5 Polymer Micelles as Drug Carriers

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5.1 Introduction

It has long been recognized that improving one or more of the intrinsic adsorption, distribution, metabolism, and excretion (ADME) properties of a drug is a critical step in developing more effective drug therapies. As early as 1906, Paul Ehrlich proposed altering drug distribution by conjugating toxic drugs to “magic bullets” (antibodies) having high affinity for cancer cell-specific antigens in order to both improve the therapeutic efficacy of cancer while decreasing its toxicity.[1] Since that

time, it has become clear that directly improving intrinsic ADME through modifications of the drug is limited or precluded by structural requirements for activity. In other words low molecular mass drugs are too small and have only limited number of atomic groups that can be altered to improve ADME, which often adversely affects drug pharmacological activity. In turn the modifications of many low molecular mass drugs aimed to increase their pharmacological activity often adversely affect their ADME properties. For example, the potency and specificity of drugs can be improved by the addition of hydrophobic groups.[2] The associated decrease in water solubility, however, increases the likelihood of drug aggregation, leading to poor absorption and bioavailability during oral administration[2] or lowered systemic bioavailability, high local toxicity, and possible pulmonary embolism during intravenous administration.[3]

Although there have been considerable difficulties for improving some existing drugs through chemical modifications the problem became even more obvious with the development of high-throughput drug discovery technologies. Almost half of lead drug candidates identified by high-throughput screening have poor solubility in water and are abandoned before the formulation development stage.[4] In addition newly synthesized drug candidates often fail due to poor bioavailability, metabolism and/or undesirable side effects, which together decrease the therapeutic index of the molecules. Further, a new generation of biopharmaceuticals and gene therapy agents are emerging based on novel biomacromolecules, such as DNA and proteins. The use of these biotechnology-derived drugs is completely dependent on efficient delivery to the critical site of the action in the body. Therefore, drug delivery research is essential in the translation of newly discovered molecules into potent drug candidates and can significantly improve therapies of existing drugs.

Polymer-based drugs and drug delivery systems emerged from the laboratory bench in the 1990's as a promising therapeutic strategy for the treatment of certain devastating human diseases.[5, 6] A number of polymer therapeutics are presently on the market or undergoing clinical evaluation to treat cancer and other diseases. Most of them are low molecular weight drug molecules or therapeutic proteins that are chemically linked to water-soluble polymers to increase drug solubility, drug stability, or enable targeting to tumors.

Recently, as a result of rapid development of novel nanotechnology-derived materials, a new generation of polymer therapeutics has emerged which uses materials and devices of nanoscale size for the delivery of drugs, genes, and imaging molecules.[7-12] These materials include polymer micelles, polymer-DNA complexes ("polyplexes"), liposomes, and other nanostructured materials for medical use, that are collectively called nanomedicines. Compared to first generation polymer therapeutics, the new generation nanomedicines are more advanced. They entrap small drugs or biopharmaceutical agents, such as therapeutic proteins and DNA, and can be designed to trigger the release these agents at the target site. Many nanomedicines are constructed using self-assembly principles, such as spontaneous formation of micelles or interpolyelectrolyte complexes driven by diverse molecular interactions (hydrophobic, electrostatic, etc.). This chapter considers polymeric micelles among as an important example of the new generation of nanomedicines, which perhaps is also among most advanced toward clinical applications in diagnostics and treatment of human diseases.

5.2 Polymer Micelle Structures

5.2.1 Self-Assembled Micelles

Self-assembled polymer micelles are created from amphiphilic polymers that spontaneously form nanosized aggregates when the individual polymer chains ("unimers") are directly dissolved in aqueous solution (dissolution method)[13] above a threshold concentration (critical micelle concentration or CMC) and solution temperature (critical micelle

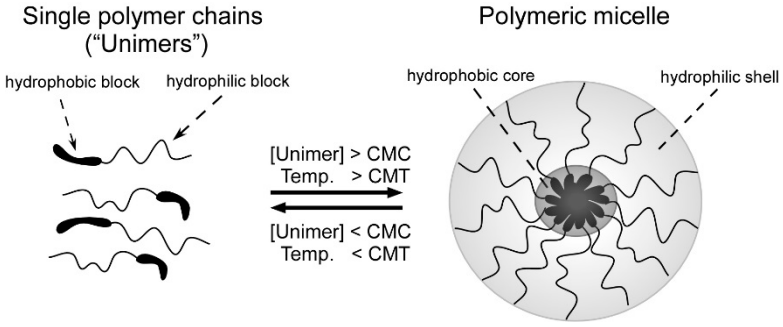

Figure 5.1 Self-assembly of block copolymer micelles.

temperature or CMT) (Figure 5.1). Amphiphilic polymers with very low water solubility can alternatively be dissolved in a volatile organic solvent, then dialyzed against an aqueous buffer (dialysis method). [14]

Amphiphilic di-block (hydrophilic-hydrophobic) or tri-block (hydrophilic-hydrophobic-hydrophilic) copolymers are most commonly used to prepare self-assembled polymer micelles for drug delivery,[9,15,16] although the use of graft copolymers has been reported.[17-19] For drug delivery purposes, the individual unimers are designed to be biodegradable[20, 21] and/or have a low enough molecular mass (<~40 kDa) to be eliminated by renal clearance in order to avoid polymer buildup within the body that can potentially lead to toxicity.[22] The most developed amphiphilic block copolymers assemble into spherical core-shell micelles approximately 10 to 80 nm in diameter[23] that consist of a hydrophobic core for drug loading and a hydrophilic shell that acts as a physical ("steric") barrier to both micelle aggregation in solution and to protein binding and opsonization during systemic administration (Figure 5.2).

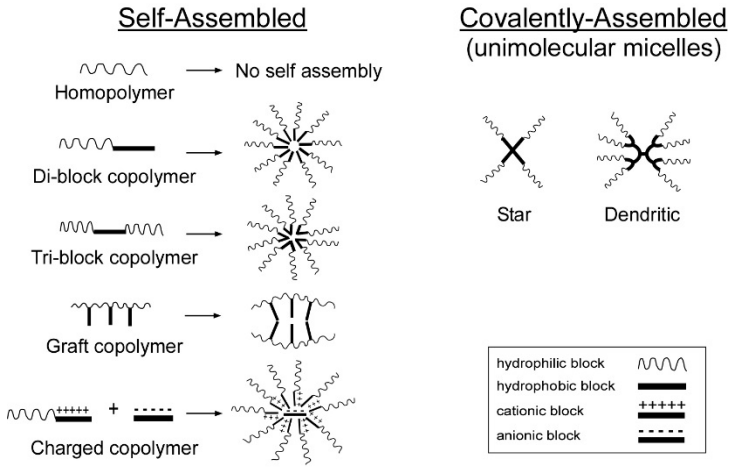

Figure 5.2 Polymer micelle structures.

The most common hydrophilic block used to form the hydrophilic shell is the FDA-approved excipient poly(ethylene glycol) (PEG) or poly(ethylene oxide) (PEO).[24] PEG and PEO consist of the same repeating monomer subunit CH_2-CH_2-O, and may have different terminal end groups depending of the synthesis procedure, example

hydroxyl group HO-(CH_2-CH_2-O)$_n$-H; methoxy group CH_3O-(CH_2-CH_2-O)$_n$-H, etc. PEG/PEO blocks typically range from 1 to 15 kDa.[16,24]

In addition to its FDA approval, PEG is extremely soluble and has a large excluded volume. This makes it especially suitable for physically interfering with intra-micelle interactions and subsequent micelle aggregation. PEG also blocks protein and cell surface interactions, which greatly decreases nanoparticle uptake by the reticuloendothelial system (RES) and consequently increases the plasma half life of the polymer micelle.[25] The degree of steric protection by the hydrophilic shell is a function of both the density and length of the hydrophilic PEG blocks.[25]

Unlike the hydrophilic block, which is typically PEG or PEO, different types of hydrophobic blocks have been sufficiently developed as hydrophobic drug loading cores.[16] Examples include for diblock copolymers: a) poly(L-amino acids), b) biodegradable poly(esters), which includes poly(glycolic acid), poly(D lactic acid), poly(D,L-lactic acid), copolymers of lactide/glycolide, and poly(ε-caprolactone) and c) phospholipids/long chain fatty acids;[26] and for tri-block copolymers: d) polypropylene oxide (in Pluronics/poloxamers).[9] The choice of hydrophobic block is largely dictated by drug compatibility with the hydrophobic core (when drug is physically loaded, as described later) and the kinetic stability of the micelle.

The self-assembly of amphiphilic copolymers is a thermodynamic and, consequently, reversible process that is entropically driven by the release of ordered water from hydrophobic blocks and is either stabilized or destabilized by solvent interactions with the hydrophilic shell. As such, the structural potential of amphiphilic copolymer unimers to form micelles is determined by the mass ratio of hydrophilic to hydrophobic blocks, which also affects the subsequent morphology if aggregates are formed.[14] If the mass of the hydrophilic block is too great, the copolymers exist in aqueous solution as unimers, whereas if the mass of the hydrophobic block is too great, unimer aggregates with non-micellar morphology are formed.[27] If the mass of the hydrophilic block is similar or slightly greater than the hydrophobic block, then conventional core shell micelles are formed.

An important consideration for drug delivery is the relative thermodynamic (potential for disassembly) and kinetic (rate of disassembly) stability of the polymer micelle complexes after intravenous injection and subsequent extreme dilution in the vascular

compartment.[28] This is because the polymer micelles must be stable enough to avoid burst release of the drug cargo (in the case of physically loaded drug) upon systemic administration and remain as nanoparticles long enough to accumulate in sufficient concentrations at the target site.

The relative thermodynamic stability of polymer micelles (which is inversely related to the CMC) is primarily controlled by the length of the hydrophobic block.[13] An increase in the length of the hydrophobic block alone significantly decreases the CMC of the unimer construct (increases the thermodynamic stability of the polymer micelle), whereas an increase in the hydrophilic block alone slightly increases the CMC (decrease the thermodynamic stability of polymer micelle).[14]

Although the CMC indicates the unimer concentration below which polymer micelles will begin to disassemble, the kinetic stability determines the rate at which polymer micelle disassembly occurs. Many diblock copolymer micelles possess good kinetic stability and only slowly dissociate into unimers after extreme dilution.[29] Thus, although polymer micelles are diluted well below typical unimer CMCs[29] (10^{-6}–10^{-7} M) after intravenous injection, their relative kinetic stability might still make them suitable for drug delivery. The kinetic stability depends on many factors, including the size of a hydrophobic block, the mass ratio of hydrophilic to hydrophobic blocks, and physical state of the micelle core.[14] The incorporation of hydrophobic drugs may also further enhance micelle stability.

5.2.2 Unimolecular Micelles

Unimolecular micelles are topologically similar to self-assembled micelles but consist of single polymer molecules with covalently linked amphiphile chains. For example, copolymers with star-like or dendritic architecture, depending on their structure and composition, can either aggregate into multimolecular micelles[30-32] or exist as unimolecular micelles.[33] Dendrimers are widely used as building blocks to prepare unimolecular micelles because they are highly-branched, have well-defined globular shape and controlled surface functionality.[34-40] For example, unimolecular micelles were prepared by coupling dendritic hypercores of different generations with PEO chains.[40,41] The dendritic cores can entrap various drug molecules. However, due to the structural limitations involved in the synthesis of dendrimers of higher generation, and relatively compact structure of the dendrimers the loading capacity

of such micelles is limited. Thus to increase the loading capacity the dendrimer core can be modified with hydrophobic block followed by attachment of the PEO chains. For example, Wang et al. recently synthesized an amphiphilic 16-arm star polymer with a polyamidoamine dendrimer core and arms composed of inner lipophilic poly(ε-caprolactone) block and outer PEO block.[42] These unimolecular micelles were shown to encapsulate a hydrophobic drug, etoposide, with high loading capacity.

Multiarm star-like block copolymers represent another type of unimolecular micelles.[42-46] Star polymers are generally synthesized by either the arm-first or core-first methods. In the arm-first method, monofunctional living linear macromolecules are synthesized and then cross-linked either through propagation using a bifunctional comonomer[47] or by adding a multifunctional terminating agent to connect precise number of arms to one center.[45] Conversely, in the core-first method polymer chains are grown from a multifunctional initiator.[43,44,46,48] One of the first reported examples of unimolecular micelles suitable for drug delivery was a three-arm star polymer composed of mucic acid substituted with fatty acids as a lipophilic inner block and PEO as a hydrophilic outer block.[44] These polymers were directly dispersible in aqueous solutions and formed unimolecular micelles. The size and solubilizing capacity of the micelles was varied by changing the ratio of the hydrophilic and lipophilic moieties. In addition, star-copolymers with polyelectrolyte arms can be prepared to develop pH-sensitive unimolecular micelles as drug carriers.[46]

5.2.3 Cross-linked Micelles

The multimolecular micelles structure can be reinforced by formation of cross-links between the polymer chains. These resulting cross-linked micelles are in essence single molecules of nanoscale size that are stabile upon dilution, shear forces and environmental variations (e.g. changes in pH, ionic strength, solvents etc.). There are several reports on the stabilization of the polymer micelles by cross-linking either within the core domain[49-53] or throughout the shell layer.[54-56] In these cases, the cross-linked micelles maintained small size and core-shell morphology while their dissociation was permanently suppressed. Stable nanospheres from the PEO-*b*-polylactide micelles were prepared by using polymerizable group at the core segment.[49] In addition to

stabilization the core polymerized micelles readily solubilized rather large molecules such as paclitaxel and retained high loading capacity even upon dilution.[50] Formation of interpenetrating network of a temperature-sensitive polymer (poly-N-isopropylacrilomide) inside the core was also employed for stabilization of Pluronic micelles.[53] The resulting micelle structures were stable against dilution, exhibited temperature-responsive swelling behavior, and showed higher drug loading capacity than regular Pluronic micelles.

Recently, a novel type of polymer micelles with cross-linked ionic cores was prepared by using block ionomer complexes as templates.[57] The nanofabrication of these micelles involved condensation of PEO-b-poly(sodium methacrylate) diblock copolymers by divalent metal cations into spherical micelles of core-shell morphology. The core of the micelle was further chemically cross-linked and cations removed by dialysis. Resulting micelles represent hydrophilic nanospheres of core-shell morphology. The core comprises a network of the cross-linked polyanions and can encapsulate oppositely charged therapeutic and diagnostic agents while a hydrophilic PEO shell provides for increased solubility. Furthermore, these micelles displayed the pH- and ionic strength-responsive hydrogel-like behavior due to the effect of the cross-linked ionic core. Such behavior is instrumental for the design of drug carriers with controlled loading and release characteristics.

5.3 Drug Loading and Release

In general, there are three major methods for loading drugs into polymer micelle cores: 1) chemical conjugation, 2) physical entrapment or solubilization, and 3) polyionic complexation (e.g. ionic binding).

5.3.1 Chemical Conjugation

Drug incorporation into polymer micelles via chemical conjugation was first proposed by Ringsdorf's group[58] in 1984. According to this approach, a drug is chemically conjugated to the core-forming block of the copolymer via a carefully designed pH- or enzyme-sensitive linker that can be cleaved to release a drug in its active form within a cell.[59,60] The polymer-drug conjugate then acts as a polymer prodrug which self assembles into a core-shell structure. The appropriate choice of conjugating bond depends on specific applications.

The nature of the polymer–drug linkage and the stability of the drug conjugate linkage can be controlled to influence the rate of drug release, and therefore, the effectiveness of the prodrug.[61-63] For instance, recent work by Kataoka's group proposed pH-sensitive polymer micelles of PEO-b-poly(aspartate hydrazone doxorubicin), in which doxorubicin, was conjugated to the hydrophobic segments through acid-sensitive hydrazone linkers that are stable at extracellular pH 7.4 but degrade and release the free drug at acidic pH 5.0 to 6.0 in endosomes and lysosomes.[63,64] The original approach developed by this group used doxorubicin conjugated to the poly(aspartic acid) chain of PEO-b-poly(aspartic acid) block copolymer though an amide bond.[65] Adjusting both the composition of the block copolymer and concentration of conjugated doxorubicin led to improved efficacy, as evidenced by a complete elimination of solid tumors implanted in mice.[66] It was later determined that doxorubicin physically encapsulated within the micellar core was responsible for antitumor activity. This finding led to the use of PEO-b-poly(aspartate doxorubicin) conjugates as nanocontainers for physically entrapped doxorubicin.[67]

5.3.2 Physical Entrapment

The physical incorporation or solublization of drugs within block copolymer micelles is generally preferred over micelle-forming polymer-drug conjugates, especially for hydrophobic drug molecules. Indeed, many polymers and drug molecules do not contain reactive functional groups for chemical conjugation, and therefore, specific block copolymers have to be designed for a given type of drug. In contrast, a variety of drugs can be physically incorporated into the core of the micelles by engineering the structure of the core-forming segment. In addition, molecular characteristics (molecular weight, composition, presence of functional groups for active targeting) within a homologous copolymer series can be designed to optimize the performance of a drug for a given drug delivery situation.[9,14] This concept was introduced by our group in the late 1980s and was initially called a "micellar microcontainer",[68] but is now is widely known as a "micellar nanocontainer".[9,10] Haloperidol was encapsulated in Pluronic block copolymer micelles,[68] the micelles were targeted to the brain using brain-specific antibodies or insulin, and enhancement of neuroleptic activity by the solubilized drug was observed. During the last 25 years,

a large variety of amphiphilic block copolymers have been explored as nanocontainers for various drugs.

Different loading methods can be used for physical entrapment of the drug into the micelles including but not limited to dialysis,[69-72] oil in water emulsification,[69] direct dissolution,[42,73,74] or solvent evaporation techniques.[75,76] Depending on the method, drug solubilization may occur during or after micelle assembly. The loading capacity of the polymer micelles, which is frequently expressed in terms of the micelle-water partition coefficient, is influenced by several factors, including the both structure of core-forming block and a drug, molecular characteristics of the copolymer, such as composition, molecular weight, and the solution temperature.[13]

Many studies indicate that the most important factor related to the drug solubilization capacity of a polymer micelle is the compatibility between the drug and the core-forming block.[9,14,77-80] For this reason, the choice of the core-forming block is the most critical. One parameter that can be used to assess the compatibility between the polymer and a drug is the Flory-Huggins interaction parameter, χ_{sp}, defined as $\chi_{sp} = (\delta_s - \delta_p)^2 V_s/kT$, where δ_s and δ_p are Scatchard-Hildebrand solubility parameters and V_s is the molecular volume of the solubilizate. It was successfully used as a correlation parameter for the solubilization of aliphatic and aromatic hydrocarbons in block copolymer micelles.[80,81] Recently, Allen's group[82] elegantly demonstrated that calculation and comparison of partial solubility parameters of polymers and drugs could be used as a reliable means to predict polymer-drug compatibility and guide formulation development. Polymer micelles possessing core-forming blocks predicted to be compatible with the drug of interest (Ellipticine), were able to increase the solubility of the drug up to 30,000 times compared to its saturation solubility in water.[82] The degree of compatibility between the drug and the core-forming block has also been shown to influence the release rate of the drug from the micelles: when the environment within the core of the micelle becomes more compatible with the drug, it results in a considerable decrease in the rate of drug release.

For a given drug, the extent of incorporation is a function of factors that also control the micelle size and/or aggregation number. Such factors include the ratio of hydrophobic to hydrophilic block length and the copolymer molecular weight. For example, the loading capacity of Pluronic micelles was found to increase with the increase in the

hydrophobic PPO block length. This effect is attributed to a decrease in CMC, and therefore, an increase in aggregation number and micelle core size. Also, but to a lesser extent, the hydrophilic block length affects the extent of solubilization: an increase in percentage of PEO in Pluronic block copolymers results in a decrease of loading capacity of the micelles.[80,83-85] For a given ratio of PPO-to-PEO, higher molecular weight polymers form larger micelles, and therefore, show a higher drug loading capacity. Therefore, the total amount of loaded drug can be adjusted as a function of the micellar characteristics as clearly was demonstrated by Nagaradjan[83] and Kozlov et al.[85] Several studies indicate that the both copolymer concentration as well as the drug to polymer ratio upon loading have a complex effect on loading capacity of polymer micelles.[79,84,86] In general, more polymer chains provide more absorption sites. As a result, solubilization is increased with polymer concentration.[82] However, the solubilization capacity was found to reach a saturation level with an increase of polymer concentration.[79] The maximum loading level is largely influenced by the interaction between the solubilizate and core-forming block, and stronger interactions enable saturation to be reached at lower polymer concentration. It was also demonstrated in the studies by Hurter and Hatton[84,86] that the loading capacity of micelles formed from copolymers with high hydrophobic content was independent of the polymer concentration. In addition, the location of the incorporated molecules within polymer micelles (micelle core or the core-shell interface) determines the extent of solubilization as well as the rate of drug release.[87,88] It has been found that more soluble compounds are localized at the core-shell interface or even in the inner shell, whereas more hydrophobic molecules have a tendency to solubilize in the micelle core.[85,87,88] The release rate of drug localized in the shell or at the interface appears to account for the “burst release” from the micelles.[87] In general, for drugs physically incorporated in polymer micelles, release is controlled by the rate of diffusion of the drug from the micellar core, stability of the micelles, and the rate of biodegradation of the copolymer. If the micelle is stable and the rate of polymer biodegradation is slow, the diffusion rate of the drug will be mainly determined by the above mentioned factors: the compatibility between the drug and core forming block of copolymer,[69,82] amount of drug loaded, the molecular volume of drug, and length of the core forming block.[89] In addition, the physical state of the micelle core and drug has a

large influence on release characteristics. It was demonstrated that the diffusion of incorporated molecules from the block copolymer micelles with glassy cores is slower in comparison to the diffusion out of the cores that are more mobile.[87]

5.3.3 Polyionic Complexation

Charged therapeutic agents can be incorporated into block copolymer micelles through electrostatic interactions with an oppositely charged ionic segment of block copolymer. Since being proposed independently by Kabanov and Kataoka in 1995,[90,91] this approach is now widely used for the incorporation of various polynucleic acids into block ionomer complexes for developing non-viral gene delivery systems. Ionic block lengths, charge density, and ionic strength of the solution affect the formation of stable block ionomer complexes and, therefore, control the amount of the drug that can be incorporated within the micelles.[8,92] The pH- and salt-sensitivity of such block ionomer micelles provide a unique opportunity to control the triggered release of the active therapeutic agent.[15,63,93-96] Furthermore, block ionomer complexes can participate in the polyion interchange reactions which are believed to account for the release of the therapeutic agent and DNA in an active form inside cells.[7] Several comprehensive reviews can be found in the literature that focus on block ionomer micelles as drug and gene delivery systems.[8,92] In addition physicochemical aspects of the DNA complexes with cationic block copolymers have been also recently reviewed.[97]

As an example, the metal-complex formation of ionic block copolymer, PEO-b-poly(L-aspartic acid), was explored to prepare polymer micelles incorporating *cis*-dichlorodiamminoplatinum (II) (CDDP),[98,99] a potent chemotherapeutic agent widely used in the treatment of a variety of solid tumors, particularly, testicular, ovarian, head and neck, and lung tumors.[100,101] The CDDP-loaded micelles had a size of approximately 20 nm. These micelles showed remarkable stability upon dilution in distilled water, while in physiological saline they displayed sustained release of the regenerated Pt complex over 50 h due to inverse ligand exchange from carboxylate to chloride. The release rate was inversely correlated with the chain length of poly(L-aspartic acid) segments in the block copolymer. The stability of CDDP-loaded micelle against salt was shown to be improved by the addition of

homopolymer, poly(L-aspartic acid), in the micelles.[102] Recently, CDDP-loaded micelles were newly prepared using another block copolymer, PEO-b-poly(glutamic acid) to improve and optimize the micellar stability, as well as the drug release profile.[103] The drug loading in the micelles was as high as 39% (w/w), and these micelles released the platinum in physiological saline at 37°C in sustained manner > 150 h without initial burst of the drug.

The principle of polyionic complexation can be also used to design new photosensitizers for photodynamic therapy of cancer. The group of Kataoka reported formation of micelles as a result of mixing of oppositely charged dendrimer porphirin and block ionomer, based on electrostatic assembly[104] or combination of electrostatic and hydrogen bonding interactions.[95,105] The micelles were stabile at physiological conditions and released the entrapped dendrimers in the acidic pH environment (pH 5.0) suggesting a possibility of pH-triggered drug release in the intracellular endosomal compartments. Overall, the photodynamic efficacy of the dendrimer porphyrins was dramatically improved by inclusion into micelles. This process resulted in a more than two orders of magnitude increase in the photocytotoxicity compared with that of the free dendrimer porphyrins.

In addition the polyionic complexation has been used to immobilize charged enzymes, such as egg white lysozyme[106] or trypsin,[107] which were incorporated in the core of polyion micelles after mixing with oppositely charged ionic block copolymer. A remarkable enhancement of enzymatic activity was observed in the core of the micelles. Furthermore the on-off switching of the enzyme activity was achieved through the destabilization of the core domain by applying a pulse electric field.[108] These unique features of the polyion micelles are relevant for their use as smart nanoreactors in the diverse fields of medical and biological engineering.

Last, but not least, a special class of polyion complexes has been synthesized by reacting block ionomers with surfactants of opposite charge resulting in formation of environmentally responsive nanomaterials, which differ in sizes and morphologies and include micelles and vesicles.[109-113] These materials contain a hydrophobic core formed by the surfactant tail groups and a hydrophilic shell formed, for example, by PEO chains of the block ionomer. These block ionomer complexes can incorporate charged surfactant drugs, such as retinoic acid, as well as other drugs via solubilization in the hydrophobic

domains formed by surfactant molecules.[114] They display transitions induced by changes in pH, salt concentration, chemical nature of low molecular mass counterions as well as temperature, and can be fine tuned to respond to environmental changes occurring in a very wide range of conditions that could realize during delivery of biological and imaging agents.[94,115] The unique self-assembly behavior, the simplicity of the preparation and the wide variety of available surfactant components that can easily produce polymer micelles with a very broad range of core properties make this type of materials extremely promising for development of vehicles for delivery of diagnostic and therapeutic modalities.

5.4 Pharmacokinetics and Biodistribution

Incorporation of a low molecular mass drug into polymer micelles drastically alters pharmacokinetics and biodistribution of the drug in the body, which is crucial for the drug action. Low molecular mass drugs after administration in the body rapidly extravasate to various tissues affecting them almost indiscriminately, and then are rapidly eliminated from the body via renal clearance often causing toxicity to kidneys.[116] Furthermore, many drugs display low stability and are degraded in the body, often forming toxic metabolites. An example is doxorubicinol, a major metabolite of doxorubicin, which causes cardiac toxicity.[117] These impediments to the therapeutic use of low molecular mass drugs can be mitigated by encapsulating drugs in polymer micelles. Within the micelles the drug molecules are protected from enzymatic degradation by the micelle shell. The pharmacokinetics and biodistribution of the micelle-incorporated drugs is mainly determined by the surface properties, size, and stability of the micelles, and is less affected by the properties of the loaded drug. The surface properties of the micelles are determined by the micelle shell. The shell from PEO effectively masks drug molecules and prevents interactions with serum proteins and cells, which contributes to prolonged circulation of the micelles in the body.[16] From the size standpoint polymer micelles fit an ideal range of sizes for systemic drug delivery. On the one hand micelles are sufficiently large, usually exceeding 10 nm in diameter, which hinders their extravasation in nontarget tissues and prevents renal glomerular excretion. On the other hand the micelles are not too large as their size usually does not exceed 100 nm. As a result micelles avoid scavenging by the mononuclear phagocytes system (MPS) in liver and spleen. To this end,

"stealth" particles whose surface is decorated with PEO are known to be less visible to macrophages and have prolonged half-lives in the blood.[64,118,119]

The contribution of the micelle stability to pharmacokintetics and biodistribution is much less understood although it is clear that micelle degradation should result in decrease of the size and drug release, perhaps, prematurely. Degradation of the micelles resulting in formation of block copolymer unimers could also be a principal route for the removal of the polymer material from the body. The molecular mass of the unimers of most block copolymers is below the renal excretion limit, i.e. less than about 20 to 40 kDa[22,120,121] while the molecular mass of the micelles, which usually contain several dozen or even hundreds of unimers molecules, is above this limit. Thus the unimers are sufficiently small and can be removed via renal excretion, while the micelles cannot. A recent study by Batrakova et al. determined pharmacokinetic parameters of an amphiphilic block copolymer, Pluronic P85 and provided perhaps first evidence that the pharmacokinetic behavior of a block copolymer can be a function of its aggregation state.[119] Specifically, formation of micelles increased the half-life of the block copolymer in plasma and decreased the uptake of the block copolymer in the liver. However, it had no effect on the total clearance, indicating that the elimination of Pluronic P85 was controlled by the renal tubular transport of unimers but not by the rate of micelles disposition or disintegration. Furthermore, the values of the clearance suggested that a significant portion of the block copolymer was reabsorbed back into the blood, probably, through the kidney's tubular membranes. Chemical degradation of the polymers comprising the micelles followed by renal excretion of the relatively low molecular mass products of degradation may be another route for the removal of the micelle polymer material from the body. This route could be particularly important in the case of the cross-linked or unimolecular micelles, micelles displaying very high stability and/or micelles composed from very hydrophobic polymer molecules that can bind and retain for the long time biological membranes and other cellular components.

The delivery of chemotherapeutic drugs to treat tumors is one of the most advanced areas of research using polymer micelles. Two approaches have been explored to enhance delivery of drug-loaded polymer micelles to the tumor sites: 1) passive targeting and 2) vectorized targeting. The passive targeting involves enhanced

permeability and retention (EPR) effect.[122,123] It is based on the fact that solid tumors display increased vascular density and permeability caused by angiogenesis, impaired lymphatic recovery, and lack of a smooth muscle layer in solid tumor vessels. As a result micellar drugs can penetrate and retain in the sites of tumor lesions. At the same time extravastion of micellar drugs in normal tissues is decreased compared to low molecular drug molecules. Among normal organs, spleen and liver can accumulate polymer drugs, but the drugs are eventually cleared via the lymphatic system. The increased circulation time of the micellar drugs should further enhance exposure of the tumors to the micellar drug compared to the low molecular mass drugs. Along with the passive targeting the delivery of micellar drugs to tumors potentially be can enhanced by modification of the surface of the polymer micelles with the targeting molecules, vectors that can selectively bind to the surface of the tumor cells. Potential vectors include antibodies, aptamers and peptides capable of binding tumor-specific antigens and other molecules diplayed at the surfaces of the tumors.[124-126]

Altered biodistribution of a common antineoplastic agent was demonstrated for CDDP encapsulated in polyionic micelles with PEO-b-poly(glutamic acid) block copolymers.[103] Free CDDP is rapidly distributed to each organ, where its levels peak at about one hour after *i.v.* administration. In contrast, in the case of the CDDP-incorporated micelles due to their remarkably prolonged blood circulation time the drug level in the liver, spleen, and tumor continued to increase up to at least 24 hours after injection. Consequently, the CDDP-incorporated micelle exhibited 4-, 39-, and 20-fold higher accumulation in the liver, spleen, and tumor, respectively, than the free CDDP. At the same time the encapsulation of CDDP into the micelles significantly decreased drug accumulation in the kidney, especially during first hour after administration. This suggested potential for decrease of severe nephrotoxicity observed with the free drug, which is excreted through the glomerular filtration and thus affects the kidney.[127]

Promising results were also demonstrated for doxorubicin incorporated into styrene-maleic acid micelles.[128] In this case as a result of drug entrapment into micelles the drug was redirected from heart to the tumor and the doxorubicin cardiotoxicity was diminished. Complete blood counts and cardiac histology for the micellar drug showed no serious side effects for *i.v.* doses as high as 100 mg/kg doxorubicin equivalent in mice. Similar results were reported for doxorubicin

incorporated in mixed micelles of PEO-b-poly(L-histidine) and PEO-b-poly(L-lactic acid) block copolymers.[129] Tissue levels of doxorubicin administered in the micellar formulation were decreased in blood and liver and considerably increased in the solid tumor, compared to the free drug. Further increase in the tumor delivery was achieved by modifying the surface of the micelles with the folate molecules. The accumulated doxorubicin levels observed using folate-modified micelles was 20 times higher than these for free doxorubicin, and 3 times higher than these for the unmodified micelles.

The first micellar formulation of doxorubicin to reach clinical evaluation stage used the micelles composed of triblock copolymer, PEO-b-poly(propylene oxide)-b-PEO, Pluronic.[130] Analysis of pharmacokinetics and biodistribution of doxorubicin incorporated into mixed micelles of Pluronics L61 and F127, SP1049C, demonstrated more efficient accumulation of micellar drug in the tumors compared to the free drug. Specifically, the areas under the curves (AUC) in the Lewis lung carcinoma 3LL M-27 solid tumors in C57Bl/6 mice were increased about two fold using SP1049 compared to the free doxorubicin. Furthermore, this study indicated that the peak levels of doxorubicin formulated with SP1049 in the tumor were delayed and the drug residence time was increased in comparison with the free doxorubicin.[130]

A clear visualization of drug delivery to the tumor site was shown for doxorubicin covalently incorporated through pH-sensitive link into polymer micelles of PEO-poly(aspartate hydrazone doxorubicin).[64] A phase-contrast image showed that the tumor blood vessels containing the micelles leaked into extravascular compartments of the tumors resulting in infiltration of the micelles into tumor sites. The micelles circulated in the blood for a prolonged time, and the AUC for micellar doxorubicin was 15-fold greater than the AUC for free doxorubicin. Furthermore, the AUC values of the micellar doxorubicin in the heart and kidney decreased compared to the free drug. Thus, the selectivity of drug delivery to the tumor compared to heart and kidney (AUC_{tumor}/AUC_{organ}) was increased by 6- and 5-folds, respectively. This may result in reduction of side effects of doxorubicin such as cardiotoxicity and nephrotoxicity. Moreover, the micellar doxorubicin showed relatively low uptake in the liver and spleen despite very long residence time in the blood.

Biodistribution of paclitaxel incorporated into biodegradable

polymer micelles of monomethoxy-PEO-b-poly(D,L-lactide) block copolymer, Genexol-PM, was compared with the regular formulation of the drug in Cremophor EL.[131] Two to three-fold increases in drug levels were demonstrated in most tissues including liver, spleen, kidneys, lungs, heart and tumor after *i.v.* administration of Genexol-PM as compared to paclitaxel. Nevertheless, acute dose toxicity of Genexol-PM was about 25 times lower than that of the conventional drug formulation, which appears to be due to a reformulation avoiding the use of Chremophor EL and dehydrated ethanol that are toxic.

Selective tumor targeting with paclitaxel encapsulated in micelles modified with tumor-specific antibodies 2C5 ("immunomicelles") was reported using Lewis lung carcinoma solid tumor model in C57Bl/6J mice.[26] These micelles were prepared from PEO-distearyl phosphatidylethanolamine conjugates with the free PEO end activated with the p-nitrophenylcarbonyl group for the antibody attachment. The amount of micellar drug accumulated in the tumor exceeded that in non-target tissue (muscles) by more than ten times. Noteworthy, the highest accumulation in the tumor was demonstrated for the micelles containing the longest PEO chains, which also had the longest circulation time in the blood. Furthermore, the immunomicelles displayed the highest amount of tumor-accumulated drug compared to the either free paclitaxel or non-vectorized micelles. It was demonstrated that paclitaxel delivered by plain micelles in the interstitial space of the tumor was eventually cleared after gradual micellar degradation. In opposite, paclitaxel-loaded 2C5 immunomicelles were internalized by cancer cells and enhance retention of the drug inside the tumor.[132]

Unexpected results were found using pH-sensitive polymer micelles of N-isopropylacrylamide and methacrylic acid copolymers randomly or terminally alkylated with octadecyl groups.[133] It was demonstrated that aluminium chloride phthalocyanine (AlClPc) incorporated in such micelles was cleared more rapidly and accumulated less in the tumor than the AlClPc formulated with Cremophor EL. Furthermore, significant accumulation in liver and spleen (and lungs for most hydrophobic copolymers) was observed compared to Cremophor EL formulation. The enhanced uptake of such polymer micelles by the cells of mononuclear phagocyte system (MPS) could be due to micelle aggregation in blood and embolism in the capillaries. Thus, it was attempted to reduce the uptake of the micelles in MPS by incorporating water soluble monomers, N-vinyl-2-pyrrolidone in the copolymer

structure.[134] The modified formulation displayed same levels of tumor accumulation and somewhat higher antitumor activity than the Cremophor EL formulation. This work serves as an example reinforcing the need of proper adjustment of the polymer micelle structure, and perhaps the need of using block copolymers that produce a defined protective hydrophilic shell to facilitate evasion of polymer micelles from MPS.

5.5 Drug Delivery Applications

The studies on application of polymer micelles in drug delivery have mostly focused of the following areas: 1) delivery of anticancer agents to treat tumors; 2) drug delivery to the brain to treat neurodegenerative diseases; 3) delivery of antifungal agents; 4) delivery of imaging agents for diagnostic applications; and 5) delivery of polynucleotide therapeutics. These areas are considered below.

5.5.1 Chemotherapy of Cancer

To enhance chemotherapy of tumors using polymer micelles four major approaches were employed: 1) passive targeting of polymer micelles to tumors due to EPR effect; 2) targeting of polymer micelles to specific antigens overexpressed at the surface of tumor cells; 3) enhanced drug release at the tumors sites having low pH; and 4) sensitization of drug resistant tumors by block copolymers.

A series of pioneering studies by Kataoka's group used polymer micelles for passive targeting of various anticancer agents and chemotherapy of tumors.[102,103,135] One notable recent example reported by this group involves polymer micelles of PEO-b-poly(L-aspartic acid) incorporating CDDP. Evaluation of anticancer activity using murine colon adenocarcinoma C26 as an *in vivo* tumor model demonstrated that CDDP in polymer micelles had significantly higher activity than the free CDDP, resulting in complete eradication of the tumor.[103] A formulation of paclitaxel in biodegradable polymer micelles of monomethoxy-PEO-b-poly(D,L-lactide) block copolymer, Genexol-PM, also displayed elevated activity *in vivo* against human ovarian carcinoma OVCAR-3 and human breast carcinoma MCF7 compared to a regular formulation of the drug in Cremophor EL.[131] In addition, anthracycline antibiotics, doxorubicin and pirarubicin, incorporated in styrene-maleic acid

micelles each revealed potent anticancer effects *in vivo* against mouse sarcoma S-180 resulting in complete eradication of tumors in 100% of tested animals.[128] Noteworthy, animals survived for more than one year after treatment with the micelle-incorporated pirarubicin at doses as high as 100 mg/kg of pirarubicin equivalent. Complete blood counts, liver function test, and cardiac histology showed no sign of adverse effects for intravenous doses of the micellar formulation. In contrast, animals receiving free pirarubicin had a much reduced survival and showed serious side effects.[136] Collectively, these studies suggested that various micelle-incorporated drugs display improved therapeutic index in solid tumors, which correlates with enhanced passive targeting of the drug to the tumor sites and decreased side effects compared to conventional formulations of these drugs.

Tumor-specific targeting of polymer micelles to molecular markers expressed at the surface of the cancer cells has been also explored to eradicate tumor cells. For example a recent study by Gao's group developed a polymer micelle carrier to deliver doxorubicin to tumor endothelial cells with overexpressed $\lambda_v\beta_3$ integrins.[137] A cyclic pentapeptide, cRGD was used as a targeting ligand that is capable of selective and high affinity binding to the $\lambda_v\beta_3$ integrin. Micelles of PEO-b-poly(ε-caprolactone) loaded with doxorubicin were covalently bound with cRGD. As a result of such modification the uptake of doxorubicin-containing micelles in *in vitro* human endothelial cell model derived from Kaposi's sarcoma was profoundly increased. In addition folate receptor often overexpressed in cancer cells has been evaluated for targeting various drug carriers to tumors.[138] This strategy has been also evaluated to target polymer micelles. For example, mixed micelles of PEO-b-poly(L-histidine) and PEO-b-poly(L-lactic acid) block copolymers with solubilized doxorubicin[129] or micelles of PEO-b-poly(DL-lactic-co-glycolic acid) block copolymer with covalently attached doxorubicin[139] each were surface modified by conjugating folate molecules to the free PEO ends. In both cases *in vitro* and *in vivo* studies demonstrated increased antitumor activity of the micelle-incorporated drug resulting from such modification. The enhanced delivery of the micellar drugs through the folate receptor and enhanced retention of the modified micelles at the tumor sites are likely reasons for the effects of these folate modifications.

Micelles conjugated with antibodies or antibody fragments capable to recognize tumor antigens were shown to improve therapeutic efficacy

in vivo over non-modified micelles.[23] This approach can result in high selectivity of binding, internalization, and effective retention of the micelles in the tumor cells. In addition, recent advances in antibody engineering allow for the production of humanized antibody fragments reducing problems with immune response against mouse antibodies.[140] For example, micelles of PEO-distearyl phosphatidylethanolamine were covalently modified with the monoclonal antibody 2C5 that binds to nucleosomes displayed at the surface of many tumor cells. The micelles were then used for incorporating various poorly soluble anticancer drugs including tamoxifen, paclitaxel, dequalinium, and chlorine e6 trimethyl ester.[26,132,141] It was shown that paclitaxel-loaded 2C5-immunomicelles could specifically recognize a variety of tumor types. The binding of these immunomicelles was observed for all cancer cell lines tested: murine Lewis lung carcinoma, T-lymphoma EL4, and human breast adenocarcinomas, BT-20 and MCF7.[141] Moreover, paclitaxel-loaded 2C5 immunomicelles demonstrated highest anticancer activity in Lewis lung carcinoma tumor model in mice compared to plain paclitaxel-loaded micelles and the free drug.[132] The increased antitumor effect of immunomicelles *in vivo* correlated with enhanced retention of the drug delivered with the immunomicelles inside the tumor.

Tumors often display low pH of interstitial fluid, which is mainly attributed to higher rates of aerobic and anaerobic glycolysis in cancer cells than in normal cells.[142,143] This phenomenon has been employed in the design of various pH-sensitive polymer micelle systems for delivery of anticancer drugs to the tumors. One approach consisted in chemical conjugation of anticancer drugs to the block copolymers through pH sensitive cleavable links that are stable at neutral pH but are cleavable and release the drug in the mildly acidic pH. For example, several groups used for this purpose hydrasone-based linking groups, to covalently attach doxorubicin to PEO-b-poly(DL-lactic-co-glycolic acid) block copolymer,[21,144] PEO-b-block-poly(allyl glycidyl ether)[145] or PEO-b-poly(aspartate hydrazone) block copolymer.[63,64] It was suggested that doxorubicin will remain in the micelles in the blood stream and will be released at tumor sites at lower pH. For example, *in vitro* and *in vivo* studies using PEO-b-poly(aspartate hydrazone doxorubicin) micelles demonstrated that the micelles display an intracellular pH-triggered drug release capability, tumor-infiltrating permeability, and effective antitumor activity with extremely low toxicity.[63,64] Overall, the animal studies suggested that such polymer

micelle drug has a wide therapeutic window due to increased afficacy and decreased toxicity compared to free doxorubicin.[64]

An alternative mechanism for pH-induced triggering of drug release at the tumor sites consists of using pH sensitive polyacids or polybases as building blocks for polymer micelles.[94,146,147] For example, mixed micelles of PEO-b-poly(L-histidine) and PEO-b-poly(L-lactic acid) block copolymers incorporate pH-sensitive poly-base, poly(L-histidine) in the hydrophobic core.[147] The core can also solubilize hydrophobic drugs, such as doxorubicin. The protonation of the polybase at acidic conditions resulted in the destabilization of the core and triggered release of the drug. This system was also targeted to the tumors through the folate molecules as described above and has shown significant *in vivo* antutumor activity and less side effects compared to the free drug.[129] Notably it was also effective *in vitro* and *in vivo* against multidrug resistant (MDR) human breast carcinoma MCF7/ADR that overexpresses P-glycoprotein (Pgp). Pgp is a drug efflux transport protein that serves to eliminate drugs from the cancer cells and significantly decreases the anticancer activity of the drugs. The micelle incorporated drug was released inside the cells and thus avoided the contact with Pgp localized at the cell plasma membrane, which perhaps contributed to increased activity of pH sensitive doxorubicin micelles in the MDR cells.

A different approach using Pluronic block copolymer micelles to overcome MDR in tumors has been developed by our group.[130,148-151] Studies by Alakhov et al. demonstrated that Pluronic block copolymers can sensitize MDR cells resulting in increased cytotoxic activity of doxorubicin, paclitaxel, and other drugs by 2-3 orders of magnitude.[148,149] Remarkably, Pluronic can enhance drug effects in MDR cells through multiple effects including 1) inhibiting drug efflux transporters, such as Pgp[149,152] and multidrug resistance proteins (MRPs);[153,154] 2) abolishing drug sequestration within cytoplasmic vesicles;[149, 153] 3) inhibiting the glutathione/glutathione S-transferase detoxification system;[154] and 4) enhancing proapoptotic signaling in MDR cells[155]. Similar effects of Pluronics have also been reported using *in vivo* tumor models.[130,150] In these studies, mice bearing drug-sensitive and drug-resistant tumors were treated with doxorubicin alone and with doxorubicin in Pluronic compositions. The tumor panel included *i.p.* murine leukemias (P388, P388-Dox), *s.c.* murine myelomas (Sp2/0, Sp2/0-Dnr), *i.v.* and *s.c.* Lewis lung carcinoma (3LL-M27), *s.c.* human

breast carcinomas (MCF7, MCF7/ADR), and *s.c.* human oral epidermoid carcinoma (KBv).[130] Using the NCI criteria for tumor inhibition and increased lifespan, Pluronic/doxorubicin has met the efficiency criteria in all models (9 of 9), while doxorubicin alone was only effective in selected tumors (2 of 9).[130] Results showed that the tumors were more responsive in the Pluronic/doxorubicin treatment groups than to doxorubicin alone. These studies demonstrated improved treatment of drug resistant cancers with Pluronics.

The mechanisms of effects of Pluronic on Pgp have been studied in the greatest detail.[151] In particular, exposure of MDR cells to Pluronics has resulted in inhibition of Pgp-mediated efflux,[149] and this overcomes defects in intracellular accumulation of Pgp-dependent drugs,[148,149,152] and abolishes the directionality difference in the flux of these drugs across polarized cell monolayers.[156-158] The lack of changes in membrane permeability with Pluronics to 1) non-Pgp compounds in MDR cells,[158,159] and 2) to Pgp probes in non-MDR cells[149,153] suggested that Pluronic effects were specific to the Pgp efflux system. These effects were observed at Pluronic concentrations less than or equal to the critical micelle concentration (CMC).[152,159] Thus, Pluronic unimers rather than the micelles were responsible for these effects. Specifically, Pluronic molecules displayed a dual function in MDR cells.[160-162] First, they incorporated into the cell membranes and decreased the membrane microviscosity. This was accompanied by inhibition of Pgp ATPase activity. Second, they translocated into cells and reached intracellular compartments. This was accompanied by inhibition of respiration,[163] presumably due to Pluronic interactions with the mitochondria membranes. As a result, within 15 minutes after exposure to select Pluronics, intracellular levels of ATP in MDR cells were drastically decreased.[160-162] Remarkably, such ATP depletion was not observed in non-MDR cells, suggesting that the Pluronic was "selective" with respect to the MDR phenotype.[160,164] Combined, these two effects, Pgp ATPase inhibition and ATP depletion, resulted in shut-down of the efflux system in MDR cells.[160-162] Notably, either component alone was insufficient. The Pgp remained functionally active when 1) ATP was restored using an ATP supplementation system in the presence of a Pluronic, or 2) when ATP was depleted, but there was no direct contact between the Pluronic and Pgp (and no ATPase inhibition). Overall these detailed studies which resulted in development of a micellar formulation of doxorubicin that is evaluated clinically, reinforce that block

copolymers comprising the micelles can serve as biological response modifying agents that can have beneficial effects in chemotherapy of tumors.

5.5.2 Drug Delivery to the Brain

By restricting drug transport to the brain, the blood brain barrier (BBB) represents a formidable impediment for treatment of brain tumors and neurodegenerative diseases, such as HIV-associated dementia, stroke, Parkinson's and Alzheimer's diseases. Two strategies using polymer micelles have been evaluated to enhance delivery of biologically active agents to the brain. The first strategy is based on modification of polymer micelles with antibodies or ligand molecules capable of transcytosis across brain microvessel endothelial cells comprising the BBB. The second strategy uses Pluronic block copolymers to inhibit drug efflux systems, particularly Pgp, and selectively increase the permeability of BBB to Pgp substrates.

An early study used micelles of Pluronic block copolymers for delivery of the CNS drugs to the brain.[68,73] These micelles were surface-modified by attaching to the free PEO ends either polyclonal antibodies against brain-specific antigen, α_2-glycoprotein, or insulin to target the receptor at the lumenal side of BBB. The modified micelles were used to solubilize fluorescent dye or neuroleptic drug, haloperidol, and these formulations were administered intravenously in mice. Both the antibody and insulin modification of the micelles resulted in enhanced delivery of the fluorescent dye to the brain and drastic increases in neuroleptic effect of haloperidol in the animals. Subsequent studies using *in vitro* BBB models demonstrated that the micelles vectorized by insulin undergo receptor-mediated transport across brain microvessel endothelial cells.[156] Based one these observations one should expect development of novel polymer micelles that target specific receptors at the surface of the BBB to enhance transport of the incorporated drugs to the brain.

The studies by out group have also demonstrated that selected Pluronic block copolymers, such as Pluronic P85 are potent inhibitors of Pgp and increase entry of the Pgp-substrates to the brain across BBB.[156,158,159,165] Pluronic did not induce toxic effect in BBB as revealed by lack of alteration in paracellular permeability of the barrier[156,158] and in histological studies using specific markers for brain endothelial

cells.[166] Overall this strategy has a potential in developing novel modalities for delivery of various drug to the brain, including selected anti-cancer agents to treat metastatic brain tumors as well as HIV protease inhibitors to eradicate HIV virus in the brain.[167,168]

5.5.3 Formulations of Antifungal Agents

The need for safe and effective modalities for delivery of chemotherapeutic agents to treat systemic fungal infections in immunocompromised AIDS, surgery, transplant and cancer patients is very high. The challenges to delivery of antifungal agents include low solubility and sometimes high toxicity of these agents. These agents, such us amphotericin B, have low compatibility with hydrophobic cores of polymer micelles formed by many conventional block copolymers. Thus to increase solubilization of amphotericin B the core-forming blocks of methoxy-PEO-b-poly(L-aspartate) were derivatized with stearate side chains.[169-172] The resulting block copolymers formed micelles. Amphotericin B interacted strongly with stearate side chains in the core of the micelles resulting in efficient of entrapment the drug in the micelles and subsequent sustained release in the external environment. As a result of solubilization of amphotericin B in the micelles the onset of hemolytic activity of this drug toward bovine erythrocytes was delayed relative to that of the free drug.[171] Using a neutropenic murine model of disseminated candidas, it was shown that micelle-incorporated amphotericin B retained potent in vivo activity. Pluronic block copolymers were used by the same group for incapsulation of another poorly soluble antifungal agent, nystatin.[172] This is a commercially available drug that has shown potential for systemic administration, but has never been approved for that purpose due to toxicity issues. The possibility to use Pluronic block copolymers to overcome resistance to certain antifungal agents has been also demonstrated.[173-176] Overall one should expect further scientific developments using polymer micelle delivery systems for treatment of fungal infection.

5.5.4 Delivery of Imaging Agents

Efficient delivery of imaging agents to the site of disease in the body can improve early diagnostics of cancer and other diseases. The studies

in this area using polymer micelles as carriers for imaging agents were initiated by the group of Torchilin.[177] For example, micelles of amphiphilic PEO-lipid conjugates were loaded with ^{111}In and gadolinium diethylenetriamine pentaacetic acid-phosphatidylethanolamine (Gd-DTPA-PE) and then used for visualization of local lymphatic chain after subcutaneous injection into the rabbit's paw.[178] The images of local lymphatics were acquired using a gamma camera and a magnetic resonance (MR) imager. The injected micelles stayed within the lymph fluid, thus serving as lymphangiographic agents for indirect MR or gamma lymphography. Another polymer micelle system composed of amphiphilic methoxy-PEO-b-poly[epsilon,N-(triiodobenzoyl)-L-lysine] block copolymers labeled with iodine was administered systemically in rabilts and visualized by X-ray computed tomography.[179] The labeled micelles displayed exceptional 24 hr half-life in the blood, which is likely due to the core-shell architecture of the micelle carriers that protected the iodine-containing core. Notably, small polymer micelles (< 20 nm) may be advantageous for bioimaging of tumors compared to PEG-modified long-circulating liposomes (ca. 100 nm). In particular, the micelles from PEO-distearoyl phosphatidyl ethanolamine conjugates containing ^{111}In-labeled model protein were more efficacious in delivery of the protein to Lewis lung carcinoma than larger long-circulating liposomes.[180] Overall, polymer micelles loaded with various agents for gamma, magnetic resonance, and computed tomography imaging represent promising modalities for non-invasive diagnostics of various diseases.

5.5.5 Delivery of Polynucleotides

To improve the stability of polycation-based DNA delivery complexes in dispersion block and graft copolymers containing segments from polycations and nonionic water-soluble polymers, such as PEO, were developed.[90,181,182] Binding of these copolymers with DNA results in the formation of micelle-like block ionomer complexes (“polyion complex micelles”) containing hydrophobic sites formed by the polycation-neutralized DNA and hydrophilic sites formed by the PEO chains. Despite neutralization of charge, complexes remain stable in aqueous dispersion due to the effect of the PEO chains.[183] Overall the PEO modified polycation-DNA complexes form stable dispersions and do not interact with serum proteins.[183,184] These systems were used successfully for intravitreal

delivery of an antisense oligonucleotide and suppression of gene expression in retina in rats.[185] Furthermore, they displayed extended plasma clearance kinetics and were shown to transfect liver and tumor cells after systemic administration in the body.[186-188] In addition there is a possibility of targeting of such polyplexes to specific receptors at the surface of the cell, for example, by modifying the free ends of PEO chains with specific targeting ligands.[189-191] Alternatively, to increase binding of the complexes with the cell membrane and the transport of the polynucleotides inside cells the polycations were modified with amphiphilic Pluronic molecules.[192,193] One recent study has shown a potential of Pluronic-polyethyleneimine-based micelles for *in vivo* delivery of antisense oligonucleotides to tumors and demonstrated sensitization of the tumors to radiotherapy as a result of systemic administration of the oligonucleotide-loaded micelles.[194]

5.6 Clinical Trials

Three polymer micelle formulations of anticancer drugs have been reported to reach clinical trials. The doxorubicin-conjugated polymer micelles developed by Kataoka's group[195] have progressed recently to Phase I clinical trial at the National Cancer Center Hospital (Tokyo, Japan). The micelle carrier NK911 is based on PEO-b-poly(aspartic acid) block copolymers, in which the aspartic acid units were partially (ca. 45%) substituted with doxorubicin to form hydrophobic block. The resulting substituted block copolymer forms micelles that are further noncovalently loaded with free doxorubicin. Preclinical studies in mice demonstrated higher NK911 activity against Colon 26, M5076, and P388 compared to the free drug. Moreover, NK911 has less side effects resulting in less animal body and toxic death than the free drug.[196]

The Pluronic micelle formulation of doxorubicin has been most advanced clinically. Based on the *in vivo* efficacy evaluation Pluronic L61 was selected for clinical development for treatment of MDR cancers. The final block copolymer formulation is a mixture of 0.25% Pluronic L61 and 2% Pluronic F127 formulated in isotonic buffered saline.[130] This system contains mixed micelles of L61 and F127 with an effective diameter of ca. 22 to 27 nm and is stable in the serum. Prior to administration doxorubicin is mixed with this system, which results in spontaneous incorporation of the drug in the micelles. The drug is easily released by diffusion after dilution of the micelles. The formulation of

doxorubicin with Pluronic, SP1049C is safe following systemic administration based on toxicity studies in animals.[130] A two-site Phase I clinical trial of SP1049C has been completed.[197] Based on its results the dose-limiting toxicity of SP1049C was myelosuppression reached at 90 mg/m^2 (maximum tolerated dose was 70 mg/m^2). The phase II study of this formulation to treat inoperable metastatic adenocarcinoma of the esophagus is close to completion.[198]

Finally, Phase I studies were reported for Genexol-PM, a Cremophor-free polymer micelle-formulated paclitaxel.[199] Twenty-one patient entered into this study with lung, colorectal, breast, ovary, and esophagus cancers. No hypersensitivity reaction was observed in any patient. Neuropathy and myalgia were the most common toxicities. There were 14% partial responses. The paclitaxel area under the curve and peak of the drug concentration in blood were increased with the escalating dose suggesting linear pharmacokinetics for Genexol-PM.[199]

5.7 Conclusions

About two decades have passed since the conception of the polymer micelle conjugates and nanocontainers for drug delivery. During the first decade only few studies were published, however, more recently the number of publications in this field has increased drastically. During this period novel biocompatible and/or biodegradable block copolymer chemistries have been researched, the block ionomer complexes capable of incorporating DNA and other charged molecules have been discovered, the pH and other chemical signal sensitive micelles have been developed. Many studies focused on use of polymer micelles for delivery of poorly soluble and toxic chemotherapeutic agents to the tumors to treat cancer. There has been considerable advancement in understanding of the processes of polymer micelle delivery into the tumors including passive and vectorized targeting of the polymer micelles. Notable achievements also include the studies demonstrating the possibilities for overcoming multidrug resistance in cancer and enhance drug delivery to the brain using block copolymer micelles systems. Overall it is clear that this area has reached a mature stage, which is reinforced by the fact that several human clinical trials using polymer micelles for cancer drug delivery have been initiated. At the same time it is obvious that the possibilities for delivery of the diagnostic and therapeutic agents using polymer micelles are extremely broad and

one should expect further increase in the laboratory and clinical research in this field during the next decade. Targeting polymer micelles to cancer sites within the body will address an urgent need to greatly improve the early diagnosis and treatment of cancer. Capabilities for the discovery and use of targeting molecules will support the development of multifunctional therapeutics that can carry and retain antineoplastic agents within tumors. This will also be instrumental in developing novel biosensing and imaging modalities for the early detection of cancer and other devastating human diseases.

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