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# Pharmacokinetics and Biodistribution of Nanoparticles

**Shyh-Dar Li[†] and Leaf Huang***

*Division of Molecular Pharmaceutics, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599*

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**Abstract:** Nanoparticles show their promise for improving the efficacy of drugs with a narrow therapeutic window or low bioavailability, such as anticancer drugs and nucleic acid-based drugs. The pharmacokinetics (PK) and tissue distribution of the nanoparticles largely define their therapeutic effect and toxicity. Chemical and physical properties of the nanoparticles, including size, surface charge, and surface chemistry, are important factors that determine their PK and biodistribution. The intracellular fate of the nanoparticles after cellular internalization that affects the drug bioavailability is also discussed. Strategies for overcoming barriers for intracellular delivery and drug release are presented. Finally, future directions for improving the PK of nanoparticles and perspectives in the field are discussed.

**Keywords:** Nanoparticles; pharmacokinetics; biodistribution; drug release

## 1. Introduction

Nanotechnology has been increasingly employed in drug delivery. By nanosizing a formulation, we can increase the drug dissolution rate, leading to enhanced drug absorption and bioavailability. Using the nanoparticles to deliver drugs, improved tissue selectivity can be achieved due to the selective uptake of nanoparticles in certain tissues. Nanoparticles can be employed to provide improved protection or reduced renal clearance for easily degraded or short half-life drugs, such as small peptides and nucleic acids, for a prolonged pharmacological effect. However, nanoparticles may also enhance the delivery of drugs to certain tissues and thus, cause new side effects. The pharmacokinetic profiles of the parent drug and the drug encapsulated in the nanoparticles are often different. Therefore, it is very important to monitor the pharmacokinetics (PK) and biodistribution of nanoparticles to understand and predict their efficacy and side effects. The PK profile of the nanoparticles is mainly determined by their chemical and physical properties, such as size, charge, and surface chemistry. In this work, factors that control the biofate of nanoparticles are discussed with examples and approaches for improving drug release in the target tissues and cells are compared. Since liposomes are the most well studied and characterized nanoparticles for drug delivery, most of the examples in this review are from the research using liposomes. However, general rules can be applied for other types of nanoparticles, including polymer-based, silica-based, and hybrid nanoparticles. In fact, the rapid rise of the field of nanoparticles for drug delivery benefits from decades of research with liposomes. Finally, remaining challenges are emphasized with future directions.

## 2. PK Study

PK study involves measuring drug concentrations in all major tissues after drug administration over a period of time until the elimination phase. It is necessary to monitor the drug concentration long enough to fully describe the behavior of the drug or nanoparticles in vivo (usually 3 × half-life). The PK profile in the blood can be fitted using various programs to obtain key PK parameters that quantitatively describe how the body handles the drug or nanoparticles. Important parameters include $C_{max}$ (maximum concentration), $t_{1/2}$ (half-life), Cl (clearance), AUC (area under the curve), and MRT (mean resident time, average time that a molecule of a drug stays in the body). When a drug formulation shows prolonged blood circulation, an increased $t_{1/2}$, a reduced Cl, an increased AUC, and an increased MRT are usually observed. On the other hand, if a formulation is quickly

* To whom correspondence should be addressed: 2316 Kerr Hall, 311 Pharmacy Lane, Chapel Hill, NC 27599. Telephone: (919) 843-0736. Fax: (919) 966-0197. E-mail: leafh@unc.edu.

[†] Current address: Moores Cancer Center, University of California at San Diego, La Jolla, CA 92093-0819.



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eliminated from the body, a low $t_{1/2}$, a high Cl, a low AUC, and a low MRT are often obtained. PK data not only can help describe but also can help predict the behavior or profile of the drug or nanoparticles. PK data are often used in deciding the dose and dose regimen for maintaining a desirable blood concentration for improved therapeutics with minimal side effects. The blood concentration of drugs is highly correlated with their efficacy and toxicity in most cases, especially for free drugs. However, to gain insight into how the body handles the drug formulation and how the formulation may affect the efficacy and adverse effects, it is essential to obtain the tissue distribution information for the drug. A high level of accumulation of a drug in the target tissue often results in an enhanced therapeutic effect, and oppositely, a large amount of drug distributed to nontarget organs may cause unwanted toxicity. PK and tissue distribution studies that allow investigators to screen formulations for a given drug are extremely important during drug development. By optimizing the drug formulation, investigators can improve the drug delivery to the target tissue and reduce drug distribution to the nontarget tissues to obtain increased therapeutic activity with minimal side effects.

## 3. Comparison of PK of Free Drugs and Drugs Encapsulated in Nanoparticles

When a hydrophilic drug is intravenously (i.v.) injected into the body, without much protein binding, the drug is often quickly eliminated from the blood by renal filtration into the urine. In the case of a hydrophobic drug, the renal clearance is significantly reduced compared to that of the hydrophilic drugs due to an increased level of serum protein binding. The hydrophobic drugs are often transformed into hydrophilic metabolites in the liver and excreted into the bile or eliminated into the urine. When the drugs are encapsulated in nanoparticles, the drugs are protected from metabolizing enzyme in the liver before they are released and as well as from renal clearance due to the increased size. The cutoff size for renal excretion is approximately 5.5 nm according to recent research using quantum dots,[1] and the nanoparticles are usually much larger. Reduced liver metabolism and renal clearance of drugs encapsulated in the nanoparticles often result in prolonged blood circulation with an increased chance of accumulation in the target tissue.

## 4. Tissue Selectivity of Nanoparticles

The limited pore size of the endothelial wall in the tissue is the primary delivery barrier for nanoparticles but also allows selective accumulation in certain tissues. Unlike small molecule drugs that can diffuse through the capillary wall into the tissue, nanoparticles rely on the gaps between the endothelium to pass through the barrier. Tissues with a leaky endothelial wall usually contribute significant uptake of nanoparticles, including tumor, liver, spleen, and bone marrow. The increased rate of tumoral uptake of nanoparticles is based on a phenomenon termed the "enhanced permeability and retention" (EPR) effect[2] due to the increased capillary permeability in the tumor tissue. The enhanced uptake in the liver, spleen, and bone marrow is largely attributed to the macrophages residing in the tissues, which are responsible for clearing particulates and macromolecules circulating in the blood. When nanoparticles are i.v. administered, a variety of serum proteins bind to the surface of the nanoparticles, which are recognized by the scavenger receptor on the macrophage cell surface and internalized, leading to a significant loss of nanoparticles from the circulation.[3] The serum proteins binding on the nanoparticles are also termed "opsonins", and the macrophages contributing the major loss of injected dose are also known as the reticuloendothelial system (RES) or mononuclear phagocyte system (MPS). Minimizing protein binding is the key point for developing a long circulation nanoparticle formulation.

## 5. Factors That Influence PK of Nanoparticles

Opsonization is the major factor that induces MPS uptake of nanoparticles, and therefore, surface characteristics of nanoparticles greatly influence their PK. Generally, nanoparticles that have a mean diameter of approximately 100 nm with a neutral and hydrophilic polymer-extended surface exhibit prolonged blood circulation and an increased level of tumor delivery.

**5.1. Surface Modification with Polyethylene Glycol Reduces the Rate of MPS Uptake and Prolongs the Circulation Half-Life of Nanoparticles and the Encapsulated Drugs.** To minimize opsonization, the most commonly used strategy is to conjugate the polyethylene glycol (PEG) polymer onto the surface of the nanoparticles, which is a relatively inert hydrophilic polymer that provides good steric hindrance for preventing protein binding. Investigators have demonstrated that PEGylation reduces the rate of MPS uptake and increases circulation half-life for various types of nanoparticles, including liposomes,[4] polymer-based nanoparticles,[5] and hybrid nanoparticles.[6–9] Sadzuka et al. also studied the PK of the encapsulated drugs formulated in either plain liposomes or PEGylated liposomes.[10] The AUC of the blood PK profile of the drug formulated in the PEGylated liposomes was 6-fold higher than that of the non-PEGylated formulation and 36-fold higher than that of the free drug. The rate of MPS uptake in the liver for the drug encapsulated in the non-PEGylated liposome was 3-fold higher than that of the PEGylated formulation. This resulted in a 3-fold higher

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rate of tumoral uptake for the drug encapsulated in the PEGylated liposomes compared to that in the non-PEGylated liposomes. The increased level of tumoral accumulation of the PEGylated formulation was correlated with its antitumor efficacy, which was superior to that of the free drug as well as the drug formulated in the non-PEGylated liposomes. Similar results were also reported by other groups.[5,11,12] Although several materials have been developed to mimic the effect of PEG for reducing the degree of opsonization,[13–15] PEGylation is still the most commonly used approach. It is also noted that PEGylation not only reduces the rate of MPS uptake but also weakens the interaction between the nanoparticles and the target cells, which often causes inefficient intracellular delivery. Several strategies have been developed to address this problem, which is discussed in section 6.

It is known that a high degree of PEGylation may change the balance of hydrophilicity and hydrophobicity of the nanoparticles, which often results in instability of the nanoparticles. This is especially the case for lipid-based nanoparticles, such as liposomes. DSPE-PEG is the most commonly used lipid with which to PEGylate liposomal formulations, which is known to disrupt the integrity of the lipid membrane due to its detergent-like properties when used at a high concentration.[16] Usually, 5 mol % modification by PEG on the particle surface is common for producing a reduced rate of RES uptake while maintaining the stability of the lipid-based formulation. Recently, our group has found that the LPD (liposome−polycation−DNA) nanoparticles composed of two lipid bilayers were resistant to an increased degree of PEGylation (S.-D. Li and L. Huang, unpublished data). LPD nanoparticles were prepared by mixing cationic liposomes, a polycationic peptide (protamine), and nucleic acids at a fixed ratio. The self-assembled nanoparticles are approximately 100 nm in diameter. The detailed structure of the LPD nanoparticle was demonstrated by cryo-TEM (transmission electron microscopy),[17] showing that the nucleic acid was complexed by protamine to form a compact core, which was coated with two cationic lipid bilayers. The inner bilayer was supported by charge−charge interaction of the cationic lipids and the negatively charged complex core. Our data showed that the outer lipid bilayer was stripped off during the incubation with an increased concentration of the micelle solution of DSPE-PEG and DSPE-PEG was inserted into the outer leaflet of the inner lipid bilayer, resulting in 10.6 mol % DSPE-PEG presented on the particle surface. This led to a complete charge shielding ($\zeta$ potential $= -5.6 \pm 4.5$ mV) and abolishment of liver sinusoidal uptake in the isolated liver perfusion experiment, which may explain the extremely high rate of tumor uptake of the formulation (70−80% of the injected dose/g of tissue).[7]

**5.2. Nanoparticles That Have a Mean Diameter of Approximately 100 nm Show Prolonged Blood Circulation and a Relatively Low Rate of MPS Uptake.** Liu et al. have investigated the biodistribution of liposomes of different sizes (30−400 nm) in blood, liver, spleen, and tumor.[18] They i.v. injected the radioisotope-labeled liposomes into the mice and examined the recovered dose in those tissues 4 h later. It was found that approximately 60% of the injected liposomes between 100 and 200 nm in size were detected in the blood, while only 20% of the ID for liposomes greater than 250 nm or less than 50 nm in size was recovered in the blood. For liver uptake, liposomes of approximately 100 nm exhibited only 20% ID accumulation, and particles greater than 250 nm in size had ~25% ID found in the liver. Approximately 60% ID was recovered in the liver for liposomes with a diameter of less than 50 nm, which was smaller than the pore size of liver fenestrae (100 nm), and easily penetrated through the endothelial wall, resulting in

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an enhanced liver uptake. In the case of spleen uptake, liposomes less than 100 nm in size exhibited minimal spleen uptake, whereas an increase in particle size led to an increase in the rate of spleen uptake. For the liposomes approximately 400 nm in size, 40−50% ID was found in the spleen 4 h after injection. The tumoral uptake data were tightly correlated to those of the blood PK, in which liposomes with a diameter between 100 and 200 nm showed a 4-fold higher rate of uptake in the tumor compared to the liposomes greater than 300 nm or less than 50 nm in size.

Allen's group also demonstrated a similar result, in which liposomes with a diameter of approximately 120 nm exhibited a 10−20-fold increase in the rate of tumoral uptake compared to those with a diameter of approximately 170 nm.[19] In this particular tumor model, the cutoff size range was even narrower. Torchillin et al. found that using micelles with a mean diameter of 10 nm to deliver paclitaxel did not show any EPR effect compared to the free drug.[20] However, when the micelles were modified with a targeting antibody, significantly improved tumoral uptake was discovered. For very small particles, they can easily pass through the leaky capillary wall in the tumor but can also be easily pushed out from the tumor into the blood. Therefore, small particles have good permeability but poor retention. After conjugation with a targeting ligand, the retention in the tumor was greatly enhanced, leading to improved tumoral uptake.

Figure 1 summarizes the effect of size on the tumor targeting of nanoparticles. The research into the influence of size on PK was mainly studied in the liposome field since liposomes of a certain size can be prepared with a narrow size distribution by the membrane extrusion method.

**5.3. Neutral Nanoparticles Exhibit a Decreased Rate of MPS Uptake and Prolonged Blood Circulation Compared to the Charged Ones.** Levchenko et al. prepared liposomes with different charge status approximately 200 nm in size and studied their tissue distribution in the mice over a period of time.[21] They demonstrated that the rate of clearance from the blood was significantly higher for the negatively charged liposomes ($\zeta$ potential $\sim -40$ mV) than for neutral liposomes ($\zeta$ potential $\pm$ 10 mV). The negatively charged liposomes also showed an increased rate of MPS uptake in the liver compared to the neutral liposomes, indicating that phagocytic cells favored the uptake of negatively charged particles and, thus, increased the rate of clearance of particles from the blood. The surface charge could be shielded by PEGylation as shown by Levchenko and colleagues, who found that the $\zeta$ potential of the negatively charged liposomes was reduced to approximately −15 mV. This led to a significantly reduced rate of liver uptake and prolonged blood circulation.

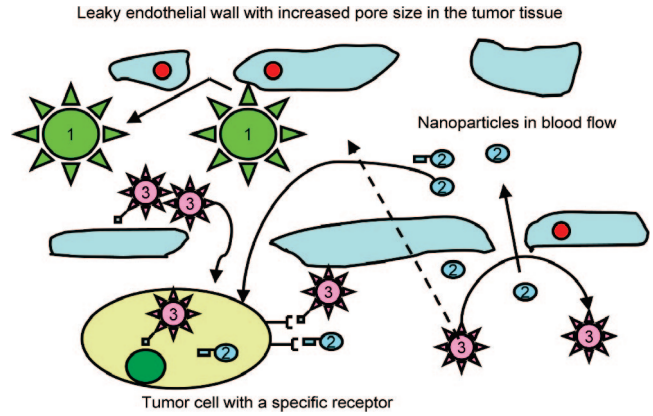


***Figure 1.*** Summary of the effect of the size of nanoparticles on tumor accumulation and uptake. The endothelial wall in the tumor tissue has an increased pore size for selective penetration of macromolecules and nanoparticles. When the nanoparticles are larger than the cutoff size of the endothelial gap, the nanoparticles (1) exhibit a reduced level of tumor accumulation and uptake due to the lack of permeability. If the nanoparticles are too small, they (2) can easily enter the tumor tissue through the gaps but can also readily come out, resulting in little tumor accumulation. However, tumor retention can be improved by conjugating a targeting ligand to the nanoparticles (2 with flag), which also enhances intracellular uptake. Nanoparticles of the optimal size (3) exhibit enhanced permeability and retention (EPR) for an increased level of tumor accumulation. Conjugation of a targeting ligand simply increases the rate of intracellular delivery of the nanoparticles (3 with flag) but not the overall level of tumor accumulation, which is dependent on the EPR effect.

Positively charged particles have been known to form aggregates in the presence of the negatively charged serum proteins once i.v. administered.[22] The aggregates are large and often cause transient embolism in the lung capillaries. The dissociated particles subsequently redistribute to the liver.[22] Thus, positively charged nanoparticles often exhibit a rapid blood clearance phase with a large dose accumulating in the lung and the liver. Similarly, PEGylation is also the most commonly used approach to improving the PK of cationic nanoparticles, including lipoplex,[23] polyplex,[24] and LPD nanoparticles.[6–9,25]

The effect of charge on PK was mostly studied with liposome-based formulations, which can be prepared by using

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different lipid components with the control of size and other chemical and physical properties.

**5.4. PK of Polymersomes with Worm-Shaped Structure.** Discher and colleagues have developed worm-shaped nanoparticles composed of a diblock copolymer.[26] The nanoparticles circulate in the mouse blood with a very surprisingly long half-life, i.e., 5 days. Subsequent studies using cultured macrophages revealed that the worm-shaped nanoparticles experience a strong drag force by the fluid flow such that the macrophage can not engulf them before they are carried away by the flow. This is probably the mechanism underlying the ultraprolonged circulation time.

**5.5. Mini Summary.** MPS is the major contributor for the clearance of nanoparticles. Reducing the rate of MPS uptake by minimizing the opsonization is the best strategy for prolonging the circulation of the nanoparticles. Approaches for improving the PK of nanoparticles include maintaining the size around 100 nm, keeping the $\zeta$ potential within 10 mV, and grafting PEG onto the surface of nanoparticles. The FDA-approved nanoparticle formulation for cancer therapy, Doxil (liposomal doxorubicin), fits all of these criteria. However, non-PEGylated and negatively charged nanoparticles can be used for intentionally targeting MPS (i.e., for fungal infection), where AmBisome (liposomal amphotericin B) is a good example.

## 6. Release of Drug from Nanoparticles: Challenges and Strategies

To create pharmacological efficacy, the encapsulated drug must be released from the nanoparticles to the target cells. This is a major challenge in the advanced drug delivery studies. Taking Doxil as an example, although it is an effective carrier for delivering doxorubicin to the tumor tissue, only a modest increase in antitumor activity was observed.[27,28] The major reason is the low rate of release of the drug from Doxil both in the blood circulation and in the tumor tissue.[27–29] High-level tumor accumulation of nanoparticle formulation does not directly correlate to the bioavailability of the drug to the tumor, which is more dependent on the rate of drug release.[27,30] Laginha and colleagues have shown that the tumor bioavailability for Doxil was only 40−50%.[31] Moreover, Doxil suffers from new side effects, including hand-foot syndrome and mucositis,[32,33] which could be consequences of the slow release of the drug.[34,35] Although Doxil showed no acute cardiotoxicity,[27,30] these new side effects limit its maximum tolerated dose (MTD).[27] Another typical example is the liposomal cisplatin formulation (SPI-077). In clinical trials, investigators found that SPI-077 accumulated substantially in the tumor tissue but exhibited no antitumor effect.[36–40] Cisplatin cannot diffuse through the intact

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liposomal membrane[27] and, thus, predominantly stays inside the PEGylated liposome and displays little activity.[27]

Maintaining the high stability of the nanoparticles in the circulation while obtaining a sufficient local drug bioavailability in the target tissue remains challenging. An increased level of attention has been paid to designing a formulation with a triggered-release mechanism,[27,28,30] in which a nanoparticle formulation can release the encapsulated drug after accumulating in the target tissue.[27] Optimally, the rate of release of drug from the nanoparticles can be controlled to match the pharmacokinetic profile of the nanoparticles and pharmacodynamic profile of the drug.[28,30] There are three approaches: (a) conjugating a targeting ligand to increase the rate of intracellular delivery and bioavailability of the drug, (b) incorporating a pH sensitive component in the formulation to facilitate the escape of the drug from the endosomal compartment, and (c) triggering drug release locally by a physical method at the target tissue.

**6.1. Increase the Rate of Intracellular Delivery by Conjugating a Targeting Ligand on the Surface of Nanoparticles.** A targeting ligand conjugated to the surface of nanoparticles can recognize and bind with the receptor expressed on the target cell surface, which later triggers receptor-mediated endocytosis, resulting in an increased level of intracellular delivery of the formulation. This approach is particularly useful for delivering drugs with low membrane permeability, such as DNA, oligonucleotides, and siRNA. Several active targeting strategies (antibodies, carbohydrates, peptides, and small molecule ligand[41]) have been developed and showed promising results. As shown by Park and colleagues, the PK and tissue distribution of the nontargeted liposomes and immunoliposomes (liposomes coated with a targeting antibody) were similar to each other, while the intracellular delivery of the drug was significantly enhanced with the immunoliposomes.[42] Immunoliposomes showed further improved antitumor efficacy compared to the nontargeted liposomes.[43] Coating of a targeting ligand on nanoparticles does not increase the level of accumulation of the drug in the target tissue but increases the rate of intracellular delivery, which was also reported by other groups using polymer-based nanoparticles[44] and LPD nanoparticles.[6] Drug accumulation in the tissue is highly dependent on the EPR effect.[42]

There are still some unsolved challenges using the targeting ligand approach. First, with targeted nanoparticles having slow internalization after binding to the first encountered target cells, it blocks the binding of more targeted nanoparticles subsequently extravasated.[27] Second, antibody-conjugated nanoparticles exhibit greater blood clearance.[45,46] Third, internalization by receptor-mediated endocytosis is usually through the endosome/lysosome pathway, in which a great percentage of the drug is trapped in the organelle or degraded.[27] Therefore, targeted nanoparticles have not always exhibited a significantly increased therapeutic efficacy compared to the nontargeted ones.[47]

**6.2. Increase the Rate of Escape of Drug from the Endosome/Lysosome To Improve Bioavailability.** The pH of the endososme/lysosome can be as low as 5, which allows a triggered release of the drug from the nanoparticles by a pH-dependent mechanism. pH-triggered release can be introduced by using an acidic lipid[27,48] as the component to stabilize the liposomes consisting of DOPE (dioleoylphosphatidylethanolamine). DOPE cannot form liposomes by itself because of its tendency to form inverted micellelike structure. However, stable liposomes can be prepared by mixing a micelle-forming lipid (such as oleic acid[28,48] and CHEMS[28,48]) with DOPE. At physiological pH (~7.4), the carboxylic group in oleic acid or CHEMS ($pK_a \sim 5$) is ionized and micelle-forming. It stabilizes the bilayer phase of DOPE. When the pH is below 5, the acidic lipid is protonated and loses its micelle forming property. It destabilizes the bilayer composed of DOPE, leading to drug release. Collins and Huang demonstrated that the pH sensitive liposomes released almost 100% drug content when the pH was below 5, while only 10% of the drug was released from the non-pH sensitive liposomes.[49] When toxin was encapsulated in the pH sensitive immunoliposomes, the cytotox-

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icity was greatly enhanced compared to that of the immunoliposomes.[49] However, this strategy cannot be applied for in vivo drug delivery since opsonization stabilizes the formulation which is no longer pH sensitive.[50]

Usually, polymer-based nanoparticles exhibit improved endosomolytic activity compared to the lipid-based nanoparticles. The polymer that exhibits endosomolytic activity usually contains multiple neutral amine groups with a p$K_a$ of approximately 6.5, such as crowded tertiary amines, histidine,[44] and imidazole.[23] At physiological pH, the amine groups stay neutral, while in the endosome (pH 5−6), the amine groups are protonated, resulting in a reduced free proton concentration and an increased pH in the endosome. Due to the continuous influx of proton and its counteranion, chloride, into the endosome to maintain the low pH, the osmotic pressure in the endosome rises rapidly. Eventually, the endosome ruptures, and nanoparticles are released. The process is called the proton sponge effect[51] and has been experimentally verified.[52]

**6.3. Increase the Rate of Drug Release Locally at the Target Tissue by an Internal or External Triggering Mechanism.** The third strategy is to locally trigger release of the drug from the nanoparticles in the target tissue, as demonstrated with different approaches.[27] Release of the drug from the nanoparticles triggered by low pH,[48,53,54] temperature,[55–57] light,[48,58] and endogenous enzymes[27–29,59] has been conducted.

As mentioned earlier, PEGylation greatly weakens the interaction between the cells and nanoparticles. Therefore, one can conjugate a cleavable PEG moiety to the surface of a nanoparticle to stabilize the formulation. Once the nanoparticles reach the target tissue, a cleavage mechanism can be triggered for de-PEGylation, resulting in instability of the nanoparticles and drug release. Similarly, pH-triggered release can be introduced by using an acid-labile PEG-conjugated lipid[54] as the liposome component to stabilize the liposome consisting of DOPE. At low pH (∼5), PEG is cleaved from the liposomes, leading to the loss of the hydrophilic headgroup, destabilization of the liposomes, and drug release.[54] Unfortunately, the pH of the interstitial fluid (even in tumor or inflamed tissue) rarely declines below pH 6.5, and this approach is thus ineffective.[60]

Liposomes with a light-triggered release mechanism can be prepared by the use of lipids that either isomerize, fragment, or polymerize upon photoexcitation,[48,58] which causes formation of defects in the lipid membrane and leakage of the drug. Nevertheless, this method is not feasible in vivo due to poor penetration of the externally illuminated light.[27] This method also requires localization of the tumor, which cannot be applied for the metastatic disease.

Needham and colleagues introduced a heat-triggered release (hyperthermia) into a new liposomal formulation, which consists of regular lipids and a lyso-phospholipid.[55–57] The lyso-phospholipid is kinetically trapped in the lipid membrane in the gel phase at 37 °C but is concentrated in the bilayer defects at the phase transition temperature (41 °C),[57] which significantly increases the permeability of the lipid membrane and leads to a rapid drug release.[57] This approach has been successfully demonstrated in vivo[56] and is now in clinical trials.[55] Similar to the light triggered-release, this method relies on the localization of the tumor and cannot be used to treat metastatic cancer. The hyperthermia approach may be limited to accessible tumors that cannot be removed by surgery.[27]

Enzyme-triggered release utilizes an enzyme that is upregulated in the tumor tissue to cleave the lipids in the liposomal membrane and destabilize the formulation.[27,59] The enzyme has to be (a) overexpressed in the tumor tissue, (b) secretory, and (c) present at a low concentration in the circulation. Several enzymes fulfill the requirements and have been utilized for triggering release of drug from liposomes, including elastase,[59,61] alkaline phosphatase,[59,62] phospho-

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lipase A2 (PLA2),[27–29] transglutaminase,[63] and phospholipase C.[64] Among all, the approach employing PLA2 is the most successful one and has produced improved antitumor activity in vitro and in vivo.[27–29] However, some limitations may hinder the application of this approach. Taking the PLA2 approach as the example, the limitations include the following: (a) not all tumors secrete sufficient enzyme in the interstitium, (b) large individual variations of the drug release rate may be observed since local enzyme concentrations vary among the patients, (c) PLA2 cannot degrade phospholipids when the lipid membrane contains more than 20 mol % cholesterol,[27] which is often required for a stable formulation,[65] and (d) human PLA2 type IIA is inactive for neutral liposomes, which is favorable for prolonged blood circulation and tumor targeting.[27]

Similarly, polymeric micelles composed of amphiphilic block copolymers can be rationally designed to release a drug via a change in the hydrophilicity and hydrophobicity balance of the polymer induced by an external trigger, including temperature, pH, chemical or enzymatic hydrolysis of side chains, oxidation−reduction processes, and light.[66] For example, Frechet et al. reported a pH sensitive block copolymer of PEG and poly(aspartic acid) functionalized with trimethoxybenzylidene acetals as an acid labile linkage.[67] Cyclic benzylidene acetals increased the hydrophobicity of the core via stacking of the aromatic rings and masked the polarity of the diol by the acetal groups. The micelles were stable at physiological pH, but once the pH of the solution reached 5, the acetal bonds were hydrolyzed and diol groups were generated, which increased the hydrophilicity of the hydrophobic segment of the polymer. This led to disintegration of the micelles and drug release.

**6.4. Mini Summary.** A variety of strategies for improving the bioavailability of the drug encapsulated in the nanoparticles have been developed. However, except for the hyperthermia method, all of the approaches are still in preclinical studies. A good EPR effect may be a prerequisite for these approaches since the trigger release mechanism usually exists or is applied locally in the tumor tissue. The mechanism of the antitumor effect from the thermal sensitive liposomes combined with hyperthermia treatment is not fully understood at present. Since most of the drugs are released in the microvessel in the tumor, antiangiogenesis is expected to play a major role. Moreover, hyperthermia itself can also induce stress for tumor cells, which can further enhance the antitumor efficacy.

## 7. Promise, Challenges, and Future Direction

Utilizing the EPR effect, nanoparticles has shown the promise of delivering drugs into tissues with leaky vessels, including tumors and inflamed sites. Uptake by MPS in the liver, spleen, and bone marrow represents the classical passive targeting of the nanoparticles. Physical and chemical properties, including size, charge, and surface chemistry, greatly influence the PK and the tissue distribution of the nanoparticles. By manipulating these factors (size of approximately 100 nm, $\zeta$ potential within 10 mV, and PEGylation), the rate of MPS uptake of the nanoparticles can be greatly reduced and blood circulation can be prolonged, allowing the nanoparticles to have an increased chance to distribute to the target tissue (i.e., tumor). However, the majority of the ID for nanoparticles is still lost to MPS uptake, typically leaving 2−10% ID being distributed to the target tissue. It is anticipated that nanoparticles larger than 50 nm exhibit less efficient tissue penetration. However, as discussed earlier, nanoparticles with a diameter of less than 50 nm experience enhanced liver uptake, which may result in liver toxicity. This has been an unsolved challenge in this field. Another major research effort has been placed on how to control the release of drug from the nanoparticles since only released drug is bioavailable. If one can control the drug release locally in the target tissue, the tissue penetration problem may no longer be a critical issue, especially for small molecular drugs. Despite the existence of different strategies for triggered drug release, success has been limited. Currently, there are several nanoparticle-based formulations in clinical trials showing weakened side effects or improved efficacy, but none of them replaces the market of the free drug. In addition to the unsolved issues regarding the limited tissue targeting and drug release, the high cost of the nanoparticle-based formulation restricts its widespread use. Developing a cost-effective nanoparticle formulation is a sizable challenge.

There has been an increased level of interest in targeting vascular endothelial cells in diseased tissue to achieve improved drug delivery. It has been demonstrated that the proteomics of the endothelium is different and unique in every tissue and tissue specific targeting can be realized by targeting the microvessel.[68] Additionally, endothelial cells directly facing the blood are more accessible for treatment compared to the diseased cells hiding behind the endothelial

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barrier. Finally, caveolae-mediated transcytosis is a common mechanism for endothelial cells to transport a substance from the blood into the tissue, which may be a good mechanism for overcoming the endothelial barrier. Oh and colleagues immunized mice with the extract of the caveloae proteins isolated from the rat lung endothelium and produced an antibody.[69] They have shown that the aminopeptidase P antibody specifically targeted nanoparticles to the caveolae of rat lung endothelium.[69] The caveolae then operated as a pump, transporting the antibody-conjugated nanoparticles from the blood across the endothelium into the lung tissue. An approximately 80% injected dose/g of tissue was achieved within 30 min with minimal uptake in other tissues. This new approach opens a new window for improving the tissue targeting of nanoparticles. Instead of using the EPR effect of the nanoparticles that is passive and has limited capacity, targeting the caveolae with a tissue specific ligand utilizes the active transport system that is continuously functioning.

Simberg and colleagues used in vivo phage display technology to identify a targeting peptide that bound to the meshwork of clotted plasma proteins in the tumor tissue, which was not found in normal tissue.[70] When the peptide-modified nanoparticles were i.v. injected into the tumor-bearing mice, the targeted nanoparticles acted like platelets and clotted the microvessels in the tumor in 2 h. This strategy provides an alternative for tissue-targeted delivery, in which the nanoparticles stayed in the microvessel in the tumor. Potentially, the nanoparticles can be loaded with a drug and slowly release it locally.

There has been significant effort spent on developing a long circulation nanoparticle formulation for the past decade. However, prolonged circulation also means slow tissue accumulation of the nanoparticles (including in the target tissue) and very slow drug release, which is a disadvantage as discussed earlier. A future direction in the nanoparticle field is to utilize an active targeting system that either delivers nanoparticles from the blood into the target tissue by endothelial transcytosis or keeps the nanoparticles in the microvessels of the target tissue, which usually occurs within a few hours.[69,70] The blood circulation time of those targeted nanoparticles is short as they accumulate in the target tissue very quickly. Therefore, a rapid release formulation (within a few hours) will be more desirable for the new type of delivery system.

It is anticipated that the emergence of new nanotechnologies will facilitate the development of new delivery systems that not only specifically deliver a drug to the target tissue but also release it locally and efficiently. In addition, inexpensive but safe materials have to be explored to reduce the cost of the nanotechnology-based therapy so that a greater patient population can benefit.

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