

## Targeted Drug Delivery to Tumor Cells Using Colloidal Carriers

---

Sushma Kommareddy and Mansoor Amiji

### 1. Introduction

Advances in biotechnology and medicine have provided an opportunity for the development of a number of carrier systems for tumor-targeted delivery of anticancer drugs and genes. Tumors are an uncontrolled mass of proliferations of a single malignant cell arising from mutations, which are either inherited or caused by environmental factors (1). In order to reach the tumor cells, systemically administered drugs have to overcome a number of obstacles, which may include rapid metabolism and clearance of drugs from the body, physiological barriers in transportation of the drugs from the site of administration to the tumor cells, drug resistance, and toxicity of the anticancer drugs to normal cells (2–4). The high interstitial pressure and absence of lymphatic drainage were also reported to cause problems in distribution of the drug in the solid tumor (5).

Most of the solid tumors have increased levels of vascular permeability factors (VPF) (6), vascular endothelial growth factor (VEGF) (7), bradykinin (8), nitric oxide (NO) (9), and peroxy nitrite, which enhance the permeability of the tumor vasculature. These VEGFs secreted from the blood vessels promote angiogenesis, a complex process that involves endothelial-cell division and migration, and selective degradation of vascular basement membrane and extracellular matrix (ECM) (10). The blood capillaries of the tumor become dense, disorganized, and tortuous. The resulting vasculature in the tumor was found to be heterogeneous leading to a nonuniform distribution of the drug. Based on the permeability studies by Jain and co-workers (11,12), solid tumors were found to have a well-vascularized periphery, an intermediate semi-necrotic region, and a central necrotic region. The vascular permeability was found to vary from one tumor to the other, within the tumor in terms of both temporal and spatial distribution, and during tumor growth and regression (13). Thus, the microenvironment of the tumor was found to play an important role in the delivery of drugs to the cancer cells. An improper supply of oxygen and nutrients was also observed owing to lack of normal vasculature. The hypoxia and production of lactic acid lead to acidic conditions within the tumor mass (14).

Dvorak et al. (15–17) suggested a pathway, which is interconnected by vesiculovacuolar organelles (VVO). These structures are comprised of 12–20 vesicles or vacuoles that are interconnected with each other and with the endothelial cell by means of fenestrae

that open and close by diaphragms. The size of the VVOs was found to be in the range of 50 nm to 415 nm in diameter (18). The VVOs provide a pathway for the transportation of plasma proteins into tissues. In case of tumor cells the increase in secretion of VEGF, VPF, and other cytokines (19,20) was found to enhance the number of VVOs and fenestrae that in turn increase the permeability of tumor vasculature.

Targeting of drugs to the tumor can be achieved either by passive or active mechanisms. Specialized polymeric or colloidal carriers with target-specific ligands, such as antibodies, attached to their surface are used for active targeting. Passive tumor delivery of macromolecules and colloidal particles carrying drugs is achieved primarily by the enhanced permeability of the tumor vasculature and lack of lymphatic drainage. These processes are together termed as the “enhanced permeability and retention (EPR) effect.” Using macromolecular conjugates of anticancer drugs, Maeda et al. (21–23) were the first to describe the EPR effect of solid tumor. The EPR effect for passive targeting to tumor was later extended to nanoparticles, liposomes, and other colloidal carriers (24). For the EPR effect, the carrier systems used are not target specific and act only to transport the drug to the site of action. In this chapter, we will limit our discussion to the colloidal carrier systems used for passive targeting. These include polymeric conjugates, nanoparticles, liposomes, and micellar systems (Table 1).

## 2. Polymeric Conjugates

Polymeric conjugated systems are most widely used for the delivery of anticancer agents. Low-molecular-weight drugs penetrate readily through the cellular membrane and cause cytotoxic effects to healthy tissues (25). In order to overcome this limitation, the drug is conjugated with a polymeric carrier. There was a significant inhibition of pinocytotic uptake when macromolecular conjugates of the drug were used as compared to the free drug (26). The polymer-drug conjugates were found to accumulate passively in solid tumors by the EPR effect. The polymer conjugates are also used to enhance the solubility and half-life of the drug in the systemic circulation. Finally, multidrug resistance (MDR) can be overcome by using the conjugated systems as the macromolecular drugs enter the cells through a different pathway as compared to the small-molecule drugs (27).

The polymeric carriers used for delivery of anticancer drugs are broadly divided into synthetic and natural systems. The synthetic polymers are nonimmunogenic, but many do not degrade in the biological environment. These include poly(*N*-[2-hydroxypropyl]-methacrylamide) (HPMA), poly(styrene-co-maleic anhydride), poly(divinylether-co-maleic anhydride) (DIVEMA), and poly(ethylene glycol) (PEG). Natural biodegradable polymers include poly(amino acids) and proteins, hyaluronic acid, dextran, and chitosan are the ones used most often for the preparation of polymer-drug conjugates.

### 2.1. HPMA Conjugates

HPMA is a biocompatible, synthetic polymer, which is used systemically to deliver anticancer drugs to tumor tissue. The polymer is synthesized in such a way that any drug containing an aliphatic amino group can be conjugated by aminolysis reaction (28). Low-molecular-weight anticancer agents penetrate readily through the cellular membrane, but the HPMA copolymer conjugates are too big and enter the cell by nonspecific endocytotic uptake. This decreases the toxicity of the drug to the normal tissues and increases the targeting of the drug to the tumor by increasing the plasma residence time.

**Table 1**  
**Classification of Carriers Used in Tumor-Targeted Drug Delivery**

Carrier System	Components
Polymer conjugates	<i>N</i> -(2-hydroxypropyl)-Methacrylamide Styrene-Maleic Anhydride- Neocarzinostatin (SMANCS) Poly(ethylene glycol) (PEG) Poly(L-glutamic acid) Hyaluronic acid Dextran Chitosan
Polymeric nanoparticles	Poly(D,L-lactide-co-glycolide) Polyalkylcyanoacrylates Chitosan Gelatin
Liposomes and related systems	Conventional liposomes Lipoplexes Stealth liposomes Niosomes
Polymeric micelles	Poly(ethylene glycol) or poly (ethylene oxide) block co polymers Thermoresponsive micelles

In addition, the biocompatibility and low immunogenicity of the HPMa copolymers makes it more useful as carriers of anticancer agents (29). Table 2 gives the list of HPMa copolymer-drug conjugates that have entered Phase I/II clinical trials (30–33).

When anticancer drugs are conjugated with HPMa copolymer through a glycyphenylalanyl glycol (GFLG) oligopeptide spacer, the drug release occurs by hydrolytic degradation of the spacer in an acidic environment. These types of polymer conjugates are particularly attractive for tumor targeting, owing to low pH around the tumor (pH ~6.5), and were found to be more effective than those containing nondegradable GG spacer. The antitumor activity of doxorubicin conjugates was evaluated in mice containing EL-4T lymphoma. Free doxorubicin and free polymer precursor were also given along with the 0.15 M sodium chloride as controls. When compared to the free drug, the doxorubicin-polymer conjugates were found to have a greater reduction in tumor volume and an increase in survival time of more than 80 d in 40% of the treated animals (34). HPMa-doxorubicin conjugate, called PK1, is currently under Phase II clinical trial in the United Kingdom. The Phase I clinical trial results showed that the circulation time of the drug conjugates increased when compared to the free doxorubicin. Reduced toxicity and increased antitumor activity in refractory tumors was also observed with the conjugates (30). These polymer conjugates were found to decrease the elimination rate and increase the tumor residence time of the drug. When injected intravenously into rats bearing Walker sarcoma, daunomycin-HPMa conjugate was found to be four times more concentrated in the tumor than the free drug (35). Duncan et al. (36) evaluated the activity of HPMa-GFLG-daunomycin conjugates in mice bearing L1210 leukemia. Results showed that the polymer conjugates containing

**Table 2**  
**HPMA Copolymer Drug Conjugates Under Clinical Trial**

Polymer-drug conjugate	Reference
HPMA-doxorubicin	(30)
HPMA-paclitaxel	(31)
HPMA-camptothecin	(32)
HPMA-platinate	(33)

biodegradable GFLG linkage had a significant increase in the mean survival time relative to the free drug. In addition, HPMA has been conjugated with cisplatin and glendamyacin (33,37).

HPMA copolymer containing a degradable tetrapeptide GFLG was also used to deliver anticancer agents to multidrug resistant cells that expressed P-glycoprotein (P-gp) (38). In order to examine the efficacy of the conjugated drug in resistant cells, internalization of the HPMA copolymer-doxorubicin conjugates was compared in both sensitive and resistant cells of ovarian carcinoma cell line (A2780). Upon measurement of the intracellular fluorescence signal, the results showed that there was an increase in the concentration of the polymer-bound doxorubicin in resistant cells as compared to the free drug. HPMA copolymer bearing GFLG was also used as carrier for anticancer agents like cathepsin B (39) and elliciptine (40). The anticancer activity of these polymer drug conjugates was evaluated in mice bearing B16F10 melanoma. The results indicate an increased tumor accumulation of the drug (4.2-fold) and decreased cytotoxicity to healthy tissues. HPMA-GFLG copolymer conjugates of mesochlorin  $e_6$  monoethylenediamine were used in the treatment of ovarian cancer (41).

## **2.2. Poly(Styrene-Co-Maleic Anhydride) Conjugated With Neocarzinostatin (SMANCS)**

Neocarzinostatin (NCS) is an anticancer drug consisting of two components—a protein component (apo-NCS) and a nonprotein component (NCS-chr)—that causes breaks in double-stranded DNA, leading to cell death (42). NCS consists of 112 amino acids with two disulfide bridges and a prosthetic chromophore (43,44). NCS is conjugated with styrene-co-maleic anhydride copolymer resulting in a total molecular mass of 15,000 Daltons. This further binds to albumin in the plasma resulting in a macromolecule of 83,000 Daltons. The macromolecular conjugate was found to achieve increased concentrations of the drug in tumor mass as compared with free NCS (45). In addition, the blood circulation time in mice increased from 2 min with the free drug to 18 min with the macromolecular conjugate. It was observed that prolonged circulation of the drug resulted in an increased concentration of the drug in tumor tissues. Compared to the free drug, an eightfold increase in concentration of NCS was observed upon intravenous administration of the drug conjugate (23,46).

Konno et al. (47) treated primary hepatoma patients with SMANCS. Of the 44 patients treated, 95% of them showed a decrease in tumor volume. The mean survival time increased to 18 mo in the polymer conjugate-treated group as compared with 3.7 mo for the control group. Nine patients showed 40–99% decrease in tumor mass over a period of 1–5 mo. In a similar study, the same authors also treated 24 patients with tumors other than primary hepatoma (48). Of those patients who could be evaluated,

regression in tumor growth was observed in six (of nine) with metastatic liver cancer, one (of three) unresectable gall bladder, and in all four with lung adenocarcinoma. In both trials, fever was the most common side effect observed. In addition, abdominal pain (in 20% cases) and moderate leukocytosis (in 65% cases) were also observed. No adverse effects were observed in lung, heart, or the brain.

NCS was also conjugated with DIVEMA in order to decrease the toxicity and achieve higher tumor concentrations. Hirano et al. (49) prepared conjugates of doxorubicin using DIVEMA by reacting amino group of the drug with anhydride group of the copolymer. When these conjugates were injected into mice bearing an intraperitoneal tumor, a significant increase in survival time (up to 60 d) was observed when compared with the free drug (less than 21 d).

### 2.3. Poly(Ethylene Glycol) Conjugates

Poly(ethylene glycol) (PEG) has a general structure of  $\text{HO}-(\text{CH}_2\text{CH}_2\text{O})_n-\text{CH}_2\text{CH}_2-\text{OH}$ . The polymer has a polyether backbone that is chemically inert and terminal hydroxyl groups that can be activated for conjugation with different types of drugs. The high aqueous solubility, lack of toxicity, and ability to decrease immunogenicity makes PEG a very attractive carrier polymer for anticancer agents (50). Paclitaxel is one of the most widely used anticancer drugs. The in vivo activity, solubility, and stability of paclitaxel are improved by linking the drug covalently to monomethoxy-PEG. The PEG derivatives of paclitaxel were found to have significant antitumor activity in mice bearing HA-29 (colon), A549 (lung), and SKOV3 (ovarian) tumors. Upon administration of the conjugates in vivo in mice, the total body clearance and uptake by liver and spleen were reduced (51,52). PEG conjugation with camptothecin was found to enhance the toxicity of the drug in both sensitive and drug resistant human ovarian carcinoma cells. Compared to the nonbiotinylated conjugate, the biotinylated PEG-camptothecin conjugate was found to further enhance the activity of the drug. The increase in toxicity by as much as 60 times in sensitive cells and 30 times in resistant cells was attributed to increased uptake of these conjugates by sodium-dependent multivitamin transporter (53). The presence of amino acid or peptide spacer was found to increase the stability of the PEG conjugates against alkaline degradation. The tripeptide spacer glycyl-L-valyl-L-phenylalanine was found to be more active than the phenylalanine and tryptophan derivatives. This resulted in decreased toxicity of the drug to healthy cells (54).

Various proteins and enzyme conjugates that act as anticancer agents are also prepared using PEG. L-Asparaginase is an enzyme that converts amino acid L-asparagine to L-aspartic acid. It acts as an anticancer agent by depleting the essential amino acid L-asparagine in tumor cells. Conjugation of this enzyme with PEG was found to prevent the proteolytic degradation and enhance the activity (55). In a clinical study conducted in 31 patients with acute lymphoblastic leukemia (ALL), conjugation of L-asparaginase with PEG was found to increase the plasma half-life from 20 h to 357 h. Absence of hematological toxicity and reduced hypersensitivity were also observed (56). In another clinical trial with 21 patients having non-Hodgkin's lymphoma (NHL), severe nausea and vomiting were observed in 50% of the cases. However, the absence of hematological toxicity makes the PEG-L-asparaginase conjugate very useful in combination therapy (57). Similarly, PEG conjugates of arginine deaminase are used in the treatment of hepatoma (MH134), colon carcinoma (colon 26), sarcoma (S180), and melanoma (B16) (58). Xanthine oxidase (XO) was found to exhibit anticancer activity by generating cytotoxic oxygen radicals. The use of XO was limited owing to its high binding capac-

ity to blood vessels, which results in vascular damage. Conjugation of XO with PEG was found to decrease toxicity and enhance the anticancer activity (59).

#### **2.4. Poly(L-Glutamic Acid) Conjugates**

Poly(L-glutamic acid) was widely used to decrease the toxicity and enhance the antitumor activity of the anticancer agents (60). Singer et al. (61) used the poly(L-glutamic acid) in order to improve the solubility and anticancer activity of camptothecin. The polymer was conjugated with the drug through  $\gamma$ -carboxylic acid side chain. The conjugations of the drug to the polymer, with and without glycine linker, were evaluated and found to have similar antitumor activity against B-16 melanoma. An increase in the molecular weight of the polymer was found to increase the antitumor activity owing to increased residence time in the tumor and decreased renal clearance. When the activity of conjugated camptothecin was evaluated against athymic mice bearing human lung cancer tumors NCI-H460, a threefold decrease in the tumor volume was found relative to the control. The biodegradable conjugate of poly(L-glutamic acid) and paclitaxel, prepared under similar conditions, is under Phase-II clinical trials in the United States. Biodistribution studies carried out in mice bearing B16 melanoma and OCA-1 ovarian tumors show greater accumulation of the paclitaxel-polymer conjugate as compared with the free drug.

#### **2.5. Hyaluronic Acid Conjugates**

Hyaluronic acid (HA) is a nonsulfated glycosaminoglycan that is abundant in synovial fluid and ECM (62). HA conjugated with polymeric carriers and anticancer drugs is used to target tumor cells expressing HA receptor. Prestwich and co-workers (63) prepared *N*-(2-hydroxypropyl) methacrylamide (HPMA)-HA conjugates for the delivery of the anticancer drug doxorubicin. The results of the in vitro cell-culture experiments show that the HPMA-HA-doxorubicin conjugates have greater cytotoxicity than HPMA-doxorubicin conjugates against HBL-100 (human breast cancer), SKOV-3 (ovarian cancer), and HCT-116 (colon cancer) cell lines expressing the HA receptor. This was attributed to a more efficient receptor-mediated uptake of the macromolecular drug by the tumor cells as compared to nonspecific uptake of HPMA-doxorubicin conjugates. Similar results were obtained using HA-paclitaxel conjugates with selective toxicity against tumor cells (breast, colon, and ovarian) expressing HA receptors and minimal toxicity against mouse fibroblast cell line, which does not express HA receptor (64).

#### **2.6. Dextran Conjugates**

Dextran is a biodegradable polysaccharide widely used as a carrier of anticancer agents. Okuno et al. (65) prepared carboxymethyldextran-camptothecin conjugate with a triglycine spacer. When injected intravenously into rats bearing Walker sarcoma-256 tumor, the conjugate was found to have increased blood circulation, resulting in an increased accumulation of the drug in the tumor. These conjugates were used to treat mammary carcinoma (MX-1), lung carcinoma (IX-1), gastric (St-4), and colorectal (HT-29) tumors, which are refractory to camptothecin. Carboxymethyldextran conjugates with cisplatin are used in the treatment of human osteogenic sarcoma and human ovarian carcinoma in athymic mice and embryonic carcinoma in Balb/c mice (66). Bernstein et al. (67) showed that doxorubicin can be conjugated with oxidized dextran, thus producing greater antitumor activity than the free drug.

## 2.7. Chitosan Conjugates

Chitosan is a biodegradable co-polymer of *N*-acetyl-D-glucosamine and D-glucosamine linked together by a  $\beta$  (1–4) glycosidic linkage. Chitosan, obtained by alkaline deacetylation of chitin, is usually greater than 70% deacetylated and has a molecular weight in the range of 50,000 to 2 million Daltons (68). Sato et al. (69) synthesized *N*-succinyl chitosan and glycol chitosan conjugates of mitomycin C. They also evaluated the antitumor activity of these drug-polymer conjugates in vivo against P388 leukemia and sarcoma 180. The drug conjugates of glycol-chitosan were found to have a significant increase in the survival time against P388 leukemia. In case of sarcoma 180 inoculated subcutaneously, the drug conjugates of *N*-succinyl chitosan were found to have greater tumor-growth suppression. When compared with the free drug, both the polymer conjugates were found to exhibit increased capacity for suppression of tumor growth.

## 2.8. Other Polymeric Conjugates

The potential use of aspartic acid polymers and pyrrolo [2, 1-c] [1, 4] benzodiazepine-(PBD) polyamide conjugates as carriers of anticancer agents were evaluated in human cancer cell lines (70,71). Ferrocene-containing anti-neoplastic agents that are covalently bound to aspartic-acid polymers were tested for their cytotoxicity against EMT-6 cancer cell lines. The PBD dimers were found to be more effective against colon, melanoma, renal, and breast cancer cell lines. In both the cases, an increase in the activity was observed with an increase in the chain length of the polymer.

## 3. Polymeric Nanoparticles

Nanoparticles are colloidal systems of submicron size ( $<1 \mu\text{m}$ ). They may be classified as either nanospheres, in which the drug is dispersed throughout the system, or nanocapsules, in which the drug is entrapped in a cavity surrounded by a polymer layer. Sometimes the drug may also be adsorbed to the surface of the nanoparticles (72,73). Because of their small size, nanoparticles extravasate into the tumor cells and are used as carriers for the delivery of anticancer agents. This increases the efficiency of drug targeting and reduces the cytotoxicity of the drug in healthy tissues. From the histological examinations, it was proved that the nanoparticles first reach the extravascular compartments and are then taken up by tumor cells by endocytosis (74).

Polymeric nanoparticles can be prepared either by emulsion polymerization with monomers or by solvent precipitation of formed polymers. Emulsion polymerization is done in organic solutions of the monomers and the formed polymeric nanoparticles are precipitated in the presence of aqueous surfactant solutions with controlled stirring. This method does not involve high temperatures and can be used for encapsulation of heat-sensitive compounds. The solvent precipitation method involves gradual addition of a nonsolvent to the polymer solution under controlled stirring conditions in order to precipitate the nanoparticles.

The polymer used for nanoparticle preparation should be biocompatible and biodegradable, as accumulation of nonmetabolizable materials in vivo results in toxicity. The conventional nanoparticles, like many other colloidal systems, are engulfed predominantly by the cells of the reticuloendothelial system (RES) upon systemic administration. Surface modification of the conventional nanoparticles with PEG and other water-soluble polymers can result in long-circulating nanoparticles. The hydrophilic

nature of these surface-modified particles reduces their binding capacity to proteins (opsonins), resulting in reduced uptake by the RES.

### **3.1. Poly(D, L-Lactide-Co-Glycolide) (PLGA) Nanoparticles**

PLGA is a biocompatible and biodegradable polymer, which is used for the encapsulation of lipophilic drugs. The polymeric nanoparticles are used for sustained release of paclitaxel, doxorubicin, and cisplatin. Yoo et al. (75) prepared polymeric nanoparticles by conjugating the hydroxyl group of doxorubicin with carboxyl group of PLGA. The nanoparticles containing conjugated doxorubicin were found to exhibit sustained drug release. The *in vitro* studies using HepG2 cell line and *in vivo* antitumor activity showed that doxorubicin conjugated with PLGA nanoparticles has greater antitumor activity than free doxorubicin. Feng et al. (76) prepared PLGA-poly(L-lactic acid) (PLA) nanoparticles, which can act as carrier for administration of paclitaxel, a highly hydrophobic anticancer drug. In addition, PLGA-mPEG nanoparticles are used as carriers of cisplatin (77). The nanoparticles were prepared by the double-emulsion method. The *in vitro* studies showed that the rate of degradation and the rate of release increased with increase in mPEG content. The *in vivo* studies conducted in BALB/C mice showed an increase in the circulation time of cisplatin-containing PLGA-mPEG nanoparticles. Biodegradable nanoparticles of PLA were prepared by emulsification-diffusion technique using propylene carbonate. A significant decrease in the size of the nanoparticles was observed when stabilizing agents, like poloxamers, are used in the concentration range of 0.5–5% (w/v). pH of the external phase, internal phase to external phase volume ratio, and stirring speed were found to influence the particle size. These biodegradable nanoparticles were found to have potential for drug targeting to solid tumors (78).

PLGA nanoparticles were also prepared to encapsulate plasmid DNA for the delivery of gene constructs to the tumors. Sustained release of DNA from the nanoparticles with high encapsulation efficiency was obtained. DNA is highly negatively charged and hydrophilic, hence it is combined with a cationic agent, such as dimethyldioctylammonium bromide (DDAB), and encapsulated in PLGA (79). Cohen et al. (80) prepared PLGA nanoparticles that can protect the plasmid DNA from nuclease degradation. The DNA associated with the nanoparticles was found to be potent for a longer period of time than the liposomal DNA. However, the cell culture studies using NIH-3T3 cells showed that the transfection efficiency of DNA delivered in these particles was lower than with liposomes. Maruyama et al. (81) prepared graft co-polymers of poly(L-lysine) and PLA as carriers of DNA. PLA nanoparticles are negatively charged owing to the terminal carboxyl group. Association of the PLA with poly(L-lysine) resulted in positively charged particles, which could bind to the negatively charged DNA. The nanoparticles of the graft copolymer were obtained by solvent evaporation method or diafiltration method in which the nanoparticles are obtained by dialysis followed by centrifugation. The size of the nanoparticles and the adsorption capacity of DNA to the nanoparticles were found to vary with the concentration of the graft copolymer.

### **3.2. Poly(Alkylcyanoacrylates) Nanoparticles**

Poly(alkylcyanoacrylates) are biodegradable polymers that are widely used for the delivery of anticancer drugs. One of the major advantages of using poly(alkylcyanoacrylates) is the ease of polymerization, because the monomers do not require any energy for polymerization (82). The most widely used poly(alkylcyanoacrylates) are poly-

(butylcyanoacrylate) (PBCA), poly(hexylcyanoacrylate) (PHCA), poly(isohexylcyanoacrylate) (PIHCA), poly(isobutylcyanoacrylate) (PIBCA) and poly(methylcyanoacrylate) (PMCA). The molecular weight of these polymers, the residence time of the polymer in the plasma, as well as biodegradation kinetics depends on the alkyl chain length (83). This was further proved by the experiments of Couvreur et al. (82) that the heavier polymer (PHCA) would be cleared more slowly than the lighter one (PIBCA). It was also observed that a large proportion of the nanoparticles were taken up by the Kupffer cells in the liver. However, in the presence of a lung tumor, these nanoparticles are localized preferentially in the lung tissue as compared with the liver. The increase in efficiency of the anticancer drugs loaded in the polymeric nanoparticles was explained by Brasseur et al. (84) conducted experiments that showed slow release of the drug from the nanoparticles that resulted in an increase in the bioavailability and cytotoxic activity of the drug at the tumor site.

Soma et al. (85) prepared doxorubicin-loaded PIBCA nanoparticles for tumor-targeted delivery. An increase in antitumor activity and a decrease in clearance were observed for the doxorubicin nanoparticles. There was also a decrease in the concentration of the drug in the heart, indicating a reduction in the cardiotoxic effects of the drug. Similar effects were observed by Verdun et al. (86) using doxorubicin-loaded PIHCA nanoparticles. The biodistribution of the free and bound dactinomycin to polymethyl (PMN), polyethyl (PEN), and polybutyl cyanoacrylate nanoparticles was compared by Couvreur et al. (82) It was observed that PBN significantly increased the concentrations of the drug in liver (64-fold), spleen (44-fold), and lungs (4.7-fold) when compared to the free drug. It was also found that dactinomycin-loaded PMN nanoparticles resulted in a significant reduction in tumor volume against S250 sarcoma in rats. Similar experiments conducted using vinblastine-loaded nanoparticles showed that there was an increased accumulation in the spleen (21-fold) followed by lungs (fourfold) and liver (1.7-fold). Simeonova et al. (87) prepared PBCA nanoparticles associated with anticancer drug vinblastine. Intraperitoneal injection of these nanoparticles into mice resulted in a significant reduction in leucopenia caused by vinblastine.

PIHCA nanoparticles were also used to overcome MDR, which is the main cause of failure in systemic cancer therapy. In order to overcome the problem of low drug accumulation, Cuvier et al. (88) evaluated the effect of nanoparticles loaded with doxorubicin in five different MDR cell lines. An increase in the cytotoxicity of the cells was explained by an increase in drug concentration and an enhancement of contact time with the tumor cells. The nanoparticles of doxorubicin were tested in the C6 rat glioblastoma cell lines with varying degrees of resistance (89). It appeared that the reversal of resistance by the doxorubicin nanoparticles depended on the nature of the resistance.

The half-life and distribution of the nanoparticles was altered by coating them with surfactants like polysorbate 80, poloxamer, and poloxamine (90–93). The surfactants were found to induce steric repulsion, which reduced the uptake of these nanoparticles by the mononuclear phagocyte system (MPS). Surface coating of the nanoparticles with polysorbate 80 (Tween® 80) significantly altered the biodistribution of the drug. Doxorubicin concentration decreased in the heart, whereas there was a 60-fold increase in the brain with the use of nanoparticles. The author concluded that these nanoparticles could be used for delivery of drugs to the tumors in the brain. When compared to liposomes, poly(alkylcyanoacrylate) nanoparticles coated with poloxamine were found to deliver the anticancer drug mitoxantrone more efficiently resulting in a greater reduction in tumor growth in mice bearing B16 melanoma.

Poly(alkylcyanoacrylate) nanoparticles were also used to deliver antisense oligonucleotides to the tumor cells (94). These nanoparticles increased the bioavailability of the nucleotides by preventing their degradation by nucleases. The cyanoacrylates are negatively charged so they are combined with a cationic polymer (e.g., diethylaminoethyl dextran) or cationic detergent (e.g., hexadecyltrimethylammonium bromide [CTAB]), which facilitates the binding of the oligonucleotides by electrostatic interactions. From the *in vitro* studies using Vero, U937, and HBL100ras1 cell lines, the uptake of the oligonucleotide nanoparticles was found to be 50-fold higher with the nanoparticles than from the oligonucleotides solution (95). The *in vivo* studies conducted in OF1 mice showed that there was an increase in the biological half-life of the nanoparticle-delivered oligonucleotides. The nanoparticles were distributed preferentially in the liver after systemic administration and this significantly limits their use as colloidal carriers (96,97). Schwab et al. (98) used oligonucleotides adsorbed to poly(alkylcyanoacrylate) nanoparticles to inhibit tumor cells resulting from Ha-ras mediated mutations in nude mice. In mice implanted with HBL100ras1 cells, tumor-growth inhibition was achieved at the oligonucleotide concentrations that were approx 100-fold less than when free oligonucleotide were used.

Kattan et al. (99) conducted clinical studies of doxorubicin-loaded PIHCA nanoparticles in 21 patients with refractory solid tumors. Doppler-echocardiography showed complete absence of cardiotoxicity in patients receiving the nanoparticle formulations. However, both dose-limiting granulocytopenia as well as allergic reactions were observed when the drug was given by a 10-min intravenous infusion. Although the doxorubicin PIHCA nanoparticles had a higher therapeutic effect according to the authors as compared with the free drug, the effects could not be confirmed owing to the small number of patients and low cumulative dose.

### 3.3. Chitosan Nanoparticles

The positively charged chitosan nanoparticles were used as colloidal carriers of doxorubicin. The encapsulated efficiency of the cationic anticancer drug was improved by conjugating it with dextran sulfate prior to encapsulation in chitosan microspheres. The *in vitro* studies using human melanoma A375 cells and C26 murine colorectal carcinoma cells indicated that doxorubicin nanoparticles had cytotoxic activity equivalent to that of free doxorubicin (100). Mitra et al. (101) prepared chitosan nanoparticles incorporated with dextran-doxorubicin conjugate. The *in vivo* antitumor activity of the dextran-conjugated doxorubicin nanoparticles was evaluated in BALB/C mice implanted with J774A.1 macrophage tumor cells. The results showed that the animals treated with nanoparticle formulation had an increased survival time and greater tumor regression as compared with the free drug.

Chitosan nanoparticles are also used for the delivery of DNA to the tumor cells. Kabbaj et al. (102) prepared *Mycobacterium pheli* DNA-incorporated chitosan nanoparticles for inhibition of cancerous-cell division. The nanoparticles prevented degradation of DNA by nucleases resulting in a 20-fold increase in the transfection efficiency as compared with that of naked DNA. Mao et al. (103) prepared chitosan-DNA nanoparticles of 100–250 nm diameter by the complex coacervation process. On conjugation with transferrin and KNOB protein (cloned from adenovirus-fiber protein), the nanoparticles were reported to have significantly higher transfection efficiency in HEK293 (fourfold) and HeLa cells (130-fold). The distribution profile of the nanoparticles *in vivo* was obtained following *iv* injection of the nanoparticle suspen-

sion through the tail vein of AKR/J mice. A significant fraction of the administered dose of the nanoparticles was taken up by the liver and the kidneys. PEG surface modification of these nanoparticles was found to enhance their circulation time.

### 3.4. Gelatin Nanoparticles

Gelatin is a biocompatible and biodegradable polymer obtained by hydrolysis of collagen. It is a polyampholyte, which gels below 35–40°C. Because there are many different Bloom strengths of gelatin available, a wide range of nanoparticles can be made by salt-induced complex coacervation (104). Troung-Le et al. (105,106) prepared DNA-containing gelatin nanoparticles by the complex coacervation process. The cellular uptake of these nanoparticles was enhanced by conjugation with transferrin, and incorporation of calcium or chloroquine. The transfection efficiency of the DNA-gelatin nanoparticles in 9HTEo cells (human tracheal epithelial cells) was proved by expression of the cystic fibrosis transport regulator (CFTR) protein in more than 50% of the cells. Intramuscular injection of these nanoparticles into mice indicates that transfection efficiency was higher with the nanoparticles as compared to DNA-complexed with lipofectamine. Coester et al. (107) prepared avidin-conjugated gelatin nanoparticles by the desolvation method as carriers of biotinylated antisense drug derivatives. Additionally, Kaul et al. (108,109) prepared 250–400 nm PEG-modified gelatin nanoparticles by solvent-displacement method for encapsulation and delivery of hydrophilic macromolecules (e.g., DNA) to solid tumors. The *in vitro* studies using BT-20 cells (human breastcancer cell line) show that these nanoparticles can be used as carriers for gene delivery.

### 3.5. Other Polymeric Nanoparticles

Sharma et al. (110) prepared paclitaxel-containing poly(vinylpyrrolidone) (PVP) nanoparticles by reverse micro-emulsion method. The *in vivo* anti-tumor activity of these nanoparticles was evaluated in C57B1/6 mice bearing murine melanoma (B16F10). When compared to the free paclitaxel, these nanoparticles had increased survival time and regression in tumor volume. Kim et al. (111) prepared methoxyPEG and poly( $\epsilon$ -caprolactone) (McPEG/PCL) nanoparticles loaded with the anticancer drug paclitaxel. The size of the optimized nanoparticles was less than 100 nm and had a paclitaxel load of 20%. The *in vivo* studies conducted in ICR mice showed that the polymer used had very low toxicity. The authors concluded that these nanoparticles have significant potential as carriers of anticancer drugs.

Additionally, poly( $\gamma$ -benzyl-L-glutamate)/PEO (PBLG/PEO) block copolymer nanoparticles were prepared for targeting of the anticancer drug doxorubicin (112). The drug release from the PBLG/PEO nanoparticles was dependent on the molecular weight of the hydrophobic component of the block copolymer. The circulation time of the adriamycin nanoparticles was found to be threefold higher than that of the free drug. Potineni et al. (113) recently developed poly(ethylene oxide) (PEO)-modified poly( $\beta$ -amino ester) nanoparticles (100–150 nm in diameter) for encapsulation of hydrophobic anticancer drugs like paclitaxel. The cell-uptake studies using PEO-modified poly( $\beta$ -amino ester) nanoparticles encapsulated with fluorescent dye (rhodamine-123) indicate that the particles were taken up by the tumor cells (BT-20) by nonspecific endocytosis. Being pH-sensitive, the poly( $\beta$ -amino ester) nanoparticles are expected to spontaneously release the encapsulated payload in the low pH environment of the tumor or the endosome/lysosome complex. Thunemann et al. (114) devel-

oped retinoic acid and poly(ethylene imine) nanoparticles using different molecular weight of the polymer. The size of the nanoparticles was found to decrease with increasing molecular weight of PEI.

## 4. Liposomes and Related Systems

### 4.1. Conventional Liposomes

First described by Bangham et al. (115), liposomes are phospholipid vesicles composed of a bilayered lipid membrane. These vesicles are extensively used as drug carriers because of their ability to carry hydrophilic drugs in their aqueous compartment and lipophilic drugs in the bilayered membrane. Depending on the method of preparation and the lipids used the size of the liposomes range from 0.05 to 5.0  $\mu\text{m}$  (116). Liposomes used for drug-delivery applications are usually made from natural lipids such as phosphatidylcholine, phosphatidylglycerol, and cholesterol.

There are three major types of liposomes. The multi-lamellar vesicles (MLV) consist of several concentric bilayers of phospholipids. MLV range in size from 1.0–5.0  $\mu\text{m}$  and are prepared by hydrating the lipid film with an aqueous solution. MLV can encapsulate up to 5% efficiency of the hydrophilic drug and up to 10% efficiency of the hydrophobic drug (117). The small unilamellar vesicles (SUV) range from 30 to 50 nm and could not be used for encapsulation of hydrophilic drugs because of low encapsulation efficiency (~1%) (118). The large unilamellar vesicles (LUV) range from 50 to 100 nm and are used to encapsulate hydrophilic drugs. Drugs are incorporated into liposomes by encapsulation, partitioning, or reverse loading (116).

Liposomes have been used as carriers of a number of anticancer drugs. The drug-carrying vesicles reach the tumor tissue through the leaky vasculature and are retained in the interstitial space by passive targeting mechanism. The colloidal drug-carrying vesicles are recognized as foreign particles by MPS and are trapped by the cells of the RES (119). Hence, these liposomes can be used to target tumors present in liver, spleen, and the bone marrow. The liposomes of different compositions and the results of their therapeutic activity in various animal tumor models are presented in Table 3 (120–130).

Liposome formulations composed of distearoylphosphatidylcholine and cholesterol have unique advantages over those containing egg phosphatidylcholines because they are more resistant to leakage of entrapped drug. This is owing to the presence of lipids, whose phase-transition temperature is much higher than the physiological temperature (131). Daunorubicin encapsulated in liposomes, now marketed as DaunoXome<sup>®</sup>, was the first drug to enter into clinical trials. The liposomal formulation is composed of distearoylphosphatidylcholine and cholesterol in a 2:1 molar ratio. In one study, comparison of daunorubicin associated with the lipid bilayer and the citrate salt of the drug entrapped in the aqueous core, showed that the core-containing drug had higher stability, better biodistribution profile, and was more effective than the drug associated with the bilayers (132).

In the preclinical studies conducted in mice bearing P1798 lymphosarcoma, it was found that DaunoXome<sup>®</sup> resulted in greater mean survival time (18.5 d) than free daunorubicin (13 d) at a dose of 30 mg/kg (133). Money-Kyrle et al. (134) conducted Phase I/II trials in 25 patients with advanced AIDS-associated Kaposi's sarcoma (KS). In this study, it was found that DaunoXome<sup>®</sup>, given at 40 mg/m<sup>2</sup> dose, resulted in a partial response of 40%. In another study with patients who had not received previous treatment, a 57% response rate was observed (135). When compared to the free

**Table 3**  
**Therapeutic Efficacy of the Conventional Liposome-Associated Anticancer Drugs in Animal Tumor Models**

Drug	Liposomes	Tumor	Host	Dose	Results	Reference
Actinomycin D	PC:C:PA 7:2:1	AKR-A ascites $1 \times 10^6$ cells	AKR mice	Ip 1.0 $\mu$ g from d 2–6	Prolonged survival compared with drug	(120)
Adriamycin	PC:C:SA 18:4:5	Ridgeway osteosarcoma	AKR mice	Iv 0.8 mg/kg kg on d 1	Less effective than drug	(121)
	PC:PS:C 10:4:1	P388 ip $1 \times 10^5$ cells	BD/F <sub>1</sub> mice	Ip 4 mg/kg on d 1	As effective as drug and less cardiotoxic	(122)
	PC:C:SA 10:4:3	Lewis lung sc $1 \times 10^5$ cells	CD/F <sub>1</sub> mice	Iv 4 mg/kg on d 8, 10, 12	As effective as drug	(122)
	PC:C:DCP 7:2:1	Ehrlich ascites sc	ICR mice	Ip 1.25 mg/kg on d 7, 8, 9	Reduction in tumor weight compared to control	(123)
	PC:C:SA 7:2:1	$2 \times 10^6$ cells				
Cisplatin	PS:PC:C 3:7:10	J6456 lymphoma iv $10^6$ cells	BALB/c mice	iv 6 mg/kg on d 3, 7, 10, 24	Increase in mean survival time	(124)
	DSPC:DPPC C 7:1	Sarcoma 180 sc $5 \times 10^5$ cells	MMR1 mice	Iv 0.7–1.2 mg/kg on, d 7 followed by 1 hr hyperthermi	Delay in tumor growth	(125)
Daunomycin	PC:C:PA 7:2:1	L1210 iv $10^5$ cells	B-60/F <sub>1</sub> mice	Iv 5–80 mg/ kg on d 1	Increase in mean survival time at high doses	(126)
Methotrexate	PC:C:SA 3.25:2.25:2	L1210 ip $10^5$ cells	DBA/2 mice	Ip or iv 25– 50 mg/kg on d 1	As effective as free drug	(127)
	PC:C:SA 4:3:1	P-1798 lymphosarcoma	CD/F <sub>1</sub> mice	Ip 1 mg/kg on d 10–13	Reduction in tumor weight	(128)
	DPPC:DSPC C 7:3	L1210 sc $0.5\text{--}20 \times 10^6$ cells	B6D2/F <sub>1</sub> mice	Iv 3 mg/kg on d 1, 2, 3	Delay in tumor growth	(129)
	DPPC:C:SA A 34:23:10	Hepatoma 129 ip $10^6$ cells	C3H mice	Iv or Ip 2–3 mg/kg on d 1	Iv treatment was ineffective, ip treatment increases the mean survival time	130

C, cholesterol; DCP, dicetylphosphate; DSPC, distearylphosphatidylcholine; PC, phosphatidylcholine; PA, phosphatidic acid; PS, phosphatidylserine; SA, sterylamine; ip, intraperitoneal; iv, intravenous; sc, subcutaneous.

daunorubicin, DaunoXome® was also found to result in a 35-fold increase in the area under the curve (AUC) in plasma concentration vs time profile. Although these studies showed an absence of cardiotoxicity with DaunoXome, moderate myelosuppression was observed.

Gill et al. (136) conducted a Phase III trial in 232 patients with AIDS-related KS and compared the therapeutic efficacy of DaunoXome (40 mg/m<sup>2</sup>) with a standard ABV regimen of doxorubicin (10 mg/m<sup>2</sup>), bleomycin (15 U), and vincristine (1 mg). A 25% response rate (3 complete and 26 partial of 116 treated) was observed for DaunoXome and 28 % (1 complete and 30 partial of 111 treated) with ABV. No significant difference in the response rates and survival times were observed in the treatments. A higher incidence of alopecia and neuropathy were observed in patients treated with ABV and grade 4 neutropenia was observed in patients treated with DaunoXome. The aforementioned preclinical and clinical studies indicate the efficacy of DaunoXome, which is now approved for treatment of KS in HIV-positive patients in several countries, including the United States.

To investigate the effect of cationic charge on tumor targeting potential, Campbell et al. (137) prepared liposomes using 1, 2-dioleoyl-3-trimethylammonium propane (DOTAP), a cationic lipid, for the delivery of paclitaxel, a poorly soluble anticancer drug. The cationic lipid was combined with varying ratios of dimyristylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), and distearoylphosphatidylcholine (DSPC). It was observed that the stability of the formulations increased with an increase in the concentration of DOTAP, reaching a maximum when the lipid concentration was 30–50 mol%.

Liposomes-encapsulated drugs can also be used in the treatment of MDR tumors. The drug resistance is caused mainly by efflux of drugs in tumor cells by the P-gp pump. Rahman and co-workers (138,139) prepared doxorubicin-entrapped liposomes containing cardiolipin, which interacts with the P-gp pump and prevents the drug efflux. The major disadvantage of the formulation was that the phagocytes of the RES recognize cardiolipin and clear the vesicles from blood circulation in a relatively short period of time.

Even though the small-sized liposomes have the advantage of longer circulation than large ones, they are not capable of carrying large quantities of the drug (140). The large multilamellar liposomes have the problem of aggregation in the lungs (141). In addition, these conventional liposomes are internalized rapidly by the macrophage-rich RES and cleared from the systemic circulation (142,143). In order to overcome the rapid clearance problem, specialized liposomes like the PEG-coated, long-circulating, and cationic liposomes are being used to achieve better passive targeting to the tumor tissues.

## 4.2. Lipoplexes

Lipoplexes are cationic lipid-DNA complexes that are most widely used in gene delivery to tumor cells. They are formed by electrostatic interaction between the positively charged lipid and negatively charged DNA (144). Lipofectin and other cationic lipids are under clinical trials. Lipofectin is a cationic lipid composed of 1:1 (w/w) ratio of *N*-[1-(2, 3-dioleoyloxy) propyl]-*N,N,N*-trimethylammonium chloride (DOTMA) and dioleylphosphatidylethanolamine (DOPE). Other cationic lipids commonly used are 2,3-dioleoyloxy-*N*-[2-(sperminecarboxamido)ethyl]-*N,N*-dimethyl-1-propanaminium trifluoroacetate (DOSPA), dioctamido-decylamidoglycyl spermine (DOGS), 1,2-

dimyristyloxypentyl-3-dimethylhydroxyethyl ammonium bromide (DMRIE), 1,2-(dioleoyl-3-trimethylammonium) propane (DOTAP), dimethyl dioctadecylammonium bromide (DDAB), and  $3\beta$ -(*N,N*-dimethylaminoethane)carbamoyl cholesterol (DC-chol) (145–148).

Reimer et al. (149) prepared cationic liposomes using dioleoyldimethylammonium chloride and dioleoylphosphatidylethanolamine in a 1:1 ratio in order to deliver chloramphenicol acetyl transferase (CAT) gene to tumor cells. Upon ip administration of these liposomal formulations to a murine model bearing B16/B16 tumor, a highly variable gene expression was observed ranging from 100 mU/g to 2000 mU/g tumor. Small tumors did show much higher transfection efficiency relative to larger tumors. In order to minimize the toxic effects of lipoplexes in the systemic circulation, intratumoral delivery has been suggested (150).

### 4.3. Long-Circulating (Stealth®) Liposomes

Modification of conventional liposomes by hydrophilic polymers, like PEG, enhances the repulsive interactions between the colloidal particles and prevents opsonization by the macrophages of the RES. This results in long circulating liposomes, also called Stealth® liposomes (151), sterically stabilized liposomes (152), or cryptosomes (153). The mechanism of long circulation of the polymer-coated liposomes was explained by Torchillin and co-workers (154–156). The authors suggested that the coated polymer is in constant motion and exists as a cloud above the liposome surface, thus protecting the liposome from opsonization and recognition by RES cells. The high hydrophilicity, flexibility, and low immunogenicity were found to make PEG most acceptable for surface modification of the liposomes (157–159). Other synthetic polymers that can serve as protective coating on liposomes include poly(acrylamide) and poly(*N*-vinyl pyrrolidine) (160,161). These long circulating liposomes were found to deliver anticancer drugs to tumor tissues by passive targeting due to the EPR effect.

The accumulation of long-circulating liposomes in tumors was studied by Ning et al. (162) by injecting fluorescently labeled, long-circulating liposomes into rats with dorsal skin-flap window chambers and having mammary adenocarcinoma. Long-circulating liposomes made from egg phosphatidylcholine:cholesterol:disteoylphosphatidylethanolamine-PEG 2000: *N*-(6-tetramethylrhodamine-thiocarbamoyl)-1, 2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (TRITC-DHPE, a fluorescent label) in a weight ratio of 60:33:5:2 were used in the study. Within 90 min after injection, the long-circulating liposomes accumulated at three- to fourfold greater concentration than the conventional liposomes in the tumor mass. In addition, after 90 min, about 60% of the long-circulating liposomes were found to remain in systemic circulation, as compared with only 20% of the conventional liposomes.

Vincristine is an anticancer drug that acts by binding to the microtubules (163). Conventional liposomal formulations of the vincristine were found to be no more effective than the free drug (0.9 mg/kg). At higher doses, the liposomal formulation was found to be toxic owing to the high lipid content (164). In order to enhance the tumor targeting and anticancer activity of the drug, vincristine was encapsulated in PEG-coated liposomes. In vivo studies of the drug entrapped long-circulating liposomes containing methoxyPEG-disteoylphosphatidylethanolamine, hydrogenated soybean

phosphatidylcholine, and cholesterol in a weight ratio of 5:56:39 were conducted in various tumor models (165–167). The results in Table 4 show an increased mean survival time in leukemia models and reduction in tumor growth in colon carcinoma and mammary carcinoma models.

Doxorubicin, an anthracycline antineoplastic drug, is most widely used in the treatment of many different forms of human cancers. The clinical use of this drug is limited by a dose-dependent cardiotoxicity, myelosuppression, and stomatitis. The early studies using the conventional liposomes containing doxorubicin showed the problems of drug targeting to the tumor tissue and rapid clearance by RES. These drawbacks can be overcome by using long-circulating liposomes.

Doxil® is the long-circulating liposomal formulation of doxorubicin marketed by Liposome Technology, Inc. (Menlo Park, CA). The lipid bilayer of Doxil is made of hydrogenated soybean phosphatidylcholine, cholesterol, and PEG (molecular-weight 1900–2000)-modified distearylphosphatidylethanolamine in a weight ratio of 56:5:39. The formulation has doxorubicin at a concentration of 2 mg/mL and the lipids at 16 mg/mL (168). The published results of preclinical studies conducted in animals show the effectiveness of Doxil over free doxorubicin (169–171). The results, summarized in Table 5, prove that Doxil has longer circulation time and greater inhibition of tumor growth than free doxorubicin.

Clinical trials of the Doxil formulation were conducted in patients with different types of cancer. In a Phase III clinical trial of patients with AIDS-related KS, Doxil was compared with a combination therapy of doxorubicin, bleomycin, and vincristine (ABV) (172). The drugs were administered every 14 d in 6 cycles. There was no significant difference in the survival times of the patients. However, a higher incidence of alopecia, nausea, and vomiting was observed in patients treated with ABV regimen as compared with Doxil. The reduction in toxicity was found to be a major advantage of Doxil over the conventional ABV regimen.

Lyass et al. (173) conducted clinical trials of Doxil in breast-cancer patients who were previously treated with doxorubicin at a dose of less than 400 mg/m<sup>2</sup>. The results indicate a high incidence of mucositis and palmar-plantar erythrodysesthesia. No significant cardiotoxicity was observed in these trials, except for the occurrence of congestive heart failure in one patient who had prior mitoxantrone and radiotherapy. Partial response (five patients) and complete response (two patients) was observed in patients who were present in different treatment groups. In all the preclinical and clinical trials of Doxil, a high incidence of stomatitis was observed. Stomatitis is also a dose-limiting toxicity of continuous doxorubicin infusion.

The sterically stabilized liposomes are also used to encapsulate other anticancer drugs including cisplatin. SPI-077, a liposomal formulation of cisplatin, with a lipid composition hydrogenated soybean phosphatidylcholine: cholesterol: PEG (2000)-modified distearylphosphatidylethanolamine in a weight ratio 51:5:44 is currently in clinical trials. The preclinical studies indicate inhibition of tumor growth in colon C26 carcinoma and Lewis lung carcinoma models when compared with the delayed tumor growth observed in the treatment with cisplatin and carboplatin (174,175).

Long-circulating liposome combined with local hyperthermia is used to maximize tumor targeting of the anticancer drugs (176). The combination enhances the cytotoxic effects of the drug against tumor cells. Liposomes with the aforementioned properties were prepared by using DPPC, DSPC, distearylphosphatidylethanolamine-monomethoxypolyethylene glycol succinimidyl succinate of molecular weight 2000

**Table 4**  
**Therapeutic Efficacy of Long-Circulating Liposomes Loaded With Vincristine**

Tumor	Host	Dose	Results	Reference
P388 Lymphocytic leukemia 10 <sup>6</sup> cells iv or ip	B6D2F1 mice	Single dose of 1.3 mg/kg or 2.0 mg/kg iv or ip after 24 h	Increase in mean survival time (MST); MST of ip tumor is greater than MST of iv tumor	(165)
L1210 leukemia 10 <sup>6</sup> cells ip	DBA/2J mice	Single dose 0.5– 3 mg/kg after 24 h	Increase in survival time 17–117% for the given dose range	(166)
C26 colon carcinoma 4 × 10 <sup>5</sup> cells sc	BALB/c mice	Three weekly doses of 1.3 or 2 mg/kg starting on d 6 or 10	Reduction in tumor growth with stealth liposomes when compared with the delay in tumor growth of the free drug	(165)
MC2B mammary carcinoma 1 mm <sup>3</sup> piece implanted sc	C3H/HE mice	Three weekly injections of 1.0 or 1.3 mg/kg starting on d 3	Reduction in tumor growth with 1.3 mg/kg dose of the liposome formulation being more effective	(167)

ip, intraperitoneal; iv, intravenous; sc, subcutaneous.

**Table 5**  
**Therapeutic Efficacy of Doxorubicin-Encapsulated in Long-Circulating Liposomes (Doxil®) in Athymic Mice Xenografted With Human Tumors**

Tumor	Dose	Results	Reference
Human ovarian carcinoma HEY sc or iv	6 mg/kg on day 1, 8 and 15 iv or ip	Inhibition of tumor growth with 15 out of 18 treated mice being tumor- free when compared with 5 out of 18 in case of free adriamycin	(169)
Pancreatic adenocarcinoma AsPC-1 sc	3 mg/kg on d 1, 8, 15, 22, and 29 iv	Inhibition of tumor growth and more effective than free adriamycin (7/20 Vs 1/20). The doxorubicin could be detected in the tumor long after treatment (168 h) when compared with free adriamycin (24 h).	(170)
Prostatic carcinoma PC-3	6 mg/kg on d 1, 8, 15, and 22 iv	More effective than free adriamycin in inhibiting tumor growth	(171)

ip, intraperitoneal; iv, intravenous; sc, subcutaneous.

(DSPE-PEG-Osu 2000), and cholesterol. The activity of the thermosensitive liposomes with entrapped doxorubicin was studied in mice bearing colon 26 tumor. When compared to conventional thermosensitive liposomes, the long-circulating thermosensitive liposomes resulted in 2–2.5 times higher drug levels in the tumor tissue upon application of heat (42°C).

Tumor targeting is also enhanced by combining the unique properties of long-circulation and pH sensitivity. Slepishkin et al. (177) have prepared sterically stabilized, pH-sensitive liposomes using cholesteryl hemisuccinate (CHEMS), DOPE, and PEG-derivatized phosphatidylethanolamine (PEG-PE). The pharmacokinetics of the  $^{131}\text{In}$ -labeled liposomes was studied by injecting them iv in rats. The sterically stabilized, pH-sensitive liposomes were found to have enhanced tumor accumulation (100-fold) and longer circulation time ( $t_{1/2} = 11.1$  h) when compared with the pH-sensitive liposomes, which are rapidly cleared within the first 30 min.

#### 4.4. Niosomes

Niosomes are nonionic surfactant vesicles (NSV) formed by hydration of a single alkyl chain nonionic surfactant with cholesterol or a steroid (178). These vesicles are considered to be an alternative to liposomes and are capable of entrapping water-soluble drugs, altering their distribution and metabolic stability. Niosomes are used to encapsulate a wide range of anticancer drugs such as doxorubicin, vincristine, methotrexate, pentoxifyline, and 5-fluorouracil (5-FU).

Uchegbu et al. (179) prepared doxorubicin-loaded niosomes using sorbitan monostearate (Span® 60), cholesterol and choleth-24 (24 oxyethylene cholesteryl ether) in a weight ratio of 45:45:10. The niosomal formulation, at doxorubicin dose of 5 mg/kg and 10 mg/kg, was injected iv into female NMRI mice bearing subcutaneously (sc) implanted MAC15A tumor. The results showed an increase in the area under the plasma concentration-time curve (sixfold) and tumor level-time curve (50%) as compared to the control doxorubicin administered as an aqueous solution. However, an increase in drug metabolites was observed in the liver with no significant difference in drug concentration in the heart.

Parthasarathi et al. (180) prepared niosomal encapsulated vincristine using nonionic surfactant (Span® 40) and cholesterol in a 1:1 ratio. The antitumor activity was evaluated by injecting the formulation containing 0.5 mg/kg and 1.0 mg/kg of vincristine into BALB/c mice bearing Sarcoma-180 and Ehrlich ascites. The results indicate 110% increase in life span of the animals receiving the niosomal formulation relative to the control. Diarrhea, severe weight loss, and muscular weakness, which are characteristic of vincristine, were not observed in animals receiving the niosomal formulation of the drug.

Hao et al. (181) evaluated the encapsulation efficiency of niosomes prepared by evaporation sonication method using different kinds of Spans (20, 40, 60, and 80). Span 60 was found to have the highest encapsulation efficiency. This study also showed that the encapsulated formulation provided prolonged release of colchicine and 5-FU over a 24-h period. Niosomes were also found to alter the metabolic profile of methotrexate by preventing the rapid formation of 7-hydroxy methotrexate.

#### 5. Micellar Systems

Micelles are defined as colloidal dispersions with a size ranging from 5 to 50 nm. Micelles are made from amphipathic polymer molecules that have hydrophilic and hydrophobic regions or segments (182). At a low concentration, these compounds exist

as single molecules or unimers. As the concentration in aqueous solution increases, the amphipathic polymer chains aggregate to form three-dimensional (3D) structures or micelles. The concentration of the polymer at which the micelles are formed is known as the critical micelle concentration (CMC). Polymeric micelles may be formed from diblock, triblock, or graft copolymers (183–185). In aqueous systems, these micelles consist of an inner hydrophobic core and an outer hydrophilic shell. An increase in the length hydrophobic segment was found to be associated with a decrease in the CMC value (186). Hydrophobic anticancer drugs can be entrapped into the core of the micelle and can result in an improvement in the solubility and bioavailability of these drugs. For drug-delivery applications, it is important that the CMC value of the polymeric molecules is low enough so as to preserve the micellar structure even under high dilutions, such as after systemic administration. For this reason, amphiphilic-block copolymers that form micellar structures at low concentrations are used for drug-delivery applications.

Drug delivery to the tumor tissue using micellar carriers is achieved by passive targeting based on the EPR effect. Passive targeting followed by an increase in temperature, acidic conditions, or ultrasound may also enhance the release of anticancer drugs at the tumor site (182). One of the major advantages of micellar delivery systems is the presence of the hydrophobic core that can entrap and retain hydrophobic drugs. In addition, relative to liposomes and nanoparticles, micellar carriers usually have much smaller diameter and thus provide a far more efficient tumor uptake. When compared to the free drug, an increase in circulation time was achieved by using the polymeric micelles. The increased circulation time allows for the drug to accumulate in target tissue. In contrast to many surface-active agents, polymer micelles are preferred as carriers of anticancer drugs owing to their high loading capacity and long circulating properties (187,188). However, the circulation time of micellar structures is usually shorter than that of long-circulating liposomes. This is because micelles are smaller than long-circulating liposomes and are eliminated at a faster rate. Micellar systems also reduce the toxicity and enhance the antitumor activity of the drugs (189).

### **5.1. Poly(Ethylene Glycol) Block Copolymer Micelles**

PEG and PEO are water-soluble polymers used as hydrophilic blocks in the preparation of diblock and triblock copolymers. The block copolymers are synthesized by anionic polymerization or by ring-opening polymerization. The outer shell of the block copolymers is formed by the hydrophilic polymers (PEG or PEO) and the inner core is formed by the hydrophobic polymers such as poly(L-aspartic acid) (PAsp), poly( $\beta$ -benzyl L-aspartate) (PBLA), Poly( $\gamma$ -benzyl-L-glutamate) (PBLG), poly(D-lactic acid) (PLA), poly(propylene oxide) (PPO), and distearoylphosphatidylethanolamine (DSPE) (Table 6). The hydrophilic nature of the outer shell prevents the interaction of the micelles with the MPS and, thus increases the circulation time after systemic administration.

PEG or PEO is a nonionic homopolymer of ethylene oxide that is distinguished based on the molecular weight (i.e., <20,000 Daltons are PEG). The total molecular weight of these polymers depends on the average number of oxyethylene groups present in the molecule. The chemical composition and structures the polymers PEG and PEO are the same except for the presence of an extra hydroxyl group in PEG. Hence PEG and PEO are discussed together.

**Table 6**  
**Some Examples of Block Copolymers of Poly(Ethylene Glycol)**  
**or Poly(Ethylene Oxide) Using for Tumor-Targeted Drug Delivery**

Block Copolymer	Reference
Poly(ethylene glycol)-poly(L-aspartic acid)	(190)
Poly(ethylene glycol)-poly( $\beta$ -benzyl L-aspartate)	(192)
Poly(ethylene glycol)-poly(D,L-lactic-co-glycolic acid)	(209)
Methoxypoly(ethylene glycol)-poly(D,L-lactide)	(210)
Poly(ethylene glycol)-phosphatidyl ethanolamine	(214)
Poly(ethylene oxide)-poly(aspartic acid)	(197)
Poly(ethylene oxide)-poly( $\beta$ -benzyl L-aspartate)	(200)
Poly(ethylene oxide)-poly( $\gamma$ -benzyl glutamate)	(213)
Poly(ethylene oxide)-poly(propylene oxide)	(203)

#### 5.1.1. PEG-Poly(L-Aspartic Acid) or PEO-Poly(L-Aspartic Acid)

Yokoyama et al. (190–193) prepared polymeric micelles from PEG-poly(L-aspartic acid) (PEG-PAsp) block copolymer. The anticancer drug doxorubicin was incorporated into the micelles by chemical conjugation and physical entrapment. Physical entrapment of the drug resulted in the formation of a doxorubicin dimer, which contributed to the stability and slow release of the drug. The incorporation of the drug in the micelles also resulted in an increase in the maximum tolerated dose. This caused a significant regression in tumor volume upon systemic administration. The antitumor activity was evaluated in mice bearing different solid tumors (C26, P388, C38, M 5076, MKN-45, and MX-1). A significant reduction in weight loss and regression in tumor volume were observed in all of the tumor models. It was also observed that the physically entrapped drug contributed positively towards the antitumor activity as compared to the covalently attached drug. The increased half-life of the doxorubicin and the small size of the micelles (50 nm) were found to further increase the potential of these systems as carriers of anticancer drugs. Cisplatin-containing micelles of PEG-PAsp block copolymer were prepared by ligand-substitution reaction. The size of the micelles (20–100 nm) was found to increase with an increase in size of PAsp segment. These micelles were found to be highly stable upon dilution (194). When compared to the free cisplatin, the micelles were found to have an increased antitumor activity and reduced nephrotoxic activity in nude mice bearing MKN45, a human gastric cancer xenograft tumor (195).

Nakanishi et al. (196) prepared a micellar carrier NK911, which is a block copolymer of PEG-PAsp. The conjugated doxorubicin was found to increase the hydrophobicity of the core and the entrapped doxorubicin contributed to the anticancer activity. High-performance liquid chromatography (HPLC) analysis of the doxorubicin concentrations in the plasma and tumor tissue, after systemic administration, showed that there was a significant enhancement of antitumor activity with the NK911 micelles (40 nm). The authors concluded that these micelles were passively targeted to the tumor mass by the EPR effect. The antitumor activity of the doxorubicin-loaded NK911 and free doxorubicin was also determined by the *in vivo* experiments conducted on CDF1 mice inoculated with four sc tumors (C26, M5076, Lu-24, MX-1) and one intravenous tumor (P388). The results of the *in vivo* experiments given in Table 7 and Table 8, show that

**Table 7**  
**Antitumor Activity of NK911 and Doxorubicin in Murine Tumor Models**

Dose (mg/kg)	Mouse colon 26 carcinoma		Mouse M5076 sarcoma		Mouse P388 leukemia	
	NK911	DOX	NK911	DOX	NK911	DOX
	T/C (%) <sup>a</sup>	T/C (%)	T/C (%) <sup>b</sup>	T/C (%)	ILS (%) <sup>c</sup>	ILS (%)
30.0	2.4	10.3	19.2	—	379	199
24.0	14.4	21.2	30.5	26.8	390	290
19.2	31.6	33.7	35.4	54.1	203	73
15.4	50.6	48.5	34.4	72.5	68	58

<sup>a</sup>T/C (%) - (Tumor volume of treated mouse / Tumor volume of control mouse) × 100 on d 13.

<sup>b</sup>T/C (%) - (Tumor volume of treated mouse / Tumor volume of control mouse) × 100 on d 14.

<sup>c</sup>ILS (%) - Increased life span: [(mean survival time of treated mice / mean survival time of control mice) - 1] × 100 on d 60.

Adapted from ref. 196.

doxorubicin in NK911 micelles provides better antitumor activity as compared with the free drug. The micellar systems presently are undergoing clinical trials in Japan.

Yokoyama and co-workers (197,198) developed doxorubicin-conjugated PEO-PAsp micelles and also entrapped additional doxorubicin by physical means. Any unconjugated doxorubicin remaining in the micelles was removed by dialysis and a known amount of the drug was entrapped. Micelles prepared by this method were found to have 100 times lower in vitro cytotoxic activity than the free doxorubicin against P388D<sub>1</sub> cells. It was also observed that PEO-PAsp micelles resulted in increased survival time and reduced cytotoxicity when given intraperitoneally to mice bearing P388 leukemia.

#### 5.1.2. PEG-Poly( $\beta$ -Benzyl L-Aspartate) or PEO-Poly( $\beta$ -Benzyl L-Aspartate)

Yokoyama et al. (192) evaluated the potential of PEG-poly( $\beta$ -benzyl L-aspartate) (PEG-PBLA) block copolymer micelles and its derivatives as carriers of an investigational anticancer drug KRN 5500. The polymers were synthesized by ring-opening polymerization and were formed by partial hydrolysis and cetyl ester substitution of the  $\beta$ -benzyl L-aspartate units. KRN 5500 was incorporated by dialysis, followed by sonication, which resulted in drug-containing micelles (71 nm in diameter). Kataoka et al. (199) prepared doxorubicin-loaded micelles of PEG-PBLA by the emulsion method to enhance the stability of the drug and provide sustained release. The dimer derivatives of doxorubicin thus formed enhanced the stability and sustained release. The accelerated release of doxorubicin at pH 5.0 showed that the micelles were pH-sensitive and would be beneficial for tumor-targeting, because the pH around the tumor is much lower than the systemic circulation. Intravenous injection of these micelles into mice with C26 tumor indicated higher anticancer activity and long circulation as compared with free doxorubicin.

Micelles of PEO-PBLA have also been used as carriers of anticancer drugs (200–202). These micelles provide an opportunity for drug targeting and decrease toxicity. For instance, in the case of doxorubicin-loaded PEO-PBLA micelles (approx 40 nm in diameter), entrapment of the drug was found to enhance the stability of the drug.

**Table 8**  
**Anti-Tumor Activity of NK911 and Doxorubic Against Subcutaneously Implanted Tumors**

Dose (mg/kg)	Human Lu-24 lung cancer		Human MX-1 breast cancer	
	NK911 T/C (%) <sup>a</sup>	DOX T/C (%)	NK911 T/C (%)	DOX T/C (%)
18.0	23.5	—	30.7	—
14.4	35.5	28.3	42.2	48.3
11.5	54.7	48.1	67.6	63.1

<sup>a</sup>T/C (%) = (Tumor volume of treated mouse / Tumor volume of control mouse) × 100 on d 14.

Adapted from ref. 196.

### 5.1.3. PEO-PPO-PEO Triblock Copolymers (Pluronics® or Poloxamers)

The PEO-PPO-PEO triblock copolymers are commercially available as Pluronics® or poloxamers from BASF Corporation (Parsippany, NJ). The PPO segment forms the inner hydrophobic core and the two PEO chains form the outer hydrophilic shell (203). There are more than 30 different types of PEO-PPO-PEO triblock copolymers available with varying PPO and PEO chain lengths. The CMC of these copolymers also depends on the chemical composition. These triblock copolymers form micelles that are highly hydrophilic and escape the RES, which results in long circulation time. Hence these polymeric micelles were used to increase the efficiency of anticancer drugs like camptothecin (187) and doxorubicin (204). However, a decrease in efficiency of the Pluronic systems was observed at concentrations above the CMC (44–46). Drug release from the micelles was enhanced by the application of ultrasound at the tumor site (205).

It was also observed that the Pluronic micelles could be used to deliver anticancer agents to MDR tumors. The Pluronic effects on the drug resistance mechanisms include inhibition of drug efflux transporters and glutathione/glutathione S-transferase detoxification system. These energy-dependent mechanisms were inhibited by ATP depletion induced by Pluronic block copolymer (206,207). Batrakova et al. (208) evaluated the anticancer activity of Pluronic L-61, P-85, and F-108 micelles carrying epirubicin (EPI) and doxorubicin (DOX) in animals bearing murine leukemia (P388) and daunorubicin-sensitive (Sp2/0) and resistant (Sp2/0[DNR]) myeloma tumors grown *sc*. This study has shown that the Pluronic micelles are associated with a greater than 150% increase in survival time between treatment and control groups. The formulations EPI/P85 and DOX/L61 were found to inhibit tumor growth in more than 90% of the animals and complete disappearance of tumor growth in 33–50% of animals bearing Sp2/0 tumors. An increase in the therapeutic activity of the micellar drug was observed with an increase in the hydrophobicity of the copolymer as a result of increasing the PPO segment length.

### 5.1.4. Miscellaneous Diblock Copolymers of PEG and PEO

Yoo et al. (209) prepared biodegradable polymeric micelles from the diblock copolymer of PEG and PLGA. Doxorubicin was incorporated into the micelles by conjugating the amino group of the drug with the hydroxyl group of PLGA. The micelles containing conjugated doxorubicin were found to exhibit sustained release to a greater extent than the micelles with entrapped drug. The cytotoxic activity of the micelles

against HepG2 cells was found to be greater than the free drug. Micelles of the diblock copolymer of methoxyPEG and poly(D,L-lactide) (MPEG-PLA) are used as carriers of the anticancer drug paclitaxel (210). The diblock copolymer is formed by ring-opening polymerization of the monomers. The antitumor activity of the micelles was tested *in vitro* in different cell lines (OVCAR-3, MCF7, Hs578T, SKMES, and HT-29) (211,212). The biodistribution studies of paclitaxel in mice bearing B16 melanoma showed that the concentration of micellar paclitaxel is two to three times higher than the free drug in tissues, including liver, spleen, kidneys, lungs, heart, and tumor. Micellar paclitaxel administered in mice bearing SKOV-3 tumor (human ovarian cancer) and P388 leukemia showed a significantly enhanced activity than did the free drug.

Doxorubicin-loaded polymeric micelles of PEO-PBLG were prepared by dialysis and ultrafiltration (diafiltration) method. The size of the micelles formed was found to increase with an increase in PBLG content (20 nm to 70 nm). The release rate of the drug was reduced with an increase in PBLG chain length and the amount of incorporated doxorubicin (213).

#### 5.1.5. PEG-Diacyllipids

PEG-phosphatidylethanolamine conjugates are used to prepare highly stable micelles, which can act as carriers for the delivery of different hydrophobic anticancer drugs (214–216). The phosphatidylethanolamine used is highly hydrophobic owing to the presence of two long-chain fatty acyl groups and PEG is highly hydrophilic. These PEG-lipid conjugates can form a micellar system that are very stable and have a very low CMC value in the  $10^{-5}$  molar range. PEG of varying chain lengths was used to prepare micelles 7–35 nm in diameter. An increase in the circulation time and selective tumor accumulation was observed upon *iv* administration of the micellar system into mice bearing *sc* Lewis lung carcinoma and EL4 lymphoma (217). Weissig et al. (216) have compared the biodistribution of the PEG-distearoylphosphatidylethanolamine (PEG-DSPE) micelles with PEG-modified liposomes using mice bearing subcutaneous Lewis lung carcinoma. It was observed that the micelles containing the model protein, soybean trypsin inhibitor, accumulated in the tumor to a greater extent than the long-circulating liposomes. This could be explained by the fact that the cut-off size of the vascular pores in Lewis lung carcinoma is small and permits micelles (5–50 nm) more efficiently than long-circulating liposomes (>100 nm) by passive targeting.

#### 5.2. Thermoresponsive Micelles

Block copolymers of poly(*N*-isopropylacrylamide) (NIPAAm) are used in the preparation of thermoresponsive micelles along with poly(D,L-lactide) (218), polystyrene (219), poly(polybutylmethacrylate) (220), poly(dimethylacrylamide) (221), and alkylterminated PIPAAm (222). NIPAAm is a water-soluble polymer, which undergoes a phase-transition from hydrated to dehydrated state (cloud point) with a slight change in solution temperature in the range of 35°–38°C. Diblock copolymers of PIPAAm, obtained by ring-opening polymerization, were used in the preparation of thermoresponsive micelles, which act as carriers of anticancer drugs. The anticancer drug doxorubicin was incorporated into the micelles by the dialysis method. The swollen core formed owing to the presence of water was found to increase the doxorubicin-loading capacity (221). Passive targeting by the EPR effect followed by an increase in temperature at the tumor site would enhance the release of the drug from the micelles. These micelles were associated with a reduction in the cytotoxicity and an increase in therapeutic efficiency of the anticancer drugs.

### 5.3. Other Polymeric Micelles

Miwa et al. (223) synthesized *N*-lauryl-carboxymethyl-chitosan (LCC), which is used as a carrier of the anticancer drug paclitaxel. Chitosan, a hydrophilic polymer of D-glucosamine, cannot form micelles and so LCC was synthesized by attaching lauryl groups to the primary amino groups of D-glucosamine in carboxymethyl chitosan. An increase in the solubility of paclitaxel was observed upon incorporation in the micellar structure. The *in vitro* studies against KB cells indicate an increase in the anticancer activity of the entrapped paclitaxel relative to the free drug.

## 6. Conclusions

Passive targeting of anticancer drugs to solid tumors using colloidal-carriers systems provides a unique opportunity to develop a newer generation of safer drugs. Pre-clinical and clinical results presented in this chapter show that the different types of colloidal carriers can improve the therapeutic benefits of anticancer drugs when directed to its intended target. A very promising observation in colloidal delivery of anti-cancer agents is the opportunity to overcome MDR as the transport of the macromolecules or particulate carrier into the cells bypasses the P-gp efflux pump present in the cell membrane. Biocompatibility of the carrier system is the most important issue that needs to be considered in developing these systems for tumor targeting.

## References

1. Seymour WL. Systemic cancer therapy using polymer-based prodrugs and progenes. In: Dumitriu S, ed. *Polymeric Biomaterials*, 2nd edition, New York, NY, Marcel-Dekker, Inc., 2001, pp. 843–850.
2. Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. *Nature* 2001;7:987–989.
3. Teicher BA. *Drug Resistance in Oncology*. New York, NY, Marcel Dekker, 1994.
4. Yuan F. Transvascular drug delivery in solid tumors. *Semin Radiat Oncol* 1998;8:164–175.
5. Jain RK. Delivery of molecular and cellular medicine to solid tumors. *Microcirculation* 1997;4:1–23.
6. Senger DR, Galli SJ, Dvorak AM, et al. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science* 1983;219:983–985.
7. Leung DW, Cachianes G, Kuang WJ, et al. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 1989;246:1306–1309.
8. Maeda H, Matsumura Y, Kato H. Purification and identification of (hydroxypropyl) bradykinin in ascitic fluid from a patient with gastric cancer. *J Biol Chem* 1988;263:16051–16054.
9. Maeda H, Noguchi Y, Sato K, Akaike T. Enhanced vascular permeability in solid tumor is mediated by nitric oxide and inhibited by both new nitric oxide scavenger and nitric oxide synthase inhibitor. *J Cancer Res* 1994;85:331–334.
10. Folkman J, Shing Y. Angiogenesis. *J Biol Chem* 1992;267:10931–10934.
11. Munn LL, Koenig GC, Jain RK, Melder RJ. Kinetics of adhesion molecule expression and spatial organization using targeted sampling fluorimetry. *Biotechniques* 1995;19:622–631.
12. Jain RK. Transport of molecules across tumor vasculature. *Cancer Metastasis Rev* 1987;6:559–593.
13. Jain RK, Safabakhsh N, Sckell Y, et al. Endothelial cell death, angiogenesis, and microvascular function following castration in an androgen-dependent tumor: role of VEGF. *Proc Natl Acad Sci USA* 1998;95:10820–10825.
14. Tannock IF, Rotin D. Acid pH in tumors and its potential for therapeutic exploitation. *Cancer Res* 1989;49:4373–4384.
15. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability and angiogenesis. *Am J Pathol* 1995;146:1029–1039.

16. Kohn S, Nagy JA, Dvorak AM. Pathways of macromolecular tracer transport across venules and small veins: structural basis for hyperpermeability of tumor blood vessels. *Lab Invest* 1992;67:596–607.
17. Dvorak AM, Kohn S, Morgan ES. The vesiculo-vacuolar organelles (VVO): a distinct endothelial cell structure that provides transcellular pathway for macromolecular extravasation. *J Leukoc Biol* 1996;59:100–115.
18. Feng D, Nagy JA, Hipp J. Vesiculo-vacuolar organelles and the regulation of venule permeability to macromolecules by vascular permeability factor, histamine and serotonin. *J Exp Med* 1996;183:1981–1986.
19. Roberts WG, Palade GE. Increased vascular permeability and endothelial fenestration induced by vascular endothelial growth factor. *J Cell Sci* 1995;108:2369–2379.
20. Roberts WG, Palade GE. Neovasculature induced by vascular endothelial growth factor is fenestrated. *Cancer Res* 1997;57:765–772.
21. Maeda H, Matsumura Y. Tumorotropic and lymphotropic principles of macromolecular drugs. *Crit Rev Ther Drug Carrier Syst* 1989;6:193–210.
22. Maeda H. SMANCS and polymer-conjugated macromolecular drugs: advantages in cancer chemotherapy. *Adv Drug Delivery Rev* 1991;6:181–202.
23. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumorotropic accumulation of proteins and the antitumor agent SMANCS. *Cancer Res* 1986;46:6387–6392.
24. Muggia F. Doxorubicin-polymer conjugates: further demonstration of the concept of enhanced permeability and retention. *Clin Cancer Res* 1999;5:7–8.
25. Duncan R. Drug-polymer conjugates: potential for improved chemotherapy. *Anticancer Drugs* 1992;3:175–210.
26. Duncan R. Selective endocytosis. In: Robinson JR, ed. *Sustained and Controlled Drug Delivery Systems*. New York, Marcel Dekker, 1987, pp. 581–621.
27. Luo Y, Prestwich GD. Cancer-targeted polymeric drugs. *Curr Cancer Drug Targets* 2002;2:209–226.
28. Kopecek J, Bazilova H. Poly[N-(2-hydroxypropyl) methacrylamide] 1. Radical polymerization and copolymerization. *Eur Polym* 1973;9:7–14.
29. Seymour WL, Duncan R, Kopeckova P, Kopecek J. Daunomycin and adriamycin-N-(2-hydroxypropyl) methacrylamide copolymer conjugates: toxicity reduction by improved drug-delivery. *Cancer Treatment Rev* 1987;14:319–327.
30. Vasey PA, Kaye SB, Morrison R, et al. Phase I clinical and pharmacokinetic study of PK1 [N-(2-hydroxypropyl) methacrylamide copolymer doxorubicin]: first member of a new class of chemotherapeutic agents-drug-polymer conjugates. *Clin Cancer Res* 1999;5:83–94.
31. Merum Terwogt JM, Ten Bokkel Huinink WW, Shellens JH, et al. Phase I clinical and pharmacokinetic study of PNU166945, a novel polymer-conjugated prodrug of paclitaxel. *Anticancer Drugs* 2001;12:315–323.
32. Caiolfa VR, Zamal M, Fiorini A, et al. Polymer bound camptothecin: initial biodistribution and antitumor activity studies. *J Control Release* 2000;65:105–120.
33. Gianasi, E., Wasil, M., Evagorou, E.G., Keddlé, A., Wilson G, Duncan R. HEMA copolymer platinate as novel antitumor agents: *in vitro* properties, pharmacokinetics and antitumor activity. *Eur J Cancer* 1999;35:994–1002.
34. Etrych T, Jelinkova M, Rihova B, Ulbrich K. New HEMA copolymers containing doxorubicin bound via pH-sensitive linkage: synthesis and preliminary *in vitro* and *in vivo* biological properties. *J Control Release* 2001;73:89–102.
35. Cassidy J, Duncan R, Morrison GJ, et al. Activity of N-(2-hydroxypropyl) methacrylamide copolymers containing daunomycin against rat tumor model. *Biochem Pharmacol* 1989;38:875–879.
36. Duncan R, Kopeckova P, Strohalm J, et al. Anticancer agents coupled to N-(2-hydroxypropyl) methacrylamide copolymers. II. Evaluation of daunomycin conjugates *in vivo* against L1210 leukemia. *Br J Cancer* 1988;57:147–156.
37. Kasuya Y, Lu ZR, Kopeckova P, et al. Synthesis and characterization of HEMA copolymer-aminopropylgeldanamycin conjugates. *J Control Release* 2001;74:203–211.

38. Omelyanenko V, Kopecekova P, Gentry C, Kopecek J. Targetable HPMA copolymer-adriamycin conjugates. Recognition, internalization and subcellular fate. *J Control Release* 1998;53:25–37.
39. Satchi R, Connors TA, Duncan R. PDEPT: polymer-directed enzyme prodrug therapy. I. HPMA copolymer-cathepsin B and PK1 as a model combination. *Br J Cancer* 2001;85:1070–1076.
40. Searle F, Gac-Breton S, Keane R, et al. N-(2-hydroxypropyl) methacrylamide copolymer-6-(3-aminopropyl)-elliciptine conjugates. Synthesis, in vitro, and preliminary in vivo evaluation. *Bioconjug Chem* 2001;12:711–718.
41. Tijerina M, Kopecekova J, Kopecek J. The effects of subcellular localization of N-(2-hydroxypropyl) methacrylamide copolymer-Mce6 conjugates in a human ovarian carcinoma. *J Control Release* 2001;74:269–273.
42. Seymour LW. Passive tumor targeting of soluble macromolecules and drug conjugates. *Crit Rev Ther Drug Carrier Syst* 1992;9:135–187.
43. Kuromizu K, Tsunasawa S, Maeda H, et al. Reexamination of the primary structure of an antitumor protein, neocarzinostatin. *Arch Biochem Biophys* 1986;246:199–205.
44. Kuromizu K, Abe O, Maeda H. Location of the disulfide bonds in the antitumor protein, neocarzinostatin. *Arch Biochem Biophys* 1991;286:569–573.
45. Maeda H, Takeshita J, Yamashita A. Lymphotropic accumulation of an antitumor antibiotic protein neocarzinostatin. *Eur J Cancer* 1980;16:723–731.
46. Maeda H, Matsumura Y. New tactics and basic mechanisms of targeting chemotherapy in solid tumors. In: Kimura K, Carter SK, Ota K, Pinedo HM, eds. *Cancer Chemotherapy: Challenges for the Future*. Tokyo, Excerpta Medica, 1989, pp. 239–260.
47. Konno T, Maeda H. Targeting chemotherapy of hepatocellular carcinoma: arterial administration of SMANCS/Lipiodol. In: Okada K, Ishak KG, eds. *Neoplasms of the Liver*. New York, NY, Springer-Verlag, 1987, pp. 276–291.
48. Konno T. Targeting anticancer chemotherapy for primary and secondary liver cancer using arterially administered oily anticancer agents. In: Kimura K, ed. *Cancer Chemotherapy: Challenges for the Future*. Tokyo, Excerpta Medica, 1987, pp. 299–311.
49. Hirano T, Ohashi S, Morimoto S, et al. Synthesis of antitumor-active conjugates of adriamycin or daunomycin with the copolymer of divinyl ether and maleic anhydride. *Makromol Chem* 1986;187:2815–2824.
50. Zalipsky S. Functionalized poly(ethylene glycol) for preparation of biologically relevant conjugates. *Bioconjug Chem* 1995;6:150–165.
51. Greenwald RB, Gilbert CW, Pendri A, et al. Drug delivery systems: water soluble taxol 2'-poly(ethylene glycol) ester prodrugs: design and in vivo effectiveness. *J Med Chem* 1996;39:424–431.
52. Pendri A, Conover CD, Greenwald RB. Antitumor activity of paclitaxel-2'-glycinate conjugated to poly(ethylene glycol): a water-soluble prodrug. *Anti-Cancer Drug Design* 1998;13:387–395.
53. Minko T, Paranjpe PV, Qiu B, et al. Enhancing the anticancer efficacy of camptothecin using biotinylated poly(ethylene glycol) conjugates in sensitive and multidrug-resistant human ovarian carcinoma cells. *Cancer Chemother Pharmacol* 2002;50:143–150.
54. Calcetti P, Monfardini C, Sartore L, et al. Preparation and properties of monomethoxy poly(ethylene glycol) doxorubicin conjugates linked by an amino acid or a peptide spacer. *Farmaco* 1993;48:919–932.
55. Keating MJ, Holmes R, Lerner S, Ho DH. Asparaginase and PEG asparaginase-past, present and future. *Leuk Lymphoma* 1993;10:153–157.
56. Ho DH, Brown NS, Yen A, et al. Clinical pharmacology of polyethylene glycol-asparaginase. *Drug Metab Dispos* 1986;14:349–352.
57. Kurtzberg J, Moore JO, Scudicry D, Franklin A. A phase II study of polyethylene glycol (PEG) conjugated L-asparaginase in patients with refractory acute leukemia's. *Proc AACR* 1988;29:213.
58. Duncan R, Spreafico F. Polymer conjugates. Pharmacokinetic considerations for design and development. *Clin Pharmacokinet* 1994;27:290–306.

59. Sawa T, Wu J, Akaike T, Maeda H. Tumor-targeting chemotherapy by a xanthine oxidase-polymerconjugate that generates oxygen-free radicals in tumor tissue. *Cancer Res* 2000;60:666–671.
60. Li C. Poly(L-glutamic acid)-anticancer drug conjugates. *Adv Drug Delivery Rev* 2002;54(5):695.
61. Singer JW, Bhatt R, Tulinsky J, et al. Water-soluble poly(L-glutamic acid)-glycamptothecin conjugates enhance camptothecin stability and efficacy in vivo. *J Control Rel* 2001;74:243–247.
62. Luo Y, Prestwich GD. Hyaluronic acid-N-Hydroxysuccinate: a useful intermediate for bioconjugation. *Bioconjug Chem* 2001;12:1085–1088.
63. Luo Y, Bernshaw NJ, Lu ZR, et al. Targeted delivery of doxorubicin by HPMa copolymer-hyaluronan bioconjugates. *Pharm Res* 2002;19:396–402.
64. Luo Y, Prestwich GD. Synthesis and selective cytotoxicity of a hyaluronic acid-antitumor bioconjugate. *Bioconjug Chem* 1999;10:755–763.
65. Okuno S, Harada M, Yano S, et al. Complete regression of xenografted human carcinomas by camptothecin analogue-carboxymethyl dextran conjugate. *Cancer Res* 2000;60:2988–2995.
66. Avichezer D, Schechter B, Arnon R. Functional polymers in drug delivery: carrier-supported CDDP (cisplatin) complexes of polycarboxylates-effect on human ovarian carcinoma. *React Funct Polym* 1998;36:59–69.
67. Bernstein A, Hurwitz E, Maron R, et al. Higher antitumor efficacy of daunomycin when linked to dextran: in vivo and in vitro studies. *J Natl Cancer Inst* 1978;60:379–384.
68. Hejazi R, Amiji MM. Chitosan based delivery systems: physicochemical properties and pharmaceutical applications. In: Dumitriu S, ed. *Biomaterials*. Quebec, Marcel Dekker, 2001, pp. 213–237.
69. Sato M, Onishi H, Takahara J, et al. In vivo drug release and antitumor characteristics of water soluble conjugates of mitomycin C with glycol-chitosan and N-succinyl-chitosan. *Biol Pharm Bull* 1996;19(9):1170–1177.
70. Swarts JC, Swarts DM, Neuse EW, et al. Polyaspartamides as water-soluble drug carriers. part I: Antineoplastic activity of ferrocene-containing polyaspartamide conjugates. *Anticancer Res* 2001;21:2033–2037.
71. Reddy BS, Damayanthi Y, Lown JW. Design synthesis and in vitro cytotoxicity studies of novel pyrrolo [2,1-c] [1,4] benzodiazepine (PBD)-polyamide conjugates and 2, 2'-PBD dimers. *Anti-Cancer Drug Design* 2000;15(3):225–238.
72. Kreuter J. Drug targeting with nanoparticles. *Eur J Drug Metab Pharmacokinet* 1994;3:253–256.
73. Brigger I, Dubernet C, Couvreur P. Nanoparticles in cancer therapy and diagnosis. *Adv Drug Delivery Rev* 2002;54:631–651.
74. Couvreur P, Dubernet C, Puisieux F. Controlled drug delivery with nanoparticles: current possibilities and future trends. *Eur J Pharm Biopharm* 1995;41:2–13.
75. Yoo HS, Lee KH, Oh JE, Park TG. In vitro and in vivo anti-tumor activities of nanoparticles based on doxorubicin-PLGA conjugates. *J Control Release* 2000;68:419–431.
76. Feng SS, Huang GF, Mu L. Nanospheres of biodegradable polymers: a system for clinical administration of an anticancer drug paclitaxel (Taxol). *Ann Acad Med Singapore* 2000;29:633–639.
77. Avgoustakis K, Beletsi A, Panagi Z, et al. PLGA-mPEG nanoparticles of cisplatin: in vitro nanoparticle degradation, in vitro drug release and in vivo drug residence in blood properties. *J Control Release* 2002;79:123–135.
78. Quintanar-Guerrero D, Fessi H, Allemann E, Dolker. Influence of stabilizing agents and preparative variables on the formation of poly(D, L-lactic acid) nanoparticles by an emulsification-diffusion technique. *Int J Pharm* 1996;143:133–141.
79. Hirose S, Muller BG, Mulligan RC, Langer R. Plasmid DNA encapsulation and release from solvent diffusion nanospheres. *J Control Release* 2001;70:231–242.
80. Cohen H, Levy RJ, Gao J, et al. Sustained delivery and expression of DNA encapsulated in polymeric nanoparticles. *Gene Ther* 2000;7:1896–1905.
81. Maruyama A, Ishihara T, Kim JS, et al. Nanoparticles DNA carrier with poly(L-lysine) grafted polysaccharide copolymer and poly(D,L-lactic acid). *Bioconjug Chem* 1997;8:735–742.

82. Couvreur P, Grislain L, Lenaerts V, et al. Biodegradable polymeric nanoparticles as drug carrier for antitumor agents. In: Guiot P, Couvreur P. eds. *Polymeric Nanoparticles and Microspheres*. Boca Raton, FL, CRC Press, 1986, pp. 27–93.
83. Couvreur P, Kante B, Roland M, Speiser P. Adsorption of antineoplastic drugs to polyalkylcyanoacrylate nanoparticles and their release characteristics in a calf serum medium. *J Pharm Sci* 1979;68:1521.
84. Brasseur F, Couvreur P, Kante B, et al. Actinomycin-D adsorbed on polymethylcyanoacrylate nanoparticles: increased efficiency against experimental tumor. *Eur J Cancer* 1980;16:1441.
85. Soma CE, Dubernet C, Barratt G, et al. Ability of doxorubicin-loaded nanoparticles to overcome multidrug resistance of tumor cells after their capture by macrophages. *Pharm Res* 1999;16:1710–1716.
86. Verdun C, Brasseur F, Vrancks H, et al. Tissue distribution of doxorubicin associated with polyisohexylcyanoacrylate nanoparticles. *Cancer Chemother Pharmacol* 1990;26:13–18.
87. Simeonova M, Ilarionova M, Ivanova T, et al. Nanoparticles as drug carriers for vinblastine. Acute toxicity of vinblastine in a free form and associated to polybutylcyanoacrylate nanoparticles. *Bulgarian Acad Sci Acta Phys et Pharm Bulg* 1991;17:43–48.
88. Cuvier C, Roblot-Treupel L, Millot JM, et al. Doxorubicin-loaded nanospheres bypass tumor cell multidrug resistance. *Biochem Pharmacol* 1992;44:509–517.
89. Bennis S, Chapey C, Couvreur P, Roberts J. Enhanced cytotoxicity of doxorubicin encapsulated in poly-isohexylcyanoacrylate nanospheres against multidrug resistant tumor cells in culture. *Eur J Cancer* 1994;30A:89–93.
90. Gulyaev AE, Gelperina SE, Skidan IN, et al. Significant transport of doxorubicin into brain with polysorbate 80-coated nanoparticles. *Pharm Res* 1999;16:1564–1569.
91. Illum I, Davis SS. The organ uptake of intravenously administered colloidal particles can be altered using a non-ionic surfactant (poloxamer 338). *FEBS Lett* 1984;167:79–82.
92. Reszka R, Beck P, Fichtner I, et al. Body distribution of free, liposomal and nanoparticle-associated mitoxantrone in B16-melanoma-bearing mice. *J Pharmacol Exp Ther* 1997;280:232–237.
93. Beck P, Kreuter J, Reszka R, Fichtner I. Influence of polybutylcyanoacrylate nanoparticles and liposomes on the efficacy and toxicity of the anticancer drug mitoxantrone in murine tumor models. *J Microencapsul* 1993;10:101–114.
94. Zobel HP, Junghans M, Maienschein V, et al. Enhanced antisense efficacy of oligonucleotides adsorbed to monomethylaminoethylmethacrylate methylmethacrylate copolymer nanoparticles. *Eur J Pharm Biopharm* 2000;49:203–210.
95. Zimmer A. Antisense oligonucleotide delivery with polyhexylcyanoacrylate nanoparticles as carriers. *Methods: Comp Meth Enzymol* 1999;18:286–295.
96. Nakada Y, Fattal E, Foulquier M, Couvreur P. Pharmacokinetics and biodistribution of oligonucleotide adsorbed onto poly(isobutylcyanoacrylate) nanoparticles after intravenous administration in mice. *Pharm Res* 1996;13:38–43.
97. Fattal E, Vauthier C, Aynie I, et al. Biodegradable polyalkylcyanoacrylate nanoparticles for the delivery of oligonucleotides. *J Control Release* 1998;53:137–143.
98. Schwab G, Chavany C, Duroux I, et al. Antisense oligonucleotides adsorbed to polyalkylcyanoacrylate nanoparticles specifically inhibit mutated Ha-ras-mediated cell proliferation and tumorigenicity in nude mice. *Proc Natl Acad Sci USA* 1994;91:10460–10464.
99. Kattan J, Droz JP, Couvreur P, et al. Phase I clinical trial and pharmacokinetic evaluation of doxorubicin carried by polyisohexylcyanoacrylate nanoparticles. *Invest New Drugs* 1992;10:191–199.
100. Janes KA, Fresneau MP, Marazuela A, Fabra A. chitosan nanoparticles as delivery systems for doxorubicin. *J Control Release* 2001;73:255–267.
101. Mitra S, Gaur U, Ghosh PC, Maitra AN. Tumor targeted delivery of encapsulated dextran-doxorubicin conjugate using chitosan nanoparticles as carrier. *J Control Release* 2001;74:317–323.
102. Kabbaj M, Phillips NC. Anticancer activity of mycobacterial DNA: effect of formulation as chitosan nanoparticles. *J Drug Target* 2001;9:317–328.

103. Mao H, Roy K, Troung-Le VL, et al. Chitosan-DNA nanoparticles as gene carriers: synthesis characterization and transfection efficiency. *J Control Rel* 2001;70:399–421.
104. Farrugia CA, Groves JM. Gelatin behaviour in dilute aqueous solution: designing a nanoparticulate formulation. *J Pharm Pharmacol* 1999;51:643–649.
105. Truong-Le VL, Walsh SM, Schweibert E, et al. Gene transfer by DNA-gelatin nanospheres. *Arch Biochem Biophys* 1999;361:47–56.
106. Truong-Le VL, August JT, Leong KW. Controlled gene delivery by DNA-gelatin nanospheres. *Hum Gene Ther* 1998;9:1709–1717.
107. Coester C, Kreuter J, Briesen HV, Langer K. Preparation of avidin-labelled gelatin nanoparticles as carriers for biotinylated peptide nucleic acid (PNA). *Int J Pharm* 2000;196:147–149.
108. Kaul G, Amiji MM. Long-circulating poly(ethylene glycol)-modified gelatin nanoparticles for intracellular delivery. *Pharm Res* 2002;19:1062–1068.
109. Kaul G, Potinini A, Lynn DM, et al. Surface-modified polymeric nanoparticles for tumor-targeted delivery. *surFacts Biomaterials* 2002;7:1–5.
110. Sharma D, Chelvi TP, Kaur J, et al. Novel taxol formulation: polyvinylpyrrolidone nanoparticle-encapsulated taxol for drug delivery in cancer therapy. *Oncol Res* 1996;8:281–286.
111. Kim SY, Lee YM. Taxol-loaded block copolymer nanospheres composed of methoxy poly(ethylene glycol) and poly(epsilon-caprolactone) as novel anticancer drug carriers. *Biomaterials* 2001;22:1697–1704.
112. Oh I, Lee K, Kwon HY, Lee YB, et al. Release of adriamycin from poly(gamma-benzyl-L-glutamate)/poly(ethylene oxide) nanoparticles. *Int J Pharm* 1999;181:107–115.
113. Potinini A, Lynn DM, Langer R, Amiji MM. Poly(ethylene oxide)-modified poly(beta-amino ester) nanoparticles as a pH-sensitive biodegradable system for paclitaxel delivery. *J Control Release* 2003;86:223–234.
114. Thunemann AF. Polyethyleneimine complexes with retinoic acid: structure, release profile and nanoparticles. *Macromolecules* 2000;33:6878–6885.
115. Bangham AD, Standish MM, Warkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. *J Mol Biol* 1965;13:238–252.
116. Chrai SS, Ahmad I. Liposomes (a review). Part one: manufacturing issues. *BioPharm* (2001);Nov:10–13.
117. Juliano RL, Stamp D. Interactions of drugs with lipid membranes. Characteristics of liposomes containing polar or non-polar antitumor drugs. *Biochim Biophys Acta* 1979;586:137–145.
118. Robert KY, Zee-Cheng, Cheng CC. Delivery of Anticancer drugs. *Meth Find Exp Clin Pharmacol* 1989;11:439–529.
119. Oku N, Tokudome Y, Asai T, Tsukada H. Evaluation of drug targeting strategies and liposomal trafficking. *Curr Pharmaceut Design* 2000;6:1669–1691.
120. Gregoriadis G, Necrunjun ED. Treatment of tumor bearing mice with liposome-entrapped actinomycin D prolongs their survival. *Res Commun Chem Pathol Pharmacol* 1975;10:351–362.
121. Kaye SB, Ryman BE. The fate of liposome-entrapped actinomycin-D in vivo and its therapeutic effect in a solid murine tumor. *Biochem Soc Trans* 1980;8:107–108.
122. Rahman A, Kessler A, More N. Liposomal protection of adriamycin induced cardiotoxicity in mice. *Cancer Res* 1980;40:1532–1537.
123. Shinozawa S, Maki Y, Oda T. Tissue distribution and antitumor effect of liposome entrapped doxorubicin (adriamycin) in Ehrlich ascites solid tumor bearing mice. *Acta Med Okayama* 1981;35:395–405.
124. Gabizon A, Dagan A, Goren D, et al. Liposomes as in vivo carriers of adriamycin: Reduced cardiac uptake and preserved antitumor activity in mice. *Cancer Res* 1982;42:4734–4739.
125. Yatvin MB, Muhlensiepen II, Porschen W, et al. Selective delivery of liposome associated cis-dichloro-diammineplatinum (II) by heat and its influence on tumor uptake and growth. *Cancer Res* 1981;41:1602–1607.
126. Fitchner I, Reszka R, Elbe B, Arndt D. Therapeutic evaluation of liposome encapsulated daunoblastin in murine tumor models. *Neoplasma* 1981;28:141–149.
127. Kimelberg HK, Atchison ML. Effects of entrapment of liposomes on the distribution, degradation and effectiveness of methotrexate on its chemotherapeutic efficacy in solid rodent tumors. *Ann NY Acad Sci* 1978;308:395–409.

128. Kosloski MJ, Rosen F, Miliholland RJ, Papahadjopoulos D. Effect of lipid vesicle (liposome) encapsulation of methotrexate on its chemotherapeutic efficacy in solid rodent tumors. *Cancer Res* 1978;38:2848–2853.
129. Weinstein JN, Magin RL, Cysyk RL, Zaharko DS. Treatment of solid L1210 murine tumors with local hyperthermia and temperature sensitive liposomes containing methotrexate. *Cancer Res* 1980;40:1388–1395.
130. Patel KR, Jonah MM, Rahman YE. In vitro uptake and therapeutic application of liposome-encapsulated methotrexate in mouse hepatoma. *Eur J Cancer Clin Oncol* 1982;18:833–843.
131. Forssen EA, Proffitt RT. Design and development of long circulating liposomal daunorubicin for in vivo targeting of solid tumors: DaunoXome®. In: Woodle M, Storm G. eds. *Long Circulating Liposomes: Old Drugs, New Therapeutics*. New York, NY, Springer-Verlag and Landes Bioscience, 1998, pp. 74–96.
132. Forssen EA, Coulter DM, Proffitt RT. Selective in vivo localization of daunorubicin small unilamellar vesicles in solid tumors. *Cancer Res* 1992;52:3255–3261.
133. Forssen EA, Ross ME. DaunoXome treatment of solid tumors: preclinical and clinical investigations. *J Liposome Res* 1994;4:481–512.
134. Money-Kyrle JF, Bates F, Ready J, et al. Liposomal daunorubicin in advanced Kaposi's sarcoma: a phase II study. *Clin Oncol* 1993;5:367–371.
135. Presant CA, Scolaro M, Kennedy P, et al. Liposomal daunorubicin treatment of HIV-associated Kaposi's sarcoma. *Lancet* 1993;341:1242–1243.
136. Gill PS, Wernz J, Scadden DT, et al. Randomized phase III trial of liposomal daunorubicin (DaunoXome) versus doxorubicin, bleomycin, vincristine, (ABV) in AIDS-related Kaposi's sarcoma. *J Clin Oncol* 1996;14:2353–2364.
137. Campbell R, Balasubramanian SV, Straubinger RM. Influence of cationic lipids on the stability and membrane properties of paclitaxel-containing liposomes. *J Pharm Sci* 2001;90:1091–1105.
138. Thierry AR, Rahman R, Dritschilo A. A new procedure for the preparation of liposomal doxorubicin: biological activity in multidrug-resistant tumor cells. *Cancer Chemother Pharmacol* 1994;35:84–88.
139. Gokhale PC, Radhakrishnan B, Husain SR, et al. An improved method of encapsulation of doxorubicin in liposomes: pharmacological, toxicological and therapeutic evaluation. *Br J Cancer* 1996;74:43–48.
140. Freeman AI, Mayhew E. Targeted drug delivery. *Cancer* 1986;58:573–583.
141. Rustum Y, Dave C, Mayhew E, Papahadjopoulos D. Anti-tumor effects of liposome-entrapped cytosine arabinoside against mouse L1210 leukemia: Role of liposome type and route of administration. *Cancer Res* 1979;39:1390–1395.
142. Gabizon A. Liposome circulation time and tumor targeting: implications for cancer chemotherapy. *Adv Drug Deliv Rev* 1995;16:285–294.
143. Dass CR, Walker TL, Burton MA, Decruz EE. Enhanced anticancer therapy mediated by specialized liposomes. *J Pharm Pharmacol* 1997;49:972–975.
144. Furgeson DY, Cohen RN, Mahato RL, Kim SW. Novel water insoluble lipoparticulates for gene delivery. *Pharm Res* 2002;19:382–390.
145. Anwer K, Kao G, Proctor B, Rolland A, Sullivan S. Optimization of cationic lipid/DNA complexes for systemic gene transfer to tumor lesions. *J Drug Target* 2000;8:125–135.
146. Meyer O, Kirpotin D, Hong K, et al. Cationic liposomes coated with polyethylene glycol as carriers for oligonucleotides. *J Biol Chem* 1998;273:15621–15627.
147. Birchall JC, Kellaway IW, Millis SN. Physico-chemical characterization and transfection efficiency of lipid-based gene delivery complexes. *Int J Pharm* 1999;183:195–207.
148. Whitmore M, Li S, Huang L. LPD lipoplex initiates a potent cytokine response and inhibits tumor growth. *Gene Ther* 1999;6:1867–1875.
149. Reimer DL, Kong S, Monck M, et al. Liposomal lipid and plasmid DNA delivery to B16/BL6 tumors after intraperitoneal administration of cationic liposome DNA aggregates. *J Pharmacol Exp Ther* 1999;289(2):807–815.
150. Dass CR. Biochemical and biophysical characteristics of lipoplexes pertinent to solid tumor gene therapy. *Int J Pharm* 2002;241:1–25.

151. Allen TM. Stealth<sup>TM</sup> liposomes: Avoiding reticuloendothelial uptake in liposomes. In: Lopez Berestain G, Fidler IJ, eds. *Liposomes in the Therapy of Infectious Diseases and Cancer*. New York, NY, Allen R. Liss, 1989, pp. 405–441.
152. Papahadjopoulos D, Allen TM, Gabizon A, et al. Sterically stabilized liposomes-improvements in pharmacokinetics and antitumor therapeutic efficacy. *Proc Natl Acad Sci USA* 1991;88:11460.
153. Blume G, Ceven G. Liposomes for the sustained release in vivo. *Biochim Biophys Acta* 1990;1029:91.
154. Torchilin VP, Papisov MI. Why do polyethylene glycol-coated liposomes circulate so long? *J Liposome Res* 1994;4:725–739.
155. Torchilin VP. How do polymers prolong circulation time of liposomes. *J Liposome Res* 1996;6:99–116.
156. Torchilin VP, Omelyanenko V, Papisov MI, et al. Poly(ethylene glycol) on the liposome surface: on the mechanism of polymer-coated liposomes longevity. *Biochem et Biophys Acta* 1994;1195:11–20.
157. Pang SNJ. Final report on the safety assessment of polyethylene glycols (PEGs)-6, -8, -32, -75, -150, -14M, -20M. *J. Am College Toxicol* 1992;12:429–456.
158. Yamaoka T, Tabata T, Ikada Y. Distribution and tissue uptake of poly(ethylene glycol) with different molecular weights after intravenous administration in mice. *J Pharm Sci* 1994;83:601–606.
159. Zalipsky S. Chemistry of polyethylene conjugates with biologically active molecules. *Adv Drug Deliv Rev* 1995;16:157–182.
160. Klibanov AI, Maruyama A, Torchilin VP, Huang L. Amphipathic polyethylene glycols effectively prolong the circulation time of liposomes. *FEBS Lett* 1990;268:235–237.
161. Torchilin VP, Shtilman M, Trubetskoy VS, et al. Amphiphilic vinyl polymers effectively prolong liposome circulation time in vivo. *Biochem Biophys Acta* 1994;1195:181–184.
162. Ning ZW, Daphane D, Rudolph TL, et al. Increased microvascular permeability contributes to preferential accumulation of stealth liposomes in tumor tissue. *Cancer Res* 1993;53:3765–3770.
163. Zhou XJ, Rahman R. Preclinical and clinical pharmacology of vinca alkaloids. *Drugs* 1992;44:1.
164. Layton D, Trouet A. A comparison of the therapeutic effect of free and liposomally encapsulated vincristine in leukemic mice. *Eur J Cancer* 1980;16:945.
165. Allen TM, Newman MS, Woodle M, et al. Pharmacokinetics and antitumor activity of vincristine encapsulated in sterically stabilized liposomes. *Int J Cancer* 1995;62:199–204.
166. Mayer LD, Bally MB, Laughery H, et al. Liposomal vincristine preparations which exhibit decreased drug toxicity and increased activity against murine L1210 leukemia. *Cancer Res* 1990;50:575.
167. Vaage J, Donovan D, Mayhew E, et al. Therapy of mouse mammary carcinomas with vincristine and doxorubicin encapsulated in sterically stabilized liposomes. *Int J Cancer* 1993;54:959.
168. Ceh B, Winterhalter M, Fredrik P, et al. stealth liposomes: from theory to product. *Adv Drug Deliv Rev* 1997;24:165–177.
169. Vaage J, Donovan D, Mayhew E. Therapy of human ovarian carcinoma xenografts using doxorubicin encapsulated in sterically stabilized liposomes. *Cancer* 1993;72:3671–3675.
170. Vaage J, Donovan D, Uster P. Tumor uptake of doxorubicin in polyethylene glycol-coated liposomes and therapeutic effect against a xenografted human pancreatic carcinoma. *Br J Cancer* 1997;75:482–486.
171. Vaage J, Barbera-Guillem E, Abra R. Tissue distribution and therapeutic effect of intravenous free or encapsulated liposomal doxorubicin on human prostate carcinoma xenografts. *Cancer* 1994;3:1478–1484.
172. Northfelt DW, Dezube BJ, Thommes JA. PEGylated-liposomal doxorubicin versus doxorubicin, bleomycin and vincristine in the treatment of AIDS-related Kaposi's sarcoma: results of a randomized phase III clinical trial. *J Clin Oncol* 1998;16:2445–2451.
173. Lyass O, Uziely B, Ben-Yosef R. Correlation of toxicity with pharmacokinetics of PEGylated liposomal doxorubicin (Doxil) in metastatic breast carcinoma. *Cancer* 2000;89(5):1037–1047.

174. Working P. Preclinical studies of lipid complexes and liposomal drugs. AMPHOTECTM DOXILTM and SPI-077. In: Lasic DD, Papahadjopoulos D, eds. *Medical Applications of Liposomes*. Amsterdam, Elsevier, 1998, pp. 605–624.
175. Newman MS, Colbem GT, Working P, et al. Comparative pharmacokinetics, tissue distribution and therapeutic efficiency of cisplatin encapsulated in long circulating pegylated liposomes (SPI-077) in tumor bearing mice. *Cancer Chemother Pharmacol* 1999;46:155–165.
176. Maruyama K. Enhancement of doxorubicin by encapsulating in long circulating thermosensitive liposomes combined with local hyperthermia. In: Woodle MD, Storm G, eds. *Long Circulating Liposomes: Old Drugs, New Therapeutics*. New York, Springer-Verlag and Landes Bioscience, 1998; pp. 97–109.
177. Slepishkin VA, Simoes S, Dazin P, et al. Sterically stabilized pH-sensitive liposomes. Intracellular delivery of aqueous contents and prolonged circulation in vivo. *J Biol Chem* 1996;272:2382–2388.
178. Baillie AJ, Florence AT, Hume LR, et al. The preparation and properties of niosomes-non-ionic surfactant vesicles. *J Pharm Pharmacol* 1985;37(12):863–868.
179. Uchegbu IF, Double JA, Turton JA, Florence AT. Distribution, metabolism and tumoricidal activity of doxorubicin administered in sorbitan monostearate (Span 60) niosomes in the mouse. *Pharm Res* 1995;12(7):1019–1024.
180. Parthasarathi G, Udupa N, Umadevi P, Pillai GK. Niosome encapsulated Vincristine sulfate: improved anticancer activity with reduced toxicity in mice. *J Drug Target* 1994;2:173–182.
181. Hao Y, Zhao F, Li N, et al. Studies on high encapsulation of colchicine by a niosome system. *Int J Pharm* 2002;244:73–80.
182. Torchilin VP. Structure and design of polymeric surfactant-based drug delivery systems. *J Control Release* 2001;73:137–172.
183. Yokoyama M, Miyauchi M, Yamada N, et al. Polymer micelles as novel drug carrier: Adriamycin-conjugated poly(ethylene glycol)-poly(aspartic acid) block copolymer. *J Control Release* 1990;11:269–278.
184. Rapoport N, Herron JN, Pitt WG, Pitina L. Micellar delivery of doxorubicin and its paramagnetic analogue ruboxyl, to HL-60 cells: effect of micelle structure and ultrasound on the intracellular drug uptake. *J Control Release* 1999;58:153–162.
185. Winnik FM, Davidson AR, Hamer GK, Kitano H. Amphiphilic poly(N-isopropylacrylamide) prepared by using a lipophilic radical initiator: synthesis and solution properties in water. *Macromolecules* 1992;25:1876–1880.
186. Kwon GS, Yokoyama M, Okano T, et al. Biodistribution of micelle-forming polymer-drug conjugates. *Pharm Res* 1993;10:970–974.
187. Cortesi R, Esposito E, Maietti A, et al. Formulation study for the antitumor drug camptothecin: liposomes, micellar solutions and microemulsion. *Int J Pharm* 1997;159:95–105.
188. Singla AK, Garg A, Aggarwal D. Paclitaxel and its formulations. *Int J Pharm* 2002;235:179–192.
189. Kataoka K, Harada A, Nagasaki Y. Block copolymer micelles for drug delivery: design, characterization and biological significance. *Adv Drug Deliv Rev* 2001;47(1):113–131.
190. Yokoyama M, Fukushima S, Uchida R, et al. 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* 1998;50:79–92.
191. Yokoyama M, Miyauchi M, Yamada N, et al. Characterization and anticancer activity of the micelle forming polymeric anticancer drug adriamycin-conjugated poly(ethylene glycol)-poly(aspartic acid) block copolymer. *Cancer Res* 1990;50:1693–1700.
192. Yokoyama M, Satoh A, Sakurai Y, et al. Incorporation of water-insoluble anticancer drug into polymeric micelles and control of their particle size. *J Control Release* 1998;55:219–229.
193. Yokoyama M, Okano T, Sakurai Y, et al. Toxicity and antitumor activity against solid tumors of micelle-forming polymeric anticancer drug and its extremely long circulation in blood. *Cancer Res* 1991;51:3229–3236.
194. Nishiyama N, Kataoka K. Preparation and characterization of size-controlled polymeric micelle containing cis-dichlorodiammineplatinum (II) in the core. *J Control Release* 2001;74:83–94.

195. Mizumura Y, Matsumura Y, Hamaguchi T, et al. Cisplatin incorporated polymeric micelles eliminate nephrotoxicity, while maintaining antitumor activity. *Jpn J Cancer Res* 2001;92(3):328–336.
196. Nakanishi T, Fukushima S, Okamoto K, et al. Development of polymer micelle carrier system for doxorubicin. *J Control Release* 2001;74:295–302.
197. Yokoyama M, Okano T, Sakurai Y, Kataoka K. 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* 1994;32:269–277.
198. Kataoka K, Kwon GS, Yokoyama M, et al. Block copolymer micelles as vehicles for drug delivery. *J Control Release* 1993;24:119–132.
199. Kataoka K, Matsumoto T, Yokoyama M, et al. Doxorubicin-loaded poly(ethylene glycol)-poly(beta-benzyl-L-aspartate) copolymer micelles: their pharmaceutical characteristics and biological significance. *J Control Release* 2000;64:143–153.
200. Kwon GS, Naito M, Yokoyama M, et al. Block copolymer micelles for drug delivery: loading and release of doxorubicin. *J Control Release* 1997;48:195–201.
201. Kwon GS, Naito M, Yokoyama M, et al. Physical entrapment of adriamycin in AB block copolymer micelles. *Pharm Res* 1995;12(2):192–195.
202. Kwon GS, Naito M, Yokoyama M, et al. Micelles based on AB block copolymers of poly(ethylene oxide) and poly(beta-benzyl L-aspartate). *Langmuir* 1993;9:945–949.
203. Kabanov AV, Batrakova EV, Melik-Nubarov NS, et al. A new class of drug carriers: micelles of poly(oxyethylene)-poly(oxypropylene) block copolymers as micro containers for drug targeting from blood in brain. *J Control Release* 1992;22:141–158.
204. Venn A, Li S, Mandeville R, et al. Hyper sensitizing effect of pluronic L61 on cytotoxic activity, transport and subcellular distribution of doxorubicin in multi-drug resistant cells. *Cancer Res* 1996;56:3626–3629.
205. Rapoport N, Munshi N, Pitina L, Pitt WG. Pluronic micelles as vehicles for tumor-specific delivery of two anticancer drugs to HL-60 cells using acoustic activation. *Polymer Reprints* 1997;38:620–621.
206. Kabanov AV, Batrakova EV, Alakhov VY. Pluronic block copolymers for overcoming drug resistance in cancer. *Adv Drug Deliv Rev* 2002;54(5):759–779.
207. Kabanov AV, Batrakova EV, Alakhov VY. Pluronic block copolymers as novel polymer therapeutics for drug and gene delivery. *J Control Release* 2002;82:189–212.
208. Batrakova EV, Dorodnych TY, Klinskii EY, et al. Anthracycline antibiotics non-covalently incorporated into the block copolymer micelles: *in vivo* evaluation of anti-cancer activity. *Br J Cancer* 1996;74:1545–1552.
209. Yoo HS, Park TG. Biodegradable polymeric micelles composed of doxorubicin conjugated PLGA-PEG block copolymer. *J Control Release* 2001;70(1–2):63–70.
210. Zhang X, Jackson JK, Burt HM. Development of amphiphilic diblock copolymers as micellar carriers of taxol. *Int J Pharm* 1996;132:195–206.
211. Kim SC, Kim DW, Shim YH, et al. *In vivo* evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy. *J Control Release* 2001;72:191–202.
212. Zhang X, Burt HM, Von Hoff D, et al. An investigation of the antitumor activity and biodistribution of polymeric micellar paclitaxel. *Cancer Chemother Pharmacol* 1997;40:81–86.
213. Jeong YI, Nah JW, Lee HC, et al. Adriamycin release from flower-type polymeric micelle based on star-block copolymer composed of poly(gamma-benzyl L-glutamate) as the hydrophobic part and poly(ethylene oxide) as the hydrophilic part. *Int J Pharm* 1999;188:49–58.
214. Gao Z, Lukyanov AN, Singhal A, Torchilin VP. Diacyllipid-polymer micelles as nanocarriers for poorly soluble anticancer drugs. *Nano Lett* 2002;2(9):979–982.
215. Trubetskoy VS, Torchilin VP. Use of polyoxyethylene-lipid conjugates as long circulating carriers for delivery of therapeutic and diagnostic agents. *Adv Drug Deliv Rev* 1995;16:311–320.
216. Weissig V, Whiteman KR, Torchilin VP. Accumulation of protein-loaded long-circulating micelles and liposomes in subcutaneous Lewis lung carcinoma in mice. *Pharm Res* 1998;15:1552–1556.

217. Lukyanov AN, Gao Z, Mazzola L, Torchilin VP. Polyethylene glycol-diacyllipid micelles demonstrate increased accumulation in subcutaneous tumors in mice. *Pharm Res* 2002;19:1424–1429.
218. Kohori F, Sakai K, Aoyagi T, et al. Control of adriamycin cytotoxic activity using thermally responsive polymeric micelles composed of poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide)-b-poly(D,L-lactide). *Colloids Surfaces B: Biointerfaces* 1999;16:195–205.
219. Cammas S, Suzuki K, Sone Y, et al. Thermo-responsive polymer nanoparticles with a core shell micelle structure as site-specific drug carriers. *J Control Release* 1997;48:157–164.
220. Chung JE, Yokoyama M, Yamato M, et al. Thermo-responsive drug delivery from polymeric micelles constructed using block copolymers of poly(N-isopropylacrylamide) and poly-(butylmethacrylate). *J Control Release* 1999;62:115–127.
221. Kohori F, Yokoyama M, Sakai K, Okano T. Process design for efficient and controlled drug incorporation into polymeric micelle carrier systems. *J Control Release* 2002;78:155–163.
222. Chung JE, Yokoyama M, Suzuki K, et al. Reversibly thermo-responsive alkyl terminated poly(N-isopropylacrylamide) core shell micellar structures. *Colloids Surfaces B: Biointerfaces* 1997;9:37–48.
223. Miwa A, Ishibe A, Nakano M, et al. Development of novel chitosan derivatives as micellar carriers of taxol. *Pharm Res* 1998;15:1844–1850.