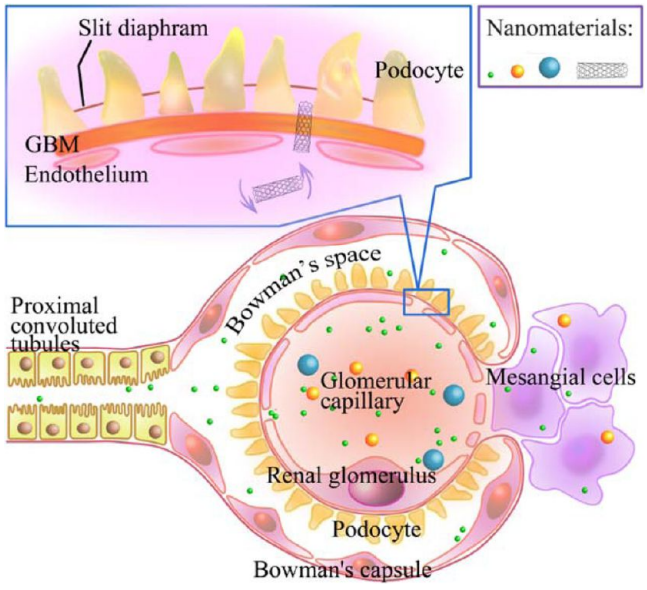

FIGURE 4. Glomerular filtration of NMs *in vivo*. NMs larger than the pore size in the GBM but less than the size of endothelial pore can enter the glomerular mesangium and accumulate in the mesangial cells or across the endothelial pore and deposit in the GBM.[41] NMs smaller than the pore size of the slit diaphragm can be filtered via glomerulus. High degrees of alignment of CNTs perpendicular to the glomerular filter increase the probability of CNTs moving through the glomerulus.[44]

using QDs with zwitterionic cysteine demonstrated the size threshold for glomerular filtration of QDs was about 5.5 nm. The QDs less than 5.5 nm were effectively excreted in the urine, while renal excretion was prevented when the diameter was above this value.[42] This filtration-size threshold is similar to that of conventional molecules to some extent; however, for most NMs, their size and charge *in vivo* is dynamically changing and may differ completely from their primitive size and charge. For instance, protein adsorption has a profound effect on the hydrodynamic diameter and surface charge of NMs thus in turn influences the renal filterability and likely shifts the route of excretion from kidney to liver. Our previous work indicated that because the anionic charged QDs absorbed serum albumin and γ-globulin and formed aggregations of several hundred nanometers, the aggregated QD particles could not be filtered via the glomerulus and were mainly metabolized in the liver.[34]

Traditionally, the molecular weight (MW) cutoff for glomerular filtration is about 70 kDa. Plasma albumin with a MW of 69 kDa passes through the glomerulus in minute quantities. However, it has been reported that CNTs with high MW (150−750 kDa) could be eliminated via urine from the body.[43] Thus, in the case of NMs, the traditional MW threshold seems inconsistent. Wang and colleges[43] reported the renal elimination of 75% iodine-125-labeled

hydroxylated SWCNTs (mean length 340 nm, MW avg 600 kDa) within 11 days following intraperitoneal injection in mice. This is an important first result to indicate that some NMs, like SWCNTs with ultrahigh MW, might be excreted in urine in a similar manner to small molecules. It is noteworthy that CNTs in the longitudinal dimension largely exceed the size of glomerular pores, therefore, it is suggested that there might be some special mechanism that mediates CNT filtration within the glomerulus. Ruggiero et al.[44] found that longitudinal SWCNTs could be highly oriented with blood flow, aligning with the long axis directly toward endothelial fenestrations, thereby increasing the probability of entry through the pores and into the glomerulus.

Under conditions when NMs deposit in kidney and are unable to be filtered by the glomerulus, they often induce severe kidney damage. The glomerular mesangial cells, glomerular capillary bed, and proximal tubular epithelial cells are considered important target sites for nephrotoxicity after exposure to NMs. It was reported that NMs with a 75 ± 25 nm diameter could target the mesangium through the endothelial pore and accumulate in multiple clusters within the phagocytic-type mesangial cells.[41] Our previous work in mice showed that oral exposure to copper nanoparticles induced grave morphological and pathological damages in the renal proximal convoluted tubule, such as reducing karyons, degeneration, and irreversibly massive necrobiosis in epithelial cells of the renal proximal convoluted tubules.[30]

4. Conclusion and Outlook

The novel properties of NMs that make them attractive may also present unwanted exposure risks for human health. Physicochemical characterization of NMs is paramount to correlate their fate *in vivo* with their nanoproperties. In most of the existing work, characterization is only performed prior to exposure to living cells or animals. However, in the native biomicroenvironment, the NM surface that is in contact with the biological milieu is in a dynamic exchange with biomolecules,[19] and thereby the properties of NMs continually vary during exposure. The status of NMs *in vivo*, such as particle wrapping, surface free energy releases, phase transformations, restructuring, degradation, dissolution, agglomeration, and deagglomeration, cannot be accurately described by the parameters determined *ex situ*. However, it remains a challenge to track fully the changes in NM characteristics during the entire metabolic process due to the lack of appropriate techniques.

In vitro approaches and models for analyzing the biological effects of NMs could better interpret the overarching information of experimental data, and extract general rules that can be applied to studies of nanotoxicity, design, modification, and applications. In this Account, some schemes referring to the circulation and clearance of NMs are extrapolated from the *in vitro* outcomes. Yet, it should be kept in mind that sometimes the extrapolation is not straightforward. Therefore, predictive mechanisms arising from *in vitro* findings should be validated through *in vivo* tests. Simulations based on PBPK and QNSAR modeling possess potential in categorizing NMs, predicting their *in vivo* results, and determining whether the *in vivo* tests are warranted or need to be redesigned.[45−47]

The new and unexpected circulation and clearance patterns of NMs can be attributed to nonspecific interaction with biological structures because of their physical properties (size and shape), biopersistence, specific interaction with biomolecules through their surface properties, or release of toxic ions through dissolution in the biomicroenvironment.[48] Quantitative study on the metabolism of NMs *in vivo* is of great concern in toxicological investigation and pharmaceutical screening. The development of *in situ*, "living" techniques for characterization, real-time dynamic techniques for quantitation, *in vitro* assays, and the adaptation of PBPK and QNSAR modeling to NMs will streamline future *in vivo* studies and optimize the design and clinical translation of NMs.

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BIOGRAPHICAL INFORMATION

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Yuliang Zhao is Professor in Chemistry and Physics. He moved to Chinese Academy of Sciences from RIKEN (Japan) as a Hundred

Elite Professor in 2001. He is mainly focused on the biomedical effects of nanostructures and nanoscale materials, including the biomedical functions of nanomaterials, the toxicological effects of nanomaterials and establishing standard procedures for safety assessment of nanoproducts, surface chemistry of nanoparticles and their novel properties, and molecular dynamics using theoretical simulation of the dynamic processes of the interplay between nanosystems and biosystems.

Weiyue Feng graduated from Fudan University of Applied Chemistry in 1989 and received her Ph.D. from the Institute of High Energy Physics, Chinese Academy of Sciences, in 1998. She is currently professor of inorganic chemistry and biological chemistry. Her primary fields of research include chemical biology, metallomics/metalloproteins, and biological and toxicological study of nanomaterials.

FOOTNOTES

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The authors declare no competing financial interest.

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