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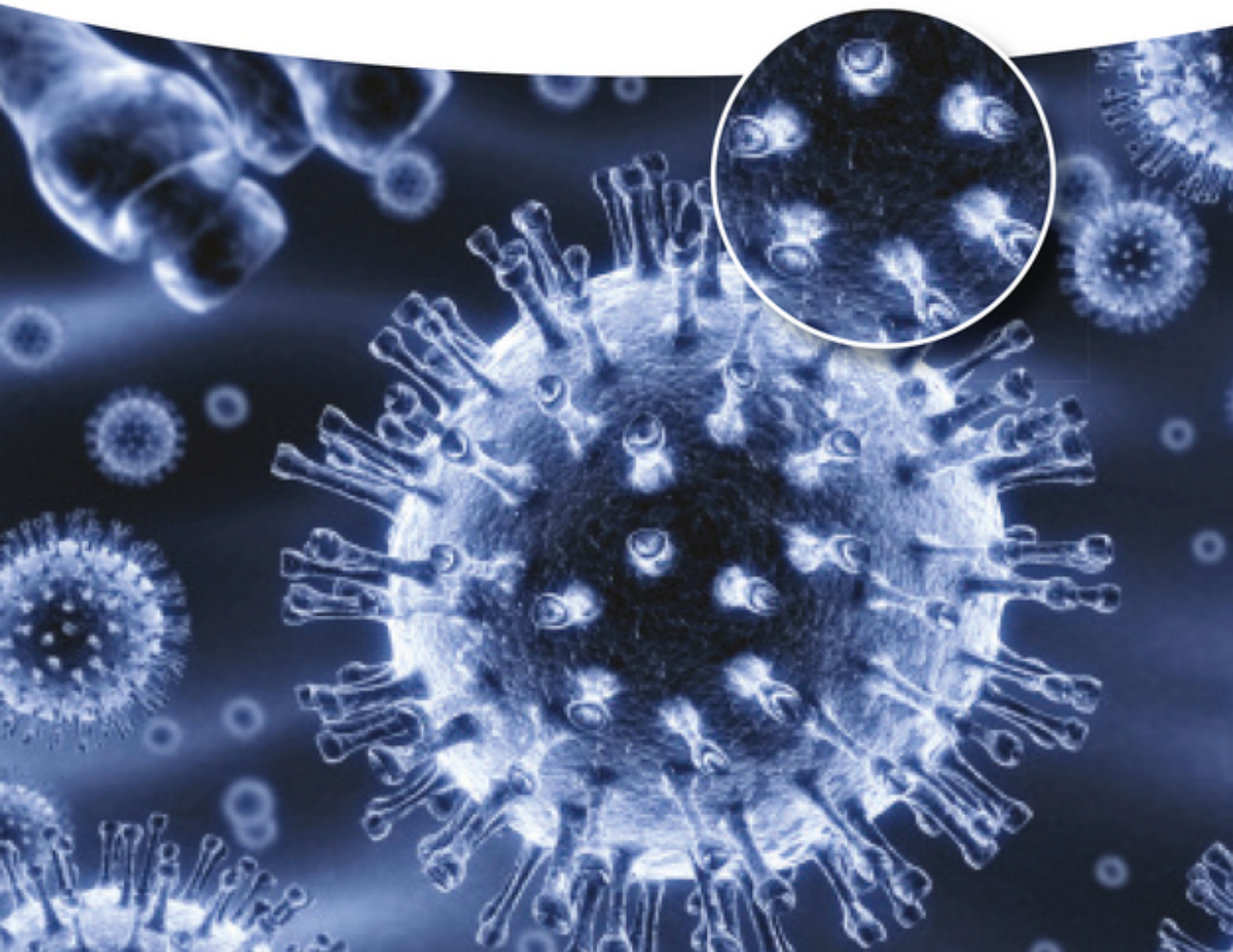
Biomedical Nanomaterials



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WILEY-VCH
Verlag GmbH & Co. KGaA

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Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Bibliographic information published by the Deutsche Nationalbibliothek
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at <http://dnb.d-nb.de>.

Print ISBN: 978-3-527-33798-9
ePDF ISBN: 978-3-527-69443-3
ePub ISBN: 978-3-527-69441-9
Mobi ISBN: 978-3-527-69442-6
oBook ISBN: 978-3-527-69439-6

Typesetting SPi Global, Chennai, India
Printing and Binding

Printed on acid-free paper

Contents

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1
Pharmacokinetics and Pharmacodynamics (PK/PD) of Bionanomaterials

Ergang Liu, Meng Zhang, and Yongzhuo Huang

1.1
Introduction

Nanomaterials (NMs) refer to synthetic or naturally occurring substances with size ranging from 1 to 1000 nm. The concept of "nanomaterial" was proposed by Feynman 50 years ago in the field of physics [1], which has since unveiled an era of nanotechnology. NMs contain merely tens to thousands of atoms, and are characterized by the surface and quantum size effects that are distinct from the bulk matters, and have thus gained wide applications in various areas [2]. For example, in medical application, nanotechnology has attracted specific attention in cancer therapy and diagnosis, largely due to the proposal of enhanced permeation and retention (EPR) effect by Maeda and coworkers; they demonstrated that nanosized macromolecules displayed a preferential retention in tumor site due to the leaky vasculatures [3, 4]. The EPR effect-associated nanomedicine composed of various natural or synthetic entities in the nanoscale, which have been developed to deliver drugs/imaging agents to the tumors based on the passive targeting effect [5]. Later, in order to further increase the transport efficiency, antibodies or targeting ligands with high binding affinity to tumor-overexpressed surface antigens or receptors have been applied to conjugate onto the surface of NMs to achieve the so-called active targeting [6].

NMs can also be applied in formulation development because of their capability to improve solubility [7], drug permeation [8], and drug stability [9]. Pharmaceutical nanotechnology may thus help improve druggability of those active molecules that are otherwise considered to be unsuitable for formulation development for clinical use due to unfavorable properties such as poor solubility and low permeation to the lipid bilayer membranes [10].

The emerging nanomedicine has greatly promoted drug development, and a good number of NM-based medicine or diagnostic agents have entered clinical trials, most in the field of cancer therapy, in which the NM-based delivery strategies are characterized by EPR effect for achieving tumor targeting. However, in spite of the enhanced permeability of the tumor vasculature, not all types of NMs

Biomedical Nanomaterials, First Edition. Edited by Yuliang Zhao and Youqing Shen.

could benefit from EPR effect to achieve a substantial targeting efficiency [11]. The *in vivo* ADME (absorption, distribution, metabolism, and excretion) behaviors of NMs vary because of the difference of the surface properties, size, and charges of the NMs, as well as their compositions, often leading to inconsistent therapeutic outcomes in animal studies [12].

On this account, investigation of "what the body does to NMs" may help us with a better understanding of the *in vivo* fate. We herein present a brief introduction of the commonly utilized NMs in pharmaceutical research, the anatomic features of the body and tumor, and the physiochemical natures of NMs that affect the *in vivo* fate. The established PK/PD models for simulating the *in vivo* ADME behavior of NMs will also be introduced. We hope this summary would give a glimpse into the complicated *in vivo* processes and provide helpful information for the rational design of NM-based drug delivery systems.

1.2 Commonly Utilized NMs in Pharmaceutical Research

NMs can be categorized into different groups based on certain classification. To make it simple, we use the natural/synthetic classification in this chapter because the natural/synthetic NMs are generally disposed by the body in different ways. Moreover, inorganic NMs characterized by the hard-core structure bear unique physical characteristics (magnetism, thermal response to radiation, optical features, etc.) [13, 14], and are discussed as an independent section. Other resources such as cell-based NMs (e.g., RBCs [15] and MSCs [16]) and components from microbes (e.g., inactivated virus envelope [17] and TAT [18]), are usually utilized with preservation of their original natures, which are thereby discussed as a complimentary to this classification.

1.2.1 Natural NMs

Natural NMs have been widely investigated because of their biodegradability and compatibility to human body. As known, lipids, proteins, carbohydrates, and nucleic acids are highly biodegradable in the body. Phospholipids are one of the most widely applied natural resources to build the nanocarriers such as liposomes and solid lipid nanoparticles (SLNs) [19]. Polysaccharides, including a variety of carbohydrates with different structures and functional groups, can be utilized to build different types of nanoparticles. Protein-based NMs (typically, serum proteins such as albumin [20], high-density lipoprotein (HDL) [21], and lactoferrin [22]) are often utilized as drug carriers.

1.2.1.1 Lipid-Based NMs

Lipid-based NMs include liposomes [19], SLNs, micelles [23], and nanoemulsions [24] (Figure 1.1). The main components of liposomes are phospholipids. In aqueous solution, the phospholipids will self-assemble into a bilayer structure

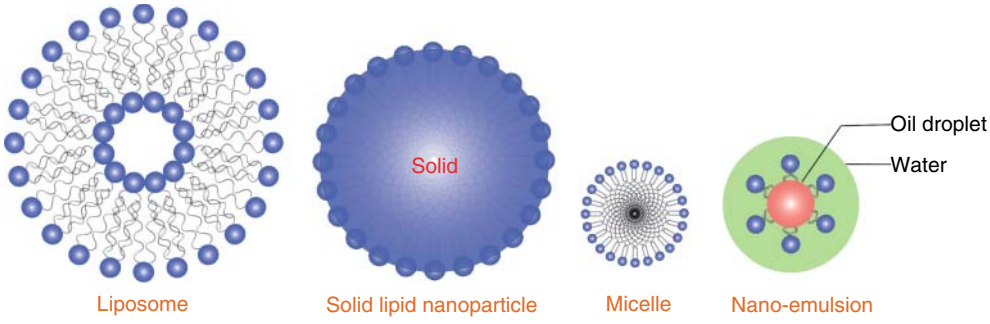

Figure 1.1 Schematic illustration of lipid-based NMs.

that functions as drug carriers with hydrophilic drugs encapsulated inside the interior, whereas hydrophobic drugs in bilayer [19]. One-tail lipids are inclined to form micelles in aqueous media [25], whereas using steric acid or oleic acid supplemented with surfactants to stabilize the solid/liquid lipids normally results in nanoparticles [26] or emulsions [27].

1.2.1.2 Protein-Based NMs

Protein-based drug carriers have been widely used in pharmaceutical industry. However, owing to the concerns of protein immunogenicity (e.g., OVA, which has been utilized as adjuvant for immune activation [28]), endogenous serum-rich proteins with low immunogenicity and long half-life such as albumin, high-density lipoprotein, and lactoferrin have distinct advantages [20–22]. As a case in point, albumin-bound paclitaxel nanoparticles (Abraxane®) have attained great market success [29]. In general, proteins can either be processed to form nanoparticles [30] or directly coupled with drugs by physical adsorption or via covalent bonds [20]. In certain instances, the protein carriers are further modified with targeting ligands to achieve specific delivery [31].

1.2.1.3 Polysaccharide-Based NMs

Polysaccharides originate from animal, plant, or bacterial sources. In general, the physicochemical properties of polysaccharides are governed by monosaccharide unit and the overall molecular weight [32]. The high molecular weight molecules, such as heparin and hyaluronic acid, show strong affinity to water molecules, and thus form hydrogels that have been widely applied for local administration because of their biocompatibility and sustained drug release functions [33]. The ionic polysaccharides can bind with molecules of the opposite charge, and the interaction normally leads to decreased solubility and the formation of nanoparticles [34].

1.2.2 Synthetic NMs

Although NMs based on naturally occurring materials have the advantages of biocompatibility and wide availability, structure modification is difficult

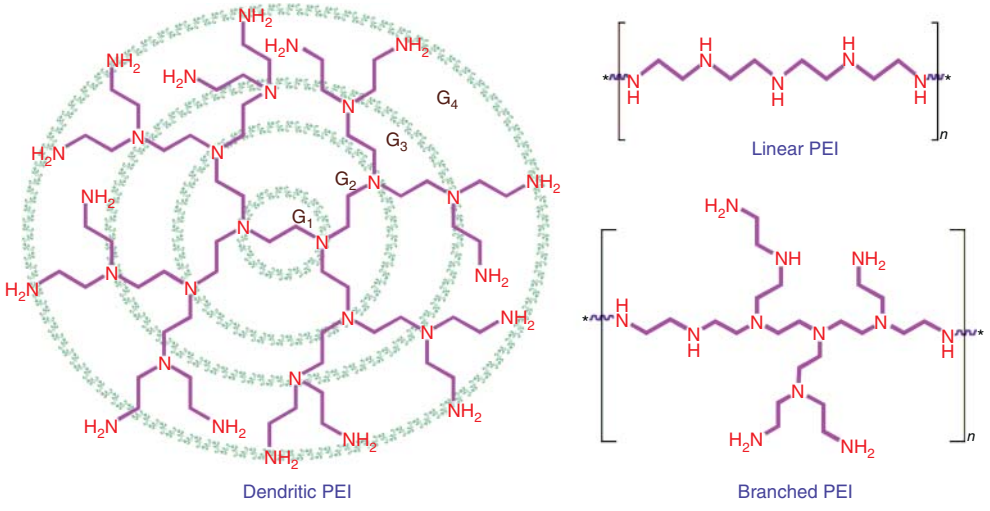

Figure 1.2 Various forms of PEI.

to process to tailor their functions to satisfy the needs from pharmaceutical application.

By contrast, synthetic polymers can be much more flexibly designed for a specific application. For example, by using the pH-sensitive synthetic materials, the NMs could release drugs in a pH-dependent manner for achieving tumor-targeting delivery [35], because the rapidly prolific neoplastic tissues normally secrete more lactose from the hyperactive anaerobic glycolysis, leading to a decreased pH in tumor microenvironment [36].

1.2.2.1 Diversity of Synthetic NMs in Forms

Synthetic NMs can be tailored for different purposes. For instance, polyethyleneimine (PEI) can be synthesized in the forms of linear, branched, or dendritic structures (Figure 1.2) [37]. Synthetic polymers can be fabricated into various types of NMs such as nanoparticles, micelles, and nanocubes. For example, poly(lactic-*co*-glycolic acid) (PLGA) can be made into widely applied nanoparticles [38], microspheres [39], and micelles [40].

NMs are often used as carriers for small molecular drugs. Drugs are loaded into NMs by encapsulation or via a covalent linkage, in order to improve the PK profiles and achieve targeting delivery to a specific site.

1.2.2.2 Drug Release Behaviors

Drug release from NMs is governed by the physiochemical properties of the drug and NMs. Burst release is often seen for the hydrophobic drugs in capsulation by liposomes, while sustained release for the hydrophilic drugs in liposomes, which are slow to diffuse across the lipid bilayers [41]. However, it is more complex to

investigate the drug release profile when NMs are injected to the body, and knowledge of the *in vitro in vivo* correlation (IVIVC) is still insufficient.

In order to reduce the unwanted drug exposure, a number of strategies have been developed to achieve a site-specific release of the loaded cargos.

pH-sensitive NMs

The slightly acidic tumor environment and endosome's even lower pH have attracted extensive interests in the application for designing NMs with the ability to respond to pH changes during the delivery. This strategy has been intensively explored by employing the polycationic dendrimers such as PEI and PAMAM. Acidic pH could cause the electrostatic repulsions between side chains in these polyamines because of the protonation of amino groups. As a result, the dendrimers swell in response to the acidic condition and the abrupt pH drop – the so-called "proton sponge" effect [42]. After engulfed by the cells, the swelling of NMs can lead to endosome rupture, and thus is favorable for intracellular drug release.

Another strategy is to use pH-sensitive linkage (e.g., hydrazone bonds) for cross-linking and building the NMs [43]. The NMs disassemble in a pH-dependent pattern, thus triggering drug release in acidic environments.

Redox-sensitive NMs

Besides the decreased pH, the rapidly growing tumor is also characterized by the intracellular reducibility due to the increased level of glutathione (GSH) [42]. The redox-sensitive NMs (e.g., NMs built via disulfide linkage) can display an accelerated drug release once entering the tumor cells.

Enzyme-sensitive NMs

Tumor-associated proteases have been widely investigated for their application as biomarkers in cancer diagnosis, prognosis, and therapy. Overexpression of tumor-associated proteases (e.g., MMP-2 [44], MMP-9 [45], and legumain [46]) in tumors provides ideal targets for the design of "smart" NMs with controlled release. A general strategy is to use a specific substrate peptide to modify the NMs, and the cleavage of the peptide would trigger drug release or cellular uptake.

Thermo/radio wave-sensitive NMs

Other stimulus-responsive NMs can respond to external physical stimulation (such as localized heating and electromagnetic radiation) and have been applied in drug delivery [47]. Specificity of this strategy is largely dependent on the precise control of the applied stimuli at the target sites.

1.2.3 Inorganic NMs

Inorganic NMs are distinguished from the organic NMs (soft matters) with the hard cores. In order to avoid aggregation in aqueous media, the inorganic cores are typically modified with surfactants or hydrophilic polymers to form a core – shell structure [48]. Of note, the *in vivo* biofate of inorganic NMs is greatly affected by the surface characteristics of the coating materials [49].

Inorganic NMs exhibit many unique physical properties – for example – fluorescence (quantum dots), superparamagnetism (iron oxide nanoparticles), photothermal effect (gold nanorods, carbon nanotubes), or special optical properties (silver nanoparticles) [13, 50]. These properties of the inorganic NMs have been utilized in cancer diagnosis and treatment applications.

1.2.4
Other NMs

Together with the rapid development of NMs, knowledge of NMs has accumulated. Proteins, in terms of the size, can also be viewed as bionanomaterials, which are rich in the body. As a case in point, albumin (MW 67 kDa) with a diameter around 7 nm [51] can serve as a unique "protein carrier" for drugs. Another example is the red blood cells (RBCs), with diameter from several to tens of micrometers [15], which may be regarded as a type of "microliposomes" to deliver therapeutic macromolecules. Moreover, even the protein capsids of a virus (size <100 nm) can be used as a "nanocapsule" [52]. Inspired by biomimetics, these physiologically originated NMs have been explored as novel carriers for drug delivery.

Moreover, there is another form of nanodrugs – the nanocrystals of hydrophobic drugs [53]. Such nanodosage forms can solve the solubility problem, and furthermore improve the PK profiles.

1.3
In vivo Biodistribution and the Evolving Targeting Principles for NMs

The targeting strategies have been mostly employed in cancer therapy areas. In general, there are two types of targeting strategies: the passive targeting via EPR effect and the active targeting mediated by antibodies or ligands that can specifically bind with receptors on cancer cells. Recently, with the growing knowledge of tumor physiology, the tumor microenvironments (e.g., acidic pH and the overexpressed proteases) have been used as a target for cancer drug delivery [6]. A combination of the targeting strategies by using multifunctional NMs has also been investigated for achieving improved specific targeted delivery and controlled release [54]. The ultimate goal is to increase drug concentration in tumor while reducing its exposure to the healthy organs.

1.3.1
Organ Distribution versus Cell-Specific Targeting

Ideally, NMs should be able to deliver the cargo drugs specifically into a target site. The *in vivo* fate of NMs is determined by a combination of multiple factors, including particle size, shape, and surface characteristics [55]. It is thus difficult to assess the overall targeting efficiency from *in vitro* data (e.g., cellular uptake by a