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reviews

Factors Affecting the Clearance and Biodistribution of Polymeric Nanoparticles

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Abstract: Nanoparticle (NP) drug delivery systems (5−250 nm) have the potential to improve current disease therapies because of their ability to overcome multiple biological barriers and releasing a therapeutic load in the optimal dosage range. Rapid clearance of circulating nanoparticles during systemic delivery is a critical issue for these systems and has made it necessary to understand the factors affecting particle biodistribution and blood circulation half-life. In this review, we discuss the factors which can influence nanoparticle blood residence time and organ specific accumulation. These factors include interactions with biological barriers and tunable nanoparticle parameters, such as composition, size, core properties, surface modifications (pegylation and surface charge), and finally, targeting ligand functionalization. All these factors have been shown to substantially affect the biodistribution and blood circulation half-life of circulating nanoparticles by reducing the level of nonspecific uptake, delaying opsonization, and increasing the extent of tissue specific accumulation.

Keywords: Biodistribution; circulation half-life; polymeric nanoparticles

Introduction

Nanoparticles have drawn increasing interest from every branch of medicine[1–5] for their ability to deliver drugs in the optimum dosage range, often resulting in increased therapeutic efficacy of the drug, weakened side effects,[6,7] and improved patient compliance. Today, there are several examples of nontargeted NPs currently used in clinical practice (Doxil[8–11] and Daunoxome[12]) and in clinical development (Cyclosert).[13] Early success of these lipid-based vesicular drug delivery nanoparticles has led to the investigation and development of many different compositions of polymeric nanoparticles, including polymeric micelles, dendrimers, drug conjugates, and polypeptide- and polysaccharide-based nanoparticles. Among these, Genexol-PM [methoxy-PEG-poly(D,L-lactide)Taxol] is the first polymeric micellar nanoparticle in phase II clinical trials in the United States.[14,15] Generally, clinical success correlates well with pharmacological and toxicological parameters. Blood circulation residence, maximal tolerated dose (MTD), and selectivity are the most important factors for achieving a high therapeutical index and corresponding clinical success. Polymeric nanoparticles are defined by their morphology and polymer composition in the core and corona (Figure 1). The therapeutic load is typically conjugated to the surface of the

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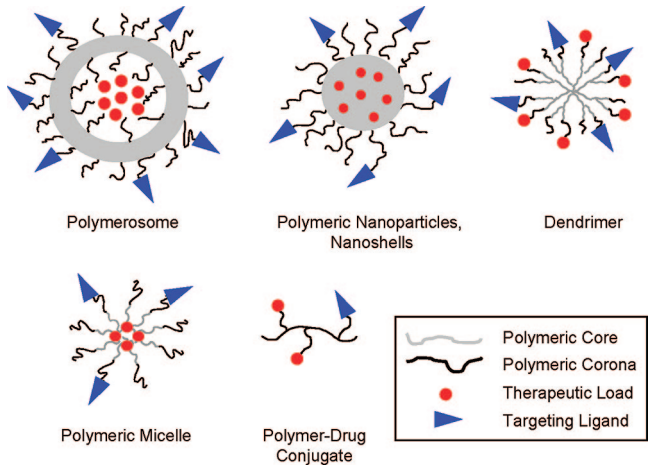

Figure 1. Nanoparticle platforms for drug delivery. Polymeric nanoparticle platforms are characterized by their physicochemical structures, including polymerosome, solid polymeric nanoparticle, nanoshell, dendrimer, polymeric micelle, and polymer−drug conjugates.

nanoparticle, or encapsulated and protected inside the core. The delivery systems can be designed to provide either controlled release or a triggered release of the therapeutic molecule.[16,17] The nanoparticle surface can then be functionalized by various methods to form the corona. Surface functionalization can be utilized to increase residence time in the blood, reduce nonspecific distribution, and, in some cases, target tissues or specific cell surface antigens with a targeting ligand (peptide, aptamer, antibody/antibody fragment, small molecule). For instance, it is well established that hydrophilic polymers, most notably poly(ethylene glycol) (PEG), can be grafted, conjugated, or absorbed to the surface of nanoparticles to form the corona, which provides steric stabilization and confers "stealth" properties such as prevention of protein absorption.[18,19] Surface functionalization can address the major limiting factor for long-circulating nanoparticle systems, which is protein absorption. Proteins adsorbed on the surface of the nanoparticle promote opsonization, leading to aggregation and rapid clearance from the bloodstream.[20–23] The resultant rapid clearance is due to phagocytosis by the mononuclear phagocyte system (MPS) in the liver and splenic filtration. Typically, the majority of opsonized particles are cleared by a receptor-mediated mechanism in fewer than a few minutes due to the high concentration of phagocytic cells in the liver and spleen, or they are excreted.[21] Thus, over the past 20 years, numerous approaches to improving nanoparticle blood residence and accumulation in specific tissues for the treatment of disease have been developed. In this review, we will discuss the effects of physiological tissue defects (high permeability) and polymeric nanoparticle physicochemical properties on their biodistribution and clearance. Specifically, polymeric composition, nanoparticle size, pegylation, surface charge, and targeting functionality will be discussed.

Overcoming Biological Barriers: Effect of Physiological Defects

Numerous biological barriers exist to protect the human body from invasion by foreign particles. These barriers

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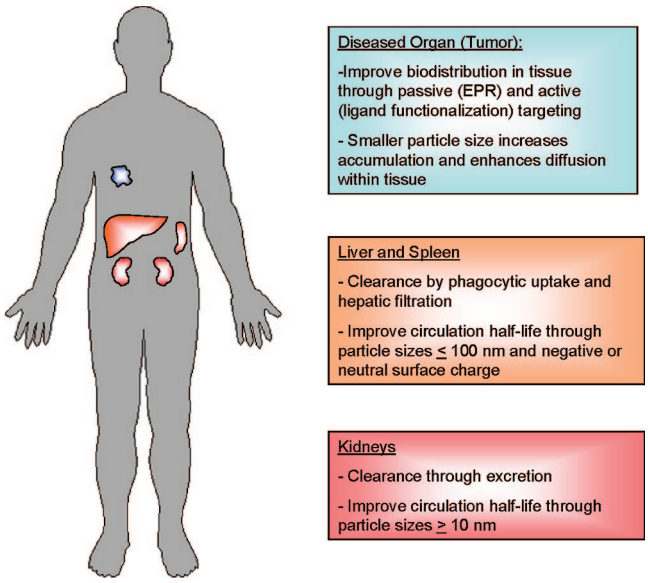

Figure 2. Biodistribution and clearance of polymeric nanoparticles. Tissue defects, stealth properties, targeting, and the size of the nanoparticles are major factors affecting the biodistribution and clearance of polymeric nanoparticles.

include cellular and humoral arms of the immune system as well as mucosal barriers among others. These barriers must be overcome in order for nanoparticles to reach their target (Figure 2). Due to their unique size, and amenability to surface functionalization to incorporate the desired characteristics, nanoparticles are particularly well suited to overcoming these barriers. This is especially true in the case of abnormal neovascularization. Blood vessels are responsible for delivering molecules, nutrients, and oxygen to organs throughout the body. Endothelia composing the blood vessels have been classified as continuous, fenestrated, or discontinuous, depending on the morphological features of the endothelium. The continuous endothelium morphology appears in arteries, vessels,[24,25] and the lungs.[26] In contrast, fenestrated endothelium[27] appears in glands,[28] digestive mucosa, and kidney. Fenestrae have an octagonal symmetry with radial fibrils interweaving in a central point forming pores of approximately 60 nm. Discontinuous endothelium is a characteristic of the liver (fenestrea of 50−100 nm)[29] and bone marrow. Endothelial cells from the blood vessels are able to respond to the physiological environment, resulting in angiogenic activity. Angiogenesis[30,31] is well-characterized for cancers[32] as well as ocular and inflammatory diseases,[33] with antiangiogenesis compounds commonly used for therapy.[34,35] Angiogenesis during tumor growth results in defective hypervasculature and a deficient lymphatic drainage system, which has given rise to the concept of passive targeting of nanoparticles to tumors through the "enhanced permeability and retention" (EPR) effect.[36,37] The EPR is a unique feature which allows macromolecules or drug delivery nanoparticles (cutoff size of >400 nm) to preferentially accumulate and diffuse in tumor tissues.[38] Long-circulating drug delivery nanoparticles are able to extravasate into tumor tissues, accumulate, and release the therapeutic drug locally in the extracellular area. Similarly, abnormal neovascularization or angiogenesis as well as enhanced vascular permeability are major causes of many ocular disorders, including age-related macular degeneration (AMD), retinopathy of prematurity (ROP), ischemic retinal vein occlusions, and diabetic retinopathy (DR), causing irreversible vision loss. In ocular disorders, the angiogenic process appears to be due to a stimulus response for retinal neovascularization. The stimulus can be tissue hypoxia, inflammatory cell infiltration, increased local concentration of cytokines (VEGF, PDGF, FGF_5 TNF, IGF etc.). The result

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is the formation of new vessels, which disrupt the organizational structure of the neural retina or break through the inner limiting membranes into the vitreous.[39] Many other disorders are also characterized by angiogenesis or vasculature defects such as obesity,[40] asthma,[41] diabetes,[42,43] and multiple sclerosis.[44,45] The development of new imaging systems and medical knowledge about physiological defects is leading to novel therapeutic approaches using nanoparticle drug delivery systems. It is now well accepted that nanoparticles are suitable for crossing biological barriers through tissue diffusion, extravasation, and escape from hepatic filtration.

Nanoparticle Properties

Composition. Historically, the administration of therapeutic agents has been limited by multiple factors, primary among these being low solubility, stability and rapid clearance. The result is a short circulation half-life and low efficacy, making frequent administration necessary. Additionally, there can be significant side effects in non-diseased tissues that adsorb therapeutic agent. These issues have led to the development of various targeting strategies aimed at increasing therapeutic index, including monoclonal antibodies and immunoconjugates. Some of the strategies are currently used in clinical practice and others are in clinical development.[46] In addition to these strategies, it has been shown that polymer−drug conjugates can substantially improve the blood residence time and weaken side effects. It is anticipated that engineered multifunctional nanoparticles can address issues with targeting as well as carry a more substantial drug payload. Many polymers have been investigated, including HPMA [*N*-(2-hydroxypropyl)methacrylamide], dextran, and polyglutamate. HPMA and polyglutamate drug conjugates represent ~35% of all the polymeric drug delivery systems in clinical development. The clinical success of AP5346, PK1, PK2, Xyotax, and CT-2106 seems to be due to their long circulation half-life, passive targeting ability, and, most importantly, lower toxicity allowing higher dosages (see Table 1). Thus far, the main impact of polymer conjugates has been to improve the pharmacokinetic parameters of drugs already in clinical use. These polymeric nanocarriers must be non-toxic, non-immunogenic, and carry sufficient amount of drug and release the drug at the optimal dose. The use of poly(ethylene glycol) (PEG) for nanoparticle surface functionalization has had led to very favorable results due to its intrinsic physicochemical properties but has had limited impact as a drug conjugate carrier due to low drug loadings. PEG polymers have low toxicity and no immunogenicity and are approved by the Food and Drug Administration (FDA) for clinical use. PEG−drug conjugates or nanoparticles functionalized with PEG chains have been described as long-circulating drug delivery systems with potential applications for systemic drug administration.[21,47] Poly(ethylene glycol) or poly(ethylene oxide) refers to an oligomer or polymer of ethylene oxide in linear or branched structures.[48] PEG is a hydrophilic polymer that can be adsorbed or covalently attached to the surface of nanoparticles. Hrkach et al. have demonstrated the formation of PLA−PEG nanoparticles in a core−corona structure with a solid core and anchored PEG chains on the surface. Their results showed for the first time that PEG chains, covalently attached to a particle surface, could exhibit flexibility similar to that of free PEG polymer dissolved in water. Furthermore, PEG has been shown to substantially reduce nonspecific interactions with proteins through its hydrophilicity and steric repulsion effects, reducing opsonization and complement activation.[49–55] The chain length, shape, and density of PEG on the particle surface have been shown to be the main parameters affecting nanoparticle surface hydrophilicity and phagocytosis. The mechanism involved in phagocytosis of opsonized nanoparticles is receptor-mediated by interaction of specific proteins absorbed on the surface of the nanoparticles with phagocytes.

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Table 1. Clinical Status of Polymeric Drug Delivery Nanoparticles under Development

composition (trade name)	status	particle size (nm)	circulation half-life	relative tissue accumulation of the drug	maximum tolerated dose (MTD)	efficacy
methoxy-PEG-poly (D,L-lactide)Taxol (Genexol-PM)	Phase II	30−60 nm	paclitaxel, 12 h (human)[61]	Genexol-PM vs Taxol: 2× more in liver, spleen, heart, and tumor (mice)[62]	390 mg/m^2 administered intravenously for 3 h every 3 weeks (human)[14]	75% of metastatic breast cancer patient showed 2 years overall survival[15]
HPMA-DACH palatinate (ProLindac, previously AP5346)	Phase II	6−15 nm	DACH-platinum, 70 h (human)[63]	NA[a]	640 mg/m^2 (initial cycle); repeated cycles of therapy were not assessed (human)[64]	NA[a]
PEG-arginine deaminase (Hepacid, previously ADI-SS PEG 20000 MW)	Phase I/II	NA[a]	arginine deaminase, 7 days (human)[65]	NA[a]	640 units/m^2 once a week (MTD > maximum feasible dose by intravenous (iv) administration)[66]	double median survival time of patients with metastatic melanoma; 47% response rate in HCC patients (n = 19)[66]
PEG-camptothecin (Prothecan)	Phase I/II	NA[a]	camptothecin, 40 h (human)[67]	camptothecin % ID/g of tissue 24 h postinjection: 3.7% in tumor, 4.41% in blood, 2.32% in liver (mice)[68]	7000 mg/m^2 administered in 1 h iv infusions every 3 weeks (human)[67]	NA[a]
Pluronic-doxorubicin (SP1049C)	Phase II	∼25 nm	doxorubicin, 3 h (human)[69]	enhanced AUC in tumor (50.8 vs 30.1) and brain (9.2 vs 5.6) compared with free doxorubicin (mice)[70]	70 mg/m^2 administered intravenously every 3 weeks for a maximum of six cycles (human)[69]	three patients over 21 showed responses to treatment[69]
polycyclodextrin camptothecin (IT-101)	Phase I	∼40 nm	camptothecin, 38 h (mice)[71]	camptothecin % ID/g of tissue: tumor = 1.3%; liver = 1.9% (24 h) (mice)[13,71]	NA[a]	preliminary data is reported to show stable disease rate in patients with solid tumors[13]
polyglutamate camptothecin (CT-2106)	Phase I/II	NA[a]	camptothecin, 44−63 h (human)[72]	NA[a]	25 mg/m^2 administered by iv infusion weekly every 3 of 4 weeks (human)[72]	less toxicity than free drug[72]
polyglutamate paclitaxel (Xyotax)	Phase III	NA[a]	paclitaxel, 100 h (human)[73]	paclitaxel % ID/g of tissue: tumor = 2.2%; spleen = 16%; liver = 8% (mice)[74]	for an iv administration, 233 mg/m^2 for patients on a two-dose weekly schedule and 177 mg/m^2 on a three-dose weekly schedule (human)[73]	response rate of 10% for 99 patients and a median time to disease progression of 2 months[75]
dextran-doxorubicin (AD-70)	Phase I	NA[a]	doxorubicin, 3−12 h (human)[76]	NA[a]	20 mg/m^2 (human)[76]	discontinued due to severe hepatotoxicity limiting the dose at 20 mg/m^2 [76]
dextran-camptothecin (DE-310)	Phase I/II	NA[a]	camptothecin, 300−400 h (human)[77]	NA[a]	9 mg/m^2 given once every 4 weeks (human)[77]	no new major toxicity compared to drug beside hepatotoxicity reported as reversible[77]
HPMA-paclitaxel (PNU166945)	Phase I	NA[a]	paclitaxel, 3−12 h (human)[78]	NA[a]	196 mg/m^2 (human)[78]	discontinued due to severe neurotoxicity[78]
HPMA-doxorubicin (PK1)	Phase II	NA[a]	doxorubicin, 93 h (human)[79]	NA[a]	320 mg/m^2 (human)[79]	hepatic toxicity at doses > 120 mg/m^2; two partial and two minor responses over 36 patients[79]
PEG-aspartic acid-doxorubicin (NK911)	Phase I	30−50 nm	doxorubicin, 1.6−4.7 h (human)[80]		67 mg/m^2, plasma clearance 400-fold higher than Doxil (human)[80]	no severe toxicity; Phase II clinical trial for pancreatic cancer[80]
HPMA-doxorubicin with galactosamine (PK2)	Phase I	∼8.4 nm	biphasic clearance with half-lives of 2.9 and 26.7 h when 120 mg/m^2 administered by 24 h infusion (human)[81]	enhanced accumulation in liver, hepatoma, and metastatic hepatoma compared with nontargeted HPMA-doxorubicin (human)[81]	160 mg/m^2 with administration by iv infusion over 1 h every 3 weeks (human)[81]	of 18 patients, three responded to treatment, with two in partial remission for >26 and >47 months[81]

[a] Not applicable.

Gref et al. were the first to report the advantages of pegylation on PLGA−PEG nanoparticles, resulting in a substantial increase in blood residence time. Apolipoproteins (A-IV and E) were found to be present on the surface of pure PLA nanoparticles in contrast to the PLA−PEG nanoparticles using gel electrophoresis analysis.[53] Although the assay did not provide a precise quantitative analysis, it was clearly shown that protein adsorption, particularly apolipoprotein J and complement protein C3, was substantially reduced on the surface of the PLA−PEG nanoparticles compared with pure PLA formulations. These results suggest that these specific apolipoproteins might also play a role in the process of opsonization of the particles. Gref et al.[53] have systematically studied the effect of PEG chain length in preventing protein adsorption on the surface of the nanoparticles. The results showed that an optimal molecular mass (M_w) range exists (between 2 and 5 kDa) in order to reduce plasma protein adsorption. The amount of protein absorbed on PLA−PEG 5 kDa was substantially reduced (∼80%) compared to the amount of nonpegylated PLA nanoparticles. PEG content as low as 0.5 wt % on the surface of the nanoparticles was able to significantly reduce the total amount of protein when compared to the nonpegylated PLA nanoparticles. The most significant reduction of protein absorption was found for pegylated particles (5 wt %). The effect of PEG on the surface of the nanoparticle in preventing protein absorption correlated with the polymorphonuclear leukocyte (PMN) and human monocyte (THP-1) uptake. Interestingly, a threshold of 1−2 nm space between the PEG chains was estimated for minimal protein absorption. Since then, many research groups have investigated the interaction of polymeric nanoparticles with serum opsonins, characterizing both uptake mechanisms and kinetics.[53,56–58] In one study, the relationship between the protein adsorption kinetics of the nanoparticles and their uptake in the liver was investigated.[56] The results showed that the rate of hepatic uptake of polystyrene nanoparticles (50 nm) was significantly higher when nanoparticles were incubated with serum prior to transfusion in the rat liver. It was suggested that the increased rate of hepatic uptake was mainly mediated by opsonization. In vitro uptake studies showed that the increased amount of complement protein C3 and immunoglobulin G (IgG) adsorbed on nanoparticles was directly reflected in the increased rate of uptake of nanoparticles by Kupffer cells. These results indicate that the amount of opsonins associated with the surface of the nanoparticles increased over time and triggered substantial hepatic agglomeration. Similarly, C. Fang et al.[59] have recently shown the effect of the molecular mass of PEG for passive targeting of stealth poly(cyanoacrylate-*co*-*n*-hexadecyl) cyanoacrylate (PHDCA) nanoparticles. As in previous studies, PEG surface modification of nanoparticles was able to dramatically reduce protein absorption as detected using quantitative assays (BCA assay). The amount of protein adsorbed was directly dependent on the molecular mass of the PEG. Medium-sized pegylated nanoparticles (100−200 nm) showed 10−40% protein absorption, and PEG 10 kDa was found to be the most efficient size of PEG as compared to PEG 2 kDa and PEG 5 kDa in preventing protein absorption. The results suggest that a dense PEG shielding over a negatively charged surface is important in preventing protein absorption. The trend in shielding properties was confirmed by the analysis of nanoparticle uptake by murine macrophages and blood clearance kinetics. Interestingly, the level of tumor necrosis factor-α (TNF-α) delivered by nanoparticles into tumor-bearing mice was clearly correlated with the shielding density. Another recent example confirmed the advantages of surface pegylation by utilizing thiolated gelatin nanoparticles (∼300 nm) to improve passive tumor targeting in an orthotopic human breast adenocarcinoma xenograft mouse model.[60] Despite the fact that these particles were twice the size of those in the previous study, the results showed a lower rate of uptake of the PEG-modified nanoparticles by the liver, indicative of the stealth properties of pegylated nanoparticles. In general, pegylated nanoparticles were found to have longer circulation time and higher levels of tumor accumulation than nonpegylated nanoparticles. In summary, much has been learned about PEG molecular mass and PEG density on nanoparticles which has led to reduced plasma protein adsorption, opsonization, and nonspecific uptake. In turn, this has resulted in increased nanoparticle circulation half-life and improved therapeutic efficacy of drugs delivered using pegylated nanocarriers.

Effect of Size. On the basis of physiological parameters such as hepatic filtration, tissue extravasation, tissue diffusion, and kidney excretion, it is clear that, along with surface composition, particle size is a key factor in the biodistribution of long-circulating nanoparticles and achieving therapeutic efficacy (Figure 2). In one study, in vivo biodistribution results of polystyrene nanoparticles with consistent composi-

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tion and varying particle sizes of 50 and 500 nm showed higher levels of agglomeration of the larger nanoparticles in the liver.[56] It was suggested that the mechanism of hepatic uptake was mediated by surface absorption of proteins leading to opsonization. However, the effect of temperature (37 °C vs 4 °C) on hepatic elimination showed unexpectedly faster uptake of the 50 nm polystyrene nanoparticles at the lower temperature. Similarly, the size of the nanoparticle was shown to have a substantial effect on the protein absorption. Small (<100 nm), medium (100−200 nm), and large (>200 nm) pegylated PHDCA nanoparticles incubated with serum protein for 2 h showed a significant correlation between particle size and protein absorption.[59]Protein absorption on small nanoparticles (80 nm) was quantified (6%) and compared to the same nanoparticle formulation with a larger size (171 and 243 nm, 23 and 34%, respectively). The effect of protein absorption on different sized nanoparticles was also confirmed with the analysis of nanoparticle uptake by murine macrophages and blood clearance kinetics. Blood clearance of the smaller nanoparticles was twice as slow as with the larger nanoparticle formulations. More importantly, the amount of drug encapsulated (TNF-α) in nanoparticles that accumulated in the tumor within 24 h was twice that of the larger nanoparticle formulation. The results suggest that it might be due to a higher surface PEG density (brushlike) on the surface of smaller nanoparticles. Rijcken et al.[81] have shown that size and polydispersity can substantially affect the biodistribution of micelles. Cross-linked micelles incubated in PBS (pH 7.4) at 37 °C for more than 3 days did not show substantial increases in size and polydispersity in contrast to non-cross-linked micelles (PD ∼ 0.5 after 10 h). Stable micelles showed a long circulation half-life of ∼8 h due to a low rate of hepatic uptake (liver ∼ 10% of the injected dose; spleen ∼ 2% of the injected dose). Similar to results with small liposomes (<100 nm), micelles were found to accumulate in the skin.

In summary, it has been consistently shown that pegylated nanoparticles smaller than 100 nm have reduced plasma protein adsorption on their surface and also have reduced hepatic filtration. Further, these small pegylated nanoparticles have a long blood residence time and a high rate of extravasation into permeable tissues, demonstrating the importance of tunable particle size and surface composition for achieving effective, targeted delivery.

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Effect of the Core. Shenoy et al.[82] investigated the biodistribution of stealth poly(β-amino ester) nanoparticles (PbAE) and poly(caprolactone) (PCL)-based nanoparticles with a similar size range of 100−200 nm. In addition, both nanoparticle formulations had a very high positive surface charge of approximately +30 mV. The results clearly showed a significantly higher level of accumulation in the heart and lung tissues for the PbAE nanoparticles. This was correlated with a lower level of accumulation in the liver as compared to PCL nanoparticles. It was suggested that a higher degree of aggregation of PbAE nanoparticles in the presence of serum proteins affected the biodistribution leading, to such high levels of accumulation in the lung. Recently, greater degrees of nanoparticle "flexibility" were hypothesized to improve the binding ability of particles on the cell surface.[83] Shell cross-linked nanoparticles (SCKs) containing partially hydrochlorinated poly(isoprene) cores were shown to undergo temperature-dependent deformation.[84] SCK nanoparticles with similar physiochemical properties (size, ca. 20 nm; ζ, ca. −25 mV) possessing a low glass transition temperature (T_g) with a fluidlike poly(methyl acrylate) (PMA) core or a high T_g with a glassy poly(styrene) (PS) core were synthesized to evaluate the effects of the rigidity of the polymeric core on the in vivo biodistribution.[84] The results showed that high-T_g poly(styrene) core nanoparticles exhibited a significantly higher blood residence time compared to the low-T_g poly(methyl acrylate) nanoparticles. The low-T_g core is expected to provide greater flexibility and an increased number of surface interactions of the nanoparticles with the tissues and biological environment. However, it was not clear if the relative rigidity or other physicochemical properties of the polymers (hydrophobicity) affected the blood residence time. The results suggested that the composition of the core has an important effect on the blood residence time. In this case, PEG surface modifications did not have a noticeable effect on kidney accumulation and clearance most probably due to the small size of the nanoparticle formulations. These results are consistent with previous work investigating the effect of the core composition on the nature of absorbed proteins using a series of block copolymers with the same PEG thickness and density.[85] Copolymers of poly(ethylene glycol) (PEG) and polyesters with increased hydrophobicity (PLGA, PLA, and PCL) were synthesized.[85] Evidence of C3 cleavage was used as a semiquantitative way to characterize the level of complement activation by naked and pegylated nanocapsules (NCs) formed with different core copolymers. The results showed that PLA NCs prepared from biodegradable polyesters act as strong activators of complement. Since PCL showed a lower level of complement activation, the reason for the stronger complement response did not seem to correlate with the hydrophobicity of the copolymer. However, pegylated NCs had significantly reduced levels of complement activation which were shown to depend on PEG chain length and density. In another study, polymeric nanoparticles coated with polysorbate were shown to be able to accumulate in the brain tissues.[86] The brain blood vessels are characterized by tight junctions between endothelial cells possessing a TEER (trans-endothelial electrical resistance) of approximately 1500−2000 Ω/cm^2 which prevents paracellular transport of molecules and represents a biological barrier for delivery of the drug to the brain. However, poly(butyl cyanoacrylate) nanoparticles coated with polysorbate 80 were able to deliver significant amounts of doxorubicin to the brain. Multiple formulations were studied, and polysorbate 80-coated nanoparticles exhibited a high doxorubicin concentration (6 μg/g) in brain tissues as compared to noncoated nanoparticles (0.1 μg/g). It was found that apolipoprotein E (apoE) binds substantially to polysorbate 80 surfactants, and the results suggest that apoE is involved in the mediated transport of the polysorbate 80-coated nanoparticles to the brain. Further explanation of the high concentration of doxorubicin drug delivered to the brain can be derived from the fact that polysorbate can act as an anchor for apoE protein binding and interact with LDL receptors expressed in the brain blood vessels. Polysorbate 80 also has been shown to have Pgp inhibition properties which could provide an additional rationale for the delivery of the doxorubicin with nanoparticles having a polysorbate 80 coating.[87] Recently, Shaw and Weissleder (in press in *Proceedings of the National Academy of Sciences of the United States of America*) have found interesting results regarding the core composition and relative toxicities of nanoparticles utilizing an in vitro, high-throughput, multidimensional analysis across varying concentrations utilizing multiple cell types and multiple assays of cellular physiology. Their generalizable, systematic approach indicates that biological effects result from the combined effects of many aspects of nanoparticle composition, including the core composition which was found to have a somewhat surprising effect on biological properties. Future work developing such structure−activity relationships may shed even more light on the complex interplay between nanoparticle properties and subsequent biological effects. In summary, it has been found that physicochemical properties of the core are critical parameters of the nanoparticle formulations. Precise modifications of these properties are able to dramatically affect the extent of interactions with blood and biodistribution of nanoparticles.

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Effect of Surface Functionality and Charge. It has been established that the physicochemical characteristics of a polymeric nanoparticle such as surface charge and functional groups can affect its uptake by the cells of the phagocytic system. It was previously shown that polystyrene microparticles with a primary amine at the surface underwent significantly more phagocytosis as compared to microparticles having sulfate, hydroxyl, and carboxyl groups. Therefore, it is well accepted that positively charged nanoparticles have a higher rate of cell uptake compared to neutral or negatively charged formulations. Nanoparticles carrying a positively charged surface are also expected to have a high nonspecific internalization rate and short blood circulation half-life. Nanoshells having a negative surface charge have shown a marked reduction in the rate of uptake. The ζ potential for nanoshells with BSA absorbed on the surface of the nanoparticle was characterized by a shift to a more negative value. However, the BSA absorption did not promote a higher rate of cell uptake. Interestingly, when the biodistribution of Tyr- and Tyr-Glu-PEG/PDLLA micelles was investigated in mice,[88] both the Tyr- (neutral) and Tyr-Glu (negatively charged) PEG/PDLLA micelles exhibited no remarkable difference in their blood clearance kinetics. The pharmacokinetic parameters suggest that all the micelle formulations are distributed mainly into the extracellular space volume of the spleen and liver. However, the anionic Tyr-Glu-PEG/PDLLA micelles showed a lower distribution (10 times lower) into the liver and spleen 4 h post-injection. Remarkably, a lower level of accumulation of Tyr-Glu-PEG/PDLLA micelles into the liver and spleen is believed to be due to synergic steric and electrostatic repulsion which decrease the rate of cellular uptake. In addition, urinary excretion was the major excretion route, confirming a low rate of hepatic uptake of the micelle formulation. Recently, thiolated gel nanoparticles ($\sim$250 nm; ca. -5 mV)[60] have had a higher level of tumor accumulation than nonthiolated nanoparticles, and it was correlated with a very short plasma half-life of $\sim$3 h, indicating a possible preferential uptake or accumulation in the tumor tissues. In addition, the thiolated nanoparticles were found to have a higher rate of uptake into the spleen. The reasons for such an impact of the thiolated functional group on the surface of the nanoparticles are not very clear, but it could be due to aggregation of nanoparticles through disulfide bond formation or reaction with thiolated molecules circulating in the blood. In summary, it is established that neutral or negatively charged surface nanoparticles have a reduced plasma protein adsorption and low rate of nonspecific cellular uptake. Thus, surface functionality is another critical parameter in controlling the development of long-circulating nanoparticles.

Effect of Active Targeting. Active targeting of nanoparticles involves the conjugation of targeting ligands to the surface of nanoparticles. These ligands can include antibodies, engineered antibody fragments, proteins, peptides, small molecules, and aptamers. The active targeting mechanism takes advantage of highly specific interactions between the targeting ligand and certain tissues or cells within the body to promote the accumulation of nanoparticles.[3,89–92] In the case of weak binding ligands, low affinity can be offset by increased avidity through the surface functionalization of multiple molecules or multivalent designs and has been shown to be a valid approach. There are several examples of FDA-approved antibodies in clinical practice today,[93,94] including Rituxan (target, CD20-positive B-cells for the treatment of non-Hodgkin's lymphoma and rheumatoid arthritis), Herceptin (target, HER-2-overexpressing breast cancer cells), Erbitux [target, epidermal growth factor receptor (EGFR) for the treatment of colorectal cancer], Iressa (target, EGFR for the treatment of non-small cell lung cancer and metastatic breast cancer), and Avastin [target, vascular epidermal growth factor (VEGF) for the treatment of metastatic colorectal, non-small lung, and breast cancers]. While the progress with monoclocal antibodies has been encouraging, they have not been shown to be curative. This has led to the development of immunoconjugates with the intent of utilizing the targeting specificity of the antibody to deliver a relatively potent drug. The development of immunoconjugates has been slow due to various challenges but has resulted in the FDA approval of three immunoconjugates[46] such as Mylotarg (conjugate of calicheamicin targeting CD33 for the treatment of acute myeloid leukemia), Zevalin (yttrium-90 radio-immunotherapy conjugates targeting CD20-positive B-cells for the treatment of non-Hodgkin's lymphoma), and tositumomab (^{131}I radio-immunotherapy conjugates targeting CD20-positive B-cells for the treatment of non-Hodgkin's lymphoma). Currently, drug delivery carriers are being functionalized with proteins, including antibodies or antibody fragments and various other targeting ligands, with the goal of both delivering a high therapeutic dose and delivering this high therapeutic dose to specific tissues or cells. Conjugation approaches for controlling the amount of targeting proteins on the surface of the nanoparticles have been developed to increase specificity and binding affinity. Research using proteins for targeting applications has led to a better understanding of the effect of stability and size of the ligand for successful targeting and clinical

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development.[95,96] Antibody molecules are very large and complex and can be expensive to manufacture relative to small-molecule drugs. In addition, antibodies have a hydrodynamic size of ~20 nm that can increase the size of the NPs. Recently, Davis et al.[97] have demonstrated the therapeutic impact of differential distribution between intracellular and extracellular tumor domains. Cyclodextran-based nanoparticles containing transferrin targeting ligand (NP size of ~70 nm) showed enhanced intracellular accumulation in a human tumor xenograft mouse model. Interestingly, the model of tumor accumulation and uptake fit the experimental data, suggesting that tumor retention time and internalization into tumor cells are the critical factors affecting accumulation of nanoparticles in targeted tissues. The same group[98] reported the first toxicology study of a targeted, polymeric nanoparticle platform in non-human primates as reported above. This type of study is crucial for answering the numerous critical questions about the toxicological response to polymeric nanoparticles circulating in the blood. The results clearly show that their intravenously injected polymeric nanoparticles were safely administered to non-human primates even after multiple administrations over 3 weeks. These promising results led to the submission of an investigational new drug application.

While targeting with proteins has been shown to be advantageous in some cases, for various reasons, it may be desirable to achieve targeting with relatively small targeting ligands that are potentially easier to manufacture. The discovery of new peptide targeting domains[99] provides the advantage of being able to utilize small synthetic molecules for active targeting. One such example is peptides which are relatively stable compared to antibodies and are also less likely to be immunogenic. Similarly, small molecules can be very attractive for use as targeting ligands, and small molecules such as folic acid or sugar molecules have been extensively used. For example, cell surface membrane lectins have been shown to be overexpressed on the surface of numerous cancer cells and are able to specifically internalize sugar molecules (lactose, galactose, and mannose).[5] Similarly, nucleic acid aptamers are able to fold into unique structures capable of binding to specific targets with high affinity and specificity.[100] Favorable characteristics of aptamers have resulted in their rapid progress into clinical applications. Our group is interested in developing this class of molecules for targeted delivery of controlled drug-releasing polymer vehicles.[101] We described the first proof-of-concept drug delivery polymeric nanoparticle utilizing aptamers as targeting ligands in vitro[102] and subsequently showed the efficacy of similarly designed nanoparticles against prostate cancer tumors in vivo.[103] In vivo results using a human prostate cancer tumor xenograft mouse model showed a significant therapeutic efficacy of aptamer-targeted nanoparticles loaded with docetaxel compared to nontargeted formulations. Targeted nanoparticles substantially reduced the size of the tumor after a single intratumoral injection, and all of the treated mice survived more than 3 months in contrast to other controls. More recently, we reported a novel strategy for facile synthesis of targeted nanoparticle formulations.[104] We have shown that targeted nanoparticles specifically accumulated in the prostate tumor xenograft mouse model (~1.8%ID/g of tissue) compared to nontargeted nanoparticles (0.5%ID/g of tissue). In summary, it has now been shown both in vitro and in vivo that targeted nanoparticles are able to agglomerate in specific tissues to improve the therapeutic efficacy, and there is a growing body of literature in this area. Clearly, active targeting provides a powerful approach to increasing the drug delivery load at a specific site for treatment.

Conclusion

The exact role of each of the proteins adsorbed on the surface of the nanoparticles in the clearance and biodistribution is still not clear besides their role in opsonization and enhanced hepatic uptake. It is generally assumed that the rapid uptake of injected nanoparticles is triggered by receptor-mediated mechanisms of absorbed proteins from the blood (opsonins) onto their surface and complement activation. However, it is not clear if a specific type, a combination of proteins, or even the protein conformation is the most important factor for a high rate of phagocytotic uptake. The efficacy of the PEG "brush" in altering the biodistribution of nanoparticles has been clearly demonstrated, and in vivo studies showed a drastic increase in blood circulation time

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with an increase in PEG surface density. It is also clear that relatively small physicochemical differences have significant biological implications in the fate of the biodistribution of nanoparticles. Pegylated nanoparticles between 10 and 100 nm in size are able to remain in the systemic circulation for hours and extravasate or diffuse into the diseased tissues by a passive targeting mechanism. Recently, targeted nanoparticles funtionalized with ligands that have high affinity and specificity have been shown to efficiently accumulate in specific tissues and dramatically increase the therapeutic efficacy of long-circulating nanoparticle drug delivery systems. Natural and synthetic polymers are now in preclinical and clinical phases for drug delivery. More importantly, targeted and nontargeted polymeric nanoparticles are now in the preclinical and clinical phases and confirm the great promise of the past 20 years of research and lessons learned from the failure of some clinical studies to increase the therapeutic index of drugs approved for clinical use.

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