

# Chapter 2

## Nanoparticles for Targeted and Temporally Controlled Drug Delivery

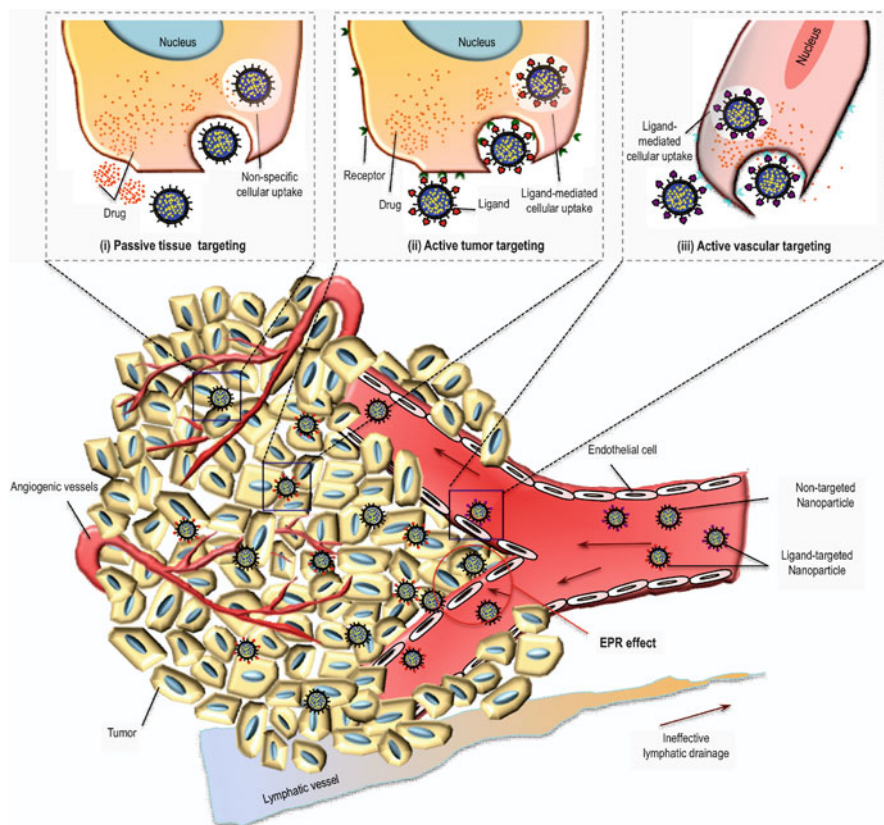
Archana Swami, Jinjun Shi, Suresh Gadde, Alexander R. Votruba, Nagesh Kolishetti, and Omid C. Farokhzad

### 2.1 Introduction

Therapeutic nanoparticle (NP) technologies have the potential to revolutionize the drug development process and change the landscape of the pharmaceutical industry [1–5]. By virtue of their unique physicochemical properties, nanoparticles have shown promise in delivering a range of molecules to desired sites in the body. Nanoparticle technologies may improve the therapeutic index of drugs by enhancing their efficacy and/or increasing their tolerability in the body. Nanoparticles could also improve the bioavailability of water-insoluble drugs, carry large payloads, protect the therapeutic agents from physiological barriers, as well as enable the development of novel classes of bioactive macromolecules (e.g., DNA and siRNA). Additionally, the incorporation of imaging contrast agents within nanoparticles can allow us to visualize the site of drug delivery or monitor the *in vivo* efficacy of the therapeutic agent [6, 7]. Thus far, over two-dozen nanotechnology products have been approved by the US Food and Drug Administration (FDA) for clinical use, and many are under clinic and preclinic development [2, 8, 9]. Interestingly, the majority of these clinically approved, first-generation nanotechnology products are comprised of liposomal drugs and polymer–drug conjugates, which are relatively simple and generally lack active targeting or controlled drug release components. To develop safer and more effective therapeutic nanoparticles, researchers have designed novel multifunctional nanoparticle platforms for cell/tissue-specific targeting, sustained or triggered drug delivery, co-delivery of synergistic drug combinations, etc. Among these functions, we believe that spatial and temporal controls in drug delivery may be critical for the successful development of next-generation nanotechnology products [5].

---

A. Swami • J. Shi • S. Gadde • A.R. Votruba • N. Kolishetti • O.C. Farokhzad (✉)  
Department of Anesthesiology, Laboratory of Nanomedicine and Biomaterials,  
Brigham and Women's Hospital, and Harvard Medical School, Boston, MA 02115, USA  
e-mail: ofarokhzad@zeus.bwh.harvard.edu



**Fig. 2.1** Schematic presentation of passive vs. active targeting of nanoparticles. (i) Nanoparticles extravasate through the leaky vasculature and preferentially accumulate through the EPR effect. In this case of “passive targeting,” the drugs may be released in the extracellular matrix and diffuse throughout the tissue for bioactivity. Some of these nanoparticles might also be taken up nonspecifically. (ii) After extravasation in the target tissue, the ligand-conjugated nanoparticles actively interact with the receptors present on target cell or tissue, resulting in cellular uptake through receptor-mediated endocytosis. This is referred as “active targeting.” (iii) The targeted nanoparticles can be equipped for vascular targeting as well by incorporating ligands specific to endothelial cell surface receptors

Spatially controlled drug delivery can be obtained by conjugating drug-encapsulated nanoparticles with targeting ligands, which could facilitate the preferential delivery of nanotherapeutics to the sites of interest while reducing undesired side effects elsewhere. Since the first description of cell-specific targeted liposomes in 1980 [10, 11], targeted nanoparticles have shown some promising clinical and pre-clinical results in the treatment of different diseases. For tumor cell targeting, the presence of targeting ligands could enhance cellular uptake and retention of drugs via receptor-mediated endocytosis, although tumor accumulation through the enhanced permeability and retention (EPR) effect [12] is largely determined by the physico-chemical properties of nanoparticles and long circulation half-life (Fig. 2.1) [3].

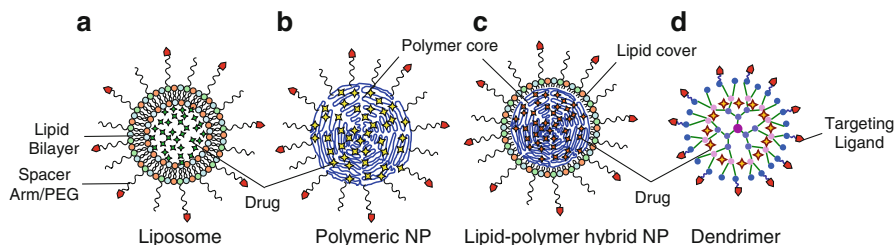
Active nanoparticle targeting is particularly essential for the delivery of biomacromolecules (e.g., DNA and siRNA) that require intracellular delivery for bioactivity [3]. In the case of vascular endothelial targeting for oncology or cardiovascular indications, ligand-mediated targeting may be critically important as nanoparticle localization is not a function of EPR (Fig. 2.1) [13–15]. In addition, efforts have been made to transport drugs across tight epithelial and endothelial barriers with nanotherapeutics (e.g., the blood–brain barrier) via ligand-mediated transcytosis [16]. More recently, targeted nanoparticles have been employed in solving the complex problems of multidrug resistance [8].

Controlled release polymer technology, resulting in the temporal control of drug exposure, has benefited virtually every branch of medicine over the past 4 decades. Many products utilizing this technology are now in clinical use, including Atridox<sup>®</sup>, Lupron Depot<sup>®</sup>, Gliadel<sup>®</sup>, Zoladex<sup>®</sup>, Trelstar<sup>®</sup> Depot, and Sandostatin<sup>®</sup> LAR [17]. Polymeric nanoparticles can encapsulate drugs and release them at sustained rates in the optimal range of drug concentration, thus enhancing the *in vivo* therapeutic efficacy, maximizing patient compliance, and facilitating the use of highly toxic, poorly soluble, or relatively unstable drugs [17, 18]. In general, drug release can be regulated by diffusion of the drug molecules through the polymer matrix or by differential surface and bulk erosion of the polymer [19]. Alternatively, drug release can be triggered by specific microenvironments in the body (e.g., changes in pH, temperature, and enzymatic activities) or manipulated by external events (e.g., electric field, magnetic field, and ultrasound) [20–22]. By further functionalization with targeting ligands, controlled release polymeric nanoparticles could deliver therapeutic agents in a spatiotemporally regulated fashion, which may be essential to many medical applications.

In this chapter, we focus on the major classes of organic nanoparticle platforms (*i.e.*, liposomes, polymeric nanoparticles, lipid–polymer hybrid nanoparticles, and dendrimers), their applications in targeted and/or temporally controlled delivery of therapeutic molecules, and their optimal design for escaping immune surveillances, as well as review the various available classes of ligands for targeted drug delivery applications.

## 2.2 Nanoparticle Platforms for Targeted and Temporal Delivery

Over the past few decades, different nanotechnology platforms were studied for their use in therapeutic applications [8, 9]. These nanoparticle platforms have been developed to enhance the pharmacological properties and therapeutic index of a myriad of drugs [1, 23]. Herein, we discuss four major classes of organic nanoparticle delivery systems, including liposomes, polymeric nanoparticles, lipid–polymer hybrid nanoparticles, and dendrimers (Fig. 2.2), which can encapsulate drugs with high loading efficiency and protect them from undesired effects of external conditions [3].



**Fig. 2.2** Schematic representation of several major therapeutic nanoparticle platforms including liposome, polymeric nanoparticles, lipid–polymer hybrid nanoparticles, and dendrimers for the targeted and/or temporally controlled delivery of drugs

### 2.2.1 Liposomal Platforms

Liposomes are artificial, single, or multilaminar vesicles made with bilayered membrane structures, composed of natural or synthetic amphiphilic lipid molecules (Fig. 2.2a). As drug delivery carriers, liposomes exhibit several unique properties including favorable safety profiles, long systemic circulation half-life, and ease of surface modifications [24]. Among the clinically validated nanotechnology products, liposomal drugs were the first nanotherapeutics to get FDA approval for clinical use. Since the approval of DOXIL<sup>®</sup> (doxorubicin liposomes) for the treatment of AIDS associated with Kaposi's sarcoma in 1995 [9], several other liposomal drugs have been approved for clinical use, and many are in the various stages of clinical development, as shown in Tables 2.1 and 2.2. In addition to small molecule drugs, liposome systems also allow for the delivery of bioactive macromolecules (e.g., DNA) for therapeutic applications [25]. For example, Allovectin-7, composed of cationic lipid-based liposomes (DMRIE–DOPE), can carry plasmid DNA encoding HLA-B7 and  $\beta$ 2 microglobulin that stimulate both innate and adaptive immune responses for cancer treatment [26].

Ligand-conjugated liposomes have also shown potential to enhance the therapeutic efficacy of drugs through targeted delivery. Three targeted liposomal systems have already entered clinical trials. MCC-465, a PEGylated liposome, is tagged with the F(ab')<sub>2</sub> fragment of the human monoclonal antibody GAH. Although this product does not appear to have progressed through development after a Phase I clinical trial, it has demonstrated superior cytotoxic activity against several human stomach cancer cells [27]. Recently, a novel *N*-glutaryl phosphatidylethanolamine (NGPE)-liposome formulation, MBP-426, which is conjugated to the human transferrin (Tf) ligand, improved the safety and efficacy of oxaliplatin through prolonging the drugs circulation time and by specifically targeting Tf receptors on tumor cells [28]. Another liposomal drug (SGT-53) also targets the Tf receptor on tumor cell surfaces by using the ligand of the anti-Tf receptor single-chain antibody fragment (Tf-R-scF<sub>v</sub>) for the delivery of the tumor suppressor gene p53 [8].

The significance of targeted liposomes is further highlighted by extensive preclinical studies. For example, the monoclonal antibody 2C5 (mAb 2C5) recognizes intact

**Table 2.1** Examples of nontargeted nanoparticles for drug delivery applications that are FDA approved or in clinical trials

Platform	Trade name	Drug	Clinical stage	Company
<b>Liposomes</b>				
PEGylated liposome	Doxil/Caelyx SPL-77	Doxorubicin	Approved	Ortho Biotech, Schering-Plough
	Myocet	Cisplatin	Phase II	Sequus Pharma.
Non-PEGylated liposome	DaunoXome	Doxorubicin	Approved	Zeneus
	Onco TCS	Daunorubicin	Approved	Gilead Sciences
	AmBisome	Vincristine	Phase III (complete)	INEX Pharma.
	LE-SN38	Amphotericin B	Approved	Gilead Sciences
	CPX-1	SN-38	Phase II	NeoPharm
	DepoCyt	CPT-11, floxuridine	Phase II	Celator Pharma.
	NX211	Cytarabine	Phase I/II	SkyePharma
	Nyotran	Lurtotecan	Phase II	Gilead Sciences
	Atragen	Nystatin	Phase I/II	Aronex Pharma.
	Aroplatin	All-trans retinoic acid	Phase II	Aronex Pharma.
	Annamycin	NDDP	Phase II	Aronex Pharma.
	L9NC	Annamycin	Phase I/II	Callisto
	Allovectin-7	Camptothecin	Phase II	Verschraegen
	EndoTAG	DNA plasmid	Phase III	Vical Inc.
	E1A gene	Paclitaxel	Phase II	MediGene
		E1A gene	Phase I	Targeted Genetics Corp.
<b>Non-PEGylated, cationic liposome</b>				
Polymeric nanoparticles	Genexol-PM	Paclitaxel	Approved in Korea	Samyang
	NK911	Doxorubicin	Phase I	Nippon Kayaku
	NK105	Paclitaxel	Phase II	Nano carrier
	NC-6004	Paclitaxel	Phase I/II	Nano carrier
	NK012	Cisplatin	Phase II	Nippon Kayaku
	CRLX101	SN-38	Phase II	Cerulean Pharma
	SPI049C	Camptothecin	Phase II	Supratek Pharma
		Doxorubicin	Phase III	

**Table 2.2** Targeted nanoparticles for drug delivery in different stages of clinical studies

Platform	Trade name	Targeting ligand	Drug	Clinical stage	Company
PEGylated liposome	MCC-465	F(ab') <sub>2</sub> fragment of human antibody GAH	Doxorubicin	Phase I	Mitsubishi Pharma.
NGPE liposome Liposome	MBP-426	Transferrin	Oxaliplatin	Phase I/II	MebioPharm
	SGT-53	Single-chain antibody fragment	p53 gene	Phase I	SynerGene therapeutics
Cyclodextrin-based polymeric NP	CALAA-01	Transferrin	siRNA	Phase I	Calando Pharma.
PEGylated PLGA NP	BIND-014	Peptide	Docetaxel	Phase I	BIND Biosciences

nucleosomes (originating from apoptotically dying neighboring tumor cells), bound to the surface of live tumor cells. Conjugation of mAb 2C5 to DOXIL liposomes resulted in improved biodistribution and cell targeting and in increased drug efficacy [29]. Liposomes carrying poly(ethylene glycol) (PEG) chains on their surface and loaded with doxorubicin have been coupled with RGD peptides to target the integrins of tumor vasculature and have demonstrated increased efficacy against C26 colon carcinoma in murine model [30]. Folate-functionalized liposomes, encapsulating fluorescent calcein and doxorubicin, and targeting the folate receptor (type- $\beta$ ), have been used for the treatment of acute myelogenous leukemia [31]. However, while liposomes are commonly explored for drug delivery applications, they do not readily allow for sustained release of therapeutic molecules, which marks a significant shortcoming of this class of nanocarriers [32].

### 2.2.2 Polymeric Nanoparticles

Polymer–drug conjugates have made a significant clinical impact by improving the pharmaceutical efficacy and dosing of a variety of already approved drugs [8, 33]; however, their drug loading efficiency may be limited by the number of conjugation sites in the polymer, and most of them lack the ability of active targeting or controlling drug release. In order to further enhance the drug loading capacity and incorporate the spatial and/or temporal control over drug delivery, many biocompatible polymeric nanoparticle platforms have been developed [34–36].

Polymeric micelles have attracted substantial attention for their remarkable potential as therapeutic carriers [37]. Polymeric micelles can be formed by self-assembly of amphiphilic polymers with two or more polymer chains of different hydrophobicity. In aqueous environments, these block copolymers can spontaneously self-assemble into core-shell nanostructures, with a hydrophobic core and a hydrophilic shell (Fig. 2.2b) [35, 37]. To date several polymeric micelles have reached different stages of clinical development, and these systems have demonstrated enhanced accumulation of therapeutic agents at the target site and/or reduced adverse effects of therapeutic agents (Table 2.1) [9, 38]. Among them, NK911 [39] and NK105 [40] utilize PEG-poly(aspartic acid) copolymer to carry and protect the anticancer agents doxorubicin and paclitaxel, respectively. Notably, NK105 was shown to reduce the reported adverse effects of paclitaxel, which include neurotoxicity, myelosuppression, and allergic reactions [40]. A cisplatin-incorporated polymeric micelle-based system, NC-6004, is being examined in Phase I/II clinical trials and has demonstrated several distinct features, including sustained cisplatin release, promoted accumulation of cisplatin in cancer cells, and reduced nephrotoxicity and neurotoxicity associated with cisplatin [41]. Another PEG-poly(glutamic acid)-based polymeric micelle, NK012, loaded with 7-ethyl-10-hydroxycamptothecin (SN-38), has been shown to exert more potent antitumor activity against various human tumor xenografts than irinotecan (CPT-11), a water-soluble prodrug of SN-38 [42]. More impressively, the nontargeted polymeric micelle composed of



poly(L-lactic acid) (PLA)-PEG (Genexol<sup>®</sup>-PM), for delivery of paclitaxel, was first approved for cancer therapy in Korea in 2007 [8] and is currently being evaluated in a clinical Phase II trial in the United States for the treatment of metastatic pancreatic cancer [43, 44].

The conjugation of polymeric nanoparticles with targeting ligands could also enable drug delivery in a spatially and temporally controlled manner, which may further enhance the therapeutic efficacy of drugs and reduce their toxic side effects. Our group has pioneered the development of aptamer-targeted polymeric nanoparticles and applied these nanoparticles to cancer therapy [14, 45–48]. For example, we have developed A10 RNA aptamer-conjugated poly(lactide-*co*-glycolide)-poly(ethylene glycol) (PLGA-PEG) nanoparticles that can recognize PSMA (prostate-specific membrane antigen), expressed on the cancer cell surface [49]. This PLGA-PEG-aptamer nanoparticle can substantially reduce tumor growth in a human prostate cancer tumor xenograft mouse model. More recently, we have reported a strategy for precisely engineering PLGA-PEG-aptamer nanoparticles with different biophysicochemical properties in a reproducible manner, whereby enabling the systematic screening of the targeted polymer nanoparticles for optimization [50]. Building on these efforts, BIND Biosciences has developed a self-assembled, targeted polymeric nanoparticle (BIND-014) and is currently evaluating this nanotherapeutic candidate in Phase I/II clinical trials for the treatment of solid tumors [51].

### 2.2.3 Lipid-Polymer Hybrid Nanoparticles

The success of polymeric nanoparticles and liposomes has also motivated the development of lipid-polymer hybrid nanoparticles (Fig. 2.2c), which could integrate the unique advantages of both polymeric nanoparticle and liposome systems, while overcoming some of their limitations. Thus far, several important lipid-polymer hybrid nanoparticles have been developed. For example, lipid-coated polymeric nanoparticles comprising a PLGA core, a PEG shell, and a lipid monolayer at the interface were recently described and characterized [13, 52–56]. The PLGA core is capable of carrying poorly water-soluble drugs, while the PEG shell helps to decrease biofouling and increase circulation half-life. The lipid monolayer that resides at the interface between PLGA core and PEG shell acts as a molecular fence, promoting drug retention and sustained release from the polymeric core [52, 56]. When compared to PLGA and PLGA-PEG nanoparticles, this lipid-coated PLGA nanoparticle allows for higher drug encapsulation, tunable and sustained drug release over a longer period of time, and excellent serum stability [56]. In another example, a liposome-enveloped PLGA nanoparticle, known as “nanocell,” was developed in a multistep manner for the effective treatment of cancers. The nanocell has a PLGA core encapsulating the PLGA-conjugated anticancer drug doxorubicin,



and a lipid multilayer shell containing the antiangiogenic agent, combretastatin. The synergistic effect of the two drugs is obtained through temporally controlled release, where combretastatin is first released to reduce vascularization, while the sustained release of doxorubicin from the nanocell directly kills the tumor cells [57].

Lipid-coated polymeric nanoparticles, developed by Zhang *et al.*, showed enhanced uptake in prostate cancer cells overexpressing PSMA antigens when conjugated with A10 aptamer, as compared to nontargeted hybrid nanoparticles [56]. More recently, Wang *et al.* have applied A10 aptamer-targeted lipid-polymer hybrid nanoparticles for the concurrent administration of a chemotherapeutic agent (docetaxel) and a radiotherapeutic agent ( $^{111}$ -indium or  $^{90}$ -yttrium), which demonstrated higher level of cellular cytotoxicity, as compared to targeted nanoparticles containing only a single agent or nontargeted nanoparticles [55]. For the treatment of injured vasculature, Chan *et al.* developed a “nanoburr” system by conjugating the lipid-coated PLGA hybrid nanoparticle with a novel peptide ligand, screened from a combinatorial library of heptapeptide ligands against human collagen IV, which represents 50% of the vascular basement membrane [13]. The peptide-conjugated “nanoburr” demonstrated efficient targeting toward vascular basement membrane, high nanoparticle accumulation in the region of injured vasculature in a rat model, and sustained drug release over 2 weeks.

## 2.2.4 Dendrimers

Dendrimers are synthetic, branched macromolecules with a well-defined chemical structure (Fig. 2.2d), consisting of an initiator core and multiple layers with active terminal groups [9, 58]. Their specific molecular structure enables dendrimers to carry various drugs via covalent conjugation to the multivalent surfaces or encapsulation in the cavities of the cores through hydrophobic interaction, hydrogen bond, or chemical linkage [59, 60]. Besides, dendrimers can also carry bioactive macromolecules such as DNA by condensing them through electrostatic interactions [61]. The rigidity and the density of the branched units of dendrimers affect drug release kinetics. By use of pH- or enzyme-sensitive linkages, stimulus-responsive dendrimers can be generated [62].

Dendrimers are emerging as an important class of nanoparticle carriers for therapeutic delivery. For example, SPL7013 (L-lysine-based dendrimer) can be used in delivering microbicide for prevention of HIV and other sexually transmitted infections (STI) [63]. Frechet *et al.* have developed a biodegradable polyester dendritic drug delivery system with different architectures and molecular weights, for the delivery of doxorubicin [64]. Dendrimers composed of poly(amidoamine) (PAMAM) polymers have also been extensively investigated for the effective delivery of small molecular drugs [65]. Besides, the cationic nature of PAMAM, dendrimers allow them to effectively deliver macromolecular drugs such as DNA across cellular and subcellular barriers (*e.g.*, cell membrane and endosome) [66]. Attaching targeting ligands to their surface could further enhance the potential of PAMAM dendrimers

in drug delivery. A case in hand is a folate-conjugated, methotrexate-loaded PAMAM(G5) dendrimer, which has demonstrated a tenfold reduction in tumor size and exhibited less systemic toxicity, compared to free methotrexate [67].

## 2.3 Optimal Design of Nanoparticles

One significant challenge for the successful development of therapeutic nanoparticles is rapid clearance during systemic delivery. When nanoparticles enter the bloodstream, the particle surface may experience nonspecific protein adsorption (opsonization), thereby making them more visible to phagocytic cells [67–69]. After opsonization, nanoparticles could be rapidly cleared from the bloodstream through phagocytosis by the mononuclear phagocyte system (MPS) in the liver and by spleen filtration [70, 71]. Therefore, the factors that could affect the clearance and biodistribution of nanoparticles, such as particle physicochemical properties and targeting ligand functionalization [68], should be carefully considered for the optimal design of therapeutic nanoparticles.

### 2.3.1 *Size*

On the basis of physiological parameters such as hepatic filtration, tissue extravasation/diffusion, and kidney excretion, it is clear that particle size plays a key factor in the long circulation and biodistribution of nanoparticles. Nanoparticles smaller than 10 nm can be rapidly cleared by the kidneys or through extravasation, while larger nanoparticles may have higher tendency to be cleared by cells of the mononuclear phagocyte system (MPS also referred to as reticuloendothelial system, RES) [4]. For example, in vivo biodistribution results of polystyrene nanoparticles with consistent composition and varying particle size of 50 and 500 nm showed higher level of agglomeration of the larger nanoparticles in the liver [72]. Another study compared different size ranges of PEGylated spherical nanoparticles (<100 nm, 100–200 nm, and >200 nm) for protein absorption, nanoparticle uptake by murine macrophages, and blood clearance kinetics [73]. It was observed that nanoparticles <100 nm have a higher potential to circulate in the blood for long periods of time and experience reduced hepatic filtration. Nanoparticle size also plays a key role in tumor accumulation through the EPR effect. Several studies have tried to determine the gap size in the leaky vasculature. For example, sterically stabilized liposomes of 100–600 nm were used for transvascular transport, and the cutoff size of the pores was estimated to be 400–600 nm in diameter [74]. In another study, the pore cutoff size was estimated to be between 7 and 100 nm at 34°C and was increased to >400 nm at 42°C, allowing all nanoparticles tested (~7 nm albumin, and 100, 200, and 400 nm liposomes) to be delivered to the tumor interstitium to some degree [75]. Therefore, to capitalize on the EPR effect and to efficiently escape from the physiological barriers, many studies advocate the optimal nanoparticle size range of approximately 10–250 nm [68].

### 2.3.2 Surface Charge

It has been established that the surface charge of nanoparticles also could affect their uptake by the MPS cells. Neutrally charged particles have demonstrated much lower opsonization rates than charged particles [76, 77]. It was found that positively charged nanoparticles generate a higher immune response (complement activation and conjugate activation) compared to neutral or negatively charged nanoparticle formulations [53]. For example, nanoparticles with a primary amine at the surface promote higher rates of phagocytic uptake when compared to those having sulfate, hydroxyl, or carboxyl groups at the surface [53, 68]. In a review study, Davis *et al.* have proposed that the optimal range of nanoparticle surface charge should be between  $-10$  and  $+10$  mV for reduced phagocytosis and minimized nonspecific interactions of nanoparticles [78].

### 2.3.3 PEGylation

Surface modification of nanoparticles with PEG, which has favorable intrinsic physicochemical properties (*e.g.*, high flexibility and hydrophilicity, and low toxicity and immunogenicity), was found to reduce nanoparticle accumulation in off-target organs such as liver and spleen [79]. A PEG shell on the nanoparticle surface shields hydrophobic or charged particles from attachment by blood proteins, leading to prolonged circulation half-life compared to non-PEGylated nanoparticles [25, 80]. The length, shape, and density of PEG chains on the nanoparticle surface largely affect its surface hydrophilicity and phagocytosis [81]. For example, at low PEG surface density, the PEG chains would be closer to the surface of the nanoparticle with a “mushroom” configuration, while as the density increases, most of the chains are extended away from the surface in a “brush” configuration, which decides the thickness of the PEG shell on the nanoparticle corona [69]. It has been postulated that the brush configuration would create more effective blocking or repulsion of opsonins than the mushroom one [80]. In addition to PEG, some other promising hydrophilic polymers are under investigation for the same purpose, including natural polymers (*e.g.*, heparin, dextran, and chitosan) and synthetic polymers (*e.g.*, poly(amino acids), poly(glycerols), poly(2-oxazolines), and some vinyl polymers) [79, 82].

### 2.3.4 Ligand Functionalization

The conjugation of targeting ligands to the surface of PEGylated nanoparticles has also been shown to affect their biodistribution [83]. Although targeting ligands could improve the cell- or tissue-specific delivery of nanoparticles, they may compromise the particle surface properties by masking the PEG layer and adversely affecting the nanoparticles’ antibiofouling properties *in vivo*. Our recent study on

the effect of ligand density has also revealed a relatively narrow window of ligand density that could result in favorable tumor targeting, while minimizing nanoparticle accumulation in the liver and spleen [50]. Thus, the successful development of targeted nanoparticle technology for efficient drug delivery strongly depends on striking a balance between cellular targeting and immune evasion.

## 2.4 Targeting Ligands

Despite their enormous potential for drug delivery, the translation of targeted nanoparticle systems has faced considerable challenges, and only a handful of candidates have made it to clinical trials (Table 2.2). The reason targeted nanoparticles have demonstrated limited success in clinical development is complex and could be multifaceted [3]. Among others, an essential aspect for the successful development of targeted nanoparticles relies on the choice of targeting ligands. Several variables that could be considered include ligand biocompatibility, cell specificity, binding affinity, and purity of the ligand [84]. Other important factors that have to be taken into account are the size and charge of the ligand molecule, and their ease of modification and conjugation to the nanoparticles. The choice of ligand, from a practical perspective, is also dependent on production cost, scalability, and stability (*e.g.*, organic solvent and high temperature stability) in mass production. In this section, we discuss five different classes of targeting ligands, including antibodies and antibody fragments, aptamers, peptides, sugars, and small molecules.

### 2.4.1 *Antibodies and Antibody Fragments*

Antibodies and antibody fragments form an important class of targeting ligands with a high degree of specificity for cellular receptors and a wide range of binding affinities and have been extensively investigated in targeted drug delivery [85]. Over the past 2 decades, the feasibility of antibody-based tissue targeting has been clinically demonstrated with several different monoclonal antibodies (mAbs) approved by the FDA [86]. The recent advances in hybridoma technology have led to the development of chimeric, humanized, and fully human mAbs to reduce their immunogenicity. The ability of engineered mAbs to target disease processes has been demonstrated by the success of several monoclonal antibody therapeutics, including cetuximab rituximab, trastuzumab, and bevacizumab [19]. mAbs have been used to direct the nanoparticle carriers in a site-specific manner. For example, mAb-conjugated PLA nanoparticles exhibited a sixfold increase in the rate of particle uptake compared with nontargeted particles [87, 88]. Additionally, J591, a mAb against PSMA, was conjugated to G5-PAMAM dendrimers and showed enhanced binding affinity for LNCaP cells, as compared to nontarget PC3 cells [89]. Nevertheless, mAb-conjugated nanoparticles encounter considerable challenges and limitations for drug

delivery, since mAb are complex and large (~150 kDa) molecules and require significant engineering at the molecular level to be effective [90, 91].

Compared to mAbs, antibody fragments have demonstrated higher potential for the engineering of targeted nanoparticles as they are smaller in size and lack the complement activation region of mAbs, while retaining the antigen binding specificity [92]. Recent advances in protein engineering have led to the development of antibody fragments such as scFv (single-chain variable fragments), Fab (fragments of antigen binding), their dimers (F(ab')<sub>2</sub> and diabody), and recombinant products [93]. Some pioneering examples of antibody fragment-targeted liposomes (immunoliposomes) in clinical trials include MCC-465 that uses F(ab')<sub>2</sub> for the targeted delivery of doxorubicin [28, 94] and SGT-53 that uses scFv to deliver tumor suppressor gene, p53 [95].

## 2.4.2 Aptamers

Nucleic acid aptamers are single-stranded DNA or RNA oligonucleotides with well-defined, three-dimensional structures. Selected by systematic evolution of ligands by exponential enrichment (SELEX), aptamers can recognize a wide variety of molecules (*e.g.*, proteins, phospholipids, sugars, and nucleic acids) with high affinity and specificity [96–98]. This SELEX process uses the concepts of evolution, diversification, selection, and replication, where a library of ~10<sup>15</sup> random oligonucleotides is enriched to identify specific aptamers that can specifically recognize the target [97]. Aptamers identified through the SELEX process can be chemically synthesized with minimal batch-to-batch variation in a fast and cost-effective manner. When compared with antibodies, aptamers exhibit lower immunogenicity and a relatively smaller size compared with ~150 kD for antibodies, which enables better tissue penetration [99–101]. To further improve on their low serum stability, aptamers can be modified by incorporating 2'-amino, 2'-fluoro, or 2'-*O*-alkyl nucleotides in their backbone [102].

To date, more than 200 aptamers against a variety of biological targets have been isolated, such as cell surface antigens, therapeutic targets, and various growth factors like VEGF [103, 104]. Most notably, the FDA approved an aptamer against VEGF<sub>165</sub>, known as Pegaptanib, for the treatment of age-related macular degeneration (AMD) [105]. The PSMA-specific aptamers have been widely used for the targeted delivery of quantum dots [106], gold nanoparticles [107], and polymeric nanoparticles [14, 46, 108]. We have recently tested A10 aptamer-conjugated PLGA–PEG nanoparticles for targeted drug delivery using prostate cancer model. These PLGA–PEG–Apt nanoparticles can enhance the therapeutic effect of anticancer drugs and reduce systematic toxicity when compared to nontargeted nanoparticles [109]. More recently DNA aptamers, generated through cell-SELEX, have been conjugated with different types of nanoparticles (*e.g.*, magnetic and gold nanoparticles) for cancer detection and treatment [110–112].

### 2.4.3 Peptides

Peptide ligands have shown significant targeting potential because of their small size, high stability, and relative ease of large-scale synthesis with excellent quality control. The development of phage display techniques [113, 114] and other screening methods has enabled the discovery of new peptide-targeting domains and the isolation of new cell-specific peptide ligands [115, 116]. Peptide-conjugated nanoparticles have been widely used for targeting cancer cells and tumor vasculature [117, 118]. For example, the peptide SP5-52 can recognize tumor neovasculature, while avoiding normal blood vessels in severe, combined immunodeficiency mice bearing human tumors. The SP5-52 peptide-linked liposome has shown to greatly enhance the therapeutic effect of doxorubicin, decrease the growth of tumor blood vessels, and enable high survival rates among human lung and oral cancer-bearing xenograft mice [119]. Recently, this system has been used to target non-small-cell lung cancer (NSCLC) cells and demonstrated increased drug accumulation in tumor tissues by 5.7-fold compared with free drugs [120].

In the case of targeting integrin receptors (e.g.,  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$ ), short peptide antagonists have been developed based on a 2-benzazepine Gly–Asp mimetic or screened from an Arg–Gly–Asp-based (RGD) peptidomimetic library [121, 122]. For example, the cyclic version of the RGD motif has demonstrated effective binding toward integrins and has been extensively investigated in targeting nanoparticles for disrupting tumor angiogenesis [123]. For intra-articular targeting and retention in cartilage, the peptide ligand WYRGRL against collagen, type II,  $\alpha 1$  (COL2A1) was used in the targeted delivery of polymeric nanoparticles [15]. We have recently screened specific targeting peptides against collagen, type IV of the basement membrane and conjugated to lipid-coated polymeric nanoparticles for vascular wall targeting [13].

### 2.4.4 Sugars

Specific sugar molecules (e.g., lactose, galactose, and mannose) can recognize lectins that are overexpressed on the surface of numerous cancer cells [124, 125]. Thus, sugar molecules represent another interesting approach to specifically target nanoparticle systems to cancer cells. For example, galactose could recognize the asialoglycoprotein receptor which is expressed on hepatocytes, and its high expression is retained on primary liver cancer cells [125]. The galactosamine-conjugated *N*-(2-hydroxypropyl) methacrylamide copolymers (HPMA) (PK2) is currently under clinical evaluation for the treatment of primary liver cancer [8]. In another study, lectin-mediated endocytosis of sugar-conjugated HPMA copolymer conjugates in three different human colon cancer cell lines suggested their potential use for targeted delivery of chemotherapeutics to colon adenocarcinoma [126]. However, to compensate for the weak binding affinity of carbohydrates, multiple or multivalent molecules should be conjugated to the surface of nanoparticles to achieve multivalent interactions. In the case of galactosylated liposomal

carriers, it was shown that the targeting efficacy depended on the galactose ligand density [127].

### 2.4.5 Small Molecules

Small molecules have also attracted considerable attention as potential targeting ligands due to their low molecular weights, low production costs, and easy conjugation with nanoparticles. The small size of this kind of targeting ligand allows the functionalization of multiple ligand molecules on single nanoparticles. Folic acid, which is essential in many metabolic processes for cell survival, has shown high specificity in recognizing folate receptors that are overexpressed in many types of tumor cells [128]. There are several examples of folate-conjugated nanoparticles in drug delivery [129, 130], including liposomes, polymeric nanoparticles, and dendrimers [67, 131–134]. These nanoparticles have demonstrated to be effective in treating ovarian, breast, lung, renal, and colon cancers [135, 136]. However, immunohistochemistry studies have shown overexpression of folate receptors in normal tissues such as the placenta and kidneys as well, raising some concerns for the translation of folate-targeted nanoparticles from bench to bedside.

The development of small molecule-targeting ligands that demonstrate a high affinity and specificity toward cellular receptors has proven to be a challenging task. One strategy to improve the targeting of small molecule-conjugated nanoparticles is through multivalent binding effects, by conjugating multiple ligands on the nanoparticle surface. Another strategy is to select small molecules with high affinity and specificity by using high-throughput screening methods. For example, using fluorescent magnetic nanoparticles, Weissleder *et al.* have recently screened several small molecular ligands from a library of 146 small molecules ( $\leq 500$  Da), which can specifically bind to endothelial cells, activated human macrophages, and pancreatic cancer cells, respectively [137].

## 2.5 Conclusions

The application of nanoparticle technologies to drug delivery has demonstrated significant impact on many areas of medicine. The approval of more than two-dozen therapeutic nanoparticle products for clinical use has generated great enthusiasm in both academia and industry, although these first-generation nanoparticle therapeutics are relatively simple and only provide clinical benefits across a narrow range of clinically validated drugs. Toward the development of next-generation nanoparticles, the introduction of controlled release properties and targeting ligands is expected to enable the development of safer and more effective therapeutic nanoparticles. With continuous advances in identifying new biomarkers and associated targeting ligands, and in engineering nanoparticle delivery systems with optimal biophysicochemical properties, it will be increasingly feasible to develop targeted and controlled release nanoparticle products as promising candidates for clinical translation.



## References

1. Allen TM, Cullis PR (2004) Drug delivery systems: entering the mainstream. *Science* 303:1818–1822
2. Wagner V, Dullaart A, Bock A-K, Zweck A (2006) The emerging nanomedicine landscape. *Nat Biotechnol* 24(10):1211–1217
3. Farokhzad OC, Langer R (2009) Impact of nanotechnology on drug delivery. *ACS Nano* 3(1):16–20
4. Petros RA, DeSimone JM (2010) Strategies in the design of nanoparticles for therapeutic applications. *Nat Rev Drug Discov* 9(8):615–627
5. Shi J, Votruba AR, Farokhzad OC, Langer R (2010) Nanotechnology in drug delivery and tissue engineering: from discovery to applications. *Nano Lett* 10(9):3223–3230
6. Cai W, Chen X (2007) Nanoplatforms for targeted molecular imaging in living subjects. *Small* 3(11):1840–1854
7. Gao X et al (2005) In vivo molecular and cellular imaging with quantum dots. *Curr Opin Biotechnol* 16(1):63–72
8. Davis ME, Chen Z, Shin DM (2008) Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat Rev Drug Discov* 7(9):771–782
9. Zhang L et al (2007) Nanoparticles in medicine: therapeutic applications and developments. *Clin Pharmacol Ther* 83(5):761–769
10. Heath T, Fraley R, Papahadjopoulos D (1980) Antibody targeting of liposomes: cell specificity obtained by conjugation of F(ab')<sub>2</sub> to vesicle surface. *Science* 210:539–541
11. Leserman LD, Barbet J, Kourilsky F, Weinstein JN (1980) Targeting to cells of fluorescent liposomes covalently coupled with monoclonal antibody or protein A. *Nature* 288:602–604
12. Maeda H (2001) The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. *Adv Enzyme Regul* 41:189–207
13. Chan JM et al (2010) Spatiotemporal controlled delivery of nanoparticles to injured vasculature. *Proc Natl Acad Sci USA* 107(5):2213–2218
14. Dhar S, Kolishetti N, Lippard SJ, Farokhzad OC (2011) Targeted delivery of a cisplatin pro-drug for safer and more effective prostate cancer therapy in vivo. *Proc Natl Acad Sci USA* 108(5):1850–1855
15. Rothenfluh DA, Bermudez H, O'Neil CP, Hubbell JA (2008) Biofunctional polymer nanoparticles for intra-articular targeting and retention in cartilage. *Nat Mater* 7(3):248–254
16. Georgieva JV et al (2011) Surface characteristics of nanoparticles determine their intracellular fate in and processing by human blood–brain barrier endothelial cells in vitro. *Mol Ther* 19(2):318–325
17. Farokhzad OC, Langer R (2006) Nanomedicine: developing smarter therapeutic and diagnostic modalities. *Adv Drug Deliv Rev* 58(14):1456–1459
18. Brigger I, Dubernet C, Couvreur P (2002) Nanoparticles in cancer therapy and diagnosis. *Adv Drug Deliv Rev* 54(5):631–651
19. Wang AZ et al (2008) Biofunctionalized targeted nanoparticles for therapeutic applications. *Expert Opin Biol Ther* 8(8):1063–1070
20. Ganta S, Devalapally H, Shahiwal A, Amiji M (2008) A review of stimuli-responsive nanocarriers for drug and gene delivery. *J Control Release* 126(3):187–204
21. Kale AA, Torchilin VP (2010) Environment-responsive multifunctional liposomes. *Methods Mol Biol* 605:213–242
22. Oh KT, Yin H, Lee ES, Bae YH (2007) Polymeric nanovehicles for anticancer drugs with triggering release mechanisms. *J Mater Chem* 17(38):3987–4001
23. Moghimi SM, Hunter AC, Murray JC (2005) Nanomedicine: current status and future prospects. *FASEB J* 19(3):311–330
24. Antimisiaris SG, Kallinteri P, Fatouros DG (2007) Liposomes and drug delivery. Wiley, New York, pp 443–533

25. Moghimi SM, Szebeni J (2003) Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein-binding properties. *Prog Lipid Res* 42(6):463–478
26. Chowdhery R, Gonzalez R (2011) Immunologic therapy targeting metastatic melanoma: Allovectin-7. *Immunotherapy* 3(1):17–21
27. Matsumura Y et al (2004) Phase I and pharmacokinetic study of MCC-465, a doxorubicin (DXR) encapsulated in PEG immunoliposome, in patients with metastatic stomach cancer. *Ann Oncol* 15(3):517–525
28. Sankhala KK, Mita AC, Adinin R, Wood L, Beeram M, Bullock S, Yamagata N, Matsuno K, Fujisawa T, Phan AT (2009) A phase I pharmacokinetic (PK) study of MBP-426, a novel liposome encapsulated oxaliplatin. *J Clin Oncol* 27(15S):2535
29. Lukyanov AN, Elbayoumi TA, Chakilam AR, Torchilin VP (2004) Tumor-targeted liposomes: doxorubicin-loaded long-circulating liposomes modified with anti-cancer antibody. *J Control Release* 100(1):135–144
30. Schifferers RM et al (2003) Anti-tumor efficacy of tumor vasculature-targeted liposomal doxorubicin. *J Control Release* 91(1–2):115–122
31. Pan XQ et al (2002) Strategy for the treatment of acute myelogenous leukemia based on folate receptor beta-targeted liposomal doxorubicin combined with receptor induction using all-trans retinoic acid. *Blood* 100(2):594–602
32. Torchilin VP (2005) Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov* 4(2):145–160
33. Greco F, Vicent MJ (2009) Combination therapy: opportunities and challenges for polymer-drug conjugates as anticancer nanomedicines. *Adv Drug Deliv Rev* 61(13):1203–1213
34. Bae Y et al (2004) Preparation and biological characterization of polymeric micelle drug carriers with intracellular pH-triggered drug release property: tumor permeability, controlled subcellular drug distribution, and enhanced in vivo antitumor efficacy. *Bioconjug Chem* 16(1):122–130
35. Chan JM, Valencia PM, Zhang L, Langer R, Farokhzad OC (2010) Polymeric nanoparticles for drug delivery. *Methods Mol Biol* 624:163–175
36. Napier ME, DeSimone JM (2007) Nanoparticle drug delivery platform. *Polym Rev* 47(3):321–327
37. Matsumura Y, Kataoka K (2009) Preclinical and clinical studies of anticancer agent-incorporating polymer micelles. *Cancer Sci* 100(4):572–579
38. Sutton D, Nasongkla N, Blanco E, Gao J (2007) Functionalized micellar systems for cancer targeted drug delivery. *Pharm Res* 24(6):1029–1046
39. Matsumura Y, Phase I (2004) Clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin. *Br J Cancer* 91:1775–1781
40. Hamaguchi T et al (2005) NK105, a paclitaxel-incorporating micellar nanoparticle formulation, can extend in vivo antitumor activity and reduce the neurotoxicity of paclitaxel. *Br J Cancer* 92(7):1240–1246
41. Wilson RHP, Adam R, Eatock J, Boddy MM, Griffin AV, Miller MR, Matsumura Y, Shimizu T, Calvert V (2008) Phase I and pharmacokinetic study of NC-6004, a new platinum entity of cisplatin-conjugated polymer forming micelles. *J Clin Oncol (Meeting Abstracts)* 26:2573
42. Hamaguchi T et al (2010) Phase I study of NK012, a novel SN-38-incorporating micellar nanoparticle, in adult patients with solid tumors. *Clin Cancer Res* 16(20):5058–5066
43. Kim T-Y et al (2004) Phase I and pharmacokinetic study of Genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies. *Clin Cancer Res* 10(11):3708–3716
44. Lee KS et al (2006) Multicenter phase II study of a cremophor-free polymeric micelle-formulated paclitaxel in patients with metastatic breast cancer (MBC). *J Clin Oncol (Meeting Abstracts)* 24(18\_suppl):10520
45. Alexis F et al (2008) HER-2-targeted nanoparticle-affibody bioconjugates for cancer therapy. *ChemMedChem* 3(12):1839–1843
46. Farokhzad OC et al (2006) Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. *Proc Natl Acad Sci USA* 103(16):6315–6320

47. Gao W, Chan JM, Farokhzad OC (2010) pH-responsive nanoparticles for drug delivery. *Mol Pharm* 7(6):1913–1920
48. Zhang L et al (2007) Co-delivery of hydrophobic and hydrophilic drugs from nanoparticle–aptamer bioconjugates. *ChemMedChem* 2(9):1268–1271
49. Farokhzad OC et al (2004) Nanoparticle–aptamer bioconjugates: a new approach for targeting prostate cancer cells. *Cancer Res* 64(21):7668–7672
50. Gu F et al (2008) Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers. *Proc Natl Acad Sci USA* 105(7):2586–2591
51. Service RF (2010) Nanoparticle Trojan horses gallop from the lab into the clinic. *Science* 330:314–315
52. Chan JM et al (2009) PLGA–lecithin–PEG core-shell nanoparticles for controlled drug delivery. *Biomaterials* 30(8):1627–1634
53. Salvador-Morales C, Zhang L, Langer R, Farokhzad OC (2009) Immunocompatibility properties of lipid-polymer hybrid nanoparticles with heterogeneous surface functional groups. *Biomaterials* 30(12):2231–2240
54. Valencia PM et al (2010) Single-step assembly of homogenous lipid-polymeric and lipid-quantum dot nanoparticles enabled by microfluidic rapid mixing. *ACS Nano* 4(3):1671–1679
55. Wang AZ et al (2010) ChemoRad nanoparticles: a novel multifunctional nanoparticle platform for targeted delivery of concurrent chemoradiation. *Nanomedicine* 5(3):361–368
56. Zhang L et al (2008) Self-assembled lipid-polymer hybrid nanoparticles: a robust drug delivery platform. *ACS Nano* 2(8):1696–1702
57. Sengupta S et al (2005) Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system. *Nature* 436:568–572
58. Paleos CM, Tsiourvas D, Sideratou Z, Tziveleka LA (2010) Drug delivery using multifunctional dendrimers and hyperbranched polymers. *Expert Opin Drug Deliv* 7(12):1387–1398
59. Lee CC, MacKay JA, Frechet MJM, Szoka FC (2005) Designing dendrimers for biological applications. *Nat Biotech* 23(12):1517–1526
60. Liu M, Kono K, Frechet MJM (2000) Water-soluble dendritic unimolecular micelles: their potential as drug delivery agents. *J Control Rel* 65(1–2):121–131
61. Xu Q, Wang CH, Pack DW (2010) Polymeric carriers for gene delivery: chitosan and poly(amidoamine) dendrimers. *Curr Pharm Des* 16(21):2350–2368
62. Gillies ER, Jonsson TB, Frechet MJM (2004) Stimuli-responsive supramolecular assemblies of linear-dendritic copolymers. *J Am Chem Soc* 126(38):11936–11943
63. McCarthy TD et al (2005) Dendrimers as drugs: discovery and preclinical and clinical development of dendrimer-based microbicides for HIV and STI prevention. *Mol Pharm* 2(4):312–318
64. Padilla De Jesus OL, Ihre HR, Gagne L, Frechet MJM, Szoka FC Jr (2002) Polyester dendritic systems for drug delivery applications: in vitro and in vivo evaluation. *Bioconjug Chem* 13(3):453–461
65. Patri AK, Kukowska-Latallo JF, Baker JR (2005) Targeted drug delivery with dendrimers: comparison of the release kinetics of covalently conjugated drug and non-covalent drug inclusion complex. *Adv Drug Deliv Rev* 57(15):2203–2214
66. Yellepeddi VK, Kumar A, Palakurthi S (2009) Surface modified poly(amido)amine dendrimers as diverse nanomolecules for biomedical applications. *Expert Opin Drug Deliv* 6(8):835–850
67. Kukowska-Latallo JF et al (2005) Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer. *Cancer Res* 65(12):5317–5324
68. Alexis F, Pridgen E, Molnar LK, Farokhzad OC (2008) Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Mol Pharm* 5(4):505–515
69. Owens DE III, Peppas NA (2006) Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *Int J Pharm* 307(1):93–102
70. Eisenstein M (2006) Protein arrays: growing pains. *Nature* 444:959–962
71. Ostuni E, Chapman RG, Holmlin RE, Takayama S, Whitesides GM (2001) A survey of structure–property relationships of surfaces that resist the adsorption of protein. *Langmuir* 17(18):5605–5620

72. Nagayama S, Ogawara K-I, Fukuoka Y, Higaki K, Kimura T (2007) Time-dependent changes in opsonin amount associated on nanoparticles alter their hepatic uptake characteristics. *Int J Pharm* 342(1–2):215–221
73. Fang C et al (2006) In vivo tumor targeting of tumor necrosis factor-[alpha]-loaded stealth nanoparticles: effect of mPEG molecular weight and particle size. *Eur J Pharm Sci* 27(1):27–36
74. Yuan F et al (1995) Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. *Cancer Res* 55(17):3752–3756
75. Kong G, Braun RD, Dewhirst MW (2000) Hyperthermia enables tumor-specific nanoparticle delivery: effect of particle size. *Cancer Res* 60(16):4440–4445
76. Roser M, Fischer D, Kissel T (1998) Surface-modified biodegradable albumin nano- and microspheres. II: effect of surface charges on in vitro phagocytosis and biodistribution in rats. *Eur J Pharm Biopharm* 46(3):255–263
77. Schwendener RA, Lagocki PA, Rahman YE (1984) The effects of charge and size on the interaction of unilamellar liposomes with macrophages. *Biochim Biophys Acta (BBA) - Biomembranes* 772(1):93–101
78. Davis ME (2009) The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: from concept to clinic. *Mol Pharm* 6(3):659–668
79. Knop K, Hoogenboom R, Fischer D, Schubert US (2010) Poly(ethylene glycol) in drug delivery: pros and cons as well as potential alternatives. *Angew Chem Int Ed* 49(36):6288–6308
80. Vonarbourg A, Passirani C, Saulnier P, Benoit J-P (2006) Parameters influencing the stealthiness of colloidal drug delivery systems. *Biomaterials* 27(24):4356–4373
81. Gref R et al (2000) ‘Stealth’ corona-core nanoparticles surface modified by polyethylene glycol (PEG): influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. *Colloids Surf B Biointerfaces* 18(3–4):301–313
82. Moghimi SM, Porter CJH, Illum L, Davis SS (1991) The effect of Poloxamer-407 on liposome stability and targeting to bone marrow: comparison with polystyrene microspheres. *Int J Pharm* 68(1–3):121–126
83. Takae S et al (2005) Ligand density effect on biorecognition by PEGylated gold nanoparticles: regulated interaction of RCA120 lectin with lactose installed to the distal end of tethered PEG strands on gold surface. *Biomacromolecules* 6(2):818–824
84. Allen TM (2002) Ligand-targeted therapeutics in anticancer therapy. *Nat Rev Cancer* 2(10):750–763
85. Torchilin VP (2008) Antibody-modified liposomes for cancer chemotherapy. *Expert Opin Drug Deliv* 5:1003–1025
86. Gabizon AA (2001) Pegylated liposomal doxorubicin: metamorphosis of an old drug into a new form of chemotherapy. *Cancer Invest* 19(4):424–436
87. Nellis DF et al (2005) Preclinical manufacture of an anti-HER2 scFv-PEG-DSPE, liposome-inserting conjugate. I. Gram-scale production and purification. *Biotechnol Prog* 21(1):205–220
88. Nobs L, Buchegger F, Gurny R, Allemann E (2004) Poly(lactic acid) nanoparticles labeled with biologically active neutravidin for active targeting. *Eur J Pharm Biopharm* 58(3):483–490
89. Patri AK et al (2004) Synthesis and in vitro testing of J591 antibody-dendrimer conjugates for targeted prostate cancer therapy. *Bioconjug Chem* 15(6):1174–1181
90. Brennan FR, Shaw L, Wing MG, Robinson C (2004) Preclinical safety testing of biotechnology-derived pharmaceuticals: understanding the issues and addressing the challenges. *Mol Biotechnol* 27(1):59–74
91. Weinberg WC et al (2005) Development and regulation of monoclonal antibody products: challenges and opportunities. *Cancer Metastasis Rev* 24(4):569–584
92. Carter P (2001) Improving the efficacy of antibody-based cancer therapies. *Nat Rev Cancer* 1(2):118–129
93. Pavlinkova G et al (2001) Effects of humanization and gene shuffling on immunogenicity and antigen binding of anti-TAG-72 single-chain Fvs. *Int J Cancer* 94(5):717–726

94. Mebiopharm Co., Ltd (2009) Safety study of MBP-426 (liposomal oxaliplatin suspension for injection) to treat advanced or metastatic solid tumors. <http://clinicaltrials.gov/ct2/show/NCT00355888>. Accessed on May 8, 2011
95. SynerGene Therapeutics, Inc. (2010) Safety study of infusion of SGT-53 to treat solid tumors. <http://clinicaltrials.gov/ct2/show/NCT00470613>. Accessed on May 8, 2011
96. Ellington AD, Szostak JW (1990) In vitro selection of RNA molecules that bind specific ligands. *Nature* 346:818–822
97. Fang X, Tan W (2010) Aptamers generated from cell-SELEX for molecular medicine: a chemical biology approach. *Acc Chem Res* 43(1):48–57
98. Tuerk C, Gold L (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* 249:505–510
99. Farokhzad OC, Karp JM, Langer R (2006) Nanoparticle-aptamer bioconjugates for cancer targeting. *Expert Opin Drug Deliv* 3(3):311–324
100. Levy-Nissenbaum E, Radovic-Moreno AF, Wang AZ, Langer R, Farokhzad OC (2008) Nanotechnology and aptamers: applications in drug delivery. *Trends Biotechnol* 26(8):442–449
101. Nimjee SM, Rusconi CP, Sullenger BA (2005) Aptamers: an emerging class of therapeutics. *Annu Rev Med* 56:555–583
102. Potti A, Rusconi CP, Sullenger BA, Ortel TL (2004) Regulatable aptamers in medicine: focus on antithrombotic strategies. *Expert Opin Biol Ther* 4(10):1641–1647
103. Shangguan D et al (2006) Aptamers evolved from live cells as effective molecular probes for cancer study. *Proc Natl Acad Sci USA* 103(32):11838–11843
104. Daniels DA, Chen H, Hicke BJ, Swiderek KM, Gold L (2003) A tenascin-C aptamer identified by tumor cell SELEX: systematic evolution of ligands by exponential enrichment. *Proc Natl Acad Sci USA* 100(26):15416–15421
105. Ng EW et al (2006) Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. *Nat Rev Drug Discov* 5(2):123–132
106. Bagalkot V et al (2007) Quantum dot-aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on bi-fluorescence resonance energy transfer. *Nano Lett* 7(10):3065–3070
107. Kim D, Jeong YY, Jon S (2010) A drug-loaded aptamer—gold nanoparticle bioconjugate for combined CT imaging and therapy of prostate cancer. *ACS Nano* 4(7):3689–3696
108. Kolishetti N et al (2010) Engineering of self-assembled nanoparticle platform for precisely controlled combination drug therapy. *Proc Natl Acad Sci USA* 107(42):17939–17944
109. Cheng JJ et al (2007) Formulation of functionalized PLGA-PEG nanoparticles for in vivo targeted drug delivery. *Biomaterials* 28(5):869–876
110. Chiu T-C, Huang C-C (2009) Aptamer-functionalized nano-biosensors. *Sensors* 9(12):10356–10388
111. Delehanty JB, Boeneman K, Bradburne CE, Robertson K, Medintz IL (2009) Quantum dots: a powerful tool for understanding the intricacies of nanoparticle-mediated drug delivery. *Expert Opin Drug Deliv* 6(10):1091–1112
112. Huang Y-F, Sefah K, Bamrungsap S, Chang H-T, Tan W (2008) Selective photothermal therapy for mixed cancer cells using aptamer-conjugated nanorods. *Langmuir* 24(20):11860–11865
113. Lam KS et al (1991) A new type of synthetic peptide library for identifying ligand-binding activity. *Nature* 354:82–84
114. Needels MC et al (1993) Generation and screening of an oligonucleotide-encoded synthetic peptide library. *Proc Natl Acad Sci USA* 90(22):10700–10704
115. McGuire MJ, Li S, Brown KC (2009) Biopanning of phage displayed peptide libraries for the isolation of cell-specific ligands. *Methods Mol Biol* 504:291–321
116. Pasqualini R, Ruoslahti E (1996) Organ targeting in vivo using phage display peptide libraries. *Nature* 380:364–366
117. Arap W et al (2002) Steps toward mapping the human vasculature by phage display. *Nat Med* 8(2):121–127
118. Lam KS, Zhao ZG (1997) Targeted therapy for lymphoma with peptides. *Hematol Oncol Clin North Am* 11(5):1007–1019

119. Lee TY, Lin CT, Kuo SY, Chang DK, Wu HC (2007) Peptide-mediated targeting to tumor blood vessels of lung cancer for drug delivery. *Cancer Res* 67(22):10958–10965
120. Chang DK, Lin CT, Wu CH, Wu HC (2009) A novel peptide enhances therapeutic efficacy of liposomal anti-cancer drugs in mice models of human lung cancer. *PLoS ONE* 4(1):e4171
121. Li J et al (2004) Fusion protein from RGD peptide and Fc fragment of mouse immunoglobulin G inhibits angiogenesis in tumor. *Cancer Gene Ther* 11(5):363–370
122. Ruoslahti E, Pierschbacher M (1987) New perspectives in cell adhesion: RGD and integrins. *Science* 238:491–497
123. Danhier F et al (2009) Targeting of tumor endothelium by RGD-grafted PLGA-nanoparticles loaded with Paclitaxel. *J Control Release* 140(2):166–173
124. Ohannesian DW et al (1995) Carcinoembryonic antigen and other glycoconjugates act as ligands for galectin-3 in human colon carcinoma cells. *Cancer Res* 55(10):2191–2199
125. Zubietta MR et al (2006) Galectin-3 expression correlates with apoptosis of tumor-associated lymphocytes in human melanoma biopsies. *Am J Pathol* 168(5):1666–1675
126. David A, Kopeckova P, Kopecek J, Rubinstein A (2002) The role of galactose, lactose, and galactose valency in the biorecognition of N-(2-hydroxypropyl)methacrylamide copolymers by human colon adenocarcinoma cells. *Pharm Res* 19(8):1114–1122
127. Managit C, Kawakami S, Nishikawa M, Yamashita F, Hashida M (2003) Targeted and sustained drug delivery using PEGylated galactosylated liposomes. *Int J Pharm* 266(1–2):77–84
128. Ross JF, Chaudhuri PK, Ratnam M (1994) Differential regulation of folate receptor isoforms in normal and malignant tissues in vivo and in established cell lines. Physiologic and clinical implications. *Cancer* 73(9):2432–2443
129. Stella B et al (2000) Design of folic acid-conjugated nanoparticles for drug targeting. *J Pharm Sci* 89(11):1452–1464
130. Park EK, Lee SB, Lee YM (2005) Preparation and characterization of methoxy poly(ethylene glycol)/poly([epsilon]-caprolactone) amphiphilic block copolymeric nanospheres for tumor-specific folate-mediated targeting of anticancer drugs. *Biomaterials* 26(9):1053–1061
131. Liu Y, Li K, Pan J, Liu B, Feng S-S (2010) Folic acid conjugated nanoparticles of mixed lipid monolayer shell and biodegradable polymer core for targeted delivery of Docetaxel. *Biomaterials* 31(2):330–338
132. Ni S, Stephenson SM, Lee RJ (2002) Folate receptor targeted delivery of liposomal daunorubicin into tumor cells. *Anticancer Res* 22(4):2131–2135
133. Pan XQ, Wang H, Lee RJ (2003) Antitumor activity of folate receptor-targeted liposomal doxorubicin in a KB oral carcinoma murine xenograft model. *Pharm Res* 20(3):417–422
134. Stephenson SM et al (2003) Folate receptor-targeted liposomes as possible delivery vehicles for boron neutron capture therapy. *Anticancer Res* 23(4):3341–3345
135. Low PS, Henne WA, Doorneweerd DD (2007) Discovery and development of folic-acid-based receptor targeting for imaging and therapy of cancer and inflammatory diseases. *Acc Chem Res* 41(1):120–129
136. Zhao X, Li H, Lee RJ (2008) Targeted drug delivery via folate receptors. *Expert Opin Drug Deliv* 5(3):309–319
137. Weissleder R, Kelly K, Sun EY, Shtatland T, Josephson L (2005) Cell-specific targeting of nanoparticles by multivalent attachment of small molecules. *Nat Biotechnol* 23(11):1418–1423

Multifunctional Nanoparticles for Drug Delivery  
Applications

Imaging, Targeting, and Delivery

Svenson, S.; Prud'homme, R.K. (Eds.)

2012, X, 374 p., Hardcover

ISBN: 978-1-4614-2304-1