Factors Controlling Nanoparticle Pharmacokinetics: An Integrated Analysis and Perspective

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Abstract

Intravenously injected nanoparticulate drug carriers provide a wide range of unique opportunities for site-specific targeting of therapeutic agents to many areas within the vasculature and beyond. Pharmacokinetics and biodistribution of these carriers are controlled by a complex array of interrelated core and interfacial physicochemical and biological factors. Pertinent to realizing therapeutic goals, definitive maps that establish the interdependency of nanoparticle size, shape, and surface characteristics in relation to interfacial forces, biodistribution, controlled drug release, excretion, and adverse effects must be outlined. These concepts are critically evaluated and an integrated perspective is provided on the basis of the recent application of nanoscience approaches to nanocarrier design and engineering. The future of this exciting field is bright; some regulatory-approved products are already on the market and many are in late-phase clinical trials. With concomitant advances in extensive computational knowledge of the genomics and epigenomics of interindividual variations in drug responses, the boundaries toward development of personalized nanomedicines can be pushed further.

INTRODUCTION

Over the past half century, a wide range of nanoparticulate systems such as liposomes, nanospheres, and nanocapsules have been used as carriers for intravenous delivery and site-specific targeting of small molecules and macromolecular (e.g., proteins, nucleic acid structures) therapeutic agents (see **Figure 1**) (1–5). Theoretically, a drug-loaded carrier can afford protection against drug degradation or inactivation en route to the target site. This may result in a further reduction of the amount of the active agent needed to obtain therapeutic efficacy and may effectively reduce drug-induced adverse effects. For instance, in the delivery of chemotherapeutic agents to solid tumors, the concept of benefit-to-risk ratio remains vital because the therapeutic window for cytotoxic agents is often small and the dose-response curve is steep (1). Indeed, cancer nanomedicines are today's success story; the therapeutic efficacy of these particulate-based systems arises from altered pharmacokinetic parameters in vasculature as well as at the target site (e.g., a solid tumor) (2–4, 6–9). This trend is continuing strongly not only in oncology but also in other therapeutic areas such as the treatment of inflammatory conditions and infarction (1, 3, 5). In addition, the sheer complexity and the know-how of particulate drug delivery design, development, and production issues offer market exclusivity to the pharmaceutical industry and reduce the threat of generic competition (10). Such technological approaches may reduce and/or prevent the rapid fall-off of revenue for proprietary nanopharmaceuticals even after patent expiration (10).

Drug loading and encapsulation efficiency, the drug release profile, and the pharmacokinetics (PK) of particulate drug carriers vary with parameters that include chemical composition, interface forces, morphology, and size (1). With the advent of nanotechnology and parallel developments in material science, sophisticated carriers that allow for simultaneous targeting, sensing, signaling, and drug release are beginning to emerge (3–5, 8, 11, 12). Such multifunctional nanoparticles are complex in nature, assembled from a combination of materials that include lipids, proteins, sugars, synthetic polymers [e.g., poly(ethylene glycol)s (PEGs), which confer nanoparticle longevity in the systemic circulation], and even metals. Some of these building components can respond to microenvironmental (e.g., pH sensitivity, enzyme sensitivity) or external (e.g., magnetic field, light) signals (**Figure 1**) (1–12). Furthermore, their size, morphology, and surface characteristics may

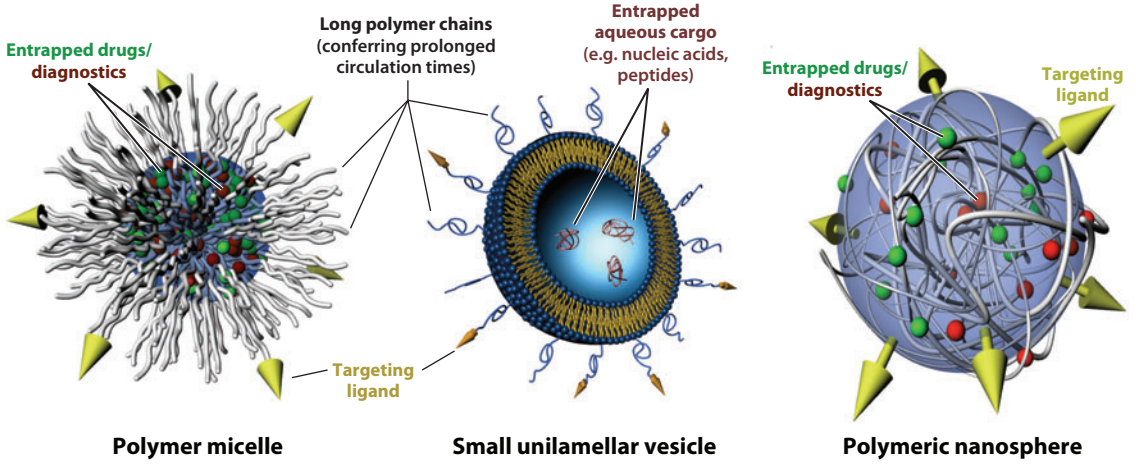

Figure 1

Schematic representation of liposomal, micellar, and polymeric nanoparticulate systems used for site-specific targeting and for controlled drug delivery and release.

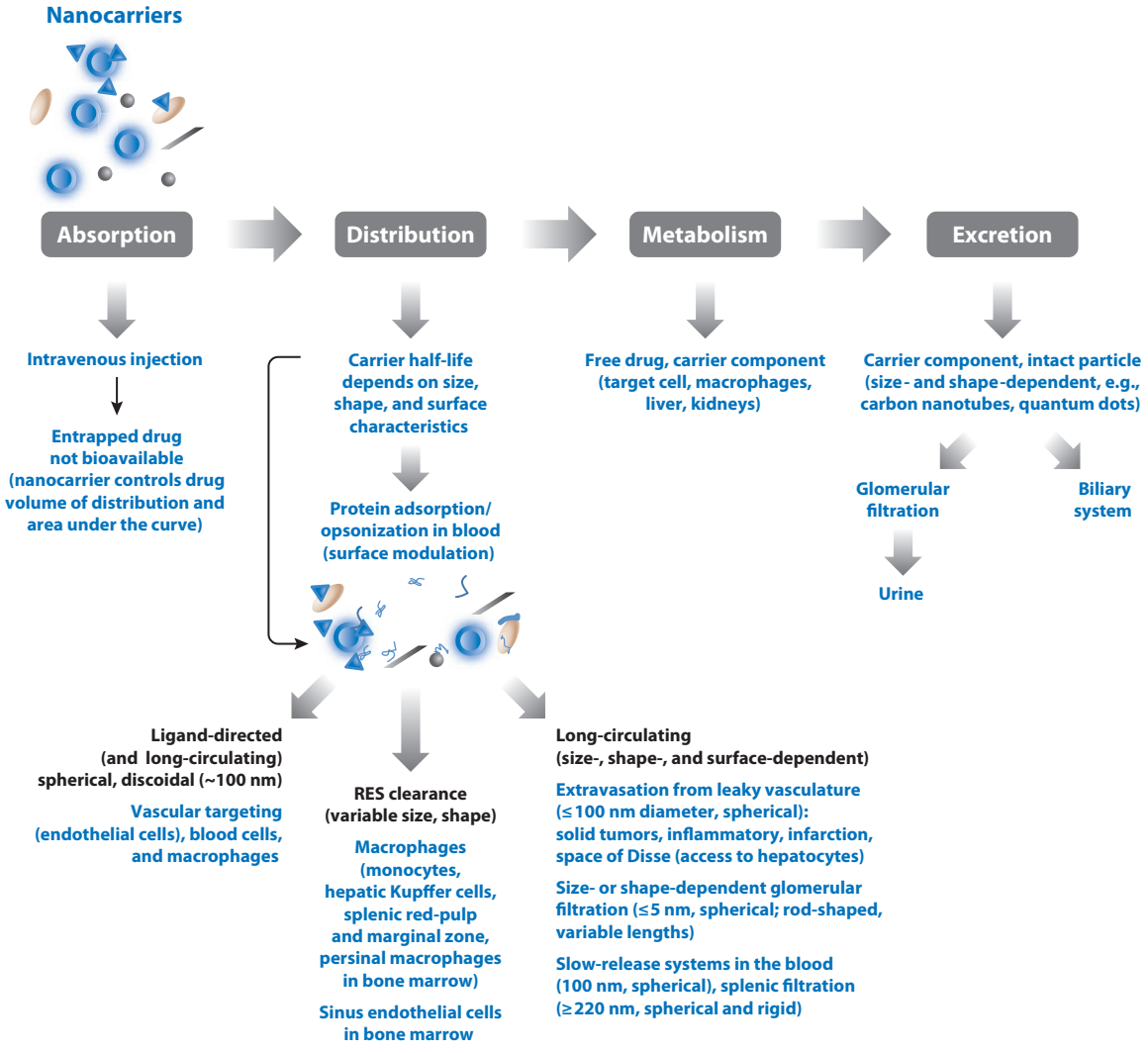

Figure 2

A general view of the influence of nanoparticle physicochemical characteristics (size, shape, and surface properties) on ADME (absorption, distribution, metabolism, excretion). Abbreviation: RES, reticuloendothelial system.

be tuned to achieve controlled PK and biological targeting on the basis of the type, developmental stage, and location of the disease (1, 3, 13–16).

The biological performance (PK profiles, target recognition, therapeutic efficacy, and adverse reactions) of intravenously injected nanoparticles is controlled by a complex array of interrelated physicochemical and biological factors (**Figure 2**) (1). The former include the particle size distribution profile, the particle shape and extent of rigidity/deformability, and the particles' surface characteristics such as chemistry and molecular architecture. Indeed, a detailed knowledge of particle characteristics both before and after intravenous administration is vital for design optimization. Although gathering such knowledge before administration is more attainable, limited technology is available to assess and follow real-time events pertaining to particle characteristics in systemic

circulation (e.g., flow properties within the blood vessels and at bifurcations in vascular and capillary systems, plasma protein–binding dynamics, particle component shedding, and dynamic size and shape changes). However, ongoing developments in nano- and microfluidic devices may offer some opportunities to study certain aspects of fluid dynamic events in vitro for better nanoparticle design and performance in the in vivo circulation (17–19). Biological determinants include not only biochemical, anatomical, or immunological barriers, but also opportunities offered by disease states for exploitation by nanoparticles (e.g., access routes, exclusive antigen/receptor expression) (1, 3). Further complexity in nanoparticle PK arises from their dosing regimens (1, 3). This is important in situations in which patients receive multiple injections at set intervals and in which PK may change after the first dose. Most researchers have studied key parameters individually (e.g., the effect of particle size, shape, or surface potential on protein binding or clearance kinetics, and the role of particle size or shape on the escape from vasculature) or in combination (e.g., the effect of size and a particular surface function on the plasma protein–binding profile and correlations with circulation half-lives) (1, 3, 20–34). These approaches are valuable within the framework of structure-performance relationships, but a more concerted approach to analysis is necessary to assess and map the dynamic interplay between physicochemical and biological factors controlling nanoparticle PK (**Figure 2**). Of particular importance is the lack of current state-of-the-art methodologies for precise (nano)material characterization, which makes interpretation of certain biological responses difficult. In this review, we reevaluate these concepts and provide an integrated perspective from current research in relation to PK.

THE PROMISCUOUS ROLE OF BLOOD PROTEINS IN CLEARANCE KINETICS

General Aspects

The physicochemical characteristics of nanoparticles, regardless of their material nature, are often changed when the nanoparticles come into contact with the blood. Studies have related the nanoparticles' interactions with proteins in blood both quantitatively and qualitatively to nanoparticle half-lives and biodistribution (1, 20, 21, 35, 36). Blood protein deposition is believed to be a dynamic event, which might increase the hydrodynamic size of nanoparticles, affect their stability (as with some vesicular and micellar carriers), and modulate the overall surface function (35, 36). The composition of the plasma protein coat is expected to differ considerably in amount and heterogeneity and in a time-dependent manner, on the basis of the original physicochemical properties of nanoparticles, the pathological state being treated, and the dosing regimen (20, 28, 33–37). Qualitatively, some of the most abundant plasma proteins such as albumin are found on the surfaces of nearly all tested nanoparticles (23, 33–37). Surface protein deposition may induce nanoparticle aggregation, and large aggregates may be trapped in the first capillary bed encountered after parenteral administration, the lungs (1). Another key consequence is surface opsonization events. A surface opsonization event is the deposition of proteins that facilitate nanoparticle recognition and clearance from the blood by circulating phagocytes as well as tissue macrophages that are in direct contact with the blood (mainly the hepatic Kupffer cells and the marginal zone and red-pulp macrophages in the spleen) (1, 3, 28, 32–35). Examples of key opsonic proteins include various subclasses of antibodies capable of mediating nanoparticle recognition by different macrophage Fc receptors. They also include complement activation products such as C3b and iC3b, which prime the surface for interaction with a plethora of macrophage complement receptors as well as other cells (e.g., platelets and erythrocytes), depending on the species (1).

Particular attention should be paid to the complement system, the most ancient defense mechanism in the body (34). This system contributes not only to nanoparticle opsonization and clearance but also to initiation of adverse effects such as anaphylaxis and cardiopulmonary disturbances (38, 39). Complement triggering and opsonization often proceeds through binding of a recognition molecule that senses the nanoparticle surface (e.g., antibody, ficolin, C-reactive protein, C1q), but nonspecific adsorption of intact C3 molecules can further initiate complement activation in the presence of complement factors B and D and accelerate nanoparticle clearance (40, 41). Additionally, surface opsonization can occur secondarily to nonspecific protein adsorption, in which conformational changes of a surface-adsorbed nonspecific protein may trigger antibody binding and/or complement activation. However, not all proteins undergo conformational changes following surface immobilization (36). The protein adsorption is often driven by entropy, a process resulting from the release of bound water molecules from the surfaces of nanoparticles (36).

Undoubtedly, the protein coat is a key determinant in controlling nanoparticle PK and secondary responses (1, 20, 21, 35, 36), but not all proteins deposited on the surfaces of nanoparticles impact their PK or pharmacodynamics. Numerous studies have tried to map out protein profiles on nanoparticle libraries, but more concerted efforts are necessary to relate these to defined biological processes and to different nanoparticle characteristics and pathological states (3, 25, 42, 43). For instance, phagocytic (or other target) cells will sense the outer corona (the outer layer of adsorbed proteins on the nanoparticle surface) (28), but the extent to which the composition/conformational state of the outer surface proteins is regulated by the type and the mode of deposition of the primary protein scaffold is unknown. Also, deposition of some known opsonic proteins (e.g., antibodies) may play no functional role in clearance kinetics because their spatial arrangement (conformation, spacing, and surface density) may not trigger complement activation and/or macrophage sensing. Conversely, nonspecific antibody deposition through the Fc domain or adsorption of certain other blood proteins may confer dysopsonic activity, leading to prolonged nanoparticle half-lives in the circulation. The dysopsonic phenomenon may further be viewed as a protective mechanism against erythrocyte lysis (e.g., by serum albumin–mediated charge neutralization of cationic poly(amidoamine) dendrimers) (44, 45) or excessive nanoparticle uptake by macrophages (46). Another interesting issue relating to protein adsorption is the ability of some nanoparticles to improve protein stability (e.g., by enhancing enzymatic activities) even under hostile conditions (11, 47, 48); this ability may be beneficial to controlled (or rapid) drug release at a designated target such as a solid tumor (11, 48). Some nanoparticles may enhance the rate of fibrillation of amyloidogenic proteins that are involved in neurological disorders such as Alzheimer's disease (AD) and Parkinson's disease, whereas others may exert antiamyloidogenic properties (49). The latter is of interest in the design of particulate agents capable of inducing the sink effect in AD (50). The sink-effect phenomenon is based on recent observations that the brain and the blood amyloid-β (Aβ) are in equilibrium through the blood-brain barrier (50). Alterations of peripheral/brain Aβ dynamics through nanoparticle-mediated peripheral sequestration of Aβ and its transport to macrophages of the reticuloendothelial system may dramatically improve AD through initiation of more Aβ release from the brain into the systemic circulation (51, 52).

Alterations and variations in plasma protein content, concentration, and activity are associated with sex, age, dietary conditions, genetic makeup, population differences, and pathophysiological conditions (53, 54). Therefore, significant variations in protein-binding patterns and responses to nanoparticles may be expected, and may have important PK consequences, but these issues have not received much attention. For instance, antiphospholipid and anticholesterol antibodies are widespread in humans and all animal species, but their specificities and titers show substantial inter- and intraspecies variation that may result in different binding patterns and responses (e.g., complement activation) to defined liposome formulations (55). The occurrence of antibodies in

humans capable of recognizing 4–5 repeat ethoxy units has also been reported (56). These antibodies may recognize the PEG coat of many long-circulating nanocarriers and alter their PK. Consequently, long-circulating drug carriers may not exhibit prolonged half-lives in individuals with high titers of such antibodies. Similarly, as a result of substantial interindividual variations in plasma concentrations of complement proteins and complement regulatory molecules such as factors H, I, C1INH, and C4BP, different degrees and patterns of nanoparticle-mediated complement activation and fixation are to be expected among individuals (53). Additional complement activation pathways, such as the C2-bypass pathway, have been observed in some patients in whom mannose-binding lectin/mannose-associated serine protease-2 directly attack and cleave C3 without formation of the corresponding C3-convertase (57). Another interesting observation is the low incidence of complement activation and complement-mediated adverse events following infusion of block-copolymer-stabilized perfluorochemical emulsions in subjects with abnormal or elevated HDL and LDL profiles (discussed in the section below) (58, 59).

Surface Curvature Versus Surface Chemistry

Cedervall et al. (37) synthesized libraries of spherically shaped polymeric nanoparticles, ranging from 70 nm to 700 nm, from random cross-linked copolymers of *N*-isopropylacrylamide and *N-tert*-butylacrylamide, and they mapped plasma protein–binding patterns. They demonstrated that the amount of bound protein varied with particle size and scaled with the amount of available surface area. The observed protein pattern was similar for all sizes. Their conclusion was that, for the stated size range, surface curvature was not a major determining factor for the relative affinities of proteins for the particles. Although this attempt did not correlate the protein fingerprint to nanoparticle clearance kinetics from the blood and/or subsequent biodistribution, the authors' conclusion ignores the possible importance of exact surface chemistry and subtle polymeric structural architectures in modulating protein binding, sequential protein addition, and subsequent protein conformation, all of which, in turn, could affect biological performance, including clearance kinetics. Their observations (37) are also in conflict with a more recent study that elegantly assessed the role of particle surface curvature in relation to antibody-binding topology and subsequent complement activation in human serum (60). In that study, serum immunoglobulin M (IgM) antibodies to dextran potently activated complement on a dextran-coated iron oxide particle 250 nm in diameter, whereas for 600-nm-sized particles, which bound IgM as effectively as did the 250-nm particle, there was substantially less complement activation on the basis of surface area (60). Remarkably, for smaller (50-nm) particles, complement activation was highly variable. These discrepancies were thought to be due to IgM geometric aspects and conformational states (for instance, consideration of the binding sites for C1q in the Cμ2 domains of IgM) and were mathematically correlated to the target surface curvature (60). The cross-sectional diameter of the planar IgM molecule in solution is 35–38 nm (61); consequently, the role of curvature becomes more significant with particles of dimensions similar to those of IgM. The degree of strain, which is a measure of the bending of the Cμ1 module of IgM and variable domains relative to the Fc_5 disk, grows rapidly for particles with diameters on the order of 50 nm (60). Therefore, for 50-nm particles, the observed responses in complement activation may be explained on the basis of variations in the affinity of IgM antibodies to dextran, with only some donor sera presenting antibodies with sufficiently high affinity to allow surface binding. For high-affinity interactions between paratopes (antigen-binding sites of an antibody) and epitopes, it seems possible to geometrically strain the IgM molecule more than would be possible for weaker interactions.

The central step in complement activation is the cleavage of the complement protein C3, which generates opsonic fragments C3b and iC3b. In accordance with crystallographic analyses of C3b

structure (62), each surface-bound C3b molecule covers an area of approximately 40 nm^2 on a nanoparticle surface. Accordingly, with small particles, the bulk of activated C3 molecules will be released into the surrounding medium rather than deposited on the particle surface. Assessment of a surface protein map in isolation may give the false impression that a smaller particle, in general, may be a poor activator of the complement when compared with a larger counterpart and when equivalent surface area is considered. Surface and morphological characteristics also play a vital role that is best illustrated in the case of spherical micelles, which not only are dynamic structures (in which there is equilibrium between monomers and micelle architecture) but also are typically below 50 nm in size (1). Some micellar species (as in PEG-phospholipid micelles) do not activate complement efficiently, whereas others [structures based on polyethylene oxide (PEO)–polypropylene oxide (PPO) block copolymers] activate complement through all three known pathways (35, 63, 64).

The role of size becomes intriguing with particles that express hydrodynamic radii smaller than that of opsonic proteins such as immunoglobulins. However, depending on surface characteristics, significant protein adsorption can still occur, as in the case of quantum dots of 5 nm in size, in which particle sizes increase two- to threefold when exposed to plasma proteins (29). Protein-binding patterns per se, however, are less likely to provide rational explanations with regard to nanoparticle PK unless they are put in the context of defined biological functionality and performance. For example, with the use of knockout models, Simberg and colleagues were able to investigate and eliminate the role of complement C3, kininogen, fibronectin, fibrinogen, mannose-binding lectin, and histidine-rich glycoprotein in the clearance kinetics of intravenously injected iron oxide nanoparticles (65).

The role of surface chemistry in protein deposition is receiving ongoing attention, but the bulk of such studies (23, 28, 33, 36, 37, 66–69) still suffers from a lack of detailed material characterization and experiments in plasma or serum (and often at low concentrations) compared with those of whole blood. This limits data interpretation and its relevance to biological performance. For example, one study compared the role of human plasma protein binding in native, amine-functionalized and carboxyl-functionalized model polystyrene nanoparticles of 50 nm and 100 nm in size (28). Although quantitative and qualitative differences in protein binding among these particles were noted, no analysis for functional-group density, clustering, or spacing was offered (28). Such analysis could have played a significant role in modulating differential protein binding and initiation of blood cascade events such as coagulation and complement through different pathways and with different consequences. How a 50-nm particle can geometrically accommodate a wide spectrum of plasma proteins of different sizes and shapes (immunoglobulins, apolipoproteins, complement system proteins, acute-phase proteins, coagulation factors, serum albumin) on its surface at the same time remains puzzling. This may be a consequence of the surface heterogeneity in a typical population of nanoparticles (and may also reflect dynamic protein adsorption-desorption processes), in which some subpopulations show affinity for specific sets of proteins but the methodologies used are capable of reporting only the bulk effect. Similarly, problems may arise from some degree of protein-mediated particle aggregation.

Other observations arising from nanoparticle incubation with plasma or serum are adsorptions of various apolipoproteins of HDL, LDL, and very-low-density lipoprotein (VLDL) origin, for which adsorbed quantities vary with the particle's physical characteristics (21, 23, 26–28, 32, 35, 46, 54, 65, 67, 70–72). Apolipoprotein adsorption has been reported with the majority of nanoparticles tested to date, whether they are macrophage prone or macrophage resistant. However, this apolipoprotein adsorption may be an artifact generated from separation procedures used to concentrate and separate nanoparticles from serum or plasma, during which contamination with lipoproteins could occur (54). Nevertheless, the biological significance of apolipoprotein

deposition is by no means clear and presumably depends on the apolipoprotein's conformation and exposure of functional determinants that may serve as ligands for lipoprotein and scavenger receptors expressed, for example, by macrophages, hepatocytes, and vascular endothelial cells (55, 73). Indeed, deposition of apolipoprotein E on some polymeric nanoparticles has been implicated in particle recognition and uptake by brain capillary endothelial cells (26, 27), but not all apolipoprotein E–decorated particles are prone to recognition by these cells. Another observation is the ability of apolipoprotein E to enhance egg phosphatidylcholine liposome uptake by hepatocytes, whereas other apolipoproteins suppressed uptake by displacing apolipoprotein E (a dysopsonic effect) (72). Yet another example is apolipoprotein binding to poly-L-lactic acid nanoparticles, which had no effect on particle uptake by human monocytes (74). Recent reports also attest to the importance of lipoproteins in the body's defenses and in particular with respect to the complement system (64, 75, 76). Apolipoproteins AI and AII are inhibitors of C9 polymerization; this inhibition can minimize the generation of complement lytic complexes (C5b–C9) and their depositions on vascular endothelial cells (77). One intriguing speculation is that the deposition of apolipoproteins AI and AII may be a natural strategy by which nanoparticle-mediated complement-derived adverse effects are minimized. Indeed, natural particles such as HDL and LDL do not seem to activate complement, and their clearance is receptor specific.

Protein deposition may alter nanoparticle PK indirectly through interaction with erythrocytes and platelets in the circulation. The role of erythrocyte and platelet complement receptors in nanoparticle transport and organ deposition is discussed above (1, 46).

Surface Architecture

Surface camouflaging of nanospheres with PEG and with block copolymers such as poloxamine 908 (a tetrafunctional PEO-PPO ethylenediamine block copolymer) is believed to circumvent the body's defenses (notably against global macrophage recognition and clearance), thus conferring greater longevity to nanospheres in the blood (1, 3). Our own studies have demonstrated that poloxamine 908–coated polystyrene nanospheres of 220 nm in size incite complement activation in human serum regardless of PEO chain configuration. However, alteration of copolymer architecture on nanospheres from a flat to a mushroom-brush configuration switches activation from the C1q-dependent classical pathway to a lectin pathway (**Figure 3**) (34). Different alterations in adsorbed polymer configuration trigger alternative pathway activation differently and, in one case, directly through the action of properdin (34), which serves as a pattern-recognition molecule and actively induces complement convertase formation (78). These observations are explainable on the basis of dynamic poloxamine 908 structure; the molecule has repetitive recognition patterns of relative polarity and hydrophobicity, and the patterns can change with changes in the density of attachment of the copolymers to the surface (34, 79). Consequently, this creates new sites for complement recognition (and perhaps other proteins) and shifts the activation pathway from one pathway of complement to another. The effect of particle curvature on combined poloxamine conformation and complement activation has not been investigated, but surface curvature and other characteristics such as surface hydrophobicity are expected to modulate PPO and PEO chain configuration (1, 79). Therefore, different patterns of complement activation may be expected. Another observation is that, in spite of fixing complement opsonic components, within the first hour of intravenous injection the majority of poloxamine 908–coated 220-nm nanospheres with PEO chains in a mushroom-brush configuration remain resistant to ingestion by the hepatic Kupffer cells (34). This strongly indicates that surface-projected PEO chains sterically prevent the binding of bound complement opsonins (e.g., C3b and iC3b) to the corresponding macrophage receptors. This possibility was studied earlier with PEG-grafted, long-circulating liposomes, which are also

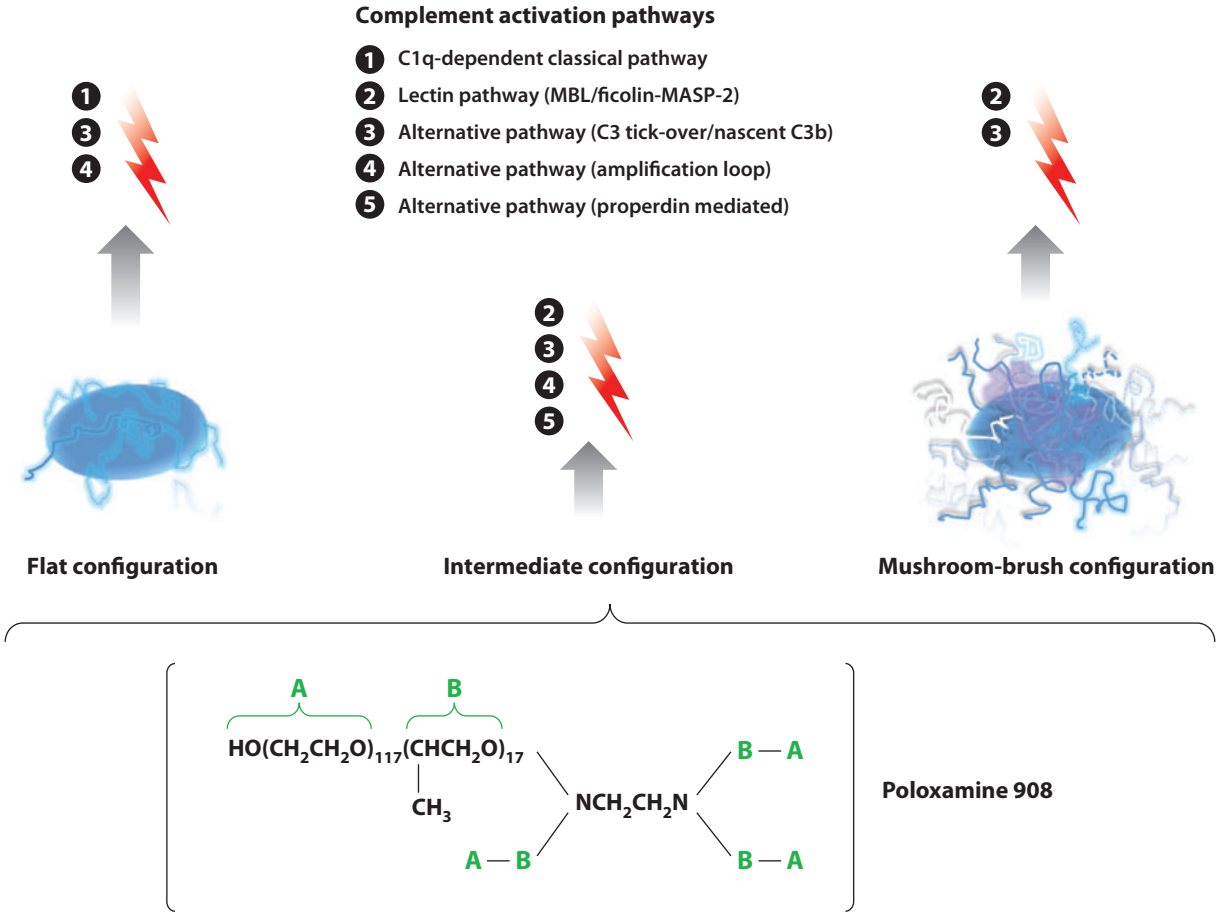

Figure 3

Conformational alteration of adsorbed poloxamine 908 on nanoparticle surfaces switches complement activation differently. Nanoparticles displaying surface polyethylene oxide (PEO) chains of the bound poloxamine 908 in flat configuration trigger complement through the classical pathway, which is mediated by the binding of the C1q protein. These particles also incite complement through the alternative pathway. When PEO configuration is changed to mushroom-brush or an intermediary conformation between flat and mushroom-brush (known as mushroom), complement activation switches from the C1q-mediated classical pathway to the lectin pathway. The latter is triggered through binding of either MBL (mannose-binding lectin) or ficolins, resulting in activation of MASP-2 (mannose-binding lectin associated serine protease-2). These alterations in PEO chain configuration also trigger the alternative pathway differently. With particles displaying the intermediate PEO configuration, alternative pathway activation further proceeds through binding of the pattern-recognition molecule properdin.

capable of triggering complement through both classical and alternative pathways (63). These are clear examples in which surface opsonization processes do not correlate with PK profiles and macrophage sequestration, and the examples thus confirm the dynamic physical, chemical, and biological interplays among surface components.

Macrophage Heterogeneity

The binding of multiple opsonic proteins and/or dynamic changes in surface opsonization processes may have important implications in nanoparticle clearance by macrophages when viewed

from the point of macrophage heterogeneity (55). Macrophages are heterogeneous in phenotype and function, even within the same organ (73, 80). Therefore, dynamic opsonization processes could indicate the presence of a recognition hierarchy, wherein a specific macrophage receptor (or population) might recognize the earliest surface changes and other receptors (or other subpopulations) might engage later. This might be demonstrated with appropriate knockout models. Such processes may ensure complete sequestration of nanoparticles from the systemic circulation by the host defense system. In line with this suggestion, Sou et al. (81, 82) recently described species differences in macrophage deposition of intravenously injected liposomes containing an anionic lipid, succinic acid. Vesicular uptake was predominantly localized to the bone marrow macrophages in rhesus monkey, rabbit, and hamster, but not in rats or mice, in which both splenic and hepatic macrophage depositions were significant. This species-dependent organ specificity of liposome sequestration resembles the species specificity of chylomicron clearance (83). It is not clear whether these observations are related to different patterns of protein binding or are directly related to differences in macrophage recognition of inherent liposomal determinants such as succinic acid.

GEOMETRIC FACTORS (SIZE VERSUS SHAPE) VERSUS BIOLOGICAL BARRIERS

Geometric parameters play a significant role in nanoparticle PK—in flow properties (margination dynamics), vascular adhesion, cellular internalization, and escape routes from vasculature (16). Most studies have examined flow properties, clearance kinetics, and escape routes from the systemic circulation in relation to spherical particles of different dimensions; only in the past decade has attention been focused on shapes other than spheres, but studies have been limited (16–19, 84–88).

Margination Dynamics and Vascular Targeting

Platelets and leukocytes are able to drift laterally in blood vessels, move in close proximity to vessel walls, and leave larger vessels in favor of smaller capillaries (89, 90). If particulate drug carriers were to exhibit similar margination dynamics (lateral drifting from the vessel core to the walls), the probability of vascular targeting would be increased significantly, particularly in small vessels and to entities decorated with a plethora of endothelial cell–specific antibodies and peptide-based and nucleic acid (aptamer)–based ligands (91). Margination dynamics could further enhance particle escape from the circulation at points where the integrity of the endothelial barrier is perturbed by inflammatory processes or by dysregulated angiogenesis (e.g., solid tumors) (1, 3). The flow of red blood cells toward the center of blood vessels has been suggested to induce plasma skimming, resulting in the formation of a cell-free plasma layer in the proximity of a vessel wall (92). The thickness of the plasma layer depends on the vessel diameter as well as the mean blood velocity and varies from a few micrometers in capillaries to tens of micrometers in arterioles (93). Mathematical modeling and experiments using in vitro fluidic systems (e.g., parallel-plate flow chambers) suggest that in a linear laminar flow, neutrally buoyant micrometer-sized spherical particles move to an equilibrium distance from the vessel wall with no lateral drift unless an external force is applied (94). The trajectories for nonspherical particles of similar dimensions are more complex; ellipsoidal particles tumble and role downstream, drifting laterally from one side of the vessel to another, and the lateral drifting velocity is directly related to the particle aspect ratio. Calculations further suggest that discoidal particles marginate far more than spherical particles, irrespective of size and density, and this may enhance targeting. Decuzzi & Ferrari as well as others (84–86, 88, 94) have further modeled and calculated the adhesive strength and adhesion

probability of particles of different sizes and shapes under linear flow conditions. However, these in vitro systems are an oversimplification of in vivo vasculature architecture and do not consider characteristics such as convolutions and bifurcations. Some of these limitations may be overcome with a novel microfluidic platform: synthetic microvascular networks. These networks simulate the vascular microenvironment by including scale, morphology, and fluidics (17–19). These systems are further amenable to protein coating or endothelial cell deposition to mimic the vessel wall in vivo, but caution should be used in the interpretation of these data because procedures used for device surface modifications may subsequently affect deposited endothelial cell morphology, phenotype, cell-cell contact, and biochemical responses. Recent studies with such networks have concentrated on examining flow and adhesion characteristics of rigid particles that have variable shapes and dimensions greater than 1 μm, which may be relevant to targeting of lung vasculature (31). Bifurcations introduced in such devices have shown significantly different flow and adhesion behaviors for rigid particles of different shapes and aspect ratios. Indeed, in parallel with in vitro fluidic attempts, in vivo studies now suggest that targeting of intracellular adhesion molecule 1 (ICAM-1) in lung vasculature can be modulated by the sizes and shapes of particles decorated with anti-ICAM-1 antibodies (31). Antibody-decorated discoidal particles with dimensions of $0.1 \times 1 \times 3$ μm remain marginally longer in murine circulation compared with 100-nm-diameter spherical particles and exhibit specific endothelial targeting, but submicrometer-sized spheres also show rapid and specific pulmonary targeting. In contrast to spherical particles, the higher surface-to-volume ratio of micrometer-sized discoidal particles may diminish dislodging forces acting on endothelium-anchored particles in the bloodstream, ensuring firm attachment (31).

An important aspect of such studies is understanding how well the surface chemistry of the particles is controlled when different shapes are obtained during synthesis. For example, surface-active molecules used in particle preparation may adhere to the particle surface in an unpredicted manner. It is important to understand the preparation processes in detail and to characterize the particles because even small changes in particle surface chemistry can lead to erroneous conclusions about the effects of particle shape and morphology on flow and target-binding properties. It is unlikely that submicrometer-sized particles will exhibit margination dynamics similar to that of platelets and leukocytes, which are substantially larger and more irregular in shape. Rather, heterogeneity in flow properties may be expected (arising from the small size) regardless of the shape factor. Further technological refinements with synthetic microvascular networks devices are required to make them suitable for studying the flow and target-binding performance of drug carriers with dimensions of less than 200 nm and for studying other interesting nanocarrier systems such as cylindrical flexible entities (e.g., filomicelles) (95). Such developments may help dramatically improve the design and precision engineering of biodegradable carriers for targeted drug delivery to vascular endothelial cells and sites beyond vasculature.

Geometric Considerations in Glomerular Filtration

The capillary endothelium of the glomerular filter of the kidney possesses nondiaphragmed fenestrations of 50–100 nm in diameter that occupy approximately 20% of the glomerular endothelial surface. The capillaries are further lined by a continuous and thick (250–350 nm) basement membrane as well as processes of podocytes, giving rise to pedicels that interdigitate with primary processes. Podocytes also possess phagocytic function. The openings between the pedicels (filtration slits) are approximately 25 nm in width and are bridged by a 4-nm-thick diaphragm. These morphological characteristics would appear to be torturous for renal filtration of particulate drug carriers, but recent studies suggest that particle shape may also play a significant role in renal clearance (30, 96). For example, intravenously injected amino-functionalized single-walled

carbon nanotubes (SWCNTs) with average lengths of 200–300 nm undergo rapid renal clearance by glomerular filtration; this process involves partial tubular reabsorption and transient translocation into the proximal tubular cell nuclei but does not involve active secretion via specific transporters (96). These observations challenge the rules and limitations surrounding renal filtration of large entities with aspect ratios ranging from 100:1 to 500:1. To pass through the capillary wall during filtration, SWCNTs must be highly oriented, with the long axis directed toward the openings. In support of this notion, recent mathematical analysis suggests that the ratio between the rate of strain in the fluid and the rotational diffusivity of SWCNTs is sufficiently large, and this results in a strong tendency for flow-induced orientation that overcomes thermal-motion-controlled random orientation (96). Generalizing such a mechanism to other entities with dimensions similar to those of SWCNTs (e.g., quantum rods, cylindrical micelles) is difficult because parameters such as the extent of rigidity/deformability and surface chemistry can still play a significant role in renal filtration. For example, cylindrically shaped micelles with lengths as long as 8 μm persist in mouse circulation up to one week after intravenous injection (95). Cationic generation 3 poly(amidoamine) Starburst dendrimers accumulate in kidneys, presumably as a result of electrostatic interaction with the negatively charged capillary endothelium of the glomerular filter and the outer surface of the podocytes, including the filtration slits (97). Other investigators have used spherical quantum dots of precise size series to define the renal filtration threshold in rodents, showing that a final hydrodynamic diameter of $\leq$5.5 nm resulted in rapid and efficient urinary excretion and elimination of quantum dots from the body (29). Although these observations are collectively interesting and challenge conventional physiological beliefs, clinical applications of nonbiodegradable and highly toxic carbon nanotubes and quantum dots still remain questionable, in spite of their remarkable physicochemical characteristics and rapid renal excretion.

Geometric Aspects of Particle Duration in the Circulation

It has long been known that surface camouflaging of nanoparticles with materials such as PEGs, various block copolymers, and hyaluronic acid can dramatically suppress Kupffer cell clearance and result in long half-lives of nanoparticles in the circulation (1, 3, 6, 7, 35, 54, 98). By virtue of prolonged circulation in the blood, engineered nanoparticles may reach accessible pathological sites (e.g., solid tumors) or ultimately target vascular elements (such as those of tumors) by conjugating targeting ligands to the distal end of surface-projected polymers (1, 3, 98). However, the duration of particle circulation in the blood is both size and shape dependent. The safe limit with nondeformable polymer-decorated spherical particles is 150 nm; larger particles are highly prone to filtration at interendothelial cell slits of venous sinuses in the spleen, whose width is approximately 200–250 nm (1, 3, 24), providing opportunities for targeting the splenic red-pulp regions, which are highly rich in macrophages (99). In contrast to long-circulating liposomes and polymeric nanospheres, surface camouflaging of rod-shaped objects such as SWCNTs with the aforementioned camouflaging polymers, either by passive adsorption or covalent functionalization, has not conferred dramatic increases in these objects' longevity in blood (100–102). This could result from nonuniform coating arising from inherent nanotube surface defects or inhomogeneity, or from a gradual buildup of the initially deposited polymers' steric repulsion to further polymer grafting or adsorption (38, 39).

Remarkable observations have been reported with soft-matter long filomicelles (2–18 μm) that exhibit prolonged circulation half-lives, often on the order of days (95). Hydrodynamic shears tend to stretch and flow-align the cylindrical filomicelles in most blood vessels and in the splenic red-pulp and marginal zones, where they easily pass through the cell slits (99). This alignment also minimizes filomicelle interactions with phagocytes by pulling off the filomicelles as they come

into contact with the phagocytes. However, following cell contact, a nanofragment of a filomicelle might break off and be taken up by macrophages in the liver and the spleen. This concept is supported by (*a*) in vivo data from filomicelle scission (at an initial shrinkage rate of ~1 μm per day) and from filomicelles' gradual buildup in macrophages and by (*b*) in vitro experiments in a flow chamber with immobilized phagocytes (95). Paclitaxel-loaded filomicelles induce apoptosis and rapidly shrink growing tumor xenografts in nude mice (95).

Particle Escape from Leaky Vasculature (Paradigms in Tumor Targeting)

Morphological investigations attest to the hyperpermeability of solid tumors to macromolecules and therapeutic nanoparticles in general, with highly heterogeneous pore cut-off sizes that range from 200 nm to 1200 nm (22). However, marked variability in endothelial permeability has been documented in different tumor types, different vessels within the same tumor, and during tumor growth, regression, and relapse (103). Pore frequency and cut-off size in tumors may be controlled by microenvironmental factors such as sex hormones and growth factors and may increase with the histological grade and malignant potential of tumors (22, 103). Experimentally, pore cut-off size can be reduced when tumors are grown in the murine cranium (22). Testosterone withdrawal in androgen-dependent mouse mammary tumors also reduces pore cut-off size from 200 nm to <10 nm within 2 days (22).

Although the exploitation of tumor vascular abnormalities for targeted drug delivery and the performance of imaging using a plethora of long-circulating nanoparticles of different dimensions and shapes have been well documented (1–9, 104, 105), serious limitations remain. Nanoparticle-based therapeutic approaches are limited to settings that precede hormone ablation therapies or other clinical interventions that may reduce transvascular transport. The chaotic blood flow in the tumor vasculature is another barrier contributing to a decrease in nanoparticle extravasation from the blood into the tumor interstitium. Already compromised by abnormal hydrostatic pressure gradients, compressive mechanical forces generated by tumor cell proliferation cause intratumoral vessels to compress and collapse (3). Also, the combination of higher-than-expected tumor interstitial pressure (which is partly due to a lack of functional lymphatic drainage) with lower intravascular pressure can seriously limit nanoparticle penetration into the tumor core (3, 103). The interstitial pressure is usually higher at the center and diminishes toward the periphery, creating a mass-flow movement of fluid away from the central region. Distribution, architectural organization, and relative levels of extracellular elements such as collagen, decorin, and other basement membrane proteins may further impede diffusion of extravasated nanoparticles. Thus, simply enhancing the circulation profiles of nanoparticles, whether by surface camouflaging or shape alteration, may not necessarily lead to an increase in tumor targeting and therapeutic efficacy. However, the effect of shape on tumor penetration of nanoparticles has not received much attention. Some of the aforementioned intratumoral barriers are not fully developed in micrometastases or in well-perfused and low-pressure regions in larger tumors; therefore, nanoparticles may provide excellent opportunities for therapeutic intervention of micrometastases and well-perfused/low-pressure regions of larger solid tumors. Attempts to modulate vascular function, such as mediation of hypertension through angiotensin II and/or vasodilation through the application of nitric oxide–releasing drugs, might further augment the enhanced permeability and retention effects in solid tumors and aid in nanoparticle extravasation (105).

Within the tumor interstitium, nanoparticulate carriers must be capable of releasing their entrapped cargo at a rate that maintains unbound drug concentrations in the therapeutic range. Fortunately, many design approaches—such as pH sensitivity, temperature sensitivity, or enzyme sensitivity of carrier components—afford controlled drug release from extravasated carriers; this

topic has been reviewed in depth elsewhere (11, 106). Moreover, various therapeutic nanoparticle engineering approaches exploit tumor cell receptor–mediated internalization processes; this strategy is expected to bypass tumor cell multidrug-efflux pumps through intracellular drug release (12). These approaches are based on the surface decoration of long-circulating carriers with ligands such as monoclonal antibodies and nanobodies (e.g., against HER-2 antigen), folate, transferrin, vasoactive intestinal peptide, and sigma-1 selective substrates. Finally, the tumor's vasculature is also its Achilles' heel; nutritionally, tumor cells are highly dependent on a functional vasculature for survival (107). Numerous advanced attempts have been made to actively target long-circulating carriers to tumor vascular endothelial cells and initiate their destruction (e.g., through drug delivery, siRNA delivery, and apoptotic gene delivery, all of which involve transcriptional targeting with killer genes such as truncated Bid). Here, particle shape may be an important factor in optimizing endothelial targeting and internalization processes.

Particle Geometry and Cellular Internalization

The effect of particle shape on cellular internalization processes is an intriguing concept because many pathogenic microorganisms such as the rod-shaped, gram-negative *Salmonella* and *Shigella* can induce their entry into nonphagocytic mammalian cells (108). Understanding these events can pave the way for the design of better nanosystems for vascular and nonmacrophage cell targeting (109, 110). Although the processes of microbial-host cell interactions are complex and involve multiple pairs of surface-recognition molecules and receptors, Gratton and colleagues (109) recently examined the effect of particle shape on nonspecific internalization by nonphagocytic cells. Their approach employed a top-down particle fabrication technique (known as particle replication in nonwetting templates) capable of generating uniform populations of nanoparticles with excellent control of size, shape, and surface chemistry. Their findings suggested that the HeLa cells could readily internalize cationic nonspherical particles (cubes and cylindrical rods) with dimensions as large as 2–3 μm through different cellular internalization pathways. The intriguing observation, however, was that the internalization of rod-shaped, high-aspect-ratio nanoparticles (diameter of 150 nm and length of 450 nm) occurred more readily and efficiently compared with the internalization of more symmetrical, low-aspect-ratio particles that had similar volumes. This was attributed to the multivalent cationic interactions with cells that are available with the higher-aspect-ratio particles because of the larger surface areas in contact with the cell membrane; however, with identically shaped negatively charged nanoparticles, no significant uptake was registered (109). Conversely, cationic rod-like nanoparticles having the same aspect ratio and smaller dimensions did not increase the number of particles internalized by the cells. Chithrani et al. (111) also examined the effect of particle design on cell uptake and reported that gold nanorods with large aspect ratios are internalized in significantly smaller proportions compared with spherical particles that have equivalent size characteristics. These studies highlight the importance of the interplay between a particle's shape and size and its defined surface chemistry for nanoparticle targeting. Further refinements and extensive studies are needed to establish the role of surface-grafted ligand conformation and density and the role of subsequent plasma protein adsorption modifications in the context of in vivo firm target cell adhesion, internalization, and intracellular processing of nonspherical drug carriers.

BIOAVAILABILITY AND REPEATED DOSING

The vast majority of studies with advanced and functional nanoparticulate systems such as carbon nanotubes, nanorods, DNA and polymer cages, and silica-based particles have not examined

drug bioavailability, volume of distribution, and many other PK parameters in relation to disease processes or after repeated administration (112). Conventional drug carriers such as liposomes, micelles, and certain polymeric nanoparticles are at the forefront of drug delivery and therapeutic research and will be for years to come because a great wealth of preclinical and clinical information is available with such systems (1–3, 7, 12, 106). Although these systems have many advantages from both manufacturing and clinical perspectives, some outstanding issues should be resolved within a therapeutic context. For example, there is still controversy as to whether approved liposomal formulations of doxorubicin exhibit efficacy equivalent to that of standard formulations of doxorubicin (7, 113). However, as a result of altered PK parameters, the frequency of doxorubicin-induced cardiotoxicity and neutropenia is lower with the liposomal formulation than with the standard formulations (113). Detailed studies with liposomes have further indicated that the extent of actively loaded doxorubicin release can be controlled through alteration of the fluidity of the liposomal bilayer. This alteration occurs when the fatty acyl chain length and/or degree of saturation of the phospholipid component is changed, resulting in a different therapeutic outcome (114). Liposomes with the fastest release rates produced the lowest tumor response, whereas those with the slowest release rates had the best therapeutic activity. Remarkably, formulations with intermediate release rates produced unexpected cardiotoxicity. Because the quantity of liposomal lipid accumulation in the tumor was comparable with all tested formulations, these studies indicate the importance of drug-leakage rates in determining benefit-to-risk ratio (114). Additionally, the loading methods of the drug can exert a significant impact on drug release profiles from liposomes even at the level of the buffer systems that are used, due to counter-ion effects.

Studies of the effect of dosing schedule and dose intensities with long-circulating (PEGylated) doxorubicin-entrapped liposomes have shown that the plasma PK of doxorubicin during each injection cycle was independent of the PK at the next cycle, at intervals between dosing of either 1, 2, or 4 weeks (114). Contrary to these observations, Ishida et al. (115) and Wang et al. (116) demonstrated that intravenous injection of a dose of drug-free PEGylated liposomes in rats elicits an IgM response in a T-cell-independent manner that could initiate the hepatic clearance of the second liposome dose through the mediation of a heat-labile opsonic factor. Notably, no IgM responses were observed when liposomes were loaded with doxorubicin, and the second PEGylated liposomal dose exhibited long residency in the systemic circulation. These altered responses likely result from doxorubicin-mediated macrophage death and inhibition of B-cell proliferation and/or the killing of proliferated B cells. Therefore, altered drug carrier PK may or may not occur after repeated injections, depending on the nature of the encapsulated cargo.

As a result of their extended time in the circulation, stealth systems (particulate drug carriers that are resistant to rapid clearance by macrophages of the reticuloendothelial system following intravenous injection and exhibit circulatory half-lives on the order of hours) with encapsulated therapeutic agents may induce new toxicity profiles. For instance, the most notable dose-limiting toxicity associated with the continuous and repeated infusion of approved PEGylated doxorubicin-entrapped liposomes is palmar-plantar erythrodysesthesia (PPE), which arises from liposome accumulation in skin capillaries and subsequent doxorubicin leakage (117). The incidence of PPE is greatest in patients receiving liposomal formulations (doxorubicin equivalent) >10–12 mg m^{-2} per week, but it can be lowered through reduction of the dose intensity or extension of the interval between subsequent dosing. Studies in mice have shown that the skin PK of one injection cycle of these liposomes is independent of the PK of the next injection cycle when the dose interval is 4 weeks, and there is little evidence of PPE symptoms (114). Although such adverse reactions can be minimized by altering dose intensity or infusion interval, these observations highlight the importance of design considerations in attaining appropriate half-lives for therapeutic nanoparticles in the systemic circulation in relation to the desired pathology. Therefore, exceedingly long

circulation profiles for nanoparticles (as those noted with filomicelles) may not necessarily improve the benefit-to-risk ratio in certain clinical settings. Similarly, short half-lives may exert detrimental effects owing to rapid elimination of nanoparticles by macrophages of the reticuloendothelial system. In cancer chemotherapy with nanoparticles, this translates to Kupffer cell destruction, thus increasing the risk of bacteremia during the period of macrophage deficiency (1).

CONCLUSIONS

It is now clear that intravenously injected nanoparticulate drug carriers provide a range of unique opportunities to increase the targeting of therapeutic agents to different sites, and promising findings have resulted from trials at the clinical level. However, levels of sophistication in design and nanoengineering may generate more heterogeneous populations of nanoparticles; consequently, this may cause difficulties in the interpretation of biological events following their administration. Definitive maps that establish the interdependency of size, shape, and surface characteristics of nanoparticles in relation to biodistribution, controlled drug release, excretion, and adverse effects are still necessary, but design considerations must be kept as simple as possible. The composite nature of many particulate drug carriers makes such studies cumbersome and difficult, but this may be overcome by improving nanoparticle characterization techniques, introducing methodologies that generate precisely defined nanoparticles, and offering the ability to independently alter one variable at a time. The protein adsorption phenomenon must be viewed carefully and in relation to nanoparticle stability and defined biological performance, with minimum extrapolation of results from one system to another. Finally, recent developments in nanofluidics, microfluidics, and related two- and three-dimensional platforms that are based on micropatterning on conducting polymers may contribute to patient-centric translational research by allowing individual processing of heterogeneous populations of cells (e.g., endothelial cells, tumor cells, and macrophages) and allowing the study of their interaction with nanoparticles in relation to sequential alteration of particulate geometry and surface characteristics under shear blood flow conditions. These approaches may further be extrapolated to nanoparticle-mediated cell-function modulation and selection in order to implement modern cell-based therapies (e.g., therapies based on immune and stem cells).

SUMMARY POINTS

1. Intravenously injected nanoparticulate drug carriers provide a wide range of unique opportunities for site-specific targeting of therapeutic agents to many areas within the vasculature and beyond. Cancer nanomedicines have shown the success of this approach. This progress continues in other therapeutic areas such as inflammatory conditions.
2. PK and biodistribution of nanocarriers are controlled by a complex array of interrelated core and interfacial physicochemical and biological factors. Size, morphology, and surface characteristics of nanocarriers can be tuned to afford controlled PK and biological targeting according to the type, developmental stage, and location of the disease.
3. The plasma protein coat remains a key determinant in controlling nanocarrier PK and secondary responses, but not all deposited proteins contribute a role. The composition of the plasma protein coat is expected to differ considerably among nanoparticles in amount and heterogeneity, and in a time-dependent manner, on the basis of the physicochemical properties of the nanoparticles as well as the pathological state and the dosing regimen.

4. Geometric parameters play a significant role in nanoparticle PK—in flow properties (margination dynamics), vascular adhesion, cellular internalization, and escape routes from vasculature—but the bulk of current data is available for spherical nanoparticles.
5. As a result of altered PK, stealth systems with encapsulated therapeutic agents may induce new toxicity profiles.

FUTURE ISSUES

1. Breaching the biological barriers may induce diseases. Should we breach biological barriers with nanoparticles? Alternatively, should we concentrate on exploiting biological opportunities offered by the disease states for rational nanocarrier engineering and targeted drug delivery?
2. Sophistication in design and nanoengineering techniques may result in the generation of more heterogeneous populations of nanoparticles. These developments may cause more difficulties in interpretation of biological events following their administration. Can we develop technologies that yield more homogeneous populations of complex nanocarriers in terms of surface chemistry, architecture, and morphology?
3. Can we generate definitive maps that establish the interdependency of size, shape, and surface characteristics of nanoparticles in relation to biodistribution, controlled drug release, excretion, and adverse effects?
4. To what extent is the composition/conformational state of the outer surface proteins regulated by the type and the mode of deposition of the primary protein scaffold? Can appropriate methodologies be developed to control these factors?
5. Can carbon nanotubes, quantum dots, and many other nonbiodegradable nanoparticles/nanomedicines reach their clinical targets? Or should future efforts concentrate on the refinement of proven biodegradable nanocarriers?
6. How important are the physical and mechanical properties of nanoparticles and nanoassemblies with respect to target binding and therapeutic performance?
7. A detailed understanding of dynamic behavior and interactive forces between therapeutic agents (and particularly macromolecular cargo) and nanocarriers is still required and remains central for optimization strategies.

DISCLOSURE STATEMENT

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