CMLS, Cell. Mol. Life Sci. 61 (2004) 2549–2559
1420-682X/04/202549-11
DOI 10.1007/s00018-004-4153-5

CMLS Cellular and Molecular Life Sciences

Review

Targeted polymeric micelles for delivery of poorly soluble drugs

V. P. Torchilin

Department of Pharmaceutical Sciences, Northeastern University, Mugar Building, Room 312, 360 Huntington Avenue, Boston, Massachusetts 02115 (USA), Fax: +1 617 373 8886, e-mail: v.torchilin@neu.edu

Received 7 April 2004; received after revision 2 June 2004; accepted 22 June 2004

Abstract. Polymeric micelles (micelles formed by amphiphilic block copolymers) demonstrate a series of attractive properties as drug carriers, such as high stability both in vitro and in vivo and good biocompatibility, and can be successfully used for the solubilization of various poorly soluble pharmaceuticals. These micelles can also be used as targeted drug delivery systems. The targeting can be achieved via the enhanced permeability and retention effect (into the areas with the compromised vasculature), by making micelles of stimuli-responsive amphiphilic block copolymers, or by attaching specific targeting ligand molecules to the micelle surface. Immunomicelles prepared by coupling monoclonal antibody molecules to p-nitrophenylcarbonyl groups on the water-exposed termini of the micelle corona-forming blocks demonstrate high binding specificity and targetability. Immunomicelles prepared with cancer-specific monoclonal antibody 2C5 specifically bind to different cancer cells in vitro and demonstrate increased therapeutic activity in vivo. This new family of pharmaceutical carriers can be used for the solubilization and targeted delivery of poorly soluble drugs to various pathological sites in the body.

Key words. Targeted drug delivery; poorly soluble drugs; micelles; amphiphilic polymers; polymer-lipid conjugates; enhanced permeability and retention; immunotargeting.

Introduction

To minimize drug degradation and loss upon administration, prevent harmful or undesirable side-effects, and increase drug bioavailability and the fraction of the drug accumulated in the pathological zone, various drug delivery and drug targeting systems are currently being developed or under development. Among drug carriers one can find soluble polymers, microparticles made of natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes and micelles [1, 2]. Each of those carrier types offers its own advantages and shortcomings, and all those carriers can be made slowly degradable, stimuli reactive (for example, pH or temperature sensitive) and even targeted (for example, by conjugating them with specific antibodies against certain characteristic components of the area of interest). In addition, drug carriers should be long circulating [3, 4] since prolonged circulation allows for maintaining the required therapeutic level of pharmaceuticals in the blood for extended time intervals. Long-circulating, high molecular weight drugs or drug-containing microparticulates can also slowly accumulate in pathological sites with affected and leaky vasculature (such as tumors, inflammations and infarcted areas) via the enhanced permeability and retention effect (EPR) and enhance drug delivery in these areas [5, 6]. In addition, prolonged circulation can help to achieve a better targeting effect for specific ligand-modified drugs and drug carriers since it increases the total quantity of targeted drug/carrier passing through the target, and the number of interactions between targeted drugs and their targets [7].

Ideally, pharmaceutical drug carriers for parenteral administration are expected to be biodegradable, have small particle size, possess high loading capacity, demonstrate prolonged circulation and accumulate in required pathological sites in the body [8]. The development of drug carriers meeting all these requirements for poorly soluble pharmaceuticals still represents a challenge. The therapeutic application of hydrophobic, poorly water soluble agents is associated with some serious problems, since low water solubility results in poor absorption and low bioavailability upon the oral administration [9]. In addition, drug aggregation upon intravenous administration might lead to such complications as embolism [10]. The formation of drug aggregates can also result in high local concentrations at the sites of aggregate deposition associated with local toxicity and lowered systemic bioavailability [11]. On the other hand, hydrophobicity and low solubility in water appear to be intrinsic properties of many drugs (such as anticancer agents, since many of them are bulky polycyclic compounds, such as camptothecin, paclitaxel and tamoxifen) [12]. This hydrophobicity helps a drug molecule to penetrate a cell membrane when the molecular target for the drug is located intracellularly [13, 14]. In addition, it has also been observed that a drug or, more generally, a biologically active molecule may need a hydrophobic group to have a sufficient affinity toward the appropriate target receptor [15, 16]. Almost a half of potentially valuable drug candidates identified by high-throughput screening technologies demonstrate poor solubility in water and, for this reason, never enter a formulation development stage [17].

To overcome the poor solubility of some drugs, certain clinically acceptable organic solvents are used in formulations [18]. More recent approaches include the use of liposomes [19] and cyclodextrins [17]. The administration of many co-solvents or surfactants causes toxicity and other undesirable side effects [20]. The use of liposomes and cyclodextrins demonstrated some promising results with certain poorly soluble drugs, although the capacity of the liposomal membrane and cyclodextrin inner cavity for water-insoluble molecules is rather limited. Another option is to use certain micelle-forming surfactants in formulations of insoluble drugs [18].

Micelles and micellization in drug delivery

Micelles represent so-called colloidal dispersions (with particle size normally within the 5–100-nm range) that belong to a large family of dispersed systems consisting of particulate matter or dispersed phase, distributed within a continuous phase or dispersion medium. They belong to a group of association or amphiphilic colloids. Such colloids are spontaneously formed under certain concentration and temperature by amphiphilic or surface-active agents (surfactants), molecules of which consist of two clearly distinct regions with opposite affinities toward a given solvent [21]. At low concentrations in aqueous medium, these amphiphilic molecules exist separately; however, as their concentration is increased, aggregation takes place within a rather narrow concentration interval. Those aggregates, known as micelles, include several dozens of amphiphilic molecules and usually have a shape close to spherical. The concentration of a monomeric amphiphile at which micelles appear is called the critical micelle concentration (CMC), while the temperature below which amphiphilic molecules exist as unimers and above as aggregates is called the critical micellization temperature (CMT). Hydrophobic fragments of amphiphilic molecules form the core of a micelle, which can solubilize poorly soluble pharmaceuticals, while hydrophilic fragments form the micelle's corona [22–24]. In aqueous systems, nonpolar molecules are solubilized within the micelle core, polar molecules will be adsorbed on the micelle surface and substances with intermediate polarity will be distributed along surfactant molecules in intermediate positions. Figure 1 shows a principal scheme of micelle formation from an amphiphilic molecule and its loading with a poorly soluble drug.

Kabanov et al. [25] suggest that an 'ideal' self-assembling drug delivery systems should spontaneously form from the mixture of drug molecules, carrier components and targeting moieties. Their size should be of around 10 nm in order to be able to penetrate various tissues and even cells. They should be stable in vivo for a sufficiently long time and should not provoke any biological reactions. They should release a free drug upon contact with target tissues or cells; and, finally, the components of the carrier should be easily removed from the body when the therapeutic function is completed.

In broad terms, micelles as drug carriers provide a set of unbeatable advantages [26–31]. The solubilization of drugs using micelle-forming surfactants (which results in formation of mixed micelles) leads to increased water solubility of a sparingly soluble drug and its improved bio-

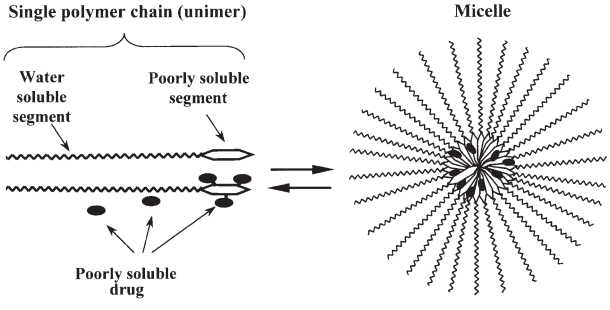

Figure 1. Micelle formation from amphiphilic unimers and drug incorporation into the micelle core by both covalent attachment to the hydrophobic fragment of the unimer and by non-covalent incorporation into the hydrophobic core micelle.

availability, reduction of toxicity and other adverse effects, enhanced permeability across the physiological barriers and substantial changes in drug biodistribution. The use of certain special amphiphilic molecules as surfactants can also introduce the property of micelle extended blood half-life upon intravenous administration [31]. Besides, micelles may be made targeted by chemical attachment of targeting moiety to their surface. In the latter case, local release of the loaded drug from the micelles in the target organ should lead to increased efficacy of the drug. On the other hand, being in a micellar form en route to the target organ or tissue, the drug is well protected from possible inactivation under the effect of biological surroundings, and itself does not provoke undesirable side effects on non-target organs and tissues. According to our analysis of the available literature, the most usual size of a pharmaceutical micelle is between 10 and 80 nm, optimal CMC value is expected to be in a low millimolar region or even lower, and the loading efficacy toward a hydrophobic drug should be between 5 and 25% wt.

Polymeric micelles

Polymeric micelles represent a class of micelles and are formed from block copolymers consisting of hydrophilic and hydrophobic monomer units. It has repeatedly been shown that amphiphilic block and graft AB-type copolymers with the length of a hydrophilic block exceeding to some extent that of a hydrophobic one can form spherical micelles in aqueous solutions [31]. The particulates are composed of the core of the hydrophobic blocks stabilized by the corona of hydrophilic polymeric chains. If the length of a hydrophilic block is too high, copolymers exist in water as unimers (individual molecules), while molecules with very long hydrophobic blocks form structures with non-micellar morphology, such as rods and lamellae [32]. The major driving force behind self-association of amphiphilic polymers is the decrease of free energy of the system due to removal of hydrophobic fragments from the aqueous surroundings with the formation of a micelle core stabilized with hydrophilic blocks exposed into water [33, 34]. The lower the CMC value of a given amphiphilic polymer, the more stable micelles are even at low net concentration of amphiphile in the medium [35]. This is especially important from the practical point of view, since upon dilution with a large volume of blood, micelles with a high CMC value may dissociate into unimers, and their content may precipitate in the blood. Numerous studies have been published developing a theoretical description of micelle formation and properties. References [27, 35, 36] represent just a few examples.

The core compartment of the pharmaceutical polymeric micelle should demonstrate a high loading capacity, a controlled release profile for the incorporated drug, and good compatibility between the core-forming block and the incorporated drug. The micelle corona should provide effective steric protection for the micelle. It should also determine micelle hydrophilicity, charge, the length and surface density of hydrophilic blocks, and the presence of reactive groups suitable for further micelle derivatization, such as attachment of targeting moieties [37–41]. These properties control important biological characteristics of a micellar carrier, such as its pharmacokinetics, biodistribution, biocompatibility, longevity, surface adsorption of biomacromolecules, adhesion to biosurfaces and targetability [1, 37–40, 42, 43].

In the majority of cases the structure of amphiphilic unimers follows a few simple rules: poly (ethylene glycol) (PEG) blocks with a molecular weight from 1 to 15 kDa are usual corona-forming blocks, and the length of a hydrophobic core-forming block is close or somewhat lower than that of a hydrophilic block [44]. Though other hydrophilic polymers may be used to make corona blocks [45, 46], PEG still remains the hydrophilic block of choice. At the same time, a variety of polymers may be used to build hydrophobic core-forming blocks: propylene oxide [39, 47], L-lysine [48, 49], aspartic acid [50, 51], β-benzoyl-L-aspartate [52, 53], γ-benzyl-L-glutamate [54], caprolactone [55, 56], D,L-lactic acid [40, 57] and spermine [58].

In some cases, phospholipid residues – short, but extremely hydrophobic due to the presence of two long-chain fatty acyl groups – can also be successfully used as hydrophobic core-forming groups [26]. The use of lipid moieties as hydrophobic blocks capping hydrophilic polymer (such as PEG) chains can provide additional advantages for particle stability when compared with conventional amphiphilic polymer micelles, due to the existence of two fatty acid acyls which might contribute considerably to an increase in the hydrophobic interactions between the polymeric chains in the micelle's core. Diacyllipid-PEG conjugates have been introduced into the area of controlled drug delivery as polymeric surface modifiers for liposomes [59]. However, the diacyllipid-PEG molecule itself represents a characteristic amphiphilic polymer with a bulky hydrophilic (PEG) portion and a very short but extremely hydrophobic diacyllipid part (see fig. 2). Similar to other PEG-containing amphiphilic block copolymers, diacyllipid-PEG conjugates were found to form micelles in an aqueous environment [60]. A series of PEG-phosphatidylethanolamine (PEG-PE) conjugates was synthesized using egg PE and N-hydroxysuccinimide esters of methoxy-PEG succinates (molecular weight of 2 kDa, 5 kDa and 12 kDa) [59]. High-pressure liquid chromatography (HPLC)-based gel permeation chromatography showed that these polymers form micelles of different sizes in water. No dissociation into individual polymeric chains was found following the

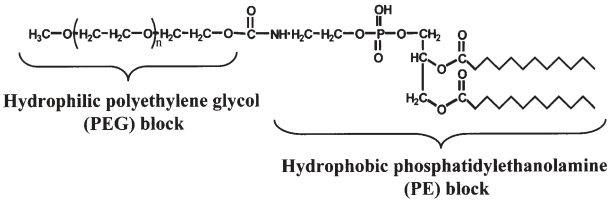

Figure 2. Chemical structure of an amphiphilic PEG-PE conjugate.

chromatography of the serially diluted samples of PEG (5 kDa)-PE up to polymer concentrations of ca. 1 μg/ml, which corresponds to a micromolar CMC value, which is at least 100-fold lower than those of conventional detergents [61]. The high stability of polymeric micelles allows for their stability upon dilution and good retention of encapsulated drugs in the solubilized form upon parenteral administration.

The process of solubilization of water-insoluble drugs by micelle-forming amphiphilic block copolymers was investigated both as a theoretical and practical problem [62, 63]. Extensive solubilization may result in some increase in micelle size due to the expansion of its core with a solubilized drug. Among other factors influencing the efficacy of drug loading into the micelle one can name the size of both core-forming and corona-forming blocks [64]. In the first case, the larger the hydrophobic block, the bigger the core size and the greater its ability to entrap hydrophobic drugs. In the second case, the increase in the length of the hydrophilic block increases the CMC value; i.e. at a given concentration of the amphiphilic polymer in solution, the smaller fraction of this polymer will be present in the micellar form and the quantity of the micelle-associated drug drops.

Drugs, such as diazepam and indomethacin [65, 66], adriamicin [50, 67–69], anthracycline antibiotics [70] and polynucleotides [71, 72] were effectively solubilized by polymeric micelles and demonstrated superior properties (as in the case of doxorubicin [73]) and lower toxicity [74] compared to free drugs. The circulation time and biodistribution of polymeric micelles formed by the copolymer of PEG and poly(aspartic acid) (PEG-b-PAA) with covalently bound adriamycin [PEG-b-PAA(ADR)] depended on the relative size of the copolymer blocks. Longer PEG blocks and shorter PAA segments favor longer circulation times and lower uptake of the micelles by the reticuloendothelial system [27, 50, 75, 76]. The whole set of micelle-forming copolymers of PEG with poly(L-amino acids) was used to prepare drug-loaded micelles by direct entrapment of a drug into the micelle core [52, 77–79]. PEG-b-poly(caprolactone) copolymer micelles were successfully used as delivery vehicles for dihydrotestosterone [80]. PEG-PE micelles can efficiently incorporate a variety of sparingly soluble and amphiphilic substances, including paclitaxel, tamoxifen, camptothecin, porphyrine, vitamin K3 and others [81– 83].

Targeted polymeric micelles

Making micelles capable of specific or, at least, preferential accumulation in desired body compartments or pathological zones (i.e. making targeted micelles) can further increase the efficiency of micelle-encapsulated pharmaceuticals (drugs and diagnostic agents). There are several approaches to achieve this. The first is the already mentioned preferential accumulation of drug-loaded micelles in areas with ‘leaky’ vasculature (tumors and infarcts) via the EPR effect. The EPR effect is based on the spontaneous penetration of long-circulating macromolecules, molecular aggregates and particulate drug carriers into the interstitium through the compromised vasculature in certain pathological sites in the body. It has been shown that the EPR effect is typical for solid tumors and infarcts [5, 6]. This approach, which can be called passive targeting, is currently under development by many research groups. Thus, micelle formulations from PEG_{750}-PE, PEG_{2000}-PE, and PEG_{5000}-PE conjugates demonstrated much higher accumulation in tumors compared to non-target tissue (muscle) in experimental Lewis lung carcinoma (LLC; tumor with a relatively small vasculature cut-off size [84, 85]) in mice. The accumulation pattern of PEG-PE micelles prepared from all versions of PEG-PE conjugates is characterized by the peak tumor accumulation times of about 5 h post-injection. The largest total tumor uptake of the injected dose within the observation period (AUC) was found for micelles formed by PEG_{5000}-PE. This was explained by the fact that these micelles had the longest circulation time and little extravasation into the normal tissue compared to micelles prepared from the ‘shorter’ PEG-PE conjugates. Micelles prepared from PEG-PE conjugates with shorter versions of PEG, however, might be more efficient carriers of poorly soluble drugs because they have a greater hydrophobic-to-hydrophilic phase ratio and can be loaded with drug more efficiently on a weight-to-weight basis. The ability of PEG-PE micelles to accumulate selectively in tumors was also confirmed with another murine tumor model, EL4 T cell lymphoma (EL4) [86]. Micelles prepared from PEG_{750}-DSPE or PEG_{2000}-DSPE demonstrated selective accumulation in this tumor as well. Some other recent data also clearly indicate spontaneous targeting of PEG-PE-based micelles into experimental tumors [87] in mice as well as into the damaged heart areas in rabbits with experimental myocardial infarction [88] (fig. 3).

Thus, the transport efficacy and accumulation of microparticulates, such as liposomes and/or micelles, in the tumor interstitium is to a great extent determined by their ability to penetrate the tumor vascular endothelium [89,

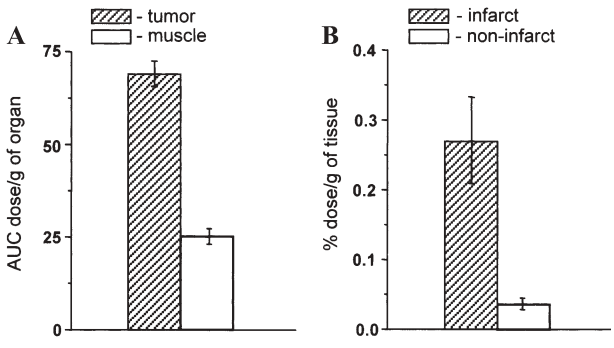

Figure 3. In vivo micelle accumulation in the pathological areas with increased vascular permeability. (*A*) Accumulation of PEG-PE micelles (as AUC) in murine Lewis lung carcinoma tumor compared to adjacent normal tissue. (*B*) Accumulation of PEG-PE micelles (as % dose per gram of tissue) in the area of experimental myocardial infarction in rabbit compared to surrounding non-infarcted myocardium.

90]. Diffusion and accumulation parameters were recently shown to be strongly dependent on the cutoff size of tumor blood vessel wall, and the cutoff size varies for different tumors [90–92]. Adriamycin in polymeric micelles was shown to be much more efficient in experimental treatment of murine solid tumor colon adenocarcionoma than the free drug [93]. Since tumor vasculature permeability depends on the particular type of the tumor [91], the use of micelles as drug carriers could be specifically justified for tumors whose vasculature has the low cutoff size (below 200 nm). Thus, the use of PEG-PE micelles for the effective delivery of a model protein drug to a solid tumor, Lewis lung carcinoma, in mice was reported [85]. In a general case, the biodistribution of a microparticulate carrier-associated anticancer drug depends on its circulation time in blood. Thus, it has been shown that long-circulating PEG-grafted liposomes demonstrate increased accumulation in implanted tumors [94]. Later, however, it was found that in some cases even the use of long-circulating liposomes could not provide their sufficient accumulation in certain tumors. Parr et al. [95] have shown that coating 100-nm liposomes with PEG did not result in increased accumulation of liposome-encapsulated drug in a subcutaneously established murine Lewis lung carcinoma. This phenomenon may be explained by the low vascular permeability (small cutoff size) of this as well as some other tumors. In those cases, drug carriers smaller in size than liposomes may provide more efficient drug delivery into tumors. Thus, the micelle-incorporated model protein (soybean trypsin inhibitor or STI, MW 21.5 kDa) accumulates to a higher extent in subcutaneously established murine Lewis lung carcinoma than the same protein in larger liposomes [85].

Another targeting mechanism is based on the fact that many pathological processes in various tissues and organs are accompanied with local temperature increase and/or acidosis [96, 97]. So, the efficiency of the micellar carriers can be further improved by making micelles capable of disintegration under the increased temperature or decreased pH values in pathological sites, i. e. by combining the EPR effect with stimuli responsiveness. For this purpose, micelles are made of thermo- or pH-sensitive components such as poly(N-isopropylacrylamide) and its copolymers with poly(D,L-lactide) and other blocks, and acquire the ability to disintegrate in target areas, releasing the micelle-incorporated drug [33, 44, 98–100]. pH-responsive polymeric micelles loaded with phtalocyanine seem to be promising carriers for photodynamic cancer therapy [101], while doxorubicin-loaded polymeric micelles containing acid-cleavable linkages provided an enhanced intracellular drug delivery into tumor cells and thus higher efficiency [102]. Thermo-responsive polymeric micelles were shown to demonstrate increased drug release upon temperature changes [103]. The penetration of drug-loaded polymeric micelles into cells (tumor cells) as well as drug release from the micelles can be enhanced by externally applied ultrasound [104].

As with other delivery systems, the drug delivery potential of polymeric micelles may be still further enhanced by attaching targeting ligands to the micelle surface. The attachment of various specific ligands to the water-exposed termini of hydrophilic blocks could be used to improve the targeting of micelles and micelle-incorporated drugs and DNA [31]. Among those ligands one can name various sugar moieties [105], transferrin [106] and folate residues [107] since many target cells, especially cancer cells, overexpress appropriate receptors (such as transferrin and folate receptors) on their surface. Thus, it was shown that galactose- and lactose-modified micelles made of PEG-polylactide copolymer specifically interact with lectins, thus modeling targeting delivery of the micelles to hepatic sites [105, 108]. Transferrin-modified micelles based on PEG and poly(ethyleneimine) sized between 70 and 100 nm are expected to target tumors with overexpressed transferrin receptors [106]. Mixed micelle-like complexes of PEGylated DNA and PEI modified with transferrin [109, 110] were designed for enhanced DNA delivery into cells overexpressing the same transferrin receptors. A similar targeting approach was successfully tested with folate-modified micelles [111]. Poly(L-histidine)/PEG and poly (L-lactic acid)/PEG block copolymer micelles carrying folate residues on their surface were shown to be efficient for the delivery of adriamycin to tumor cells in vitro, demonstrating the potential for solid tumor treatment and combined targetability and pH sensitivity [112].

Polymeric immunomecelles

Among all specific ligands, antibodies provide the broadest opportunities in terms of diversity of targets

and specificity of interaction. Several attempts to covalently attach an antibody to a surfactant or polymeric micelles (i.e. to prepare immunomicelles) have been described [31, 87, 106, 113]. Thus, micelles modified with fatty acid-conjugated Fab fragments of antibodies to antigens of brain glial cells (acid gliofibrillar antigen and alpha-2-glycoprotein) loaded with neuroleptic trifluoperazine increasingly accumulated in the rat brain upon intracarotide administration [113, 114].

By adapting the coupling technique developed for attaching specific ligands to liposomes [115], we have prepared PEG-PE-based immunomicelles modified with monoclonal antibodies. The approach uses PEG-PE with the free PEG terminus activated with a p-nitrophenylcarbonyl (pNP) group. Diacyllipid fragments of such a bifunctional PEG derivative firmly incorporate into the micelle core, while the water-exposed pNP group, stable at pH values below 6, efficiently interacts with amino groups of various ligands (such as antibodies and their fragments) at pH values above 7.5, yielding a stable urethan (carbamate) bond. All non-reacted pNP groups spontaneously hydrolyze at the same pH values. To prepare immunotargeted micelles, the antibody to be attached was simply incubated with drug-loaded micelles at pH around 8.0 (fig. 4). The micelle-attached protein was quantified using fluorescent labels or by SDS-polyacrylamide gel electrophoresis (PAGE) [87, 116]. It was calculated that 10–20 antibody molecules could be attached to a single micelle. Antibodies attached to the micelle corona preserve their specific binding ability, and immunomicelles specifically recognize their target substrates, as was confirmed by ELISA (enzyme-linked immunosorbent assay) with corresponding substrate monolayers. The analysis of micelle size and size distribution before and after attachment of various antibodies using dynamic light scattering and freeze-fracture electron microscopy demonstrated that protein attachment did not affect the size of the micelles substantially [87]. Both the original and antibody-modified micelles have a spherical shape and a uniform size of about 20 nm.

To specifically enhance the tumor accumulation of PEG-PE-based micelles, the latter have been modified with tumor-specific monoclonal antibodies [87, 116] Although anticancer antibodies are usually tumor type specific and unable to react with different tumors, earlier we showed that certain non-pathogenic monoclonal antinuclear autoantibodies with nucleosome-restricted specificity [monoclonal antibody 2C5 (mAb 2C5) being among them] recognize the surface of numerous tumor, but not normal, cells via tumor cell surface-bound nucleosomes [117, 118]. Because these antibodies bind a broad variety of cancer cells, they may serve as specific ligands for the delivery of drugs and drug carriers into tumors. The data shown in figure 5 indicate that rhodamine-labeled 2C5 immunomicelles effectively bind to the surface of several unrelated tumor cell lines: human BT20 (breast adenocarcinoma) and murine LLC (Lewis lung carcinoma) and EL4 (T lymphoma) cells. The incubation of antibody-free micelles with the same cells results in virtually no micelle-to-cell association. Drug(paclitaxel)-loaded 2C5 immunomicelles also demonstrated the same specific properties as ‘empty’ immunomicelles and effectively bound various tumor cells (fig. 5). Such specific recognition of cancer cells by drug-loaded mAb 2C5 immunomicelles results in dramatically improved cancer cell killing by such micelles. Figure 6 presents the results of in vitro experiments with human breast cancer MCF-7 cells, which showed a clearly superior efficiency of paclitaxel-loaded 2C5 immunomicelles compared to paclitaxel-loaded plain micelles or free drug.

In vivo experiments with LLC tumor-bearing mice revealed a dramatically enhanced tumor uptake of paclitaxel-loaded radiolabeled 2C5 immunomicelles compared to non-targeted micelles (fig. 7). An enhanced accumulation of 2C5-targeted micelles over plain micelles in the tumor (up to 30%) was observed both at 30 min and at 2 h post-injection, evidencing specific recognition and tumor binding of 2C5-targeted immunomicelles. These data suggest the possibility that drug-loaded immunomicelles may also be better internalized by tumor cells similar to antibody-targeted liposome [119] and, thus, deliver more drug inside tumor cells that might be achieved in the case of simple EPR effect-mediated tumor accumulation. By analyzing the absolute quantity of tumor-accu-

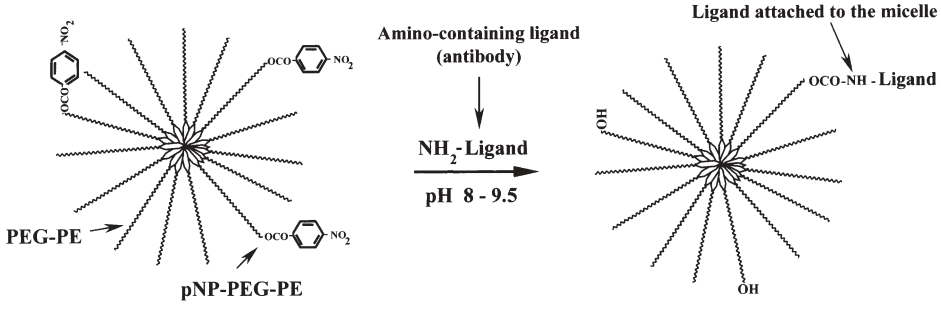

Figure 4. Antibody attachment to pNP-PEG-PE unimer via the pNP group.

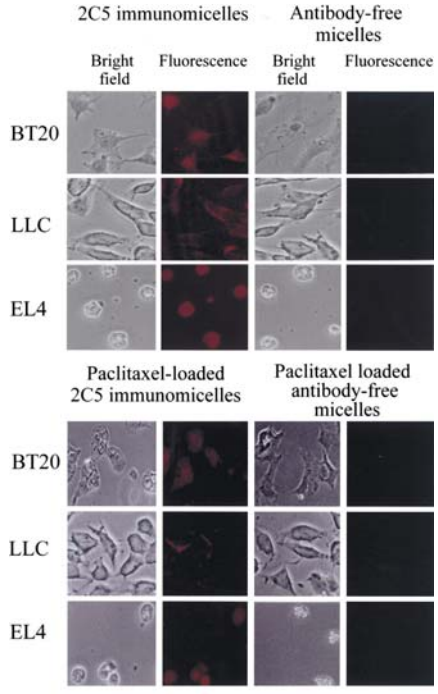

Figure 5. Binding of rhodamine-labeled empty and paclitaxel-loaded PEG-PE-based mAb 2C5 immunomicelles to tumor cells (murine Lewis lung carcinoma and EL4 T cell lymphoma, and human breast adenocarcinoma BT20) in vitro. Empty and drug-loaded immunomicelles demonstrate pronounced association with all cell lines (cell surface-bound red fluorescence); plain micelles do not bind to tumor cells.

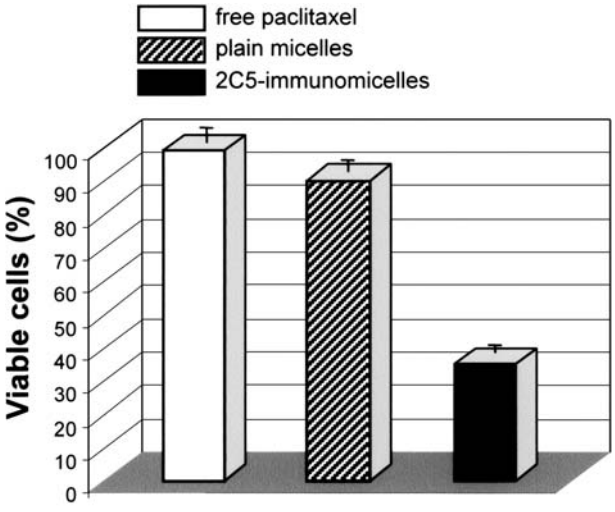

Figure 6. In vitro cytotoxicity of various preparations of paclitaxel against human breast adenocarcinoma MCF7 cells. The maximum level of cell killing is observed with the drug in mAb 2C5 immunomicelles.

mulated paclitaxel (HPLC [120]) delivered by different drug formulations, it was shown that mAb 2C5 immunomicelles were capable of bringing into tumors substantially higher quantities of paclitaxel than in the case of paclitaxel-loaded non-targeted micelles or free drug formulations (Taxol; fig. 7). The difference in tumor accumulation of paclitaxel delivered by immunomicelles compared to other taxol preparations was larger at 2 h post-injection compared to 30 min post-injection. This fact might be explained by the accumulation of non-targeted paclitaxel formulations in the interstitial space of the tumor via the EPR effect and eventual drug clearance from this site. Contrary to that, paclitaxel-loaded 2C5 immunomicelles were internalized by cancer cells and thus kept the drug inside the tumor in a way similar to what was observed with drug-loaded anti-her2 immunoliposomes [119]. Internalization by tumor cells may have high therapeutic significant for many antitumor agents. For example, a much higher tumor regression was ob-

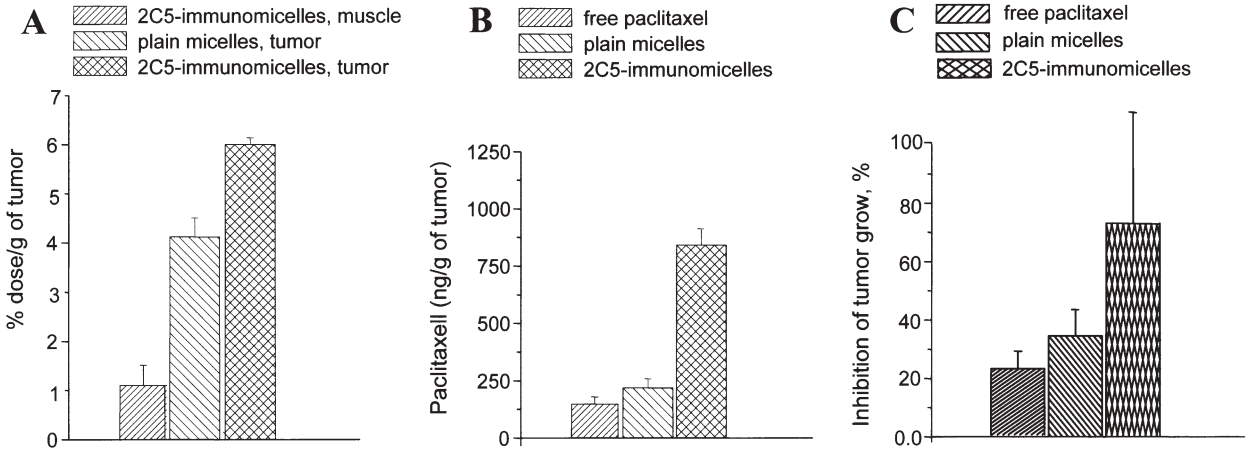

Figure 7. Paclitaxel-loaded PEG-PE-based mAb 2C5 immunomicelles in vivo. (*A*) Tumor accumulation of paclitaxel-loaded mAb 2C5 immunomicelles in Lewis lung carcinoma model in mice 2 h post- administration. (*B*) Tumor accumulation of free paclitaxel. (*C*) Inhibition of Lewis lung carcinoma growth in mice by various preparations of paclitaxel. Immunomicelles show the highest accumulation of all preparations, bring the maximum quantity of the drug into tumor and allow for maximum inhibition of tumor growth.

served with a carrier capable of intracellular drug delivery for an equal doxorubicin dose delivered to the tumor [119]. Naturally, it results in a higher therapeutic efficiency of paclitaxel-loaded mAb 2C5 micelles. The average weight of excised tumors in the group treated with paclitaxel in mAb 2C5 immunomicelles was ~0.7 g compared to 1.6 and 1.4 g in groups treated with the same quantity of paclitaxel as free drug or in plain PEG-PE micelles, respectively ($P < 0.05$ in both cases). The weight of untreated tumors was around 2.0 g (fig. 7).

In summary, stable polymeric micelles possessing an excellent ability to carry a variety of poorly soluble pharmaceuticals can be used as targeted drug delivery systems. The targeting can be achieved via the EPR effect by making micelles of stimuli-responsive amphiphilic block copolymers or by attaching specific targeting ligand molecules to the micelle surface. Immunomicelles can be prepared by coupling monoclonal antibody molecules to pNP groups on the water-exposed termini of the micelle corona-forming blocks. These micelle-coupled antibodies preserve their specific activity. Immunomicelles prepared with cancer-specific mAb 2C5 specifically bind to different cancer cells in vitro and demonstrate increased accumulation in experimental tumors in vivo. Loaded with the poorly soluble anticancer drug paclitaxel, mAb 2C5 immunomicelles demonstrate significantly increased cytotoxicity toward tumor cells in vitro and in vivo. These pharmaceutical carriers, targeted polymeric micelles, could be used for the enhanced delivery of poorly soluble pharmaceuticals to various pathological sites via a variety of mechanisms.

1 Müller R. H. (1991) Colloidal Carriers for Controlled Drug Delivery and Targeting, Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany, and CRC Press, Boca Raton, FL
2 Cohen S. and Bernstein H. (eds) (1996) Microparticulate Systems for the Delivery of Proteins and Vaccines, Marcel Dekker, New York
3 Lasic D. D. and Martin F. (eds) (1965) Stealth Liposomes, CRC Press, Boca Raton, FL
4 Torchilin V. P. and Trubetskoy V. S. (1995) Which polymers can make nanoparticulate drug carriers long-circulating? Adv. Drug Deliv. Rev. **16:** 141–155
5 Palmer T. N., Caride V. J., Caldecourt M. A., Twickler J. and Abdullah V. (1984) The mechanism of liposome accumulation in infarction. Biochim. Biophys. Acta **797:** 363–368
6 Maeda H., Wu J., Sawa T., Matsumura Y. and Hori K. (2000) Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J. Control. Release **65:** 271–284
7 Torchilin V. P. (1998) Polymer-coated long-circulating microparticular pharmaceuticals. J. Microencapsul. **15:** 1–19
8 Gref R., Minamitake J., Peracchia M. T., Trubetskoy V., Torchilin V. P. and Langer R. (1994) Biodegradable long-circulating polymeric nanospheres. Science **263:** 1600–1603
9 Lipinski C. A., Lombardo F., Dominy B. W. and Feeney P. J. (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. **46:** 3–26
10 Fernandez A. M., Van Derpoorten K., Dasnois L., Lebtahi K., Dubois V., Lobl T. J. et al. (2001) N-Succinyl-(beta-alanyl-L-leucyl-L-alanyl-L-leucyl)doxorubicin: an extracellularly tumor-activated prodrug devoid of intravenous acute toxicity. J. Med. Chem. **44:** 3750–3753
11 Yalkowsky S. H. (ed.) (1981) Techniques of Solubilization of Drugs, Marcel Dekker, New York
12 Shabner B. A. and Collings G. M. (eds) (1990) Cancer Chemotherapy: Principles and Practice, Lippincott, Philadelphia, PA
13 Yokogawa K., Nakashima E., Ishizaki J., Maeda H., Nagano T. and Ichimura F. (1990) Relationships in the structure-tissue distribution of basic drugs in the rabbit. Pharm. Res. **7:** 691–696
14 Hageluken A., Grunbaum L., Nurnberg B., Harhammer R., Schunack W. and Seifert R. (1994) Lipophilic beta-adrenoceptor antagonists and local anesthetics are effective direct activators of G-proteins. Biochem. Pharmacol. **47:** 1789–1795
15 Lipinski C. A. (2000) Drug-like properties and the causes of poor solubility and poor permeability, J. Pharmacol. Toxicol. Methods **44:** 235–249
16 Lipinski C. A., Lombardo F., Dominy B. W. and Feeney P. J. (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. **46:** 3–26
17 Thompson D. and Chaubal M. V. (2000) Cyclodextrins (CDS) – excipients by definition, drug delivery systems by function (part I: injectable applications). Drug Deliv. Technol. **2:** 34–38
18 Yalkowsky S. H. (ed.) (1981) Techniques of Solubilization of Drugs, Marcel Dekker, New York
19 Lasic D. D. and Papahadjopoulos D. (eds) (1998) Medical Applications of Liposomes, Elsevier, New York
20 Ray R., Kibbe A. H., Rowe R., Shleskey P. and Weller P. (2003) Handbook of Pharmaceutical Excipients, APhA Publications, Washington, DC
21 Mittal K. L. and Lindman B. (eds) (1991) Surfactants in Solution, vols 1–3, Plenum Press, New York
22 Lasic D. D. (1992) Mixed micelles in drug delivery. Nature **355:** 279–280
23 Elworthy P. H., Florence A. T. and Macfarlane C. B. (eds) (1968) Solubilization by Surface Active Agents, Chapman and Hall, London, UK
24 Attwood D. and Florence A. T. (eds) (1983) Surfactant Systems, Chapman and Hall, London, UK
25 Kabanov A. V., Batrakova E. V., Melik-Nubarov N. S., Fedoseev N. A., Dorodnich T. Yu., Alakhov V. Yu. et al. (1992) A new class of drug carriers; micelles poly(oxyethylene)-poly(oxypropylene) block copolymers as microcontainers for drug targeting from blood to brain. J. Control. Release **22:** 141–158
26 Trubetskoy V. S. and Torchilin V. P. (1995) Use of polyoxyethylene-lipid conjugates as long-circulating carriers for delivery of therapeutic and diagnostic agents. Adv. Drug Deliv. Rev. **16:** 311–320
27 Kwon G. S. and Kataoka K. (1995) Block copolymer micelles as long-circulating drug vehicles. Adv. Drug Deliv. Rev. **16:** 295–309
28 Kwon G. S. (1998) Diblock copolymer nanoparticles for drug delivery. Crit. Rev. Ther. Drug Carrier Syst. **15:** 481–512
29 Kabanov A. V., Batrakova E. V. and Alakhov V. Y. (2002) Pluronic block copolymers as novel polymer therapeutics for drug and gene delivery. J. Control. Release **82:** 189–212
30 Jones M. and Leroux J. (1999) Polymeric micelles – a new generation of colloidal drug carriers. Eur. J. Pharm. Biopharm. **48:** 101–111
31 Torchilin V. P. (2001) Structure and design of polymeric surfactant-based drug delivery systems. J. Control. Release **73:** 137–172

32 Zhang L. and Eisenberg A. (1995) Multiple morphologies of 'crew-cut' aggregates of polystyrene-b-poly(acrylic acid) block copolymers. Science **268:** 1728–1731

33 Kwon G. S. and Okano T. (1999) Soluble self-assembled block copolymers for drug delivery. Pharm. Res. **16:** 597–600

34 Martin A. (1993) In: Physical Pharmacy, 4th ed., pp. 396–398, Williams and Wilkins, Baltimore, MD

35 Gao Z. and Eisenberg A. (1993) A model of micellization for block copolymers in solutions. Macromolecules **26:** 7353–7360

36 Marques C. M. (1997) Bunchy micelles. Langmuir **13:** 1430–1433

37 Torchilin V. P. and Trubetskoy V. S. (1996) Biodistribution of surface-modified liposomes and particles. In: Microparticulate Systems for the Delivery of Proteins and Vaccines, chapt. 8, pp. 243–277, Cohen S. and Bernstein H. (eds), Marcel Dekker, New York

38 Gref R., Domb A., Quellec P., Blunk T., Muller R. H., Verbavatz J. M. et al. (1995) The controlled intravenous delivery of drugs using PEG-coated sterically stabilized nanospheres. Adv. Drug Deliv. Rev. **16:** 215–234

39 Kabanov A. V., Chekhonin V. P., Alakhov V. Yu., Batrakova E. V., Lebedev A. S. and Melik-Nubarov N. S. (1989) The neuroleptic activity of haloperidol increases after its solubilization in surfactant micelles. FEBS Lett. **258:** 343–345

40 Hagan S. A., Coombes A. G. A., Garnett M. C., Dunn S. E., Davies M. C., Illum L. et al. (1996) Polylactide-poly(ethelene glycol) copolymers as drug delivery systems, 1. Caracterization of water dispersible micelle-forming systems. Langmuir **12:** 2153–2161

41 Inoue T., Chen G., Nakamae K. and Hoffman A. S. (1998) An AB block copolymers of oligo(methyl methacrylate) and poly(acrylic acid) for micellar delivery of hydrophobic drugs. J. Control. Release **51:** 221–229

42 Hunter J. R. (1991) In: Foundations of Colloid Science, vol.1, Oxford University Press, New York

43 Kuntz R. M. and Saltzman W. M. (1997) Polymeric controlled delivery for immunization. Trends Biotech. **15:** 364–369

44 Cammas S., Suzuki K., Sone C., Sakurai Y., Kataoka K. and Okano T. (1997) Thermorespensive polymer nanoparticles with a core-shell micelle structure as site specific drug carriers. J. Control. Release **48:** 157–164

45 Torchilin V. P., Shtilman M. I., Trubetskoy V. S., Whiteman K. and Milstein A. M. (1994) Amphiphilic vinyl polymers effectively prolong liposome circulation time in vivo. Biochim. Biophys. Acta **1195:** 181–184

46 Torchilin V. P., Trubetskoy V. S., Whiteman K. R., Caliceti P., Ferruti P. and Veronese F. M. (1995) New sunthetic amphiphilic polymers for steric protection of liposomes in vivo. J. Pharm. Sci. **84:** 1049–1053

47 Miller D. W., Batrakova E. V., Waltner T. O., Alakhov V. Yu. and Kabanov A. V. (1997) Interactions of pluronic block copolymers with brain microvessel endothelial cells: evidence of two potential pathways for drug absorption. Bioconj. Chem. **8:** 649–657

48 Katayose S. and Kataoka K. (1998) Remarkable increase in nuclease resistance of plasmid DNA through supramolecular assembly with poly(ethylene glycol)-poly(L-lysine) block copolymer. J. Pharm. Sci. **87:** 160–163

49 Trubetskoy V. S., Gazelle G. S., Wolf G. L. and Torchilin V. P. (1997) Block copolymer of polyethylene glycol and polylysine as a carrier of organic iodine: design of a long circulating particulate contrast medium for X-ray computed tomography. J. Drug Targ. **4:** 381–388

50 Yokoyama M., Miyauchi M., Yamada N., Okano T., Sakurai Y., Kataoka K. et al. (1990) Characterization and anticancer activity of the micelle-forming polymeric anticancer drug adriamycin-conjugated poly(ethylene glycol)-poly(aspartic acid) block copolymer. Cancer Res. **50:** 1693–1700

51 Harada A. and Kataoka K. (1998) Novel polyion complex micelles entrapping enzyme molecules in the core. Preparation of narrowly-distributed micelles from lysozyme and poly(ethylene glycol)-poly(aspartic acid) block copolymer in aqueous medium. Macromolecules **31:** 288–294

52 La S. B., Okano T. and Kataoka K. (1996) Preparation and characterization of the micelle-forming polymeric drug indomethacin-incorporated poly(ethylene oxide)-poly(beta-benzyl L-aspartate) block copolymer micelles. J. Pharm. Sci. **85:** 85– 90

53 Kwon G. S., Naito M., Yokoyama M., Okano T., Sakurai Y. and Kataoka K. (1997) Block copolymer micelles for drug delivery: loading and release of doxorubicin. J. Control. Release **48:** 195–201

54 Jeong Y. I., Cheon J. B., Kim S. H., Nah J. W., Lee Y. M., Sung Y. K. et al. (1998) Clonazepam release from core-shell type nanoparticles in vitro. J. Control. Release **51:** 169–178

55 Kim S. Y., Shin I. G., Lee Y. M., Cho C. G. and Sung Y. K. (1998) Methoxy poly(ethylene glycol) and ε-caprolactone amphiphilic block copolymeric micelle containing indomethacin. II. Micelle formation and drug release behaviors. J. Control. Release **51:** 13–22

56 Allen C., Yu Y., Maysinger D. and Eisenberg A. (1998) Polycaprolactone-b-poly(ethylene oxide) block copolymer micelles as a novel drug delivery vehicle for neurotrophic agents FK506 and L-685,818. Bioconj. Chem. **9:** 564–572

57 Ramaswamy M., Zhang X., Burt H. M. and Wasan K. M. (1997) Human plasma distribution of free paclitaxel and paclitaxel associated with diblock copolymers. J. Pharm. Sci. **86:** 460–464

58 Kabanov A. V. and Kabanov V. A. (1990) Interpolyelectrolyte and block ionomer complexes for gene delivery: physicochemical aspects. Adv. Drug Deliv. Rev. **30:** 49–60

59 Klibanov A. L., Maruyama K., Torchilin V. P. and Huang L. (1990) Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes. FEBS Lett. **268:** 235–238

60 Lasic D. D., Woodle M. C., Martin F. J. and Valentincic T. (1991) Phase behavior of 'stealth-lipid' decithin mixtures. Period. Biol. **93:** 287–290

61 Duquemin S. J and Nixon J. R. (1985) The effect of sodium lauryl sulphate, cetrimide and polysorbate 20 surfactants on complex coacervate volume and droplet size. J. Pharm. Pharmacol. **37:** 698–702

62 Nagarajan R. and Ganesh K. (1989) Block copolymer self-assembly in selective solvents: theory of solubilization in spherical micelles. Macromolecules **22:** 4312–4325

63 Xing L. and Mattice W. L. (1998) Large internal structures of micelles of triblock copolymers with small insoluble molecules in their cores. Langmuir **14:** 4074–4080

64 Allen C., Maysinger D. and Eisenberg A. (1999) Nano-engineering block copolymer aggregates for drug delivery. Coll. Surf. B: Biointerf. **16:** 1–35

65 Lin S. Y. and Kawashima Y. (1985) The influence of three poly(oxyethylene)poly(oxypropylene) surface-active block copolymers on the solubility behavior of indomethacin. Pharm. Acta Helv. **60:** 339–344

66 Lin S. Y. and Kawashima Y. (1987) Pluronic surfactants affecting diazepam solubility, compatibility and adsorption from i.v. admixture solutions. J. Parenter. Sci. Technol. **41:** 83–87

67 Yokoyama M., Okano T. and Kataoka K. (1994) Improved synthesis of adriamycin-conjugated poly(ethylene oxide)-poly(aspartic acid) block copolymer and formation of unimodal micellar structure with controlled amount of physically entrapped adriamycin. J. Control. Release **32:** 269–277

68 Yokoyama M., Fukushima S., Uehara R., Okamoto K., Kataoka K., Sakurai Y. et al. (1998) Characterization of physical entrapment and chemical conjugation of adriamycin in

polymeric micelles and their design for in vivo delivery to a solid tumor. J. Control. Release **50:** 79–92
69 Yokoyama M., Satoh A., Sakurai Y., Okano T., Matsumura Y., Kakizoe T. et al. (1998) Incorporation of water-insoluble anticancer drug into polymeric micelles and control of their particle size. J. Control. Release **55:** 219–229
70 Batrakova E. V., Dorodnych T. Y., Klinskii E. Y., Kliushnenkova E. N., Shemchukova O. V., Goncharova O. N. et al. (1996) Anthracycline antibiotics non-covalently incorporated into the block copolymer micelles: in vivo evaluation of anticancer activity. Brit. J. Cancer **74:** 1545–1552
71 Kabanov A. V., Nazarova I. R., Astafieva I. R., Batrakova E. V., Alakhov V. Yu., Yaroslavov A. A. et al. (1995) Micelle formation and solubilization of fluorescence probes in poly(oxyethylene-b-oxypropylene-b-oxyethylene) solutions. Macromolecules **28:** 2303–2314
72 Kabanov A. V., Vinogradov S. V., Suzdaltseva U. G. and Alakhov V. Yu. (1995) Water-soluble block polycations as carriers for oligonucleotide delivery. Bioconj. Chem. **6:** 639–643
73 Alakhov V. Yu. and Kabanov A. V. (1998) Block copolymeric biotransport carriers as versatile vehicles for drug delivery. Expert. Op. Invest. Drugs **7:** 1453–1473
74 Matsumura Y., Yokoyama M., Kataoka K., Okano T., Sakurai Y., Kawaguchi T. et al. (1999) Reduction of the side effects of an antitumor agent, KRN5500, by incorporation of the drug into polymeric micelles. Jpn. J. Cancer Res. **90:** 122–128
75 Kwon G. S., Yokoyama M., Okano T., Sakurai Y. and Kataoka K. (1993) Biodistribution of micelle-forming polymer-drug conjugates. Pharm. Res. **10:** 970–974
76 Kwon G. S., Suwa S., Yokoyama M., Okano T., Sakurai Y. and Kataoka K. (1994) Enhanced tumor accumulation and prolonged circulation times of micelles-forming poly(ethylene oxide-aspartate) block copolymers-adriamycin conjugates. J. Control. Release **29:** 17–23
77 Kataoka K., Kwon G. S., Yokoyama M., Okano T. and Sakurai Y. (1993) Block-copolymer micelles as vehicles for drug delivery. J. Control. Release **24:** 119–132
78 Kwon G., Naito M., Yokoyama M., Okano T., Sakurai Y. and Kataoka K. (1993) Micelles based on ab block copolymers of poly(ethylene oxide) and poly(benzyl-aspartat). Langmuir **9:** 945–949
79 Kwon G. S. and Okano T. (1996) Polymeric micelles as new drug carriers. Adv. Drug Deliv. Rev. **21:** 107–116
80 Allen C., Han J., Yu Y., Maysinger D. and Eisenberg A. (2000) Polycaprolactone-b-poly(ethylene oxide) copolymer micelles as a delivery vehicle for dihydrotestosterone. J. Control. Release **63:** 275–286
81 Trubetskoy V. S. and Torchilin V. P. (1996) Polyethylene glycol based micelles as carriers of therapeutic and diagnostic agents. S.T.P. Pharma Sci. **6:** 79–86
82 Gao Z., Lukyanov A. N., Singhal A. and Torchilin V. P. (2002) Diacyl-polymer micelles as nanocarriers for poorly soluble anticancer drugs. Nano Letters **2:** 979–982
83 Wang J., Mongayt D. A., Lukyanov A. N., Levchenko T. S. and Torchilin V. P. (2004) Preparation and in vitro synergistic anticancer effect of Vitamin K3 and 1,8-diazabicyclo[5,4,0]undec-7-ene in poly(ethylene glycol)-diacyllipid micelles. Int. J. Pharm. **272:** 129–135
84 Wang J., Mongayt D. and Torchilin V. P. (2004) Polymeric micelles for delivery of poorly soluble drugs: preparation and anticancer activity in vitro of paclitaxel incorporated into mixed micelles based on poly(ethylene glycol)-lipid conjugate and positively charged lipids, J. Drug Targ., in press
85 Weissig V., Whiteman K. R. and Torchilin V. P. (1998) Accumulation of liposomal- and micellar-bound protein in solid tumor. Pharm. Res. **15:** 1552–1556
86 Lukyanov A. N., Gao Z., Mazzola L. and Torchilin V. P. (2002) Polyethylene glycol-diacyllipid micelles demonstrate increased accumulation in subcutaneous tumors in mice. Pharm. Res. **19:** 1424–1429
87 Torchilin V. P., Lukyanov A. N., Gao Z. and Papahadjopoulos-Sternberg B. (2003) Immunomicelles: targeted pharmaceutical carriers for poorly soluble drugs. Proc. Natl. Acad. Sci. USA **100:** 6039–6044
88 Lukyanov A. N., Hartner W. C. and Torchilin V. P. (2004) Increased accumulation of PEG-PE micelles in the area of experimental myocardial infarction in rabbits. J. Control. Release **94:** 187–193
89 Yuan F., Leunig M., Huang S. K., Berk D. A. Papahadjopoulos D. and Jain R. K. (1994) Microvascular permeability and interstitial penetration of sterically-stabilized (stealth) liposomes in human tumor xenograft. Cancer Res. **54:** 3352–3356
90 Yuan F., Dellian M., Fukumura M., Leunig M., Berk D. A., Torchilin V. P. et al. (1995) Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. Cancer Res. **55:** 3752–3756
91 Hobbs S. K., Monsky W. L., Yuan F., Roberts W. G., Griffith L., Torchilin V. P. et al. (1998) Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. Proc. Natl. Acad. Sci. USA **95:** 4607–4612
92 Monsky W. L., Fukumura D., Gohongi T., Ancukiewcz M., Weich H. A., Torchilin V. P. et. al. (1999) Augmentation of transvascular transport of macromolecules and nanoparticles in tumors using vascular endothelial growth factor. Cancer Res. **59:** 4129–4135
93 Yokoyama M., Okano T., Sakurai Y., Fukushima S., Okamoto K. and Kataoka K. (1999) Selective delivery of adriamycin to a solid tumor using a polymeric micelle carrier system. J. Drug Targ. **7:** 171–186
94 Papahadjopoulos D., Allen T. M., Gabizon A., Mayhew E., Matthay K. and Huang S. K. (1991) Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. Proc. Natl. Acad. Sci. USA **88:** 11460–11464
95 Parr M. J., Masin D., Cullis P. R. and Bally M. B. (1997) Accumulation of liposomal lipid and encapsulated doxorubicin in murine Lewis lung carcinoma: the lack of beneficial effects by coating liposomes with poly(ethylene glycol). J. Pharmacol. Exp.Ther. **280:** 1319–1327
96 Helmlinger G., Yuan F., Dellian M. and Jain R. K. (1997) Interstitial pH and pO2 gradients in solid tumors in vivo: high-resolution measurements reveal a lack of correlation. Nat. Med. **3:** 177–182
97 Tannock I. F. and Rotin R. (1998) Acid pH in tumors and its potential for therapeutic exploitation. Cancer Res. **49:** 4373–4384
98 Chung J. E., Yokoyama M., Aoyagi T., Sakurai Y. and Okano T. (1998) Effect of molecular architecture of hydrophobically modified poly(N-isopropylacrylamide) on the formation of thermoresponsive core-shell micellar drug carriers. J. Control. Release **53:** 119–131
99 Kohori F., Sakai K., Aoyagi T., Yokoyama M., Sakurai Y. and Okano T. (1998) Preparation and characterization of thermally responsive block copolymer micelles comprising poly(*N*-isopropylacrylamide-b-DL-lactide). J. Control. Release **55:** 87–98
100 Meyer O., Papahadjopoulos D. and Leroux J. C. (1998) Copolymers of N-isopropylacrylamide can trigger pH sensitivity to stable liposomes. FEBS Lett. 41 (1998) 61–64
101 Le Garrec D., Taillefer J., Van Lier J. E., Lenaerts V. and Leroux J. C. (2002) Optimizing pH-responsive polymeric micelles for drug delivery in a cancer photodynamic therapy model. J. Drug. Targ. **10:** 429–437
102 Yoo H. S., Lee E. A. and Park T. G. (2002) Doxorubicin-conjugated biodegradable polymeric micelles having acid-cleavable linkages. J. Control. Release **82:** 17–27

103 Chung J. E, Yokoyama M., Yamato M., Aoyagi T., Sakurai Y. and Okano T. (1999) Thermo-responsive drug delivery from polymeric micelles constructed using block copolymers of poly(N-isopropylacrylamide) and poly(butylmethacrylate). J. Control. Release **62:** 115–127

104 Rapoport N., Pitt W. G., Sun H. and Nelson J. L. (2003) Drug delivery in polymeric micelles: from in vitro to in vivo. J. Control. Release **91:** 85–95

105 Nagasaki Y., Yasugi K., Yamamoto Y., Harada A. and Kataoka K. (2001) Sugar-installed block copolymer micelles: their preparation and specific interaction with lectin molecules. Biomacromolecules **2:** 1067–1070

106 Vinogradov S., Batrakova E., Li S. and Kabanov A. (1999) Polyion complex micelles with protein-modified corona for receptor-mediated delivery of oligonucleotides into cells. Bioconj. Chem. **10:** 851–860

107 Leamon C. P., Weigl D. and Hendren R. W. (1999) Folate copolymer-mediated transfection of cultured cells. Bioconj. Chem. **10:** 947–957

108 Jule E., Nagasaki Y. and Kataoka K. (2003) Lactose-installed poly(ethylene glycol)-poly(d,l-lactide) block copolymer micelles exhibit fast-rate binding and high affinity toward a protein bed simulating a cell surface. A surface plasmon resonance study. Bioconj. Chem. **14:** 177–186

109 Ogris M., Brunner S., Schuller S., Kircheis R. and Wagner E. (1999) PEGylated DNA/transferrin-PEI complexes: reduced interaction with blood components, extended circulation in blood and potential for systemic gene delivery. Gene Ther. **6:** 595–605

110 Dash P. R., Read M. L., Fisher K. D., Howard K. A., Wolfert M., Oupicky D. et al. (2000) Decreased binding to proteins and cells of polymeric gene delivery vectors surface modified with a multivalent hydrophilic polymer and retargeting through attachment of transferrin. J. Biol. Chem. **275:** 3793–3802

111 Leamon C. P. and Low P. S. (2001) Folate-mediated targeting: from diagnostics to drug and gene delivery. Drug Discov. Today **6:** 44–51

112 Lee E. S., Na K. and Bae Y. H. (2003) Polymeric micelle for tumor pH and folate-mediated targeting. J. Control. Release **91:** 103–113

113 Kabanov A. V., Chekhonin V. P., Alakhov V., Batrakova E. V., Lebedev A. S., Melik-Nubarov N. S. et al. (1989) The neuroleptic activity of haloperidol increases after its solubilization in surfactant micelles. Micelles as microcontainers for drug targeting. FEBS Lett. **258:** 343–345

114 Chekhonin V. P., Kabanov A. V., Zhirkov Y. A. and Morozov G. V. (1991) Fatty acid acylated Fab-fragments of antibodies to neurospecific proteins as carriers for neuroleptic targeted delivery in brain. FEBS Lett. **287:** 149–152

115 Torchilin V. P., Levchenko T. S., Lukyanov A. N., Khaw B. A., Klibanov A. L., Rammohan R. et al. (2001) p-Nitrophenylcarbonyl-PEG-PE-liposomes: fast and simple attachment of specific ligands, including monoclonal antibodies, to distal ends of PEG chains via p-nitrophenylcarbonyl groups. Biochim. Biophys. Acta **1511:** 397–411

116 Gao Z., Lukyanov A. N., Chakilam A. R. and Torchilin V. P. (2003) PEG-PE/phosphatidylcholine mixed immunomicelles specifically deliver encapsulated taxol to tumor cells of different origin and promote their efficient killing. J. Drug Targ. **11:** 87–92

117 Iakoubov L. Z. and Torchilin V. P. (1997) A novel class of antitumor antibodies: nucleosome-restricted antinuclear autoantibodies (ANA) from healthy aged nonautoimmune mice. Oncol. Res. **9:** 439–446

118 Torchilin V. P., Iakoubov L. Z. and Estrov Z. (2003) Therapeutic potential of antinuclear autoantibodies in cancer. Cancer Ther. **1:** 179–190

119 Park J. W., Kirpotin D. B., Hong K., Shalaby R., Shao Y., Nielsen U. B. et al. (2001) Tumor targeting using anti-her2 immunoliposomes. J. Control. Release **74:** 95–113

120 Sharma A., Conway W. D. and Straubinger R. M. (1994) Reversed-phase high-performance liquid chromatographic determination of taxol in mouse plasma. J. Chromatogr. B: Biomed. Appl. **655:** 315–319