Amongst various cancer therapies, chemotherapy is one of the major treatment modalities along with debulking surgery (Gonzalez-Angulo et al. 2007; Matei 2007). However, cancer chemotherapy is often complicated by toxic side effects of anticancer drugs. Despite advances in the development of new anti-tumor drugs, these drugs still cause significant serious side effects (Lee et al. 2008).

However, the main problem of cancer therapy today is that the treatment is unable to focus on the tumor tissue only. While we are dealing with the tumor tissue, we are also killing the normal tissue, especially the cells in good condition (for instance marrow cells or endothelial cells of small intestine), thereby causing serious side effects. Hence, the ideal anticancer drugs may have the specificity that can get right into the tumor tissue. Also, some factors like unstable environment and high dose of drugs restrict the use of anticancer drugs. Nanoparticles can be modified or combined with some special materials to achieve the action of targeting.

Nanotechnology is an emerging branch of science dealing with cancer (Sandhiya et al. 2009). The nanoparticles used in drug delivery include liposomes, polymers, micelles, dendrimers, quantum dots, nanoshells, gold nanoparticles, paramagnetic nanoparticles, and carbon nanotubes (Mitra et al. 2006). Cancer cells have certain receptors on the surface, which can be made use of in targeting delivery of nanoparticles by attaching a monoclonal antibody or cell surface receptor ligand (Hobbs et al. 1998). Tumors are distinct from normal cells as their endothelial cells possess wide fenestrations ranging from 200 nm to 1.2 μm. The large pore size allows passage of nanoparticles into the extravascular spaces (Rawat et al. 2006). As shown in Figure 14.1, normal blood vessel is smooth and regularly growing (A–B), but the tumor blood vessels are in twists and turns and irregular (C through E). The outside of the tumor blood vessel is rough and has many indefinite pores (F–G). Hashizume et al. (2000) found that the pore size of the blood vessel is different in different kinds of tumors. Nanoparticles easily leak.
into these deviant structures of tumor blood vessels. Most nanoparticles could passively accumulated in the tumor tissue and avoid entering into normal tissues, thereby significantly reducing the side effects and targeting the tumor. Triggered release of a drug at tumor sites while maintaining a minimal release rate during circulation may result in a very desirable property in carrier design for tumor chemotherapy (Gao et al. 2005).

We developed some modalities of tumor chemotherapy aimed at circumventing side effects of the treatment via drug targeting to tumors. In this chapter, we introduce ultrasound-enhanced tumor-targeting nanoparticles and tumor pH targeting nanotechnology.

### 14.2 Ultrasound-Triggered Drug Targeting to Tumor

Present-day chemotherapy is associated with severe side effects caused by the drug-induced action on healthy tissues. In addition, in the process of chemotherapy, cells often become resistant to active chemotherapeutic agents (Rapoport et al. 2009). In the course of our previous work, we have made significant progress in site-specific tumor chemotherapy by ultrasound activated drug-loaded polymeric micelles. The micelles passively accumulate in the tumor interstitium presumably via the enhanced penetration and retention effect (EPR) (Gao et al. 2004). The biodistribution of micelle-encapsulated drug closely followed that of micelles, with a significant degree of tumor targeting (Gao et al. 2005). The use of nanomedicine methods opens new lines of attack on the problem of systemic toxicity of anticancer drugs.

#### 14.2.1 Ultrasound in Drug Delivery

Ultrasound is a type of transmission of pressure waves; its frequencies are above human hearing or above 20,000 Hz. These ultrasonic waves can be reflected, refracted (bent), focused, and absorbed. They are actual movement of molecules when the medium is compressed at high pressure and expanded at

---

**FIGURE 14.1** (See color insert.) Normal blood vessel (A–B); tumor blood vessel (C–E); the pore in the tumor blood vessel wall (F); Hematoxylin and Eosin (HE) staining of pores in tumor blood vessel wall (G).
low pressure, and thus, ultrasound can act physically upon biomolecules and cells. Ultrasonic waves are absorbed relatively little by water, flesh, and other tissues. Therefore, ultrasound can “see” into the body (e.g., diagnostic ultrasound) and can be used to transmit energy into the body at precise locations. This safe, noninvasive, and painless transmission of energy into the body is the key to ultrasonic-activated drug delivery (Pitt et al. 2004). Clinical ultrasound units being manufactured typically deliver ultrasound frequencies of 1 and 3 MHz with duty cycles ranging from 20% to 100%. Duty cycles less than 100% are usually termed pulsed ultrasound, while a 100% duty cycle is referred to as continuous ultrasound. In medicine, ultrasound is used as either a diagnostic or a therapeutic modality. The main advantage of ultrasound is its noninvasive nature; the transducer is placed in contact with a water-based gel or water layer on the skin, and no insertion or surgery is required (Rapoport 2006).

All energy-based tumor treatment modalities, including thermal treatments, require prior imaging. The field of thermal tumor therapy has advanced rapidly, particularly for high-intensity focused ultrasound (HIFU), which causes tumor ablation by coagulative necrosis of tumor tissue. Some other clinical applications of thermal therapy such as adjuvant hyperthermia with radiation therapy, drug and gene delivery, or coagulation of tumor blood vessels can be envisioned (Haar and Coussios 2007). For most systems, magnetic resonance imaging (MRI) remains the imaging modality of choice due to the possibility of combining tumor imaging with MRI thermometry, which would eventually allow development of feedback treatment control systems. However, the possibility for combining diagnostic and therapeutic ultrasound for tumor imaging and treatment appears more attractive, for reasons of simplicity, time-, and cost-effectiveness. Dual-modality ultrasound-imaging-treatment systems are expected to allow precisely controlled, ultrasound-enhanced tumor chemotherapy in a clinical environment. However, ablative techniques have a number of problems related to the precise control of heat deposition. Patient motion and breathing during the treatment are also problematic. Currently, long treatment time, incomplete treatment of large targets, a nonuniform thermal dose distribution, and unintended normal tissue damage continue to impede broad penetration of HIFU therapies into clinical practice. There is a therapeutic technique that utilizes nonthermal mechanisms of ultrasound interaction for targeted drug delivery and tumor imaging. This technique may rely on the same instruments that are used for HIFU, but driven at substantially lower ultrasound energies. In addition, thermal treatment and ultrasound-mediated drug delivery may complement each other because drug delivery works well in the highly perfused regions of a tumor (generally the periphery), while HIFU can destroy a poorly perfused tumor core. Again, tumor imaging prior to treatment is a crucial element of the proposed technique. Contrast-enhanced ultrasonic tumor imaging is still in its infancy. Development of ultrasound contrast agents and the concomitant use of harmonic ultrasound imaging have made possible imaging of liver (Wilson et al. 2000), breast (Kedar et al. 1996), and prostate tumors (Halpern et al. 2001). Also, ultrasound-stimulated acoustic emission of microbubbles has been used for color Doppler imaging of liver metastases (Marcil and Goulet 2002). Polymeric ultrasound contrast agents with targeting potential have been explored by the Wheatley group (El-Sherif et al. 2004; Lathia et al. 2004). Molecularly targeted microbubbles have been successfully used for the ultrasonic imaging of angiogenesis (Takalkar et al. 2004; Rychak et al. 2006). Bubble targeting using ultrasound radiation force has also been developed (Dayton and Ferrara 2002; Bloch et al. 2004, 2005). Combining contrast-enhanced ultrasound tumor imaging with targeted drug delivery is a challenging task. We have developed novel ultrasound-sensitive multifunctional nanoparticles composed of nanoscale polymeric micelles that function as drug carriers and nano- or microscale echogenic bubbles that combine the properties of drug carriers, enhancers of the ultrasound-mediated drug delivery, and long-lasting ultrasound contrast agents (Rapoport et al. 2007). Drug carrying, tumor targeting, and retention in the tumor volume are functions of the micelles and/or nanobubbles. Ultrasound contrast properties are provided by the microbubbles formed in a tumor volume by the coalescence of nanobubbles. The technology of ultrasound requires order of magnitude lower ultrasound energy, reducing the risk of coagulative necrosis of healthy tissues associated with HIFU.
14.2.2 Polymeric Micelles for Combining Ultrasonic Tumor Imaging

Polymeric micelles are nanoparticles formed by self-assembly of amphiphilic block copolymers; they have a size between 10 and 100 nm and core-shell structure; they are most commonly spherical in shape (Allen et al. 1999). The polymeric micelles are formed by hydrophobic–hydrophilic block copolymers at concentrations that are above their critical micelle concentration (CMC). Below the CMC, block copolymer molecules exist in solution in the form of individual molecules (unimers) or their loose, water-penetrated aggregates. At concentrations above the CMC, copolymer molecules self-assemble into dense micelles with hydrophobic cores and a hydrophilic corona. The size of micelles (~10–50 nm) and their surface properties provide for a high drug-loading capacity and long circulation time in the vascular system, which makes them attractive drug carriers (Yu et al. 1998; Rappoport et al. 2004; Rapoport 2006). Ultrasonic irradiation of the tumor triggered drug release from micelles and transiently altered cell membrane permeability, resulting in effective intracellular drug uptake by the tumor cells (Rapoport et al. 2007). The method of drug targeting to tumors is based on drug encapsulation within polymeric micelles followed by localized triggering of drug release and intracellular uptake induced by ultrasound focused at the tumor site. A rationale behind this approach is that drug encapsulation in micelles decreases the systemic concentration of free drug, diminishes intracellular drug uptake by normal cells, and provides for passive drug targeting to the tumor via the EPR effect. Drugs are encapsulated in nanoparticles (Figure 14.2) that cannot penetrate blood vessels of healthy tissues; however, they penetrate through inter-endothelial gaps and accumulate in a tumor. Liposomes, micelles, nanoemulsions, and nanobubbles are examples of such nanocarriers of drugs. The dosage forms shown in Figure 14.2 comprise polymeric micelles (small circles), nanodroplets (stars), and microbubbles (large circles); the micelles are formed by biodegradable block copolymers (e.g., poly(ethylene glycol)-block-poly(l-lactide) (PEG-PLLA) or poly(ethylene glycol)-block-poly(caprolactone) (PEG-PCL)); the droplets and bubbles are formed by perfluorocarbon (e.g., perfluoropentane (PFP) and are stabilized by the same (or another) block copolymer (Rapoport et al. 2009). Drug targeting to the tumors reduces unwanted drug interactions with healthy tissues. Upon accumulation of

FIGURE 14.2 (See color insert.) Schematic representation of a drug targeting through the defective tumor microvasculature using an echogenic drug delivery system. The system comprises polymeric micelles (small circles), nanobubbles (stars), and microbubbles (large circles). Micelles are formed by a biodegradable block copolymer (e.g., PEG-PLLA or PEG-PCL); bubbles are formed by perfluorocarbon (e.g., PFP) stabilized by the same biodegradable block copolymer. Lipophilic drug (e.g., doxorubicin) is localized in the micelle cores and the walls of nano/microbubbles. Tight junctions between endothelial cells in blood vessels of normal tissues do not allow extravasation of drug-loaded micelles or nano/microbubbles (indicated by cross). In contrast, tumors are characterized by defective vasculature with large gaps between the endothelial cells, which allow extravasation of drug-loaded micelles and small nanobubbles, resulting in their accumulation in the tumor interstitium. On accumulation in the tumor tissue, small nanobubbles coalesce into larger, highly echogenic microbubbles that release their drug load in response to therapeutic ultrasound. (From Rapoport, N. et al., J. Natl. Cancer Inst., 99, 1095, 2007; Gao, Z. et al., Ultrasonics, 48, 260, 2008.)
micelles in the tumor interstitium, effective intracellular drug uptake by the tumor cells can be induced by using ultrasonic irradiation. In vitro, ultrasound triggered drug release from micelles substantially enhanced intracellular uptake of both released and encapsulated drug. This was observed for various cell lines suggesting that the ultrasound effect on intracellular drug uptake was a general phenomenon.

A new class of multifunctional nanoparticles that combine properties of polymeric drug carriers, ultrasound imaging contrast agents, and enhancers of ultrasound-mediated drug delivery has been developed. At room temperature, the developed systems comprise perfluorocarbon nanodroplets stabilized by the walls made of biodegradable block copolymers. Upon heating to physiological temperatures, the nanodroplets convert into nano/microbubbles. The phase state of the systems and bubble size may be controlled by the copolymer/perfluorocarbon volume ratio. Upon intravenous injections, a long-lasting, strong, and selective ultrasound contrast is observed in the tumor volume indicating nanobubble extravasation through the defective tumor microvasculature, suggesting their coalescence into larger, highly echogenic microbubbles in the tumor tissue. Under the action of tumor-directed ultrasound, microbubbles cavitate and collapse resulting in a release of the encapsulated drug and dramatically enhanced intracellular drug uptake by the tumor cells. This effect is tumor selective; no accumulation of echogenic microbubbles is observed in other organs (Gao et al. 2008).

In our previous work, we developed a kind of nanoparticles which was made from PLLA-PEG. The different molecular weight of co-polymers PLLA-PEG were synthesized for engineering these nanoparticles. Nanoparticles with the size less than 1 μm were encapsulated with anticancer drugs and PFP. Because PFP’s boiling point was 29°C, the nanoparticles were stable at room temperature, but, when the temperature came up to the human normal physiological temperature (37°C), PFP evaporated into a gaseous form and the nanoparticles became nano/microbubbles. When we injected these nanoparticles into mice, after irradiation with ultrasound imaging to observe the tumor tissues, the nanoparticles were found to have strong, long-lasting imaging of microbubbles. When we used 1–3 MHz ultrasonic to irradiate the tumor tissue, the bubbles were cavitated and released drugs; in the meanwhile, the cytomembrane was pierced and could enhance drug release, killing the tumor cells effectively (Figure 14.3).

14.2.3 Evaluation of Ultrasound-Triggered Nano/Microbubbles’ Tumor-Targeting Modality

14.2.3.1 Nanodroplet/Microbubble Conversion upon Heating to Physiological Temperature

Upon heating to physiological temperatures, nanodroplets are converted into larger nanobubbles due to the vaporization of PFP inside the bubble walls (Figure 14.4). The initial nanodroplets or nanobubbles were not resolved at the 100× magnification that was the maximum available for our fluorescence microscopy system (Figure 14.5A). In addition to reversible nanodroplet/nanobubble conversion,
FIGURE 14.4  Nanodroplet/microbubble conversion on heating of the 1% PEG-PLLA/0.5% PFP system to 42°C for 5 min; the nanodroplets (691 nm) were converted into microbubbles (1.24 μm). The effect was reversible; upon cooling to room temperature, the initial size distribution shown in panel (A) was restored. (B) the particle size distribution was changed after heating to 42°C. (From Gao, Z. et al., Ultrasonics, 48, 260, 2008.)

FIGURE 14.5 Optical images of a 0.75 mg/mL Dox/0.5% PEG-PLLA/2% PFP formulation placed in a closed plastic capillary (internal diameter 340 mm) of a snake mixer slide (XXS). (A) At 26°C, nanodroplets of the initial 0.5% PEG-PLLA/2% PFP formulation were not resolved at the highest available magnification (×100). Upon heating to 37°C (B) and 50°C (C), larger bubbles grew by attraction and coalescence of small bubbles. (D) After cooling back to room temperature, the initial sample structure was not restored; a large number of small microdroplets were formed through disintegration of large microbubbles and PFP condensation. (E and F) During a second heating step to 37°C and 50°C, respectively, the growth of large microbubbles through the attraction and coalescence with small microbubbles was manifested by a progressive decrease in the number of small microbubbles. (From Gao, Z. et al., Ultrasonics, 48, 260, 2008.)
a small number of microbubbles were formed irreversibly at 37°C through coalescence of nanobubbles (Figure 14.5B). Sample overheating to hyperthermia temperatures (44°C or higher) resulted in a significant coalescence of the nanobubbles into larger microbubbles whose size and number increased with time (Figure 14.5C); this effect was irreversible, as shown in the sequence of photographs presented in Figure 14.5C through F. Large microbubbles, formed under hyperthermic conditions may potentially be useful for localized occlusion of tumor blood vessels.

### 14.2.3.2 Drug Release in the Cell

At room temperature, the nanoparticles in the formulation were not resolved by fluorescence microscopy, when heated to 46°C, the drug (doxorubicin, Dox) was localized in the bubble wall formed by the stabilizing copolymer, as indicated by fluorescence imaging (Figure 14.6A). When bubbles were incubated with cells at 37°C without the application of ultrasound, neither decrease in bubble fluorescence nor increase in cell fluorescence was observed, thus indicating strong drug retention by the microbubble wall. The application of ultrasound resulted in a decrease in bubble fluorescence and increase in cell fluorescence (Figure 14.6B through D), indicating drug transfer from the bubbles to the cells (Gao et al. 2008).

### 14.2.3.3 Nano/Microbubbles as Enhancers of Ultrasonic Drug Delivery

For a wide variety of tumor samples and ultrasound parameters, the ultrasound-mediated intracellular drug uptake was substantially enhanced in the presence of microbubbles compared to that of micelles; the effect of the microbubbles was statistically significant. A typical example is shown in Figure 14.7 (Rapoport et al. 2007).

### 14.2.3.4 Nano/Microbubbles as Ultrasound Contrast Agents

Intratumoral injections of nanobubbles produced long-lasting hyperechoic “dots” in the ultrasound scans of the MDA MB231 breast cancer or A2780 ovarian carcinoma tumors grown subcutaneously in nu/nu mice (Figure 14.8). The generation of hyperechoic sites in the tumors was also observed.
after the intravenous injections of the nanobubbles (Figure 14.9). Because the functional cutoff size of the endothelial gaps in the tumor capillaries does not exceed 1 μm (Campbell 2006), the observation of echoes in the tumors of intravenously injected mice suggested that nanobubbles were extravasated into the tumor tissue. Because high echogenicity could not be expected from the nanoscaled bubbles at 14 MHz, the observation of the echoes shown in Figure 14.9 suggested that the injected nanobubbles coalesced into highly echogenic microbubbles inside the tumor tissue.
No hyperechoic entities were observed in the kidney or liver of intravenously injected mice suggesting that the nanobubble extravasation was tumor selective.

### 14.2.4 Conclusions

Multifunctional nano/microbubble formulations have been developed for combining ultrasonic tumor imaging and ultrasound-enhanced chemotherapeutic treatment. The polymeric copolymer-stabilized PFP nanoemulsion systems undergo nanodroplet/microbubble conversion in vivo, accumulate locally in tumor tissue and coalesce into larger, highly echogenic microbubbles, which provide for long-lasting ultrasound contrast in the tumor and allow on-demand release of the encapsulated drug under the action of therapeutic ultrasound. This technique offers prospects for treating multidrug-resistant tumors that fail conventional chemotherapy, are expected to have good forehand in cancer treatment.

### 14.3 pH-Sensitive Polymeric Micelle Targeting to Tumor

#### 14.3.1 Acid Tumor pH

The difference in pH between solid tumors and normal tissue properties has been long recognized (Lee and Bae 2006). The pH of normal tissues and blood is around pH 7.4, and the intracellular pH of normal cells is around pH 7.2. However, in most tumor cells the pH gradient is reversed, the intracellular pH is higher than the extracellular pH, and the pH outside most tumors is about 7.06 (Tannock and Rotin 1989; Engin et al. 1995; Hobbs et al. 1998; Stubbs et al. 2000). As shown in Figure 14.10, when we determined tumor pH using $^1$H, $^{19}$F, $^{31}$P after implanting human cancer cells into mice, the results showed that the average tumor pH was 6.84. The main reason is that cancer cells absorb and metabolize protein much faster than normal cells. During the same time, through protein metabolism, cancer cells can produce 2 numerators’ lactic acid ($pK_a = 3.9$) and 2 numerators’ ATP; however, the normal cells only produce 1 lactic acid and 1 ATP. The cancer cells produce excess lactic acid so as to reduce pH and become acidified. The difference in pH is small but apparent as a natural signal of solid tumors for triggered release.

As we know, chemotherapy is often complicated by toxic side effects of anticancer drugs. Effective chemotherapy regimens are frequently hindered by the cross-resistance or multidrug resistance of tumor cells to one or more drugs. Developing a pH-sensitive nanotechnology that targets only the tumor tissue can be effective for treatment of drug-sensitive and multidrug-resistant tumor cells (Rapoport 2006).
Polymeric micelles have high stability, but they may still dissociate upon dilution after injection and interact with blood components (Yokoyama 1998; Moghimiet al. 2001). Considering the dose–response relationship, it was suggested that future improvement in carrier design, providing a triggerable mechanism for drug release upon reaching the tumor sites, could be the most efficacious delivery strategy (Drummond et al. 1999; Lee and Bae 2006).

### 14.3.2 Tumor pH Targeting Micelle

Triggered release of a drug from long-circulating vesicles at tumor sites while maintaining a minimal release rate during circulation, which may result in a high local dose in tumor sites and less side effects, is a very desirable property in carrier designs for tumor chemotherapy and has been proven to be effective for treating multidrug-resistant tumors. Approaches to achieve triggering systems include magnetic (Pardoe et al. 2003), thermal (Needham et al. 2000), and ultrasonic activation (Rappoprt et al. 2004) for enhanced drug release from the carriers, which were specifically designed to respond to external signals or energy. The external activation could be useful for the tumors whose location is well identified and which are accessible by external signal or energy sources. In addition, it would be technically difficult to restrict the activation only in tumor regions, and thus the external activation does not distinguish between the blood compartment and tumor cells. Nowadays, many approaches have described drug release by the cleavages of chemical bonds, which conjugate a drug to polymers by endosomal pH (Kataoka et al. 2000). It has been difficult to develop effective pH-sensitive formulations responding to tumor extracellular pH due to the lack of a proper pH-sensitive functional group in the physiological pH range (Sikic et al. 1997). Tumor tissue has an acidic environment, and it’s ideal to design polymeric micelles that have long circulation and active transport in the acidic environment of the tumor tissue. Outside of the micelle is PEG, which long circulates in vivo and so can effectively accumulate near the tumor tissues. When the micelles come into the acidic environment, because of the neutralization...
of positive and negative charges, the positive charge carriers pop up outside the micelle, so that the micelles’ active transport can happen.

Cell-penetrating proteins (CPP) are a kind of polypeptide substances that can accelerate cytomembrane transportation. A typical substance is TAT micromolecular polypeptide. TAT has a strong ability to penetrate into the cytomembrane (Torchilin et al. 2003; Torchilin 2008a,b). It was discovered by scientists in 1988 during their research on the project of AIDS virus (HIV) (Green and Loewenstein 1988). The approaches to target various solid tumors by pH include micelle systems with a triggered drug release mechanism, and exposing nonspecific cationic TAT peptide by a shielding/deshielding mechanism or by a pop-up mechanism (Lee et al. 2008). Particular polysulfonamides are negatively charged at blood pH (pH 7.4) and can be neutralized at acidic pH (tumor pH) (Carelle et al. 2002). Poly (methacryloyl sulfadimethoxine)-b-PEG is negatively charged and interacts electrostatically with TAT molecules (shielding) at blood pH. However, charge density on this polymer decreases by decreasing pH. Below pH 6.8, due to destabilized electrostatic interactions, the TAT will be deshielded. The zeta potential measurements on micelle comprising of PLLA-b-PEG-TAT demonstrated the shield/deshielding process. It was shown that the zeta potential is close to zero between pH 8.0 and 6.8, which indicates complete shielding of TAT, and from pH 6.6 to 6.0 it increased to 6.0 mV, which is close to the measured zeta potential for TAT-decorated micelles without masking. When the shielded and unshielded TAT micelles were tested for tumor cell internalization at pHs 7.4 and 6.6 by incubating for an hour, unshielded micelles were internalized into both the cells and their nucleus at pH 7.4 and 6.6. However, the micelle shielded with poly (methacryloyl sulfadimethoxine)-b-PEG was not internalized at pH 7.4, indicating TAT was masked even as it internalized into cells and the nucleus at pH 6.6. This shield/deshielding mechanism suggests that an optimized pH-targeting system with an appropriate sulfonamide polymer is feasible (Kim et al. 2005; Sethuraman and Bae 2007; Oh et al. 2008; Yin et al. 2008). The carriers were modified with acidic sulfonamide and characterized for enhanced drug release and internalization into cells at tumor pH (Na and Bae 2002; Han et al. 2003; Na et al. 2003). These self-assembled nanocarriers or micelles switched their surface properties from hydrophilic to hydrophobic by deionization of sulfonamide group at tumor pH, resulting in distribution and reorganization of the self-assembly structures. It causes enhanced drug release and interactions with cells for internalization (Figure 14.11).

The process of targeting-ligand’s exposure in the acid environment: the carrier system consists of two components, poly (l-lactic acid)-b-PEG-TAT micelles and pH-sensitive poly (methacryloyl sulfadimethoxine)-b-PEG. At normal blood pH, polysulfonamide is negatively charged, and, when mixed with TAT, polysulfonamide shields the TAT by electrostatic interaction. Only PEG is exposed to the outside, which could make the carrier long circulating. When the system experiences a decrease in pH (near tumor), polysulfonamide loses charge and detaches, exposing TAT for interaction with tumor cells. (From Sethuraman, V.A. and Bae, Y.H., J. Control. Release, 118, 216, 2007.)
14.3.3 Conclusions

Tumor pH sensitive micelle is a novel anticancer drug delivery system for overcoming limitations of conventional drug delivery system. It increases target drug accumulation at tumor site, brings less drug distribution to normal tissues and organs, and is effective for treatment of multidrug resistance. pH-sensitive micelles have good foreground and provide useful information to the drug treatment of cancer chemotherapy.

14.4 Mouse Dorsal Skinfold Window Chamber Model for Evaluation of Nanoparticle Release from Tumor Blood Vessel

The main cause of cancer treatment failure is the invasion of normal tissues by cancer cells that have migrated from a primary tumor. An important obstacle to understanding cancer invasion has been the inability to acquire detailed, direct observations of the process over time in a living system (Condeelis et al. 2005; Makale 2007). Professor Jain from Harvard University and Professor Dewhirst from Duke University developed the dorsal skinfold window chamber model. It’s a kind of model that can be observed under the fluorescence microscope. Dorsal skinfold window chamber model is a known model to visualize the distribution profiles of nanoparticles in the tumor area (Warburton et al. 2004).

Chambers made from titanium frames, which are mirror images of each other, will be implanted so as to sandwich the extended double layer of the skin. One layer of the skin will be removed in a circular area of approximately 15 mm in diameter, and the remaining layer, consisting of subcutaneous tissue, epidermis, and the striated skin muscle, will be covered with a cover slip incorporated into one of the frames. The animals will be allowed to recover for hours following the microsurgery after the cells will

![FIGURE 14.12](See color insert.) Fluorescence labeling of drug release from tumor blood vessel dorsal skinfold window chamber model (A through C); after injection of green fluorescent protein (GFP)-transfected 4T1 mammary cancer cell (D: as observed under the fluorescence, E: as observed under the white light); drug release in the blood vessel (F).
be injected. We observe the window chamber under the fluorescence microscope, so the whole processes including tumor vessel’s growth in the tissue, drug release from tumor blood vessel, drug controlling action can be directly viewed with the model (Liu et al. 2005). As shown in Figure 14.12, using a special designed microscope, we observed division and reproduction of cancer cells and the process of growth of tumor tissues. Many scientists use this model to reveal malignant tumor’s law of development and method of controlling (Jain 1991) and to evaluate drug/gene release in the tumor blood vessel (Shan et al. 2006).

We can implant both tumor tissue and tumor cell using the dorsal skinfold window chamber. Tumor cells grow in the window chamber and can be observed under the microscope. If we inject fluorescence-labeled nanoparticles, we may read the release of nanoparticles into the tumor tissue and calculate strength and area of the fluorescence to evaluate a dynamic process of the nanoparticle’s whole circuit. This model is not only very useful in the research of drug-targeting nanomaterial but also considered to be a versatile tool to study the microcirculation in health and disease (Menger et al. 2002).

References


