を目指したDDSへの応用も期待されている。ナノマテリアルを薬物の送達物質（キャリア）として用いるナノDDS医薬品開発は，2000年頃から急速に進展している。本国においては，日本発の医薬品向けのナノマテリアル開発を目指す「ナノメディシン・プロジェクト」を厚生労働省が指定型プロジェクトとして推進するなど，ナノ医薬品の開発・研究が進行している。

現在，日本では10製品のナノDDS医薬品が上市されており，用いられるナノ素材としては，主に，リポソームや水溶性高分子，高分子ミセルなどが挙げられる。これらのナノ素材は，①水溶性の高分子に薬物を化学的に結合させる方法，②高分子の集合体に薬物を包み込ませる方法，③脂質二重膜小胞に薬物を内包する方法，などにより薬物を標的部位へと到達させ，現在では主に抗がん剤に対する適用が進んでいる。さらに最近では，金属ナノ粒子のように古くから使用されていた素材を100 nm以下に微小化した素材や，フラーレンやカーボンナノチューブをはじめとするナノカーボン素材のように，新たに考案・開発された素材を応用したナノ医薬品の開発研究が進展しつつある。これらは，その調整の容易さや粒子表面修飾の多様性を有することから，各種リガンド・抗体を用いたターゲッティング能の付加による治療効果増大・副作用低減を目指した試みも精力的になされている。今後，既存のナノ素材をより洗練させるだけではなく，新規ナノマテリアルの開発が進むことで，ナノDDS医薬品産業の目覚しい発展が期待される。本稿では，紙面の都合上，新規ナノDDS医薬として期待されているナノカーボン素材について概説したい。

ナノカーボン素材は，炭素間結合を介す長い電子共役系を持つなど特殊な物性を有し，高い薬物保持能や生体内安定性，柔軟な構造（表面修飾の容易さ）といった，DDS素材としてきわめて有望な性質を発揮する。そのため，前述したように，これらナノカーボン素材を用いたDDS医薬の開発が注目を浴びており，その特性を活かした，低分子医薬，タンパク質医薬，核酸医薬の送達キャリアとしての開発が前臨床段階ではあるものの，世界中で進められている。たとえば，カーボンナノチューブ（直径は数nmと細く，その長さはマイクロメートルからミリメートルまで多様に存在する）やカーボンナノホーン（直径2～5 nm，長さが40～50 nmのチューブ状構造を示す）は，グラファイトからなるナノカーボン素材であり，薬物内包素材・ターゲッティング素材として医療・薬学分野でその応用が期待されている。これらの素材は，いずれも内腔を持つ特殊な構造を有するため，表面だけではなくその内腔に薬物を保持させ，徐放化を試みる検討が進められている。湯田坂らは，カーボンナノホーンに内包されたシスプラチンが数十時間をかけて徐放され，顕著な抗がん作用を示すことを報告している[1]。このように，腫瘍組織，炎症組織や細胞内リソソームなど低pH環境において薬剤が放出されるといったDDS機能を有するナノカーボン素材の開発も進められている。

ナノテクノロジー産物としてのフラーレン（直径1 nm程度）は，グラファイト，ダイヤモンドに次ぐ第三の炭素同素体であり，その革新的有用機能を活用し，美白剤・老化防止剤などとして広く実用化されている。フラーレンは中空の球状構造を有するため，原子を内包することが可能であり，金属元素を内包したものは金属内包フラーレンと呼ばれる。これまでに，セシウムやガドリニウムなどを内包したフラーレンが得られており，MRIの造影剤などへの応用研究が進められている。また，ナノカーボン素材特有の長い電子共役系によって，光照射で励起され，活性酸素を効率よく発生する光増感物質としての性質を有することが知られている。この性質は，がんの光線力学的治療法，フォトダイナミックセラピーに応用することが可能であり，田畑らはこの性質を利用し，*in vivo*において顕著な腫瘍退縮効果が得られることを報告している[2]。さらに，従来薬とは全く異なった作用点での抗ウイルス活性（酵素阻害活性）や抗菌活性，さらにはラジカルスポンジと呼ばれるほど圧倒的な抗炎症活性（抗酸化活性；活性酸素・ラジカル消去活性）を有しており，活性酸素が原因となり発症・悪化する各種炎症性疾患への適用も進められている。この点において筆者らは，C60フラーレンの修飾体が，炎症性腸疾患の代表的モデルマウスであるデキストラン硫酸ナトリウム（DSS）誘発大腸炎モデルマウスに対し，顕著な治療効果を発揮可能であることを先駆けて明らかとしている。その詳細な作用機序については明らかとなっていないが，C60フラーレンの修飾体がROSなどの酸化ストレス因子の産生，炎症性サイトカインの分泌を抑制する可能性を見出している。

現在，より有効な炎症性腸疾患治療薬の開発を目指し，腸管吸収性や体内動態について詳細に評価することで，腸管で最も効果を示す製剤形態などの有効性情報を収集するとともに，より高い抗酸化作用を有する他のフラーレン誘導体の探索を推進している。

## 3. ナノマテリアルの安全性に関する懸念

このように，ナノ医薬品の開発研究は世界的にも盛んに進められているが，世界的に見てもナノ医薬品の成功例はきわめて少ない。ナノ医薬品におけるボトルネックは，ナノマテリアル特有の画期的な機能が，逆に，二面性を呈してしまい，予想外の部位で未知の副作用（NanoTox）を発現する可能性を有することである。たとえば，先に紹介したカーボンナノチューブが，アスベストと同様に悪性中皮腫や肺がんなどを誘発してしまう可能性[3]や，フラーレンが体内に侵入した後，循環血中を介して脳組織へ移行し，障害性を示す可能性[4]，などが指摘されている。しかしながら，ナノ医薬品の根幹をなすナノマテリアルの安全性評価研究に関して，現状では，細胞毒性や遺伝毒性といった一部のハザード研究しかされておらず，世界的に見ても不十分かつ科学的根拠に乏しいため，ナノ医薬品の開発が遅れている1つの要因となっている。本観点から筆者らは，これまでに，有効なナノ医薬品として応用可能なナノマテリアルのスクリーニングを実施するとともに，ナノマテリアルの有効性と物性，安全性との連関評価を推進してきた。たとえば，さまざまなナノマテリアルを用いたスクリーニングにより，細胞内への薬物送達キャリアとして期待される非晶質ナノシリカが，①抗体産生誘導能の強い表皮の樹状細胞サブセットに局在する傾向を持ち，さらにその活性化などを引き起こすこと[5]，②経鼻投与することで抗原特異的免疫誘導能を示すこと[6]から，ナノマテリアルの経皮抗原キャリア・経鼻粘膜ワクチンキャリアとしての可能性を有すること，などを見出してきた。さらに，これまでの検討から，ナノマテリアルは，従来までの低分子医薬品やサブミクロン素材とは決定的に異なる体内・細胞内動態特性を有することを明らかとしている。一方で，ナノマテリアルの体内・細胞内での動態特性や有効性・安全性は，①粒子サイズ（一次粒子径，二次粒子径【分散・凝集】），②粒子形状（球状，針状など），③荷電状態（表面電荷），④水溶性・脂溶性といった親媒性（親水－疎水バランス）の4つのパラメータにより規定される[7]などの知見を得ている。そこで，筆者らが推進してきたナノ安全科学研究の中から，非晶質ナノシリカを用いた先行研究について紹介したい。

非晶質ナノシリカ（微粒二酸化ケイ素）は，局法においては従来サイズの素材と区別なく収載されており，固結防止剤などの食品添加物として日本においてもすでに，食塩やインスタント食品をはじめとした多くの食品に使用されている。また，医薬品の助剤としてすでに汎用されているのみならず，遺伝子送達キャリアなどのDDS素材としても期待され，その使用量・範囲の拡大は今後ますます進むと考えられる。筆者らはこれまでの検討から，粒子径100 nm以下の非晶質ナノシリカが，経皮・経口・経鼻投与により，生体バリアを通過し，組織内・全身血流内に移行すること，さらには，全身血流から選択的かつ効率よく胎盤に移行することを最初に見出した[8]。また非晶質ナノシリカの粒子表面性状を制御することで，胎盤への移行性はそのままに，安全性を高度に確保できること，さらには胎盤組織内の各種細胞への選択性や核内・細胞質内といった細胞内局在性を制御できることを唯一認めている[9]。本結果は，非晶質ナノシリカがこれまで送達不可能であった部位への薬物送達をも可能とする新規キャリアになり得ることを示すものであり，前述したように，筆者らも核酸送達キャリアやワクチンキャリアとしての適用を試み，興味深い知見を得つつある。そこで，粒子径70 nmの非晶質ナノシリカ（nSP70）と，対照群として粒子径300，1000 nmの従来型シリカ（nSP300，mSP1000），さらには実際に使用されている非晶質ナノシリカがさまざまな表面修飾を施されている場合があることを考慮し，nSP70の表面がアミノ基，カルボキシル基で修飾された非晶質ナノシリカ（nSP70-N，nSP70-C）を用い，物性と，体内動態・局在，ハザード発現との連関解析を実施した。なお，以後の検討では，試薬グレードの非晶質シリカを用い，各検討を実施した。各シリカを妊娠マウスに尾静脈内投与し体内動態・局在を透過型電子顕微鏡により定性的に評価した結果，nSP70のみが胎盤に集積するとともに，血液胎盤関門を通過し胎仔にまで移行することを見出

した（図2）。次に，過剰量を静脈内投与することで，非晶質ナノシリカの妊娠マウスに対するハザード同定を試みた。その結果，nSP70-N，およびnSP70-C投与群において異常が認められなかった一方で，nSP70投与群でのみ，胎仔吸収率の増加とともに，胎仔体重がコントロール群よりも10%以上減少するなど，胎仔発育不全を誘発していることが明らかとなった。なお，これら粒子の胎盤への集積や胎仔への移行，および胎仔への影響は，nSP300，およびmSP1000では認められていない。このことから，多くの非晶質ナノシリカ素材が，生殖発生毒性学的視点からも安全であるものの，一部の素材については注意を払う必要があることが示された。以上の検討で見出されたnSP70のハザードは，過剰量における検討ではあるものの，従来型非晶質シリカであるnSP300，mSP1000では認められなかったものであり，一部の非晶質ナノシリカが従来型の非晶質シリカとは異なる生体影響を誘発する可能性を示している。一方でnSP70-N，nSP70-Cは，過剰量を静脈内に投与するという実験系にもかかわらず，目立ったハザードは認められなかったことから，これらはきわめて安全性の高い素材であると考えられる。また，これらの知見は逆に，ごく一部の安全性に懸念のあるものに関しても，適切な表面修飾を施すことにより，安全性を担保できる可能性を示している。ナノマテリアルの中にも安全性が高いものとそうでないものがあることはよく知られているが，今後，安全なナノマテリアルを創製するための方法論といったナノ安全科学研究に関する情報をより多く収集することが，ナノマテリアルの安全性評価研究の最重要課題の1つであると考えている。

## 4. おわりに――ナノDDS医薬品の将来像

本総説では，ナノDDSへの適用の現状とともに，最も急がれる安全性確保に関する検討を中心に紹介した。最近では，カーボンナノ素材や非晶質ナノシリカに加え，抗酸化・抗菌活性などを有した白金や

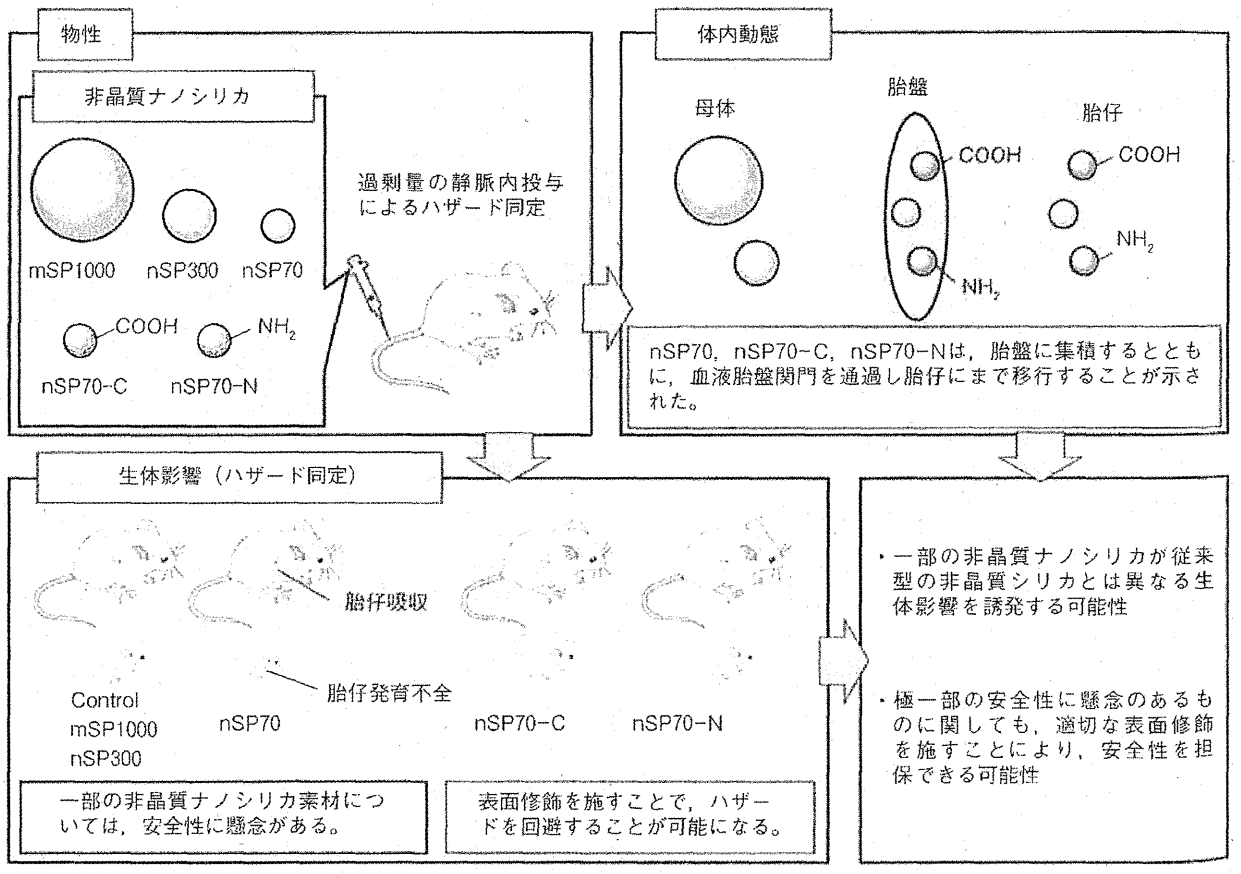


図2　生殖発生毒性学的視点からの非晶質ナノシリカの安全性評価

銀などに関して，タンパク質と同等のサブナノサイズ領域（10 nm 以下）の素材（サブナノ素材）の開発・実用化も進んでいる。しかし現状では，地球規模でナノマテリアルやサブナノ素材の安全性に警鐘が鳴らされ，OECD を含め，欧米では規制が進んでいるものの，わが国を鑑みると，薬事法・薬局方（医薬品・化粧品・医薬部外品など）をはじめとする各種法律を見ても，ナノマテリアルやサブナノ素材に言及した規制はない。さらに，これら各種法律においては，ナノマテリアルやサブナノ素材を構成する化学物質の構造式（物質名）のみで規制されているため，従前のサブミクロンサイズ（100 nm）以上の素材で安全性が確認されたものや，経験的に安全と考えられるものであれば，ナノ化・サブナノ化されたものでも自由に利用できてしまうことになる。すなわち，①ナノ化・サブナノ化によって，安全性を運命づける『動態特性や効能・効果』が，同一素材であっても，従前のサブミクロンサイズ以上の素材や分子状素材と大きく変動し得ること，②物性などによっても，ナノマテリアルやサブナノ素材に特有の性能が変動し得ること，が理解されつつあるにもかかわらず，品質管理・保障の規制・ガイドライン策定には程遠いのが現状である。したがって，今後は，物性・品質と，動態情報や安全性情報の連関解析を定量的に実施する必要があると考えられる。このように，Nano-Safety Science の視点から，ナノマテリアルの安全性情報を収集したうえで，Nano-Safety Design の視点から，安全性の高いものは実用化を推進し，安全性の低いものは表面性状制御をはじめとした適切な方策を講じて安全性を高めていくことで，ヒト健康の確保と同時に，われわれがナノテクノロジーの恩恵を享受しつつナノ産業界の発展も達成できるものと考えている。今後，ナノ開発研究とナノ安全科学研究が強固に連携し，両輪となってともに歩むことで，Sustainable Nanotechnology（いわゆる，持続可能なナノテクノロジー）に資する，地球・環境・ヒトに優しい（安全な）ナノマテリアルの創製，ひいてはナノ医薬品の開発が飛躍的に進歩することを楽しみに，筆者らも一緒にチャレンジしたい。

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# Engineered Cell Manipulation for Biomedical Application

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# Chapter 15
# The Absorption, Distribution, Metabolism, and Excretion Profile of Nanoparticles

**Yasuo Yoshioka, Kazuma Higashisaka, Shin-ichi Tsunoda, and Yasuo Tsutsumi**

**Abstract** Advances in nanotechnology have led to the recent development of many nanoparticles. With the growing commercialization of nanoparticles, opportunities for human exposure to nanoparticles will increase substantially. For the development of nanoparticles with efficacy and safety, a systematic and thorough analysis of the absorption, distribution, metabolism, and excretion (ADME) of nanoparticles is essential. In this chapter, we present the current understanding regarding the ADME profile of nanoparticles.

**Keywords** ADME • Biologic barriers • Protein corona • Safety

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## 15.1 Introduction

Nanotechnology makes it possible to design, characterize, and produce nanostructured materials by controlling their shape and size at the nanoscale. In general, nanoparticles are defined as materials whose structures have at least one dimension on the order of 100 nm or less, although there is no reason to assume that 100 nm would be an absolute threshold for changes in the physicochemical properties of these particles. Nanoparticles have various desirable properties, including enhanced electrical conductivity, tensile strength, and chemical reactivity, which are due to their increased surface area per unit weight compared with that of bulk-scale counterparts. Diverse nanoparticles such as silica nanoparticles and carbon nanotubes (CNTs) have become widespread in use through their applications in electronics, sunscreens, cosmetics, diagnostic medicines, and drug-delivery systems, among other products. In particular, the clinical applications of nanoparticles have been investigated for more than 30 years. For biomedical applications, nanoparticles including mesoporous silica nanoparticles, CNTs, quantum dots, and superparamagnetic nanoparticles have been evaluated as drug-delivery and diagnostic vehicles. For example, because of their unique mesostructural features, high drug-loading capacity, and sustained-release profiles, mesoporous silica nanoparticles are potential candidates for controllable drug-delivery agents, gene delivery vehicles, vaccine carriers, and many other biologic applications.

The increasing use of nanoparticles has prompted public concern regarding their potential toxicity. In particular, recent reports have indicated that CNTs cause mesothelioma-like lesions in mice, in a manner similar to that of asbestos-induced mesothelioma [1, 2]. Because nanoparticles have great potential to improve the quality of human life, it is essential to ensure the safety of nanoparticles for the development of safety-assessed products. The toxicity of nanoparticles is related to the dose, concentration, and duration of the exposure and their abundance and persistence in tissue. Accordingly, a systematic and thorough analysis of the absorption, distribution, metabolism, and excretion (ADME) of nanoparticles is essential as the basis for determining the potential for risk to human health. In addition, understanding of the ADME of nanoparticles is necessary not only in regard to their tissue toxicity but also their potential biomedical applications. In this chapter, we present the current understanding regarding the ADME profile of nanoparticles (particularly inorganic nanoparticles).

## 15.2 ADME via Several Exposure Routes

Various barriers prevent the entry of foreign substances, including viruses and bacteria, into the body. These same barriers modulate the access of nanoparticles and include the skin, gastrointestinal tract, and pulmonary system. Previously, only

small lipophilic molecules (<600 Da) and metallic ions (e.g., nickel and cobalt) have been able to penetrate the skin barrier. However, because of their small size, nanoparticles might be readily absorbed through the dermis of the skin and the pulmonary and gastrointestinal mucosa, thus positioning these compounds for distribution through the vascular circulation to all tissues in the body. With an average pore size of ~5 nm in mammals, the vascular endothelium presents another potential barrier to the absorption and delivery of nanoparticles, but nanoparticles smaller than this limit penetrate rapidly from blood across the endothelium and into tissue. In addition, nanoparticles potentially can translocate efficiently from blood into the liver, spleen, and bone marrow, because the discontinuous endothelium characteristic of these organs has pores of 50–100 nm in diameter. Therefore, methods for estimating the amount of the total external exposure, efficacy of absorption, and tissue biodistribution of nanoparticles are needed.

Many studies have reported that nanoparticles penetrate the biologic barriers after inhalation or oral or dermal exposure and have used qualitative and quantitative methods to assess the effect of the size and surface properties of nanoparticles on biologic behaviors. In the following sections, we discuss the absorption of nanoparticles via several exposure routes.

### 15.2.1 Dermal Exposure

Because clothing, drugs, cosmetics, and various skin care products contain nanoparticles, their contact with the skin occurs intentionally as well as accidentally. In particular, nanoparticles have been included in cosmetics and sunscreen to provide protection against ultraviolet radiation. Therefore, understanding the absorption rate of nanoparticles after exposure via the skin has garnered increasing attention during the past several years. However, whether nanoparticles actually penetrate the skin barrier in vivo is unclear, although many studies have assessed the skin penetration of nanoparticles after topical application of compounds. Several reports have stated that titanium dioxide, ZnO nanoparticles, and silver nanoparticles penetrate into the upper layers of the stratum corneum but not deeper into the viable epidermis and dermis [3–5]. In contrast, other studies showed that 40-nm, but not 750- or 1,500-nm, polystyrene nanoparticles and 40-nm silica nanoparticles can translocate to the viable epidermis in human skin explants with partially disrupted stratum corneum [6, 7]. In addition, Mortensen and colleagues showed that quantum dot nanoparticles penetrate deep into the epidermis and dermis of mice exposed to ultraviolet irradiation [8], which induces skin-barrier defects such as disruption of stratum corneum lipids and loosening of cell–cell junctions. Because consumers often apply sunscreen to sun-damaged skin, it is more important to examine the effect of ultraviolet radiation on the ability of nanoparticles to penetrate the skin.

### 15.2.2 Gastrointestinal Exposure

People in developed countries ingest an estimated $10^{12}$ to $10^{14}$ manufactured fine (diameter, 0.1–1 mm) to ultrafine (diameter, <100 nm) particles in food every day [9]. In particular, amorphous silica particles (including nanosize particles) are widely applied in food products and registered within the European Union as a food additive (E551). These particles are used mainly as thickening medium for pastes, as an anti-caking agent to maintain flow properties in powdered products, and as a carrier for fragrances and flavors in food and nonfood products [10]. However, little information about the absorption of nanosilica particles after oral exposure is available, mainly because there is no high-sensitivity method for detecting silicon in biologic tissues. One study showed the effect of size on the absorption of nanoparticles after oral administration in rats by using gold nanoparticles of different sizes (1.4–200 nm) [11]. The smallest particles had the highest absorption across intestinal membranes: after 24 h, 0.37 % of the applied 1.4-nm particles had reached the circulation. Surface charge was important in the absorption across intestinal membranes also, and more negatively charged gold nanoparticles were absorbed than were positively charged particles. Furthermore, the greatest accumulation of particles in the heart and brain after oral administration was associated with the 18-nm particles, which accumulated to even greater amounts than did the 1.4-nm particles; the precise mechanism underlying this effect is unknown. In summary, although small nanoparticles tend to be absorbed more readily than are large particles, no general assumption regarding tissue accumulation after particle absorption can yet be made.

### 15.2.3 Pulmonary Exposure

Nanoparticle-facilitated drug delivery through the lung is attractive because of the organ's large surface area. In addition, knowing the ADME profile of nanoparticles after lung exposure is particularly important, because the inhalation of nanoparticles increasingly is recognized as a major cause of adverse health effects. Gold nanoparticles 1.4 nm in diameter efficiently cross the air–blood barrier of the respiratory tract, whereas almost all 18-nm particles remain trapped in the lungs after intratracheal instillation in rats [12]. In addition, the biodistribution patterns (e.g., organ ratios) of 1.4-nm gold nanoparticles differed markedly after intravenous injection compared with intratracheal instillation. For example, the liver–blood ratio is 1.1 to 1 after instillation compared with 12.5 to 1 after intravenous injection, suggesting that 1.4-nm nanoparticles undergo some unknown chemical or biochemical transformation process during translocation through the lung. Another possibility is that the interaction of nanoparticles with alveolar fluid in the lungs during inhalation exposure or with blood proteins during intravenous injection has different effects on the surface properties of nanoparticles, leading to differences in tissue uptake. Therefore, data after intravenous injection might be unreliable for

predicting the biodistribution of nanoparticles after their passage through various barriers and may give misleading information about potential harmful effects.

Most of the biodistribution studies that have assessed nanoparticles to date occur over a relatively long time, from several hours to weeks. In contrast, Choi and colleagues used near-infrared fluorescent nanoparticles to examine biodistribution during the first hour after their administration [13]. In that study, nanoparticles with a hydrodynamic size less than 34 nm rapidly moved from the lung to mediastinal lymph nodes, and nanoparticles smaller than 6 nm rapidly moved from the lung to lymph nodes and the bloodstream, leading ultimately to renal clearance. This and other new experimental methodologies may provide new insights into the ADME profile of nanoparticles. In addition, future studies should minimize doses and examine biodistribution in more organs for prolonged periods to fully characterize the potential health effects of exposure to nanoparticles.

## 15.3 Translocation of Nanoparticles Across Internal Biologic Barriers

The blood–brain, blood–testis, and placental barriers protect particularly sensitive tissues from foreign chemicals. In this section, we discuss the translocation of nanoparticles across various internal barriers, especially the placenta.

Considerable evidence shows that, because of their physiologic immaturity, fetuses are more sensitive than are adults to numerous environmental toxins. Recurrent pregnancy loss affects 1–3 % of couples; many of these miscarriages undergo extensive—but ultimately uninformative—diagnostic testing. In addition, intrauterine growth restriction occurs in as many as 10 % of pregnancies and predisposes the child to a lifelong increased risk for hypertension, cardiovascular disorders, and renal disease, among others. Although many factors for miscarriage and intrauterine growth restriction have been suggested, the precise mechanism and mediators remain unknown. It is essential to assess the potential risk of nanoparticles to cause these pregnancy complications.

Normal placental development is required for successful embryonic growth, and placental dysfunction has been associated with miscarriage and fetal growth restriction. Some reports have warned about the potential adverse effects of nanoparticles on fetuses [14–17]. In one study, silica nanoparticles (diameter, 70 nm) and titanium dioxide nanoparticles induced miscarriage and fetal growth restriction in pregnant mice, whereas microscale silica particles did not induce these complications [15]. The observed pregnancy complications resulted from placental dysfunction, such as destruction of the placental vasculature. Notably, surface-modified silica nanoparticles (diameter, 70 nm) did not induce any pregnancy complications in mice. Furthermore, whereas maternal pulmonary exposure to carbon black during pregnancy had adverse effects on the offspring [18, 19], repeated oral administration of multi-walled CNTs during pregnancy did not cause fetal toxicities [20].

Several studies have addressed the biodistribution of nanoparticles to the fetus and placenta. For example, after intravenous injection of pregnant mice, silica nanoparticles (diameter, 70 nm) were detected in the maternal liver and placenta and the fetal liver and brain, although microscale silica particles were not noted in any of these tissues [15]. In addition, when administered by intravenous injection early during pregnancy, PEGylated single-walled CNTs reached the conceptus, whereas these nanotubes reached only the placenta and yolk sac—not the embryo—when injection occurred during later pregnancy stages [17]. Furthermore, gold nanoparticles (diameter, 13 nm) accumulated in fetuses more efficiently when pregnant mice were injected before embryonic day E11.5 than after E11.5, indicating that the stage of placental maturity influences the translocation of nanoparticles to murine fetuses [21]. This same study showed that modification of the surface of nanoparticles altered their translocation to fetuses. Therefore, the translocation of nanoparticles to fetuses during murine pregnancy is influenced both by the stage of placental maturity and by nanoparticle surface composition.

Mouse and human placentas differ in their modes of implantation, the relative importance of yolk sac placentation, and the structure (labyrinthine compared with villous) of the exchange area. For example, by using an ex vivo human placental perfusion model, Wick and colleagues showed that polystyrene nanoparticles smaller than 240 nm can cross human placental tissues to reach fetuses [22].

## 15.4 Translocation of Nanoparticles Across Cellular Barriers

Various pathways, including passive and active diffusion, enable nanoparticles to travel across external and internal barriers. For example, small nanoparticles might access the paracellular pathway by evading the tight junctions between epithelial and endothelial cells, which are considered to exclude molecules larger than 0.6–5 nm [23].

In the gastrointestinal tract, enterocytes (especially M cells) may facilitate the transport of nanoparticles through transcytosis. Chitosan nanoparticles enhance intestinal paracellular uptake by modulating tight junctions between cells [24]. Another potential mechanism for the translocation of nanoparticles in the gastrointestinal tract involves degrading enterocytes, which are shed in high numbers daily (mice, $2 \times 10^8$ cells; humans, $10^{11}$ cells) in the small intestine [25].

In human endothelial cells in vitro, iron particles induced the production of reactive oxygen species [3] and oxidative stress, consequently increasing microtubule remodeling and permeability in these cells [26]. In a particularly novel mechanism of nanoparticle transport, titanium dioxide nanoparticles have been shown to interact directly with the protein VE-cadherin at the inter-endothelial adherens junction niche to promote actin remodeling as well as internalization and degradation of VE-cadherin, thus increasing the leakiness of endothelial cells [27]. Therefore, the pathway that nanoparticles use to traverse cellular barriers likely varies depending on their material, size, and charge; these relationships need to be explored more thoroughly to fully understand the ADME profile of nanoparticles.

## 15.5 Metabolism, Degradation, Excretion, Clearance, and Biopersistence of Nanoparticles

The clearance or excretion of nanoparticles is important for assessing their long-term toxicity, because nanoparticles may be inherently stable. For example, quantum dots with the appropriate coating were retained in mice and remained fluorescent for at least 2 years [28]. In this way, the biopersistence of nanoparticles is a key factor in understanding their toxicity. The ability of cells to metabolize or degrade nanoparticles and to excrete or otherwise clear them from the body minimizes their potential toxicity. To this end, we need to learn the pathways that are responsible for breaking down nanoparticles and for removing them from the body. In addition, monitoring the concentration of nanoparticles in cells and tissues over long time periods (e.g., months) will inform us regarding the lifespan of these particles as well as their long-term biologic effects. In this section, we discuss the metabolism, degradation, excretion, clearance, and biopersistence of nanoparticles.

### *15.5.1 Excretion and Clearance of Nanoparticles*

There are two major routes for the excretion and clearance of nanoparticles: renal filtration, with excretion into the urine, and hepatobiliary processing, with excretion into the bile. In general, proteins with a hydrodynamic diameter of <5–6 nm are cleared rapidly from the body by renal filtration and urinary excretion. Choi and colleagues used quantum dots to show that the renal clearance of nanoparticles similarly is related to their hydrodynamic diameter: quantum dots smaller than 5.5 nm were excreted into urine rapidly and efficiently and eliminated from the body, but the renal clearance of quantum dots larger than 15 nm was relatively low [29]. In another study, the accumulation of 1.4-nm gold nanoparticles in the liver and spleen was significantly lower than that of 18-nm nanoparticles, and 1.4-nm gold nanoparticles were excreted by both the renal and hepatobiliary systems after intravenous injection [12]. In comparison, single-walled CNTs (average diameter, 1 nm; average length, 300–1,000 nm) and multi-walled CNTs (average diameter, 20–30 nm; average length, 500–2,000 nm) undergo rapid and effective renal clearance and urinary excretion after intravenous injection, although the precise mechanism is unknown [30].

In the reticuloendothelial system, hepatic Kupffer cells are the representative cells that have the ability to uptake particles. In general, the liver is the predominant target organ of nanoparticle accumulation as well as an important excretion route. Various studies have shown that about 4 % of the administered dose of polystyrene nanoparticles (diameter, 50 nm) was excreted as intact particles in bile within 24 h after intravenous injection, and about 30 % of the total dose accumulated in hepatocytes [31, 32].

Recent investigations have revealed a novel clearance mechanism in the immune system. Macrophages play an important role in the uptake of nanoparticles, and the biodistribution of nanoparticles (e.g., blood clearance) differed between strains of

mice with differences in global immune status [33]. In the cited study, Jones and colleagues used intravital microscopy to show that PEGylated particles (diameter, 300 nm) are cleared more slowly in Th1-prone mice than in Th2-prone mice. M2 macrophages, which are induced by Th2 cytokines and have high levels of endocytosis, were important in the enhanced clearance observed in Th2-prone mice. In addition, these results were observed in macrophages from humans, suggesting that global immune regulation might significantly affect nanoparticle clearance in humans. Furthermore, the study suggested that granulocytes, especially neutrophils, are also important in the clearance of nanoparticles, although this cell type is seldom addressed in this context [33]. In addition to their phagocytic capacity, neutrophils can release complexes of DNA and protein into the extracellular space, thus trapping pathogens at infection sites (neutrophil extracellular traps); neutrophils may trap nanoparticles in extracellular structures by a similar mechanism [34]. Clearly future studies need to address the excretion or clearance of nanoparticles targeted not only to the kidney and liver but also immune cells.

### *15.5.2 Metabolism and Degradation of Nanoparticles*

Nanoparticles had been thought to be resistant to metabolism and degradation under in vivo conditions, but several recent reports suggest that CNTs are degraded through natural enzymatic catalysis. In that regard, Kagan showed that myeloperoxidase (MPO), an abundant enzyme of inflammatory cells (neutrophils), played an important role in the oxidative biodegradation of single-walled CNTs [34]. In addition, single-walled CNTs that had been degraded by MPO in vitro failed to induce inflammatory and oxidative-stress responses after pharyngeal aspiration in mice, whereas intact nanotubes induced these responses. Consistent with these results, the clearance of single-walled CNTs from the lungs was much less effective in MPO-deficient than in wild-type mice after pharyngeal aspiration, whereas the inflammatory responses were much robust compared to wild-type mice [35]. In addition, single-walled CNTs are degraded by eosinophil peroxidase, a key oxidant-producing enzyme during inflammatory states [36]. Collectively, these findings suggest new ways to control the biopersistence of CNTs through genetic or pharmacologic manipulations. However, few studies have investigated the metabolism and degradation of nanoparticles in cells, because methods for monitoring a single particle in cells over time are currently unavailable. Given the numerous factors that might influence the metabolism and degradation of nanoparticles, methods for investigating these processes at the nanoscale are urgently needed.

### *15.5.3 Biopersistence of Nanoparticles*

The precise mechanism underlying the biopersistence of nanoparticles is not yet fully understood. To this end, Balasubramanian and coworkers found that gold nanoparticles were rapidly and consistently accumulated in the liver and spleen of

rats within 1 day after a single intravenous injection and remained at high levels at 2 months thereafter [37]. These findings are consistent with several studies showing that, regardless of their size, shape, dose, and material, intravenously injected nanoparticles accumulate in the liver and spleen and are retained in those organs for long periods of time. For example, regardless of the size of the silver nanoparticles (10 or 25 nm) to which rats were exposed orally for 28 days, the silver content in most tissues gradually decreased to control levels over the 4-month observation period [38]. However, the silver concentrations in the testes and brain did not decrease to control levels, suggesting reduced clearance of silver nanoparticles across biologic barriers such as the blood–brain and blood–testis barriers.

## 15.6 Protein Corona

When nanoparticles enter the body, they typically become coated with various proteins, thereby developing a "protein corona." The binding of proteins to nanoparticles depends on their various physical characteristics, such that the size and surface properties of nanoparticles play important roles in determining the protein corona. Some studies have suggested that these interactions with proteins actually define the biologic effects and biodistribution of nanoparticles. For example, a recent in vitro study suggested that the formation of coronas of blood proteins reduced undesirable cellular responses to and the cytotoxicity of CNTs [39]. In addition, protein coronas have proven to be a key factor in the recognition and phagocytosis of nanoparticles by macrophages. Deng and coworkers showed in vitro that negatively charged gold nanoparticles bind to and induce the unfolding of fibrinogen, consequently promoting its interaction with the integrin receptor Mac-1 [40].

Nanomaterials can be covered with biologic molecules other than proteins as well. For example, creating a "corona" of lipids has been suggested as a means to influence the cellular uptake and toxicity of nanoparticles [41, 42]. In this regard, Konduru and colleagues showed that the adsorption of phosphatidylserine onto the surface of single-walled CNTs enhanced their uptake by macrophages and dendritic cells in vitro [43]. In addition, single-walled CNTs were found to be coated with surfactant proteins and phospholipids after pharyngeal aspiration of the nanotubes in mice, and the presence of this surfactant coating enhanced the uptake of the nanotubes into macrophages in vitro [44].

The ADME of many nanoparticles in vivo likely is largely defined by the protein corona rather than the nanoparticle itself. However, little information is available that addresses relationships between differences in the ADME profiles of nanoparticles in vivo and the formation of protein coronas. In this context, one study revealed that attaching plasma proteins onto dextran-coated superparamagnetic iron oxide nanoparticles is unlikely to alter their clearance by the liver and spleen, because the plasma proteins do not mask the entire surface of the nanoparticle [45]. One key question is whether the corona present at the point of entry (e.g., blood, lung, or other) or that resulting from modification during subsequent translocation determines the biodistribution and effects of nanoparticles.

The detailed mechanism underlying the fate of the original corona as the coated nanoparticle passes through membranes and barriers and interacts with the extracellular matrix is unknown currently: the original corona may remain intact or be replaced by new biomolecules. Another important question is whether the exposure route influences the protein corona. For instance, the physicochemical changes in the protein corona that occur while nanoparticles reside in the lung or gastrointestinal tract may dramatically change their ability to cross various internal barriers. Whether the exposure route directs the formation of different coronas needs to be determined.

## 15.7 Manipulation of the ADME Profile for the Development of Nanomedicine

Foreign particles—including nanoparticles—are removed from the body by phagocytes, such as macrophages, which also recognize and avoid live, nonforeign cells. In medical applications, systemically administered nanoparticles should evade rapid clearance so that they achieve sufficient accumulation in targeted tissues and cells to yield effective local drug concentrations. One way to delay the clearance of nanoparticles is to conjugate them with polyethylene glycol (PEG) or another water-soluble polymeric modifier. The surface modification of nanoparticles with PEG decreases their uptake by macrophages and retards renal clearance, thereby prolonging the half-life of nanoparticles in vivo. The prolonged circulation of nanoparticles in the blood induces the enhanced permeability and retention effect, which is based on the leaky nature of tumor blood vessels and results in increased delivery of conjugates to tumor tissue [46, 47]. However, PEG modification of nanoparticles might hinder their uptake by and drug-delivery to diseased target cells. In this regard, Rodriguez and colleagues suggested another approach to prolonging the circulation time of nanoparticles. The membrane protein CD47, which is expressed on all cell membranes, is a marker of self that impedes the phagocytosis of nonforeign cells in mice. Nanoparticles carrying peptides designed from CD47 avoided macrophage-mediated clearance and were retained in the circulation, resulting in both enhanced tumor imaging and increased drug delivery [48]. Other homeostatic self factors might similarly be used to prevent the phagocytosis of nanoparticles and target them to specific tissues, thereby enhancing the delivery of therapeutics and imaging agents to these sites.

## 15.8 Conclusion

Engineered nanoparticles have remarkable structural diversity and adopt characteristic forms including tubes, dots, wires, fibers, and capsules. Several reports have shown that the shape of nanoparticles plays an important role in their biologic

effects in vitro. However, the in vivo behavior of nanoparticles can be influenced significantly by their physicochemical characteristics, such as particle size, surface charge, surface hydrophobicity, and particle shape. In addition, although aggregation appears to be a ubiquitous phenomenon among all nanoparticles, its influence on their ADME profiles is unclear. Furthermore, many reports have examined the effect of surface charge on the biodistribution of nanoparticles, but the results are conflicting, and a consistent rule has not yet emerged. The discrepancy among these studies likely results from the differences in the types of nanoparticles used, their charged groups, and other factors. In this regard, the complexity of the various experimental scenarios used to date complicates and perhaps even prevents the comparison of data between studies. Consequently, to collect information useful for developing general rules about the ADME profiles of nanoparticles, methodologies must be developed that facilitate the overarching interpretation of resulting experimental data. The data collected from such studies would contribute toward an improved understanding of the potential risk of nanoparticles in human health.

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Current Topics in
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# Biological Effects of Fibrous and Particulate Substances


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The environment itself can be a source of unintentional skin exposure to nanoparticles. Environmental nanoparticles in urban areas arise primarily from combustion sources such as burning coal, fuel oil, and biomass; and waste and motor vehicle emissions typically account for the greatest proportion of environmental nanoparticles [27, 28]. Comprising only a small proportion of ambient air particles by mass but a large proportion in terms of number [29], airborne nanoparticles are usually present as aggregates (diameter, ~100 nm) of very small (diameter, $\leq$10 nm) primary nanoparticles [28]. Although information regarding the health effects of environmental exposure is limited to effects after inhalation [30], skin exposure to environmental nanoparticles occurs in the same contexts in which they are inhaled.

Recent reports acknowledge that naturally occurring nanoparticles represent a previously unrecognized opportunity for exposure [31]. For example, various metal objects, including earrings and wire, spontaneously generate metal nanoparticles [32], and laundering silver-embedded textiles releases silver nanoparticles [33]. These naturally occurring metal nanoparticles are thought to arise through the nucleation of metal ions released due to chemical or photochemical reduction [32, 34].

Together, these findings suggest that we are exposed to nanoparticles via the skin merely as a consequence of our daily lives—for example, whenever we wear metal accessories or dress in clothes containing or washed with metal-embellished fabric. In addition, nanoparticles abound in nature. Various soils are naturally rich in nanoparticles, and earthquakes generate massive quantities of new soil-derived nanoparticles via mechanical grinding [31]. Although unavoidable, exposure to nanoparticles via the airways and skin is not a new problem but a long-standing facet of everyday life, at least in terms of naturally occurring nanoparticles.

## *6.2.2 Skin Penetration by Nanoparticles*

We know little regarding how naturally occurring nanoparticles or those emitted during industrial processes enter and cross the skin. However, the intense research done to confirm the safety of nanomaterials has taught us about the skin-penetrating characteristics of nanoparticles.

### 6.2.2.1 Titanium Dioxide and Zinc Oxide

Primary nanoparticles of titanium dioxide and zinc oxide are 10–20 nm in diameter, but they typically exist as 30- to 150-nm aggregates and frequently are used in cosmetics [35]. In a minipig model of human skin, inductively coupled plasma mass spectroscopy failed to detect any significant increase in the titanium concentration in the dermis or at draining lymph nodes after 22 days of sunscreen application [36]; in the same study, transmission electron microscopy (TEM) of the dermis revealed only a few titanium particles, equivalent to $10^{-6}$ to $10^{-4}$ % of the total amount applied. In another study, multiphoton microscopy of human skin in vivo 4 h after the application