

Physiologically Based Pharmacokinetic Modeling of Nanoparticles

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Nanoparticles have been intensively researched over the past several decades for applications in diverse areas, including cosmetics, foods, and medicine.^{1,2} With growth in nanoparticle applications there is an urgent need for understanding their fate within the human body.³ Nanoparticles entering the human body can be classified by two ways of exposure: unintentional and intentional. The former includes airborne pollutants and nanomaterials in working environments, or in food and cosmetic products. Intentional exposure is mostly for medical applications, especially drug delivery and imaging. For both unintentional and intentional exposure of nanoparticles, toxicology needs to be carefully evaluated. Toxicity of nanoparticles is related to concentration and duration of exposure, which necessitates a thorough understanding of the disposition of nanoparticles. For nanoparticles of medical applications, their efficacy depends on the control of their distribution within the body, which also demands a clear illustration of the concentration–time profiles in organs and tissues of interest.

Great efforts have been made in the study of the biodistribution, toxicity, and medical applications of nanoparticles. Figure 1 clearly illustrated the exponential growth of research in these areas over the past 10 years. A PubMed search on the key phrases “nanoparticles + biodistribution”, “nanoparticles + toxicity”, or “nanoparticles + cancer” carried out on July 25, 2010, resulted in 397, 2247, or 3192 references, respectively. Two-thirds of these references were published after 2007 and one-half after 2008. The trend of growth in these areas will likely continue. These studies significantly advanced the understanding of

ABSTRACT Rapid expansion of nanoparticle research demands new technologies that will enable better interpretation of experimental data and assistance in the rational design of future nanoparticles. The use of physiologically based pharmacokinetic (PBPK) models may serve as powerful tools to meet these needs. PBPK models have been successfully applied for the study of the absorption, distribution, metabolism, and excretion (ADME) of small molecules, such as drugs. Preliminary application of PBPK models to nanoparticles illustrated their potential usefulness for nanoparticle ADME research. However, due to the differences between nanoparticles and small molecules, modifications are needed to build appropriate PBPK models for nanoparticles. This review is divided into two sections, with the first discussing nanoparticle ADME research, emphasizing the interaction of nanoparticles with living systems, including transportation kinetics across biobarrriers. In the second section, the basic principles of PBPK model development are introduced, and research pertaining to PBPK models of nanoparticles is reviewed. Factors that need to be considered for developing PBPK models for nanoparticles are also discussed. Finally, perspective applications of nanoparticle PBPK models are summarized.

KEYWORDS: PBPK · nanoparticles · ADME · biodistribution · pharmacokinetics · mathematical simulation · toxicity

nanoparticle ADME within the human body (Figure 2) and also revealed many difficulties.³ There is great diversity of nanoparticle properties (such as size, surface chemistry, and composition) and experimental designs (animal models, exposure routes, targeting organs, and duration of time) as shown in Table 1.^{4–23} The complexity of experimental scenarios causes difficulty in the comparison of experimental data from one study to another. There are numerous materials, preparation methods, and surface modifications that are available and under development. This means there could be thousands of possible nanoparticle formulations available, making it difficult to screen for specific applications. More rational methodologies must be developed to interpret the overarching information of experimental data, extract general rules that can be applied to studies of nanoparticle design, toxicity, and applications.

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Physiologically based pharmacokinetic (PBPK) models of nanoparticles may assist in resolving the above-mentioned issues. PBPK models are based on the anatomical structure of the living systems, with each important organ or tissue listed as an individual compartment. All compartments are interconnected through the mass transportation among them. During the past 30 years, PBPK modeling has been successfully applied to small molecules, particularly therapeutic

agents, in ADME and toxicity studies. The applications of PBPK modeling for nanoparticles have appeared as early as 2006. More and more efforts are being made to utilize PBPK models for the advancement of nanoparticle research, and their advantages have been recognized. PBPK models have been listed as one of the current quantitative support tools for investigation of nanoparticle hazards assessment as specified in the Organization for Economic Cooperation and Development (OECD) guideline and the new European Union regulatory framework, Registration, Evaluation, and Authorization of Chemicals (REACH).²⁵

PBPK models have been applied mostly to small molecules whose ADME behaviors may differ greatly from that of nanoparticles. The ADME of small molecules is driven by diffusion, active transport, enzymatic metabolism, and excretion. Physiological processes of nanoparticles could be much more complex, involving opsonization in the blood, cellular recognition and internalization, enzymatic degradation, physical property changes, etc. These processes are not usually relevant to small molecules.²⁶ Due to these differences, thorough

understanding of the physiological processes of nanoparticles is necessary for model building.

This review summarizes the application of PBPK modeling for nanoparticles. The general ADME of nanoparticles is discussed briefly, as it has been previously discussed by others.^{3,27,28} This review will first focus on issues that are necessary for understanding and developing nanoparticle PBPK models, including the interaction between nanoparticles and living systems, the fate of nanoparticles *in vivo*, and the kinetics of nanoparticle transportation. The second section of this review addresses PBPK models for nanoparticles. The principles of PBPK models are described briefly and more detailed descriptions are referred to other reviews.^{29,30} Emphasis will be placed on the factors to be considered for model development and application and the significance to nanoparticles.

ADME OF NANOPARTICLES

A strict definition of a nanoparticle entails at least one of its dimensions less than 100 nm.³¹ However, in many works, particles within 1000 nm were also termed as nanoparticles.^{5,14,21} Quite a few studies tested particles both less and more than 100 nm without distinction, as shown in Table 1. More importantly, no evidence suggests yet that 100 nm is a critical factor that determines different ADME behavior, although size in general is one of the most important factors that may affect ADME. In this review, nanoparticles are considered to be solid particles (inorganic and polymeric nanoparticles) with a size dimension less than 1000 nm. This includes carbon nanotubes (CNT) since the diameter of a nanotube is on the order of a few nanometers despite the length, in some cases, being more than 1000 nm.³²

ADME OF NANOPARTICLES

Absorption. Absorption is the process by which nanoparticles proceed from the external site of exposure into an internal biological space. Absorption after oral, pulmonary, injection, nasal, and dermal exposures is discussed. Absorption of nanoparticles given through other routes such as ocular,³³ intravaginal,³⁴ and intratumor³⁵ are less commonly studied.

Oral Exposure. Besides degradation in the gastrointestinal (GI) tract, nanoparticles have two directions to follow: being cleared into the feces or absorbed into the body. These are two competitive processes, depending on the transportation rates of each other. Faster clearance reduces the chance of absorption, and *vice versa*. There are two major barriers for nanoparticles to enter the body: the mucus and the epithelium of the GI tract. The mucus is a layer of gel-like liquid lining the epithelium surface creating a significant barrier for macromolecules and nanoparticles to cross.³⁶ The mucus itself renews continuously by excretion and is removed from the site into the lower section of the GI tract, carrying with it any trapped nanoparticles into the feces. The epithelial cells

VOCABULARY: **opsonization** – the process by which nanoparticles are altered by opsonins so as to become more readily and more efficiently engulfed by phagocytes. Opsonins are substances in the serum of body fluids, such as plasma proteins, that can bind to the surface of nanoparticles. • **endocytosis** – the production of internal membranes from the cell membrane lipid bilayer. In so doing, plasma membrane lipids and integral proteins and extracellular nanoparticles become fully internalized into the cell. • **exocytosis** – the morphological opposite of endocytosis, describing the fusion of entirely internal membranes with the cell membrane. In so doing, this process expels nanoparticles to the extracellular space. • **phagocytosis** – a specific form of endocytosis, in which active engulfment of solid particles by the cell membrane to form an internal phagosome occurs. • **extrapolation** – the process of constructing new data points outside a discrete set of known data points.

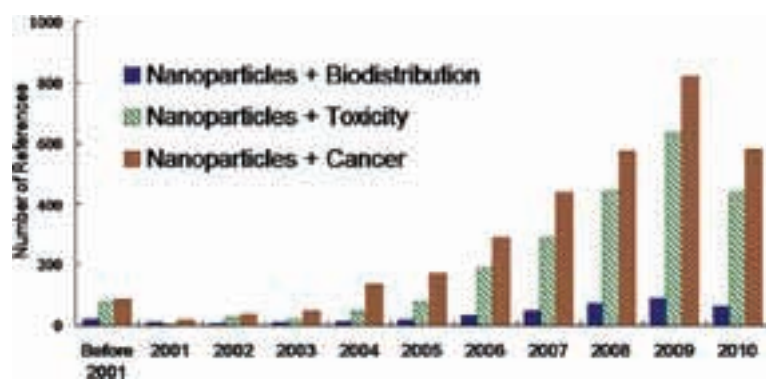


Figure 1. Distribution histogram of scientific literature on nanoparticle biodistribution or toxicity retrieved by PubMed on July 25, 2010.

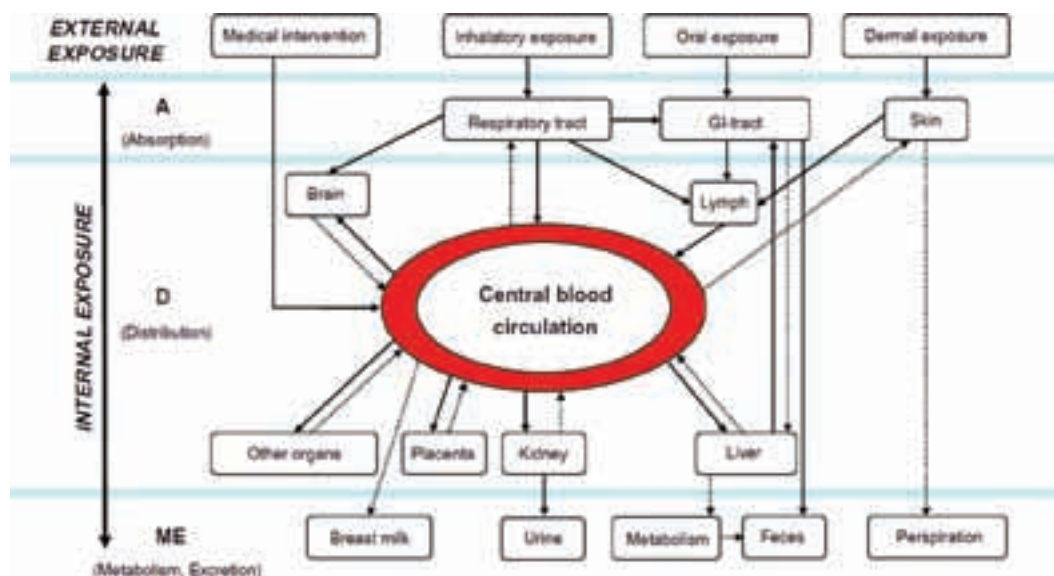


Figure 2. ADME of nanoparticles. The black lines represent confirmed routes for nanoparticles, and the dashed lines represent hypothetical routes. The transportation routes from organs back into blood are highly possible. All organs have a lymph supply, but only that of respiratory tract and GI tract were shown here. Reproduced with permission from ref 3. Copyright 2007 Elsevier.

themselves form an additional barrier for nanoparticles. Several studies in rats have shown that nanoparticles (50 nm to 200 μm) are absorbed through Peyer's patches in the wall of small intestine.³⁷ Absorption *via* intestinal enterocytes has also been demonstrated.³⁷ After entering the interstitial space of the GI tract, nanoparticles are further distributed into other organs and tissues through the blood and local lymphatic systems.³⁸

Pulmonary Exposure. Similar to oral exposure, nanoparticles inhaled into the lungs face two competitive processes: absorption and nonabsorptive clearance.³⁹ The upper section of the airway (tracheobronchial region) is protected by a mucus layer. Particles deposited in this area can be removed from the lungs by mucociliary movement or diffuse through the mucus layer to reach the epithelium cells. The lower airway, the alveolar region, is mucus free but is covered by a thin layer of surfactant and relies on macrophage phagocytosis to remove solid particles.⁴⁰ Nanoparticles could also enter the alveoli epithelium by endocytosis. The large surface area of the alveoli and the intimate air–blood contact in this region make the alveoli an advantageous site for nanoparticles to cross and enter the blood or lymphatic system underlying the alveolar epithelium.²⁷

Injections. Nanoparticles administered through noninvasive injection (subcutaneous,¹³ intramuscular,⁴¹ intradermal,⁴² and intraperitoneal)⁴³ must also be absorbed prior to distribution into other organs and tissues outside the administration site. Transportation may be primarily into regional lymph nodes followed by distribution into the blood circulation. These administration routes have commonly been investigated for delivery of nanoparticles into the respective local sites or the lymphatic system⁴³ rather than for systematic dis-

tribution. Nanoparticles in dermal tissues are likely ingested by skin macrophages and dendritic cells followed by accumulation into regional lymph nodes.²⁷ Additionally, lymphatic drainage through highly permeable lymph vessels permitting penetration of macromolecules and nanoparticles has been demonstrated.⁴⁴

Nasal Exposure. Animal studies have shown that nanoparticles deposited in the olfactory region are adsorbed into the central nervous system.⁴⁵ These studies suggest that the nasal pathway may serve as a port of entry for nanoparticles into the brain, circumventing the restrictive blood–brain barrier (BBB).⁴⁶ This is of significant interest for drug delivery to the brain in addition to the neurotoxicological impact of nanoparticles after environmental exposure by inhalation. However, the relevance of these results to humans needs to be confirmed further due to significant physiological differences between humans and the research animals.²⁷

Dermal Exposure. Nanoparticle exposure to the skin has also drawn increasing attention during the past years, due to the application of cosmetic and medical products containing nanomaterials.⁴⁷ While the skin forms a tight protective barrier for the underlying tissues, some researchers have shown that a variety of nanoparticles could penetrate through the epidermis.⁴⁸ Nanoparticles with transdermal absorption primarily collected in the lymphatic system and the regional lymph nodes.

Distribution. Numerous investigations have shown that nanoparticles distribute into nearly all tissues and organs following various administration routes.³ As expected, the concentrations in different tissues or organs differ depending on the properties of the nanoparticles and their interaction with the living system. Nanoparticles within tissues may reside in the extracellular

space, adhere on the surface of macrophages or cells of the tissue, or enter the interior of cells.⁴⁹

Blood. Protein binding to nanoparticle surfaces, termed opsonization, occurs almost instantaneously once the particle enters the blood circulation, and the physiochemical properties of these nanoparticle–protein complexes are often different than those of the original nanoparticle.^{50,51} The reaction of nanoparticles with plasma proteins has been intensively studied and has proven to be the major step to facilitate the recognition and further phagocytosis of nanoparticles by macrophages.⁵⁰ This has resulted in the design of “stealth” nanoparticles with reduced opsonization.⁵² Apart from plasma proteins, nanoparticles may also interact with blood cells. Several different nanoparticles, including gold and titanium oxide, have been identified inside human red blood cells.⁵¹

A second factor that influences nanoparticle distribution into tissues from the blood compartment is the blood vessel endothelium. Endothelia composing the blood vessels have been classified as continuous, fenestrated, or discontinuous, depending on the morphological features of the endothelium.⁵³ The fenestrated endothelium exists in glands, digestive mucosa, and kidneys and has an octagonal symmetry with radial fibrils interweaving in a central point forming fenestrae of approximately 60 nm. Discontinuous endothelium is a characteristic of the liver, spleen, and bone marrow with pores of 50–100 nm. Nanoparticles less than 60 nm may have easier access into tissues with fenestrated or discontinuous endothelium.

Nanoparticle distribution could also be influenced by the blood supply of tissues. Tissues are divided into two groups by blood supply: quickly and slowly perfused.⁵⁴ The effect of blood supply depends on the tissue blood supply and the transportation rates of nanoparticles from blood into tissues. When the blood supply is very limited, or nanoparticles distribute into tissues very fast, the blood flow may become the limiting step of nanoparticle distribution.

Reticuloendothelial System (RES). The reticuloendothelial system (also called mononuclear phagocyte system) represents a group of cells having the ability to ingest large numbers of particles.⁵⁵ They include monocytes circulating in the blood, Kupffer cells in the liver, reticular cells in the lymph nodes, bone marrow, and spleen, and fixed macrophages of various connective tissues. They are either freely circulating within the blood or fixed to various connective tissues. Proteins and other blood components that could react with nanoparticles, also called “opsonins”, act as ligands on the particle surface and facilitate their recognition and initial attachment by phagocyte receptors.

It has been noticed that nanoparticles are rapidly captured and retained by the organs comprising of the RES. Liver is the major target of nanoparticles accumulation, especially after IV administration. In the liver,

the particles are mainly retained by the Kupffer cells,⁵⁶ while the hepatocytes⁵⁷ and the liver endothelial cells⁵⁸ may play a secondary role. Accumulation of nanoparticles in the spleen is also generally high. In the spleen, the marginal zone and the red pulp macrophages are the major scavengers, while peritoneal macrophages and dendritic cells have a minor contribution.^{59,60} Bone marrow was not commonly studied in nanoparticle biodistribution studies (Table 1), but accumulation of nanoparticles in bone has been shown to be significant.¹⁹

Much effort has been made to develop nanoparticles that avoid the RES system, with the purposes of targeting nanoparticles to other tissues, especially tumors. Surface modification of nanoparticles using hydrophilic molecules, polyethylene glycol (PEG) for example, has been shown to be the most successful in reducing phagocytosis.⁵² However, even PEG-modified nanoparticles were found to primarily accumulate into liver, spleen, and bones after relatively long circulation times.⁵⁵

Lymphatic System. Lymphatic vessels are found throughout the body with the exception of cartilage, optic cornea and lens, and the central nervous system. Lymph fluid originating from the interstitial spaces between tissue cells and from within the body's cavities move into lymphatic capillaries through lymph nodes and back into the blood circulation. The overlapping nature of the lymphatic endothelial cells and loose attachment of intercellular junctions allows macromolecules, infectious organisms, and nanoparticles to gain entrance into the lymphatic circulation. Lymphatic fluid is filtered through the lymph nodes lined with macrophages which phagocytize foreign particulate agents. Nanoparticles could also accumulate after phagocytosis by macrophages located in tissues transit into the regional lymph nodes.

The lymphatic system has significant influence on nanoparticle distribution. Nanoparticles injected into muscles or under the skin are retained locally for a relatively long time,⁶¹ while a portion of the nanoparticles accumulate in the lymphatic system, trapped in lymph nodes or deposited into blood circulation. Nanoparticles that enter the local tissues after oral or pulmonary administration can produce efficient lymphatic system accumulation.³⁹ In the respiratory system, a vast network of lymphatic vessels drains both the airways and the alveolar regions and terminates in the hilary and mediastinal lymph nodes.⁶² *In vivo* studies of radio-labeled solid lipid nanoparticles revealed significant lymphatic uptake after inhalation in rats.¹⁶

Other Tissues. Lungs and GI tract could have high accumulation when they act as the administration sites.^{4,18} Due to the BBB, in most studies nanoparticles were found at very low levels or were missing in the brain. The kidneys, as the most important excretion organ, showed significant nanoparticle accumulation only in

a very few studies.⁸ The heart is routinely tested in most nanoparticle distribution studies due to its physiological importance, although in most cases it is not the destination of any significant nanoparticle accumulation. Muscles are seldom targets of nanoparticle administration and in most cases are of little interest for the studies (Table 1). However, the distribution into muscles may need to be emphasized. Due to the relative high body mass of muscles (for example, 43% of body weight for mice),⁶³ they are important for nanoparticle accumulation, even if the concentrations are low, the absolute value could be much higher. Other tissues, including the reproductive tract, skin, and glands, are seldom tested separately. The distribution into these tissues is normally low when studied. In some cases, many of these tissues are combined into one compartment termed “carcass” or “body” and analyzed together rather than individually.⁸

Tumor. Tumors are abnormal organs and consist of unique physiological features. Tumor blood vessels are leaky, tortuous, and dilated, and their endothelial cells lining have aberrant morphology with the basement membrane often being abnormal.⁶⁴ Their high vascular density, vascular leakiness, and impaired lymphatic recovery lead to an enhanced permeability and retention (EPR) effect for nanoparticles. Fast-growing, hyperproliferative tumor cells utilize glycolysis to obtain extra energy, resulting in an acidic microenvironment. Additionally, cancer cells express and release unique enzymes, such as matrix metalloproteinases, which are implicated in their movement and survival mechanisms.

Nanoparticle distribution into tumor tissues has been under intense investigation with the purposes of drug delivery and diagnosis. Both passive and active targeting approaches have been investigated. Nanoparticles can be passively targeted to tumor tissues, taking advantage of their abnormal physiological features.⁶⁵ For instance, nanoparticles of 400 nm⁶⁵ can accumulate into tumor tissues more easily than into normal tissues. To enhance accumulation into the tumor through EPR effect, nanoparticles were designed to have prolonged blood circulation by avoiding the RES. Active targeting involves the use of peripherally conjugated targeting moieties (antibody or peptide/small molecules) which recognize and bind with specific receptors on cell surfaces.⁶⁶ Tumor cells have been the focus of most actively targeted nanoparticles, although nanoparticles are also actively targeted to other cells, including hepatocytes⁶⁷ and macrophages.⁶⁸

Metabolism. In a broad meaning the metabolism of nanoparticles includes any processes that alter their physicochemical properties. The metabolism of nanoparticles depends on their composition and properties. A few examples include nanoparticles broken down in the lysosomes of macrophage cells of the RES after being internalized or the hydrolytic degradation of nanoparticles in the *in vivo* aqueous environment.

Many inorganic nanoparticles are used for medical applications (silver, gold, iron oxide, quantum dots, carbon, silica, and others). These nanoparticles are very stable and difficult to metabolize within the body. It has been shown that they could reside in the body for long time periods.⁸ One study has shown amphiphilic polymer-coated quantum dots remaining in the body for more than two years.⁶⁹ However, there are a few studies suggesting the possibility of intracellular degradation of quantum dots^{70,71} and iron oxide nanoparticles.^{72,73} Moreover, some recent reports⁷⁴ have demonstrated that CNT can be degraded by enzymes. Such lines of work are under intensive investigation by many groups, including ours.

Nanoparticles prepared using polymers, including both natural polymers (*i.e.*, albumin and chitosan), and synthesized polymers are either biodegradable (*i.e.*, poly(lactic-co-glycolic) acid (PLGA)) or nonbiodegradable (*i.e.*, polystyrene). The metabolism of these polymeric nanoparticles is through the degradation of the matrix polymers. Biodegradation of natural polymers is generally faster than that of synthesized ones. One of the advantages of polymeric nanoparticles is that their biodegradation rate can be controlled by modifying the polymer composition and molecular weight.⁷⁵

Surface coatings are commonly applied to nanoparticles to modify their surface properties and ADME profiles, including targeting to specific organs and tissues.^{76,77} Metal nanoparticles are generally coated by natural or synthesized polymers to increase their biocompatibility.^{78–80} The surface coating materials, normally distinct from the core, may be cleaved off first and degraded separately.⁸¹ See *et al.*⁸² showed that upon internalization into a wide range of mammalian cells, biological molecules attached to the nanoparticle surfaces are degraded within the endosomal compartments through peptide cleavage by the protease cathepsin L.

Nanoparticles, especially polymeric ones, may also experience noneliminating changes in physical properties resulting in the loss of their original forms. Particles could swell,⁸³ shrink,⁸⁴ dissolve,⁸⁵ or break⁸⁶ in the biological environment. These physical changes could result in the drifting of their ADME behavior.

Excretion. Nanoparticles, again depending on their properties, may be excreted directly from the body.⁸⁷ Excretion refers to nanoparticles removed from the living system without discomposition or dramatic changes in properties from their original forms. It was shown that the liver and the kidneys are the major organs responsible for nanoparticle excretion. Other excretion routes may exist, for example, through the lungs, breast milk, and sweat, but further data are needed to confirm these routes.

A proven elimination route for nanoparticles is renal clearance.⁸⁸ Nanoparticles could enter the urine by glomerular filtration or tubular secretion. The glomeru-

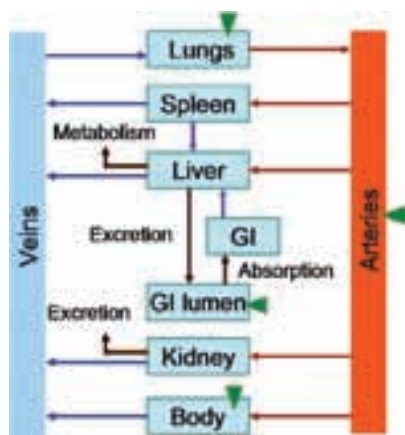


Figure 3. A typical blood flow-limited physiologically based pharmacokinetics model structure. Arrows indicate the transportation of drugs or nanoparticles. Triangles show administration routes.

lar capillary wall is composed of three layers: fenestrated endothelium, glomerular basement membrane (GBM), and glomerular epithelial cells with filtration slits. The effective filtration is determined by combined sieving effects of all the three layers.⁸⁹ The work of Choi *et al.*⁸⁸ showed that the renal clearance of quantum dots is closely related to the size of nanoparticles. According to their results, a cutoff size <5.5 nm will ensure efficient and complete renal clearance. Renal clearance of nanoparticles with larger size could be very slow but still possible.¹⁹ Our laboratory has collaborated with others to report the first studies on chemically functionalized CNT excretion *via* the kidney.²³ The intravenously injected CNT showed kidney clearance with no radioactivity left in the body within the first 24 h.

Besides the function of metabolism, the liver is also a major excretion route for nanoparticles.^{90,91} The excretion occurs across hepatocytes through the biliary production pathway. Hepatocytes were shown to have phagocytic ability, although they are not as effective as Kupffer cells in capturing nanoparticles. Furumoto *et al.*⁹² found that after intravenous administration, polystyrene nanoparticles were taken by both Kupffer cells and hepatocytes. About 4% of polystyrene nanoparticles (about 30% of the total amount distributed to the hepatocytes) was excreted in bile in the form of intact particles during 24 h after intravenous injection.

Transportation Kinetics. The kinetics of nanoparticle transportation within the living system is very complicated and still poorly understood. The most basic process nanoparticles experience within the body is interaction with various types of cells,⁹³ including epithelium, endothelium, tissue cells, and macrophages. The kinetics of the nanoparticle–cell interaction (adhesion, endocytosis, exocytosis, and diffusion) depends on the properties of both nanoparticles and individual cells. Some groups have shown that the en-

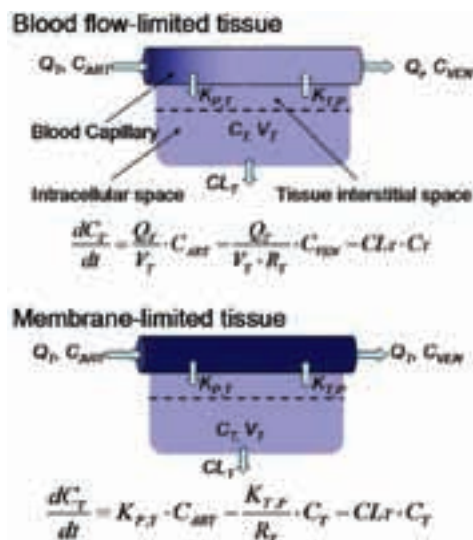


Figure 4. Diagrams and equations for a blood flow-limited tissue (up panel) and a membrane-limited tissue with the vascular membrane as the limiting membrane (down panel). C = concentration; CL = clearance; R = tissue-to-plasma partition coefficient; Q = blood flow; V = volume, K = transportation coefficient; and subscripts ART, VEN, P, and T indicate arterial, venous, plasma, and tissue, respectively. For tissues without elimination, the CL will be zero.

docytes and exocytosis of nanoparticles could be an active, energy-dependent process.^{94,95}

In the study of interaction between magnetic nanoparticles and macrophages,⁹⁶ Wilhelm *et al.* described the particle uptake kinetics as a two-step process: The first is the binding of anionic magnetic nanoparticles onto the cell surface, which was described as a Langmuir adsorption, and second is cell internalization, which was described as a saturable mechanism. Saturation of nanoparticle uptake by cells has also been shown in other studies.^{97,98} Saturation of cellular uptake of nanoparticles could be due to either the equilibrium between endocytosis and exocytosis⁹⁴ or the capacity limitation of the cells.^{99,100}

Of similar importance is the question of how fast nanoparticles could be internalized and removed from cells. Some studies reported half-lives of internalization for nanoparticles. Chithrani *et al.* studied kinetics of both endocytosis and exocytosis using gold nanoparticles with sizes from 14 to 74 nm.⁹⁷ They reported that the internalization half-lives were from 1 to 3 h, depending on the nanoparticle sizes and the cells tested. The half-lives of removal of the internalized gold nanoparticles were faster (from 0.33 to 0.75 h). Serda *et al.*⁹⁹ studied the internalization of silica nanoparticles by vascular endothelial cells and reported the half-life to be 15.7 min.

From the kinetic studies it was also revealed that the transportation of nanoparticles across cellular membranes may not be a reversible process.^{94,97} This means the kinetics and mechanisms of endocytosis and exocytosis could be different, even for the same nanoparticles and cells. Nanoparticles were within the endo-

some after internalization and could be subject to digestion or further translocation within cells. For cells that are asymmetrical, for example, the GI tract epithelium cells, nanoparticles may be internalized from the apical side and released from the basal side.¹⁰¹

There are also processes of nanoparticle transportation that do not require the interaction with cells. The clearance of nanoparticles by ciliary escalator from the airway has been extensively studied, and successful mathematical models have been developed for prediction.^{102,103} Nanoparticle penetration of mucus lining the surfaces of airway and GI tract was also studied *in vitro*, and the transportation kinetics could be quantitatively determined.³⁶

PBPK MODELS FOR NANOPARTICLES

Principles of PBPK Model Building. PBPK models have been widely and successfully applied for drug pharmacokinetic prediction, as illustrated comprehensively in the review by Nestorov.³⁰ These models separate a living system into compartments based on physiological information. Time-dependent concentrations of drugs and metabolites in these compartments are described by mathematical equations. A typical PBPK model structure is shown in Figure 3. PBPK models have advantages over traditional pharmacokinetics, which are not able to provide the mass–time profiles of individual tissues and organs. Another advantage of PBPK models is interspecies extrapolation. In many cases tissue concentration data are not from humans but only from animal models. Interspecies extrapolation allows the scale-up of the animal data to humans.¹⁰⁴

Almost all of the PBPK models developed were used to describe the kinetics within the whole body of humans or animals. These models were called “whole body” physiologically based pharmacokinetic (WBPBPK) models.^{105,106} These models generally were built centering the blood circulation, which connects other organs or tissues. WBPBPK models are generally divided into two groups: blood flow- (or perfusion-) and membrane- (or permeability-) limited.¹⁰⁷ A blood flow-limited model assumes that nanoparticle transportation into tissues is very fast, and equilibrium between blood and tissue could be reached instantly. Under such a situation, the transportation of nanoparticles into one tissue depends on its blood supply.^{108,109} In a membrane-limited model it is assumed that there could be a membrane at the capillary or cellular membrane, or both. The diagrams and the mathematical descriptions of the two models are given in Figure 4.

Besides describing the whole body, PBPK models were also developed for part of the whole body or for one organ.¹⁰² The organ is divided into a number of compartments, and the mass transfer among them are described by mathematical equations, similar to whole body models. These models were called “partial” PBPK models³⁰ in comparison to whole body models, and

blood is not necessarily included. “Partial” PBPK models could also be treated as part of a whole body model, to describe the local kinetics of mass transfer.¹⁰⁶

The development of a PBPK model generally includes the following steps: (1) specification of the model structure; (2) equation building and programming into software; and (3) estimation of model parameters and implementation. The structure of the model is generally determined based on available experimental data, the study design, and the distribution properties of the substances (drug or nanoparticles) tested.¹¹⁰ Equations are built according to the mass transfer routes and the transportation kinetics. Parameters could be determined either from literature or by estimation through data-fitting.¹¹¹ Software, both specifically developed and generally graphical, have been used for simulation.^{30,112}

Factors to be Considered. Due to the difference between the ADME behaviors of nanoparticles from that of the small molecules, there are some additional factors that need to be considered to build PBPK models for nanoparticles. All of them need to be evaluated carefully for any PBPK model but are more significant for application to nanoparticles.

Transportation Mechanisms. Appropriate description of transportation mechanisms is the foundation for prediction of nanoparticle biodistribution. A thorough understanding of nanoparticle transportation within the living systems is needed for selection and modification of transportation mechanisms. How well the model fits into the experimental data can be statistically evaluated.¹¹³ Various mechanisms have been proposed for PBPK models, including blood flow- and permeability-limited mechanisms. Modification of these basic mechanisms may be needed to better suit specific situations.

Although a blood flow-limited model worked well for small molecules in most cases, its preliminary application to nanoparticles was a mix of good and poor prediction of experimental data.^{42,108} Membrane-limited or more complicated models have yet to be attempted for nanoparticles, possibly because of poor understanding of nanoparticle transportation mechanisms. Limited knowledge is available regarding nanoparticles extravasation¹¹⁴ lymphatic washout from tissue interstitial space and returning to blood circulation,⁴⁴ binding to cell surfaces (especially for active targeting nanoparticles)¹¹⁵ and internalization into cells. Gentile *et al.*⁵³ have developed mathematical descriptions of nanoparticle margination in the blood circulation. Such work illustrates that it is very challenging to mathematically describe the processes of nanoparticle trafficking from blood circulation into tissues, within tissue interstitial space, and within cells. Similar difficulties exist for macromolecules, and relatively successful PBPK models were developed employing a two-pore model mechanism of transportation.¹¹¹ Nanoparticle PBPK modeling may benefit from these works.

Transportation Kinetics. In PBPK models, linear ordinary differential equations are often used under the assumption that linear processes are involved. When nonlinearity is assumed, nonlinear differential equations may apply. In fewer cases, algebraic equations are used for static processes, and partial differential equations are used for dispersion models.³⁰ A combination of these equations may be necessary within the same model under complex situations.

There is one very important parameter in PBPK models, the tissue/blood partition coefficient which is defined as the ratio of drug or nanoparticle concentrations in the tissue to that in the emergent venous blood of the tissue. As partition coefficients depend on the properties of the drugs or nanoparticles under study, they need to be estimated individually. Both *in vitro* and *in vivo* methods were proposed for partition coefficients estimation for small chemicals¹¹⁶ and were adopted in some PBPK modeling works of nanoparticles.⁴² By using such a parameter, it is assumed that there is the same transfer kinetics of nanoparticles from blood into tissues as from tissues back into blood circulation, and an equilibrium of concentrations between blood and tissue exists. These assumptions may not be appropriate for nanoparticles. Some researchers have already questioned the suitability of partition coefficients for nanoparticles PBPK modeling,^{24,117} and Lin *et al.*²⁴ proposed to use distribution coefficient (DC) instead.

Another issue is the effects of nanoparticles on the physiological function of organs or cells. It is reported that with exposure to particles, the physiological structures may change, leading to changes in transportation kinetics of nanoparticles. For example, tobacco smoke, which contains nanoparticles, can cause a significant impairment of the bronchial region which is mainly expressed by a successive occurrence of chronic bronchitis and a related decrease of local airway calibers and rapid decline of the mucociliary clearance efficiency.¹¹⁸ As a second example, the uptake of nanoparticles by RES could depend on the population of macrophages and the capacity of macrophage uptake.¹⁹ The functions of macrophages may be hindered by nanoparticles, resulting in reduced clearance.¹¹⁹

Effects of Lymphatic System. All of the PBPK modeling works to date for nanoparticles and for most small molecules do not include the lymphatic system. As previously noted, the lymphatic system is a major organ system of nanoparticle accumulation. Significant amounts of nanoparticles traverse into the lymphatic system, especially when given through administration routes other than IV (pulmonary,¹⁶ oral,³⁸ intradermal injections,¹²⁰ and others). If the lymphatic system was excluded from the model, in these scenarios, then significant error in simulation of the experimental data would likely occur, and the parameters obtained would be biased. Only a few PBPK models developed for macromolecules (antibodies, etc.) included the lymphatic

system.^{111,121} The mathematical description for these models is very complex, and the lymphatic system was not listed as a separate compartment.

The lymphatic system permeates throughout the entire body. Incorporation of the lymphatic system into PBPK models will result in very complicated model structures and mathematical descriptions. The influence of lymphatic system on nanoparticle distribution may further complicate simulation when considering the phagocytosis of macrophages in the lymph nodes.⁴⁴ To avoid over complexity and insolvable models, compromise in simulation accuracy may be needed. For nanoparticles administered through routes other than IV, only the regional lymph nodes draining the administration sites had high retention.¹³ Therefore, a reasonable modeling approach may be to connect only the absorption site with the lymphatic system.

Modeling the Metabolism. The metabolism of nanoparticles is also different from that of small molecules. Metabolism of small molecules consists of a series of chemical reactions. Each step produces metabolites with distinct chemistries and ADME profiles from the parent molecule. However, metabolism of nanoparticles, in most cases, is a gradual process.^{73,75} This could be explained by the fact that nanoparticles are relatively large clusters of molecules or atoms. Any changes of an individual molecule only changes the nanoparticle by a small fraction, and it may often require many such changes before properties and ADME profiles are altered.

Metabolism of molecules may be modeled by connecting PBPK models of the parent compound and the metabolites.¹²² PBPK models built for each of the metabolites, given sufficient information, can be achieved. The PBPK models of metabolites can then be connected to that of the parent molecule through the major metabolizing compartment (*i.e.*, liver). However, the metabolism of nanoparticles differs as previously mentioned. The transportation parameters for nanoparticles could drift as a result of metabolism. The changes depend upon mechanisms of degradation involved for particular particles. Exploring the mechanism of nanoparticle degradation might assist in solving this issue. A time-dependent transportation coefficient can be incorporated into the mathematical equations.²⁴

Inclusion of Tumor. Tumor tissue has been included in PBPK models developed for macromolecules¹²¹ but not for nanoparticles to date. Due to the abnormal physiological features of tumor tissues, they need to be included into PBPK models with caution. Unlike other tissues or organs, the physiological parameters, such as blood flow rates, may not be readily available from literature and need to be estimated carefully.¹²³ The mechanisms and kinetics of nanoparticles within tumor tissues could also differ from those of normal tissues. From the modeling point of view, another significant difference of tumor tissue from normal tissues is

their dramatic morphological changes with tumor growth.¹²³ This could result in changes in the transportation kinetics into and out of tumor tissues over time. The variation of tumor physiological morphology could be another cause for data diversity and then inaccuracy of modeling. It is not clear how significant these factors effect the nanoparticle distribution into tumor tissues, and further studies are needed for thorough understanding.

Current Applications. PBPK models have only recently been applied to nanoparticles over the past few years. Although available works in the literature are limited, they covered various pertinent aspects. Nanoparticle formulations of the same composition or different compositions were compared; models were based on data from both animal models and human studies; several administration routes were covered including IV, inhalation, and intradermal injection; and both “whole-body” and “partial” PBPK models were reported.

The first article of nanoparticle whole-body PBPK modeling found in the literature was published in July, 2008. Lin *et al.*²⁴ developed a blood flow-limited PBPK model to predict the concentration–time profiles of quantum dots in mice, based on experimental data collected within the same group. Contrary to commonly used tissue-to-blood partition coefficients, which they argued may not exist for nanoparticles, they developed a specific parameter, named tissue distribution coefficient (DC). The DC was considered as the ratio of the affinity of QD705 to a given tissue over that to blood; it would vary with time depending on the respective instantaneous QD705 concentrations in the blood and the individual tissues as well as the microenvironment at the tissue site. This DC value was incorporated into mass balance equations in the position of the classical partition coefficient. By using DC, the model predicted the experimental data quite well.

Lee *et al.*⁴² tried to validate whether a blood flow-limited, whole-body PBPK model could be applied across various types of quantum dots. Biodistribution data from four publications, including the one from Lin *et al.*, were collected and fitted into the model. As in most PBPK models, classical tissue-to-blood partition coefficients were used in this work. The results indicated that the model used was not sufficient to produce satisfactory simulation of quantum dots biodistribution, especially the early time points. The reasons behind this could be several. Unsatisfactory simulation, especially at early time points, may result from the utilization of an incorrect transportation mechanism. As quantum dots have quite a long plasma half-life, a permeability-limited mechanism may be better suited. Another potential reason could be the structure of the model. None of the six compartments included had the function of elimination (metabolism or excretion). Additionally, as hypothesized by the authors, the lymphatic

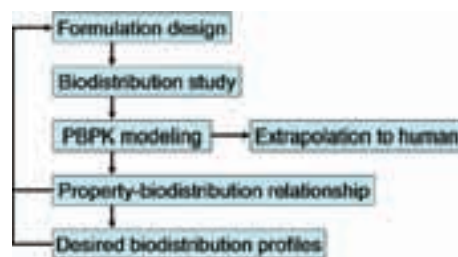


Figure 5. Schematic showing of the function of PBPK modeling in the process of nanoparticles optimization for medical applications.

system, which is not included in the model, could have a significant influence on the simulation accuracy.

A PBPK model was also developed for nanoparticles of differing composition. MacCalman *et al.*¹¹³ developed a compartmental model to simulate the nanoparticle clearance from the lungs and further translocation into the body. Experimental data of two types of nanoparticles, iridium and silver, were collected from the literature. The authors did not term their work PBPK modeling, although the essential principles were the same. The model developed in this work was a hybrid with the lungs divided further into subcompartments. The overall fitting of the simulation to the experimental data was good but not for some individual organs.

Another group, Pery *et al.* developed a PBPK model for inhaled carbon nanoparticles, based on imaging data.¹²⁴ This is the only work of nanoparticle PBPK models that is based on data collected from humans. The concentrations of nanoparticles in organs were converted from imaging by separating the radioactivity overlap of organs and tissues. This work provided a methodology to utilize imaging data for PBPK models. This method is very preferential in terms of obtaining data without collecting tissues and organs and allows continuous data collection from the same subject, enabling such human studies.

The latest application of PBPK modeling to nanoparticles is from Lankveld *et al.*¹²⁵ A PBPK model was developed to compare the kinetics of silver nanoparticles of various sizes (20, 80, and 110 nm). Although the model simulated the experimental data quite well, no clear relation between parameter values and corresponding particle diameters were concluded. The authors suggested that the kinetics were determined not only by size but also by other nanoparticle properties, such as surface charge and surface coating.

Apart from models built to describe whole-body kinetics of nanoparticles, “partial” models were developed without the involvement of blood. These “partial” models have found their application exclusively in the lungs.^{102,119,126} This is because there is a high interest in the toxicity of the airborne pollutants. These models, according to physiological functions, divide the lungs into subcompartments and describe the nanoparticle mass transfer among them. A PBPK model was

also built to describe the effects of nanoparticle exposure on the dynamics of macrophages in the lungs.¹¹⁹

PERSPECTIVES

Most PBPK models were developed for the purpose of describing experimental data. One of the most important questions regarding nanoparticle *in vivo* studies is their transportation kinetics within the body, that is, to what extent and rate they distribute among various tissues. Simulation of the experimental data could fill gaps in experimental data, including measurement and time limitations and tissues that are not feasible for detection.³ PBPK models could clearly demonstrate that all of the organs and tissues are connected to each other. Interpretation of nanoparticle ADME in any individual organ or tissue needs consideration of the influence on others. The relative importance of each model parameter could be evaluated through sensitivity analysis.¹²⁷ By PBPK modeling, the ADME of nanoparticles could be systematically described and understood, providing more insights into their *in vivo* behavior and then toxicology. By doing this, PBPK models could aid in finding factors that influence the nanoparticle distribution and further reducing toxicity and improving efficacy.

Another important application is the extrapolation of experimental data between species, tissues, exposure routes, nanoparticles, and doses.^{121,127} This means experimental data from one study could be used to predict the results under different experimental conditions. The interspecies extrapolation may be of the highest significance. The final purposes of most nanoparticle studies are their application and toxicity in humans despite *in vivo* studies being generally performed on animal models. The relevance between the data obtained from animal models to human has been a prodigious argument. PBPK models provide the possibility of scale-up data from animal models to humans, which will greatly enhance applications of nanoparticles. One of the major applications could be the dose determination of nanoparticles for humans from animal data, for example, in cancer therapy.¹¹⁷

PBPK models have been utilized to predict the activity based on available information of other similar chemical compounds, termed as quantitative structure–activity relationships (QSAR).¹²⁸ This approach could find significant application in nanoparticle design and optimization (Figure 5). Nanoparticle properties could be modified and controlled through chemical composition,¹⁹ surface engineering,⁷⁹ and preparation processes.¹²⁹ Modification of nanoparticle properties has long been explored to achieve optimized distribution. With the explosive increase of new materials and methods for nanoparticle preparation and with new products containing nanoparticles, more efficient methods for evaluation are both desired and necessary to achieve a thorough understanding of the relation be-

tween nanoparticle properties and their ADME behaviors. The enhanced ability of data extrapolation of PBPK modeling makes it the ideal tool for future application in this manner.

PBPK models have been coupled with pharmacodynamics (PBPK/PD)¹³⁰ and toxicity models.¹³¹ Similar uses can be applied for nanoparticles. Another application with high potential is for nanoparticle drug carrier delivery kinetics. The PBPK model of nanoparticles and that of the drug can be combined together, linked by the drug release kinetics from the nanoparticles. Such a model will be able to address the effects of nanoparticle distribution and drug release kinetics on the pharmacokinetics of the encapsulated drugs. Consequently, nanoparticle properties and drug release patterns can be modified accordingly to achieve optimized drug pharmacokinetics within specific tissues of interest.

To fully utilize the power of PBPK modeling for nanoparticles, some limitations need to be overcome. The first limitation is the fact that PBPK modeling requires large amounts of information.³⁰ This includes the database of physiological parameters of animals and human, the interaction of nanoparticles with the body at whole-body, organism, and cellular levels and the ADME of nanoparticles as a collection. There are already works pooling the physiological parameters together,¹⁰⁹ even particularly for PBPK modeling,¹³² but data for some relevant animal models are not yet included. For those animal models included, the list of tissues and organs are not complete. The problem with the current research works is that they are more or less arbitrarily designed, as shown in Table 1. General rules of study design are needed so the experimental data can be of greater value for PBPK modeling. Another difficulty comes from experimental data collection. For most analysis methods, tissues need to be harvested from animals in cohorts at each time point of study, resulting in a large number of animals needed for each study. A fairly large number of tissues and organs from each animal are required for PBPK modeling, further amplifying the number of samples to analyze. These limitations result in experimental designs being very time and cost consuming. Noninvasive measurement of nanoparticle biodistribution, such as imaging, could greatly reduce this difficulty.¹²⁴

A further limitation is in the research teams themselves. Development of a nanoparticle PBPK model requires cross-disciplinary work, demanding expertise in the areas of nanoparticle characterization, nanoparticle ADME, nanotoxicity, and mathematical and computational knowledge. With increasing demands, more researchers are making efforts and gaining experiences in PBPK modeling of nanoparticles. It is a fact that only a few works have been published regarding nanoparticle PBPK modeling to date, but a Google search through the Internet can retrieve dozens of related abstracts, presentations, in-plan or ongoing projects, all within

the past five years. Driven by the up-coming needs and powered with its advantages in data processing, it is expected that PBPK modeling will become a routinely used tool in nanoparticle research.

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