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Review Article

Multifunctional nanomicellar systems for delivering anticancer drugs

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Abstract: Most anticancer drugs cause severe side effect due to the lack of selectivity for cancer cells. In recent years, new strategies of micellar systems, which design for specifically target anticancer drugs to tumors, are developed at the forefront of polymeric science. To improve efficiency of delivery and cancer specificity, considerable emphasis has been placed on the development of micellar systems with passive and active targeting. In this review article, we summarized various strategies of designing multifunctional micellar systems in the purpose of improving delivery efficiency. Micellar systems compose of a multifunctional copolymer or a mixture of two or more copolymers with different properties is a plausible approach to tuning the resulting properties and satisfied various requirements for anticancer drug delivery. It appears that multifunctional micellar systems hold great potential in cancer therapy. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 102A: 2024–2038, 2014.

Key Words: micelles, stimuli responsive, controlled drug delivery, cancer therapy, tumor targeting

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INTRODUCTION

Cancer is a widespread problem although improved methods of diagnosis and treatment have recently reduced mortality considerably. Most anticancer drugs are cytotoxic to both healthy and cancer cells, so cause severe side effects. For example, the administering of doxorubicin is associated with acute side effects as well as cumulative dose-limiting cardiotoxicity.[1–3] Multifunctional micellar systems must be utilized for systemic administration to achieve selective accumulation of the chemotherapeutic drug in the cancer tissue, and subsequently to have an effective anticancer effect with a sufficient therapeutic index. Research on specific drugs and/or drug carriers that target to cancer cells is challenging. To improve efficiency of delivery and cancer specificity, considerable emphasis has been placed on the development of micellar systems with passive and active targeting. In this context, multifunctional micellar systems provide various strategies to deliver drugs efficiently to cancer cells.[4,5] Micellar systems that are formed from a multifunctional copolymer or a mixture of two or more copolymers with different properties satisfied various requirements of anticancer drug delivery.

The general micellar system for delivering anticancer drugs is based on an amphiphilic polymer with hydrophobic and hydrophilic segments. The length of the hydrophobic segment is directly related to stability. Micellar systems are core–shell structures that spontaneously formed by the thermodynamically favored aggregation of amphiphiles above the critical micellar concentration (CMC).[6] Lower values of CMC indicate better thermodynamic stability. Micellar systems exhibit lower CMC values than surfactant micelles with low molar mass because polymer chains have many points of interaction than small molecules. The tendency of micellar dissociation is related to the composition and cohesion of the hydrophobic core.[7,8] Increasing the hydrophobicity of the copolymer increases the cohesion of the hydrophobic core and reduces the CMC.[9] The drug–core interaction can also affect the stability in micellar systems in drug delivery. An encapsulated, hydrophobic drug may stabilize a micelle by hydrophobic interactions of drug and polymer in the core.[10] The segregation of the core from aqueous solution is the direct driving force of micellization. Combination of physical interactions, such as hydrophobic

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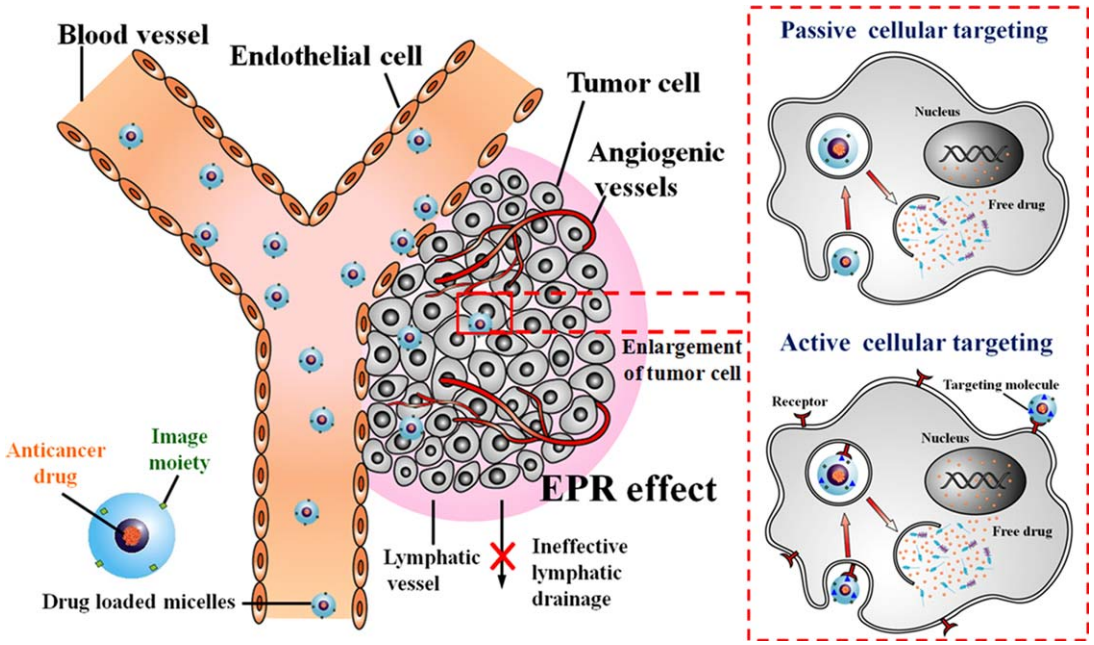

FIGURE 1. Schematic representation of drug loaded micelles (circles) with image moiety transport from injection site to tumor sites. After injection, nanocarriers (10–200 nm) demonstrated significant selective targeting of tumors of a specific size range, and the particle accumulated in solid tumors because of the tumor vasculature and ineffective lymphatic drainage (Enhanced permeability and retention (EPR) effect). Passive cellular targeting is achieved by taken up from extracellular fluid into cells by endocytosis. Active cellular targeting can be achieved by functionalizing the surface of micelles with targeting molecule that promote cell-specific recognition and binding. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

interaction,[11] electrostatic interaction,[12] stereocomplex formation,[13] π–π stacking,[14] and hydrogen bonding[15] of the component copolymers increases stability of micelle. Furthermore, chemical cross-linking of the shell[16] or the core[17] has been utilized to increase the stability of the micellar system. The structure of micellar systems can be modified to facilitate its delivery to the target site.

Progressive micellar systems commonly include other anticancer drugs that perform various functions. An ideal anticancer drug micellar system should (1) self-assemble into monodispersed drug-loaded micelles with a size of less than 200 nm; (2) exhibit favorable pharmacokinetics and tumor accumulation; (3) degrade into nontoxic components; (4) release the drug with in a particular compartment of an organ or tissue; (5) exhibit antitumor activity; and (6) prevent the adverse side effects of the anticancer drugs. This report provides an overview of modern multifunctional approaches of designing micellar systems for effective cancer therapy.

PASSIVE AND ACTIVE TUMOR TARGETING

Passive targeting

Passive targeting involves the transportation of micelles in circulating blood and then, through leaky tumor capillaries, into the tumor compartment by rapid diffusion in interstitial space (Fig. 1). Targeted drug delivery plays an important role in the treatment of cancer because the cytotoxic drugs can severely harm normal tissues. Micelles must be physically stable in circulating blood to deliver their payloads selectively at tumor sites. An effective way to improve blood stability is to reduce the rate of nonspecific recognition.[18–21] Stealth shielding on the surface of drug delivery systems is demonstrated effective in opposing opsonization and subsequent uptake by the reticuloendothelial system (RES) and increases its systemic circulation time. Without the stealth surface, particles are rapidly cleared from the blood stream and distributed into the RES of the liver, spleen and bone marrow. A micellar system exploits the characteristics of the hydrophilic and the hydrophobic segment of a copolymer. The hydrophilic segments of the micelle provide steric stability and avoid rapid uptake by the immune system, lengthening its circulation time in the body.[22–24] The hydrophobic segments of the micellar core offer some important control points for drug release. We describe the details of control points in "stimuli-responsive drug release" section.

Poly(ethylene glycol) (PEG) is the most frequently used hydrophilic segment of copolymers. PEG-conjugated micelles are hydrophilic, biocompatible, sterically stable and minimally toxic.[19,25–31] Our group found that graft micelles without PEG segments adsorb bovine serum albumin (BSA) via interaction between hydrophobic segments and albumin.[29,32] The positive effect of PEG-modified micelles in reducing protein adsorption has been demonstrated in mixed diblock and graft copolymer system.[29,32] A PEG-modified micellar system is stable, and prevents the adsorption of albumin on the surface. Accordingly, the coating of PEG chains on the surface of carriers has been demonstrated to prolong the circulation times of micelles by several orders of magnitude.[33]

Particle size is a critical factor in the design of tumor-targeting micelles. Owing to their nanoscale size, micellar systems can be administered intravenously, allowing them

to arrive at pathological sites while avoiding various biological barriers in the human body, such as limited gastrointestinal absorption and the high hepatic passing of orally administration. Following intravenous administration, carriers should deliver their payloads selectively at the target sites. Numerous investigations have demonstrated the selective targeting of designed nanostructures to tumors within the size range 10–200 nm and their accumulation within these solid tumors owing to the enhanced permeability and retention (EPR) effect. This effect was first identified by Meada's team.[34,35] Two main factors are responsible for the EPR effect. First, the angiogenic tumor vasculature, along with blood vessels in other pathological tissues, has a higher permeability than normal vasculature owing to their discontinuous endothelia. Second, lymphatic drainage is not fully developed in tumors. These characteristics cause colloidal particles to extravasate through the "leaky" endothelial layer of tumor vascular and subsequently to be retained in tumor tissues. The majority vascular pore cutoff size is between 380 and 780 nm.[36] The EPR effect in solid tumors is one of the few tumor-specific characteristics that is becoming exploited in standard antitumor drug delivery. Therefore, micellar systems must circulate in the bloodstream long enough to reach the tumor site for passive targeting.[17,37] Neoangiogenesis occurs in tumor tissue when a tumor cell cluster (nodule) reaches a size of 2–3 mm.[37] The scanning electron microscopic observations of metastatic tumor nodules in the liver indicated that vascular casts of blood vessels are obtained with plastic resin. The images also reveal that tumor nodules of size 100 μm have a highly permeable vascular bed[38] and is not observed in normal blood vasculature. The EPR effect has been observed with biocompatible macromolecules or nanoparticles with a molecular size of more than 40 kDa and up to the size of bacteria (approximately 1 μm) in most experimental solid tumor.[39] Administration of a micellar drug selectively destroyed the tumor micronodules by means of the EPR effect and did not damage normal blood vessels.[40]

Active targeting

Active targeting is an effective method to improve the selective toxicity of anticancer therapeutics.[5,41,42] Active targeting strategies using ligands at the micellar surface bind to appropriate receptors that are overexpressed by tumor cells or the tumor vasculature (Fig. 1). Targeting ligands have been developed to attach corresponding vectors, such as small organic molecules, sugar moieties, peptides, monoclonal antibodies and other ligands to the carrier surface. Below, we describe the details of micellar systems containing targeting ligands.

Folate is an example of small organic molecule for cancer targeting ligand. The folate receptor is overexpressed by a factor of 100–300 in a wide range of human tumors.[43] Kataoka and coworkers functionalized adriamycin-containing poly(ethylene glycol)-poly(aspartate-hydrazone-adriamycin) (PEG-p(Asp-Hyd-ADR)) micelles with folate and were able to demonstrate significantly increased uptake and cytotoxicity in human pharyngeal cancer cell KB cells. Folate-labeled micelles exhibited a lower *in vivo* toxicity and a higher antitumor activity over a broad range of dosages from 7.50 to 26.21 mg/kg. This range is five times as achieved with free drugs.[44] Recently, our laboratory showed folate-labeled graft-diblock mixed micelles, and carriers conjugated with folate ensure high intratumoral accumulation owing to the effect of a folate-binding protein. The inhibition of *in vivo* tumor growth demonstrates that the carriers exhibited excellent antitumor activity and a high rate of apoptosis in cancer cells.[30,45]

Sugar moieties, such as galactosamine and lactosamine, have also been used to functionalize the micellar system. Galactose residues have a high affinity for the asialoglycoprotein receptor that is overexpressed in hepatocytes.[46] Because of galactosamine ligand–receptor binding, multifunctional micelles which contain the targeting moiety, galactosamine can be internalized in cancer cells by the receptor-mediated endocytosis process and delivered to the lysosomes.[26] Zhong and coworkers[47] demonstrated that galactose-functionalized paclitaxel (PTX)-loaded interfacially cross-linked biodegradable diblock copolymer micelles can effectively target hepatic tumor cells *in vitro* as well as hepatoma *in vivo*, significantly enhancing antitumor efficacy.

Peptides are actively used as ligands in anticancer drug delivery. Cell-penetrating peptides, such as human immunodeficiency virus peptide TAT, have been utilized to deliver drug to various cells and organs in mice, and taking the cargo directly into the cell by the endocytosis pathway.[48,49] Monoclonal antibodies recognize and bind to a range of tumor antigens, interfere with signal-transduction pathways and are involved in the proliferation of cancer cells.[50] Micelles are successfully modified by incorporating antibodies and plays the role of targeting ligand and/or drug.[33] Shoichet and coworkers[51] reported upon micelles that were conjugated with anti-human epidermal growth factor receptor 2 (anti-HER2) antibodies to target HER2-overexpressing breast cancer. Micelles that were encoded with anti-HER2 antibodies supported the cell type-specific delivery of doxorubicin. These methods exploit the differences between a malignant cell and a normal cell and are promising for the active targeting of tumors.

STIMULI-RESPONSIVE DRUG RELEASE

Biodegradable polymeric micelles

Biodegradation is the chemical disruption of materials in a physiological environment where the material is degraded by enzymes or is hydrolyzed. Chemical hydrolysis can cause micellar disintegration. Polyester hydrolysis is extensively exploited in biomedical applications and is well known to be accelerated by increasing temperature and/or decreasing pH.[52,53] As mentioned earlier, the biodegradation of PEG and its attachment to the surface of micellear systems have been demonstrated to reduce significantly anticancer drug toxicity and improve biocompatibility. Hence, the most common biodegradable PEG modified micellar systems comprise a PEG hydrophilic segment and a polyester segment, such as poly(D,L-lactide) (PLA), poly(D,L-lactide-*co*-glycolic acid) (PLGA),[54] or poly(ε-caprolactone) (PCL), which are among the most investigated segments owing to their excellent biocompatibility and approval of their use in medical carriers by the U.S. Food and Drug Administration.[55] Discher and coworkers[56–59] demonstrated that the degradation and

release drug by a micellar system is associated with the nanoscale phase transition behavior of the poly(ethylene glycol)-*block*-poly(D,L-lactide) (PEG-*b*-PLA) copolymer. PLA segments are hydrolyzed at the end of the chain and result of increasing the hydrophilic/hydrophobic ratio of PEG-*b*-PLA. As hydrolysis of the PLA core proceeds, continued growth and propagation of the pores in the membranes destabilize vesicle architecture, and the carriers are ultimately dissociated into worm-like micelles. Kataoka and coworkers[60] prepared micelles from an α-acetal-poly(ethylene glycol)-poly(D,L-lactide) block copolymer (acetal-PEG-*b*-PLA) with a narrow size distribution. This micellar system has great potential as a stealth and long-circulating carrier system that is minimally taken up into the liver and spleen. PEG-*b*-PLA micelles provided an alternative to the current commercial formulations and successfully combined up to three chemotherapeutic agents in a single carrier system at clinically effective concentrations with promising stability.[61] The chemical structures of PLA and PGA are similar to each other except that the PGA lacks a methyl group, and this fact contributes to differences in their degradation kinetics.[62] PGA is less stable than PLA in the body. Degradation rate of PLGA copolymer depends on the exact ratio of PLA to PGA present therein. Poly(ethylene glycol)-*block*-poly(D,L-lactic-*co*-glycolic acid) (PEG-*b*-PLGA) has been developed for delivering anticancer drugs. For example, Langer and coworkers[63,64] prepared micelles that comprised carboxy-terminated PEG-*b*-PLGA (COOH-PEG-*b*-PLGA) copolymer and the anticancer drug docetaxel. Préat and coworkers[65] used PEG-*b*-PLGA copolymers to develop a Cremophor-free formulation that was loaded with PTX. Chen and coworkers[66] reported on a PEG-*b*-PLGA micellar system that was decorated with a DNA aptamer for the anti-glioma delivery of PTX. Anticancer drug-loaded PEG-*b*-PLGA micelles exhibit considerably higher anti-tumor efficacy and survival rates than the free drug.[63–66] PEG-*b*-PCL has also been developed as a carrier for delivering anticancer drug.[67,68] Lee and coworkers[68] prepared PEG-*b*-PCL micelles and then conjugated with folic acid to form an active targeting drug carrier. PTX-loaded micelles exhibited the sustained release of approximately 60% of loaded amount with no initial burst release. Folate-conjugated PEG-*b*-PCL micelles were selectively taken into cancer cells by folate-receptor mediated endocytosis. Rapamycin is weakly soluble in water (2.6 μg/mL) and has poor stability.[69] Kwon and coworkers[67,70] used the PEG-*b*-PCL micellar system for use in injection. Rapamycin-loaded PEG-*b*-PCL micelles released approximately 50 % of the drug within 31 h with no burst release in water. The PEG-*b*-PCL micellar system retained more of the water-insoluble drug rapamycin than free rapamycin. The advantageous properties of biodegradable polymeric micelles as drug carriers are associated with the solubilization of weakly soluble low-solubility anticancer drugs and cause them to have potential in the targeting of tumors and controlled drug release.

pH-responsive polymeric micelles

For the selective release of anticancer drugs in a tumor, micelles can be designed to respond to acidic microenvironment in solid tumors. The pH of normal tissue and blood is approximately 7.4, but the extracellular pH in a tumor is 6.5–7.2.[71,72] The triggering mechanisms of most pH-responsive micelles operate in the endosome and release the drugs into the cytoplasm. The pH thus drops markedly from 7.2–7.4 to 5.0–6.5 in the endosomes, and to 4.5 in primary and secondary lysosomes.[73,74] Endo/lysosomal pH-responsive micelles loaded with anticancer drugs have been actively developed to improve cancer therapy. Notably, after cellular uptake of micelles, anticancer drugs are released intracellularly, and then diffused into the nuclei. In contrast, free anticancer drugs typically diffuse passively into cancer cells and immediately accumulate in the nuclei. Despite delivery by a different route, anticancer drugs that are released from micellar systems accumulate in the nuclei more effectively.[75–77] The major mechanisms for inducing pH-responsive behavior involve change of the charges in the micellar system and the dissociation of pH-dependent drug-binding linkers.

Pendant ionizable groups confer pH-responsive properties. Histidine is useful in pH-responsive micellar drug delivery systems because of its buffering capacity ($pK_b = \sim 6.02$) and its imidazole ring includes a lone pair of electrons on an unsaturated nitrogen (NH of pyrrole). Bae and coworkers reported on a mixed pH-responsive micellar system [Fig. 2(A)] based on PEG-*b*-poly(L-histidine) (PEG-*b*-polyHis) and PEG-*b*-poly(L-lactic acid) (PEG-*b*-PLLA) diblock copolymers. The micelles were fairly stable under physiological conditions (pH 7.4) but exhibited enhanced release of doxorubicin from the carrier in an environment with tumor or an early endosomal pH.[78] Additionally, folate-modified mixed micelles are highly cytotoxic to drug-resistant breast cancer Michigan Cancer Foundation 7 (MCF-7) and ovarian carcinoma A2780 cell lines *in vitro* and they reverse doxorubicin-resistant of MCF-7 and A2780 tumor-xenografted mice.[79] Kim and coworkers investigated pH-sensitive micelles that comprised poly(2-hydroxyethyl methacrylate)-*b*-poly(L-histidine) (p(HEMA)-*b*-p(His)) micelles for encapsulating doxorubicin in a self-assembly process and delivering it under various pH conditions. The drug was released from the p(HEMA)-*b*-p(His) micelles in a controlled and sustained manner and that the release rate was higher at pH 5.5 than at pH 7.4.[80] In earlier work, we prepared pH-responsive mixed micelles from graft copolymer poly(2-hydroxyethyl methacrylate)-*co*-histidine-*g*-poly(D,L-lactic acid) (poly(HEMA-*co*-his)-g-PLA) and diblock copolymer PEG-*b*-PLA.[30] *In vitro* drug release studies revealed that doxorubicin was released in a pH-dependent manner by imidazole ring protonation under acidic conditions. A flexible hollow micelle system self-assembled from poly(*N*-vinylimidazole-*co*-*N*-vinylpyrrolidone)-*g*-poly(D,L-lactide) (P(NVI-*co*-NVP)-*g*-PLA) graft copolymers and mPEG-*b*-PLA diblock copolymers was formed an anticancer drug doxorubicin carrier. It exhibited an on-off switch drug release behavior. Due to the pH-sensitive structure of imidazole, doxorubicin is encapsulated in neutral surroundings and released in endosomes.[45] *In vivo* study studies of the two graft-diblock micellar system revealed specific targeting by folate

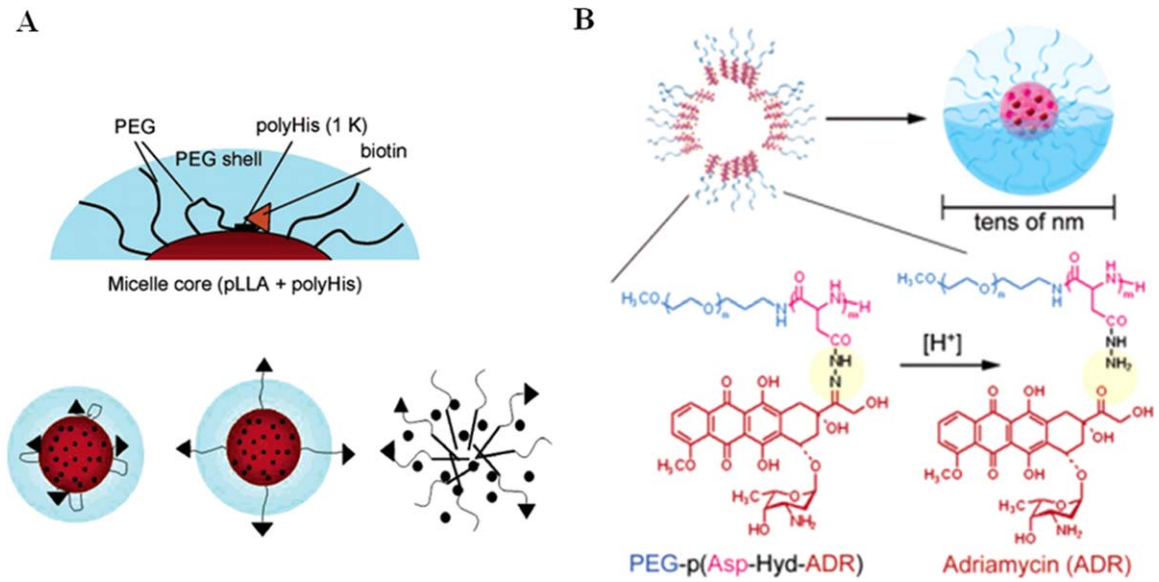

FIGURE 2. A, Schematic diagram depicting the central concept of pH induced vitamin repositioning on the micelle. While above pH 7.0, biotin that is anchored on the micelle core via a pH-sensitive molecular chain actuator (polyHis) is shielded by PEG shell of the micelle; biotin is exposed on the micelle surface ($6.5 < pH < 7.0$) and can interact with cells, which facilitates biotin receptor-mediated endocytosis. When the pH is further lowered ($pH < 6.5$), the micelle destabilizes, resulting in enhanced drug release and disrupting cell membranes such as endosomal membrane.[78] B, Preparation of tumor-infiltrating polymeric micelles was with intracellular pH-sensitivity. Micelles with tens of nm size diameter were prepared from self-assembling amphiphilic block copolymers, PEG-p(Asp-Hyd-ADR), in which the anticancer drug, adriamycin (ADR, doxorubicin), was conjugated through acid-sensitive hydrazone linkers.[86] [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

modified micelles and exhibited excellent antitumor activity. Two pH-responsive micellar systems for photodynamic therapeutic drug delivery were formed from a diblock copolymer, poly(ethylene glycol)-*b*-poly (2-hydroxyethyl methacrylate-*co*-histidine-poly(D,L-lactide) (mPEG-*b*-P(HEMA-*co*-histidine-PLA)[81] and poly(ethylene glycol)-*block*-poly(2-methoxyethyl acrylate-*co*-imidazole) (PEG-*b*-p(MEA-*co*-VIm)).[82] After acidification to pH 5.0, the micelles were protonized and precipitated gradually over time, and the drug aggregated to form a fragment. Both *in vitro* and *in vivo* studies demonstrated that the pH-responsive and dual stealth characteristics, PEGylation, and hexagonal prism structure of micelles can be exploited clinically for the safe and efficient delivery of anticancer drugs. To elucidate the effect of the release of a photosensitizer from non-pH-responsive and pH-responsive micellar systems, two new graft copolymers were designed and synthesized. They were poly(*N*-vinyl caprolactam)-*g*-poly(D,L-lactide) (P(VCL)-*g*-PLA) without a pH-sensitive segment and poly(*N*-vinyl caprolactam-*co*-*N*-vinyl imidazole)-*g*-poly(D,L-lactide) (P(VCL-*co*-VIM)-*g*-PLA) with a pH-sensitive segment.[83] Following nontoxic light treatment, protoporphyrin IX (PPIX) was observed in the nucleus of cancer cells with pH-responsive micelles, while PPIX remained in the lysosomal organism with the non-pH-responsive micelles. Polymeric micelles that are based on poly(2-ethyl-2-oxazoline)-*b*-poly(L-lactide) diblock copolymers (PEOz-PLLA) and poly(L-lactide)-*b*-poly(2-ethyl-2-oxazoline)-*b*-poly(L-lactide) (PLLA-PEOz-PLLA) triblock copolymers have been designed as anticancer drug doxorubicin carriers.[84,85] The hydrophilic segment PEOz and hydrophobic segment PLLA exhibited pH-sensitivity in aqueous solution. The results clearly indicated the colocalization of doxorubicin with acidic compartments, suggesting that the release of the drug was successfully triggered by micellar deformation in the acidic organelles. Hence, pH-dependent release profiles of micellar systems are favorable when the carriers respond to stimuli at tumor sites or are taken up via an endocytotic pathway.

Conjugated polypeptides with ionizable groups in the copolymer chain can also exhibit pH-responsive property. The drug-binding linkers can be protected by the formation of micelles in a physiological environment. The hydrazone bond is one of the drug-binding links that is present in stable copolymer-drug conjugates and shows excellent pH-sensitivity. For example, Kataoka and coworkers[86] discussed PEG-p(Asp-Hyd-ADR) micelles, in which the anticancer drug, doxorubicin, was conjugated with the hydrophobic segments via acid-sensitive hydrazone bonds [Fig. 2(B)]. Their results revealed that the micelles stably preserved drugs under physiological conditions and selectively released the conjugated drug by responding to the decrease in intracellular pH in endo/lysosomes. Ulbrich and coworkers[87] investigated a pH-responsive micellar system that was based on diblock copolymer intermediate poly(ethylene oxide)-*block*-poly(allyl glycidyl ether) (PEO-PAGE) and hydrazone-linked doxorubicin. The release profiles indicated that doxorubicin was released faster at pH 5.0 than at pH 7.4. Kwon and coworkers[88] modified PEG-p(Asp-Hyd) (Poly(ethylene glycol)-*block*-poly(aspartate-hydrazide)) by attaching either levulinic acid (LEV) or 4-acetyl benzoic acid (4AB) via

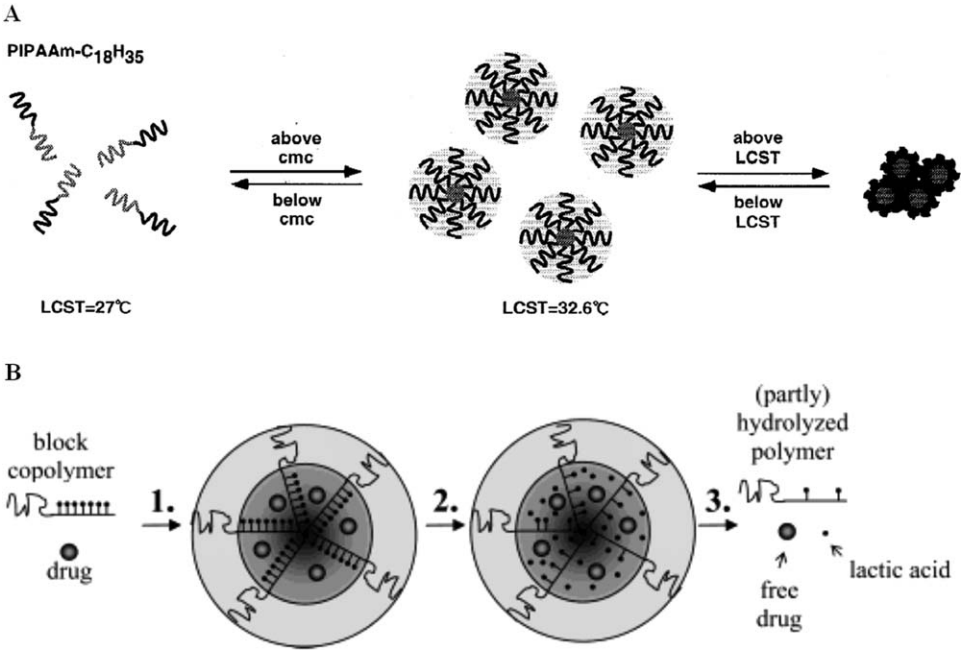

FIGURE 3. A, Micellar structures and thermoresponsive reversibility of poly(NIPAAm) copolymer.[92] B, A schematic representation of the concept of polymeric micelles with controlled instability, formed from block copolymers with hydrolytically sensitive side groups. The numbered consecutive steps are the following: (1) Self-assembly and drug loading of polymeric micelles in water above the CP. (2) Degradation and hydrophilization of the core. (3) Dissolution of the micelles and release of the drug.[101]

hydrazone bonds. A mixed micellar system that was based on PEG-p(Asp-Hyd-LEV-PTX) and PEG-p(Asp-Hyd-4AB-PTX) was assembled to deliver PTX. The pH-dependent release of PTX can be moderated by Hyd-LEV linker and assembled in the mixed micellar system. Kim and coworkers[89] synthesized poly(ethylene oxide)-hyperbranched-polyglycerol (PEO-*hb*-PG) to develop the doxorubicin delivery system, which is linked to the polymer by pH-sensitive hydrazone bonds. The pH-responsive release profiles of doxorubicin and *in vitro* cytotoxicity studies revealed that this controlled pH-responsive drug delivery system increases the efficiency of treatment of cancer. A micellar system with a copolymer-platinum conjugate prepared from a poly (oligo(ethylene glycol) methyl ether methacrylate)-*block*-poly(hydroxyethyl methacrylate) (POEGMEMA-*b*-PHEMA) diblock copolymer with a hydrazone linkage.[90] The results of micellar properties indicated that hydrazone bond was completely hydrolyzed in <4 h in slightly acidic media but was more stable at neutral pH. Accordingly, the pH-dependent release of the conjugated drug provides a strategy can be exploited in adjusting the pharmacokinetic and pharmacodynamic properties of anticancer drug delivery.

Temperature responsive polymeric micelles

To reduce the side effects of anticancer drugs, micellar systems are designed to respond to change of local temperature.[91] Temperature-responsive copolymers undergo a volume phase transition at a particular temperature that causes a sudden change in the copolymer–solvent interactions. Temperature-responsive copolymers are insoluble upon heating above their lower critical solution temperatures (LCSTs) because the hydrophobic interactions come to dominate. Poly(*N*-isopropylacrylamide) (PNIPAAm) is a well-known temperature-sensitive segment that undergoes a thermoreversible phase transition at 32.8°C [Fig. 3(A)].[92] The hydrogen bond interaction between PNIPAAm and water is stronger below the LCST. Therefore, PNIPAAm exist in a water-soluble extended chain from below LCST. PNIPAAm undergoes a reversible phase transition to a hydrophobic aggregate above the LCST. Therefore, in aqueous solution below the LCST, a hydrophobically modified PNIPAAm exhibits temperature-responsive water solubility and can form heterogeneous microstructures that are composed of hydrophilic microdomains with soluble PNIPAAm segments, as well as hydrophobic aggregated microdomains of incorporated hydrophobic segments in aqueous solution below the LCST. Copolymer with a block PNIPAAm segment had a stronger hydrophobic effect on the phase transition than did random PNIPAAm modification in the main chain of the copolymers.[93–95] Motivated by this fact, temperature-responsive copolymers with an LCST around body temperature were designed to form micellar systems for anticancer drug delivery.

Temperature-responsive polymer can be used not only as a hydrophilic shell-forming segment, but also as a hydrophobic and core-forming segment of copolymers. Okano and coworkers[96,97] prepared poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide) (P(NIPAAm-*co*-DMAAm)) as the temperature-responsive shell and used biodegradable poly(D,L-lactide) (PLA), PCL, or poly(D,L-lactide-*co*-caprolactone) (poly(LA-*co*-CL)) as the hydrophobic core. P(NIPAAm-*co*-DMAAm) with an LCST of approximately 40°C was

synthesized for temperature-responsive block polymer. The *in vitro* release profiles revealed that P(NIPAAm-*co*-DMAAm)-*b*-P(LA-*co*-CL) micelles released a considerable doxorubicin at 41°C. In contrast, P(NIPAAm-*co*-DMAAm)-*b*-PLA and P(NIPAAm-*co*-DMAAm)-*b*-PCL micelles did not exhibit temperature-dependent release profiles. Hennink and coworkers have developed a series of temperature-responsive block polymers as hydrophobic segments, these included, poly(*N*-isopropylacrylamide-*co*-*N*-(2-hydroxypropyl)methacrylamide lactate) (poly(NIPAAm-*co*-HPMAm-lactate)),[98] *N*-isopropylacrylamide (PNIPAAm),[99] and poly(*N*-(2-hydroxypropyl) methacrylamide mono/dilactate) (poly(HPMAm-mono/dilactate)).[94,100] Poly(NIPAAm-*co*-HPMAm-lactate)-*b*-PEG[94] micelles with an LCST were currently being investigated for use in an anticancer drug delivery system. PNIPAAm, however, is a nonbiodegradable polymer, and its biocompatibility is not well known at present. Hennink and coworkers found that poly(HPMAm mono/ dilactate) without NIPAAm also exhibits LCST behavior in aqueous solution. The hydrolysable lactic acid side groups cause the cloud point (CP) to increase in time. The CP of the poly(HPMAm-mono/dilactate) polymer can be tuned between 10 and 65°C by modifying the composition of the copolymer.[94,100] The removal of the lactic acid side groups in aqueous solution makes the poly(HPMAm-dilactate) more hydrophilic over time, and this change is associated with a slow increase in the CP. Therefore, polymers can be designed to be initially associated with water but then begin to dissolve after the CP increases beyond the incubation temperature. At body temperature, poly(HPMAm-dilactate) is above its CP and exists in its precipitated form, but when more than 50% of HPMAm-dilactate is converted to HPMAm-monolactate, this polymer becomes soluble at body temperature. As described, the hydrolysis of the lactic acid side groups is expected to increase the CP over time. The degraded subunit, lactic acid is an endogenous compound, which makes pHPMAm biodegradable product. Also, pHPMAm is a water-soluble polymer and has been demonstrated to be nontoxic in clinical trials. The CP of poly(HPMAm-lactate) can be well controlled by varying the length of the lactate acid side group (such as monolactate or dilactate) or the copolymer composition [Fig. 3(B)].[101] Of these polymers, poly(HPMAm-dilactate) is particularly interesting, because its CP (approximately 10°C) is well below body temperature. Poly(ethylene glycol)-*b*-poly(HPMAm-dilactate) (PEG-*b*-pHPMAmDL) are expected to form polymeric micelles at 37°C. HPMAm-dilactate gradually dissolve upon hydrolysis of the lactic acid side groups, which releases drugs loaded in the hydrophobic core into the environment.[101] PEG-*b*-pHPMAmDL has a thermoreversible LCST that is suited to the targeted delivery of PTX,[31] photosensitizers,[102] and superparamagnetic iron oxide[103] for the administration of cancer therapy or diagnosis. PTX-loaded PEG-*b*-pHPMAmDL micelles displayed comparable *in vitro* cytotoxicity against melanoma carcinoma B16F10 cells compared to the PTX standard formulation containing Cremophor EL.[31] Mice injected with the PTX loaded micelles did not show any sign of inflammation, but mice injected with free drug showed local inflammation at the site of injection.[22] The results demonstrated that PEG-*b*-pHPMAmDL micelles will be a promising delivery system.

pH- and temperature-responsive polymeric micelles

Multiresponsive micellar systems are attracting because various stimulating factors determine the controllable released profiles. Temperature- and pH-responsive micellar systems based on poly(NIPPAm) copolymers have been described. Müller and coworkers prepared poly(*N*-isopropylacrylamide)-*block*-poly(acrylic acid) (PNIPAAm-*b*-PAA), which is responsive to both temperature and pH. CP measurements revealed the formation of large aggregates at pH 4.5 and temperatures above the LCST, whereas micelles formed at pH 5−7 above the LCST.[104] Yang and coworkers[105] reported on poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide-*co*-10-undecenoic acid) P(NIPAAm-*co*-DMAAm-*co*-UA) as the pH- and temperature-responsive micelles. Dual-responsive micelles exhibited a pH-dependent LCST. These nanoparticles exhibited a stable structure in nominal physiological environment but deformed and precipitated in acidic environment, releasing the enclosed drug molecules. Zhu and coworkers[106] synthesized diblock copolymer poly(*N*-isopropylacrylamide)-*block*-poly(4-vinylpyridine) (PNIPAM-*b*-P4VP), which responds to both temperature and pH stimuli in aqueous solution. When the temperature was increased to 50°C at pH 2.8, the copolymer associated into spherical core–shell micelles, with the PNIPAM segment forming the core and the P4VP segment forming the shell. When the pH was increased from 2.8 to 6.5 at 25°C, the copolymer associated into spherical core–shell micelles with the core formed by the P4VP segment and the shell formed by the PNIPAM segment. Jiang and coworkers[107] synthesized P(*N*-isopropylacrylamide-*co*-acrylic acid)-*b*-polycaprolactone (P(NIPAAm-*co*-AA)-*b*-PCL) in the form of particles that were sensitive to both dual pH and temperature. Ma and coworkers[108] reported on the formation of dual-responsive micelles by the self-assembly of a diblock copolymer of poly(t-butyl acrylate-*co*-acrylic acid)-*b*-poly(*N*-isopropylacrylamide) (P(tBA-*co*-AA)-*b*-PNIPAM). Water is an effective solvent of PNIPAM and PAA at room temperature but a poor solvent of PtBA. The hydrophobic PtBA segments associate with each other to form a dense core that is protected by the longer soluble PAA/PNIPAM segments. Accordingly, as the temperature is increased, core–shell micelles are transformed into core–shell–corona micelles with PtBA at their core, with collapsed PNIPAM as the shell and soluble PAA as the corona.

The authors' group has developed a mixed micellar system based on graft-diblock and diblock–diblock copolymers and responds to pH and temperature. Mixed micelles were prepared from a pH- and temperature-sensitive graft copolymer, poly(*N*-isopropyl acrylamide-*co*-methacryl acid)-*g*-poly(D,L-lactide) (P(NIPAAm-*co*-MAAc)-g-PLA), a diblock copolymer methoxy poly(ethylene glycol)-*b*-poly(D,L-lactide) (mPEG-*b*-PLA) [Fig. 4(A)].[26,32] Doxorubicin was incorporated into the inner core of mixed micelles by dialysis. A drug release study determined that a change in pH (from pH 7.4

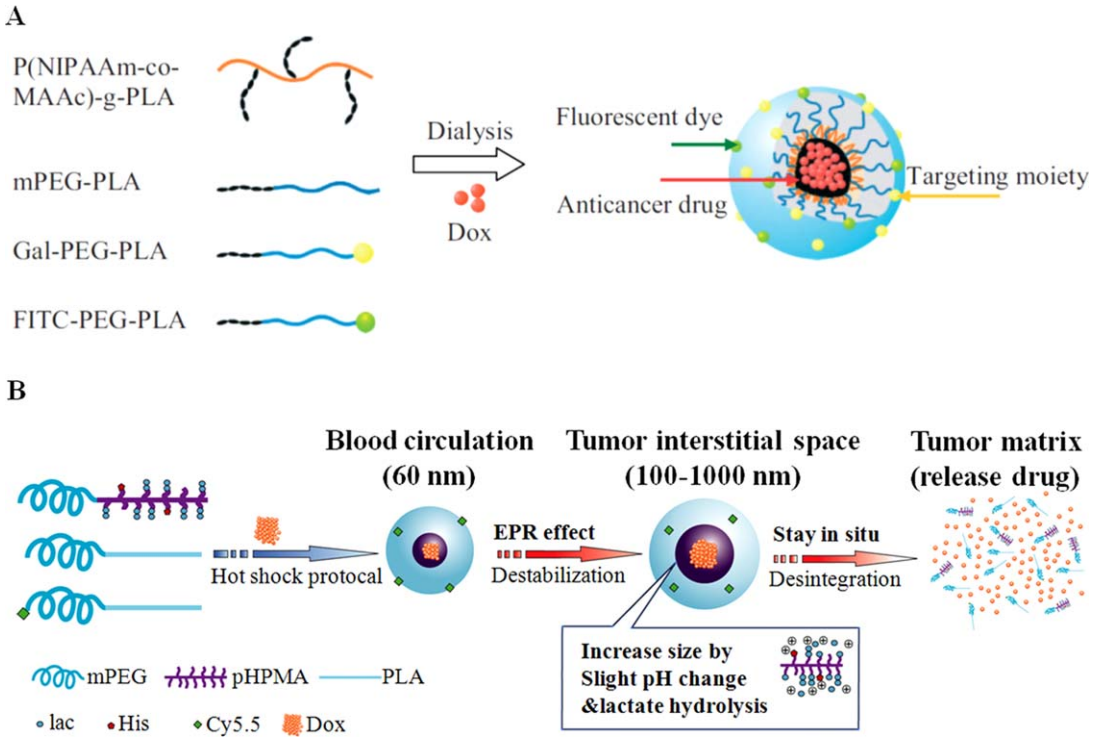

FIGURE 4. A, Schematic representation of multifunctional micelle structure made of a graft copolymer, a diblock copolymer and two functionalized diblock copolymers.[26] B, Schematic representation of mixed micelle composed of the diblock copolymers mPEG-*b*-P(HPMA-Lac-*co*-His), mPEG-*b*-PLA, and Cy5.5-PEG-PLA and loaded with doxorubicin.[76] The dual-responsive drug carrier is designed with stealth behavior in blood circulation. Micelle permeated through tumor interstitial space and maintained accumulation in tumor matrix. Finally, dual responsive micelle disintegrated and release the anticancer drug rapidly in tumor matrix. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

to 5.0) deformed the structure of the inner core from that of aggregated P(NIPAAm-*co*-MAAc), releasing a significant amount of doxorubicin. The other class of mixed micelles included a biocompatible diblock copolymer, mPEG-*b*-PLA, and a pH and temperature-sensitive diblock copolymer, methoxy poly(ethylene glycol)-*block*-poly(*N*-*n*-propylacrylamide-*co*-vinylimidazole) (mPEG-*b*-P(NnPAAm-*co*-VIm)), which was prepared using the hot shock protocol.[11] The conformational change of mPEG-*b*-P(NnPAAm-*co*-VIm) associated with a variation in temperature can influence micellar structure and stability. To improve the micellar stability of temperature-sensitive diblock copolymers, a CMC diblock copolymer, mPEG-*b*-PLA was introduced into the micellar structure to reduce the mobility of the temperature-sensitive diblock copolymers, even at temperatures below the CP of mPEG-*b*-P(NnPAAm-*co*-Vim). At pH 5.0, mixed micelles were strongly pH-sensitive and released almost 40 wt% of the doxorubicin in the first 24 h at 37°C, because imidazole group protonation deformed the micellar structure. The mixed micelle release profiles in pH 5.0 and pH 7.4 buffer solutions were also similar to those of the mPEG-*b*-P(NnPAAm-*co*-VIm) micelles, suggesting that mPEG-*b*-PLA in mixed micelles caused only a slight structural dissociation. Such a micellar system based on poly(NIPPAm) copolymers may have limited applicability because drugs released by hypothermia. The temperature-responsive segment poly (HPMAm-lactate) has great potential for delivering anticancer drugs because its hydrophobic lactate side chains are hydrolyzed at physiological temperature.[101] A mixed micelle system was established using a pH and temperature-sensitive diblock copolymer, methoxy poly(ethylene glycol)-*b*-poly(*N*-(2-hydroxypropyl) methacrylamide dilactate)-*co*-(*N*-(2-hydroxypropyl) methacrylamide-*co*-histidine) (mPEG-*b*-P(HPMA-Lac-*co*-His)) and a diblock copolymer mPEG-*b*-PLA with a low critical micelles concentration (CMC)[76,109] [Fig. 4(B)]. Particle size and stability of the mixed micelle were controlled by the copolymer content and was fine-tuned to suit the extracellular pH of the tumor. Therefore, micelles cannot diffuse throughout the tumor matrix due to the increased particle size in the tumor interstitial spaces. The *in vivo* results demonstrated a clear distribution of doxorubicin-loaded mixed micelles in tumor site and anticancer drug efficient targeting of tumor sites. We anticipate micelle systems provide a strategy for a promoting further assess the applicability of nanoparticles for cancer treatment.

Other stimuli responsive polymeric micelles

The release of an encapsulated anticancer drug can be triggered not only by pH and/or temperature, but also the other stimuli, such as light and ultrasound. The light-responsive behavior of micellar systems depends on the photochromic molecule that is attached to the polymeric backbone or to the end of the chain. Suitable photochromic

molecules, such as azobenzene,[110–112] spiropyran,[113] or stilbene[114] are incorporated into copolymers to make them susceptible to light stimuli to enable the carrier molecules to be controlled. The solubility of light-responsive segments changes under irradiation. Consequently, light-responsive micelles ideally release their encapsulated drug only upon exposure to ultraviolet, visible, or near-infrared light. Light-responsive copolymers that contain azobenzene groups have been extensively studied. For example, Kopecek and coworkers[110] synthesized *N*-(2-hydroxypropyl) methacrylamide copolymers that contained photochromic azobenzene side chains. Azobenzene can adopt an ultraviolet irradiation-induced cis-to-trans configuration that revealed the difference between the solubilities of water and oil phases, which changes its solubility in water. The related results indicate that metastable aggregates that are prepared by a gradual increase in copolymer concentration rapidly dissociated in response to ultraviolet irradiation. Zhao and coworkers[111] prepared light-responsive micelles that were based on azobenzene-containing polymethacrylate and poly(acrylic acid) (PAzoMA-*b*-PAA) copolymers. Following ultraviolet and visible light irradiation in solution, adding water to a dioxane solution of PAzoMA-*b*-PAA caused a reversible trans-to-cis change in the morphology of the micellar aggregates. One light-responsive strategy for cancer therapy is photodynamic therapy, which involves the use of a photosensitizer. Unlike chemotherapy, photosensitizer is a noninvasive therapy and has a less harmful effect on healthy tissue. The function of the photosensitizer in photodynamic therapy is to absorb a wide range of wavelengths and convert oxygen to highly reactive oxygen species (ROS) to destroy the targeted tumor cells.[115] Kataoka and coworkers[116] investigated polyion complex micelles that comprised PEG-poly(L-lysine) block copolymer for delivering dendrimer porphyrins. Irradiation under an Hg lamp (436 nm) improved the photocytotoxicity of dendrimer porphyrins-loaded micelles remarkably over that of the free drug. Zhang and coworkers[117] prepared porphyrin and galactosyl-conjugated micelles using the amphiphilic copolymer galactosyl and mono-aminoporphyrin (APP) with incorporated poly(2-aminoethyl methacrylate)-polycaprolactone (Gal-APP-PAEMA-PCL). Following treatment with these micelles, human hepatocellular liver carcinoma (HepG2) was exposed to light from a 150 W xenon lamp and filtered through a 400–700 nm long-pass filter for 20 min. The results thus obtained revealed high targeting and photodynamic therapeutic efficacy in HepG2 cells. The authors' group has developed a series of micellar systems for photodynamic therapeutic drug delivery, such as mPEG-*b*-P(HEMA-*co*-histidine-PLA),[81] PEG-*b*-p(MEA-*co*-VIm),[82] P(VCL)-*g*-PLA/PEG-*b*-PLA, and P(VCL-*co*-VIM)-*g*-PLA/PEG-*b*-PLA micelles.[83] P(VCL-*co*-VIM)-*g*-PLA/PEG-*b*-PLA micelles were incubated with adenocarcinomic human alveolar basal epithelial A549 cell for 24 h, and then treated with photoirradiation for 5, 10, 15, and 20 min under a 300 W halogen lamp (400–700 nm). ROS can be formed in both pH- and non-pH-responsive micellar systems. A dramatic difference in the phototoxicity of cancer cells achieved using these two systems) was observed in the cell viability results. Furthermore, PPIX accumulated in the nucleus when PPIX was loaded in the pH-responsive micelles after photochemical internalization.

A strategy for cancer therapy that is based on anticancer drug encapsulation in a micellar system, followed by controlled release at the tumor site that is triggered by ultrasound focused on the tumor, is developed. Ultrasound can induce the release of the drug from micellar system and target carriers to the tumor site.[118] Ultrasound of various frequencies can be applied to various parts of the tumor site. High-frequency ultrasound allows sharper focusing than low-frequency ultrasound but does not penetrate as deeply into the interior of the body. Owing to the measured decrease in the fluorescence intensity, doxorubicin is released from micelles under continuous wave or pulsed ultrasound in the frequency range of 20 kHz to 3 MHz.[119] Rapoport and coworkers[120] utilized high-frequency ultrasound (1 MHz) as an external trigger of the release of doxorubicin from Pluronic micellar systems. The results indicated that the intracellular uptake of doxorubicin increased from micellar solution after brief (15–30 s) sonication. They used low-frequency ultrasound (69 kHz) for 10 min; the cytotoxicity of the drug-loaded micelles was 53% against multidrug resistant cancer.[118] The cytotoxicity of the free drug against the multidrug-resistant cancer was only 15% without ultrasound treatment. The *in vivo* results markedly reduced the size of the colon cancer model tumor below that of the nonsonicated controls. Subsequently, Rapoport and Mohan[121] prepared PEG-*b*-PCL micelles and PEG-*b*-PCL-stabilized perfluorocarbon nanodroplets by applying ultrasound as an external trigger. Their results revealed that ultrasound induced doxorubicin trafficking into the cell nuclei, in the presence of nanoemulsions that are converted into microbubbles under ultrasound treatment. Microbubble cavitation occurs in the transient permeabilization of both plasma and nuclear membranes, increasing the therapeutic efficacy of doxorubicin-loaded nanodroplet systems. Burt, Chiao, and coworkers[122,123] reported upon the enhanced cytotoxic effects of an anticancer drug under the optimal single 10 s exposure to ultrasound in burst-mode (4 MHz, 32 W/cm^2) in prostate cancer cells PC3 and human umbilical cord. Ultrasound treatment increased the uptake of PTX-loaded PEG-*b*-PLA micelles by PC3 cells by between 50 and 160% for various concentrations of the drug. These results demonstrated that exposure to ultrasound allowed for the enhanced uptake of both hydrophilic agents (rhodamine R123, doxorubicin hydrochloride and mannitol) and hydrophobic agents (rhodamine R6G and PTX).

MULTIFUNCTIONAL MIXED MICELLES

The micellar system that was formed by mixing copolymers with different properties can be fine-tuned to satisfy the specific requirements of various applications, such as longevity, targetability, and imaging properties. Mixing a copolymer with the other copolymer which possessed a lower

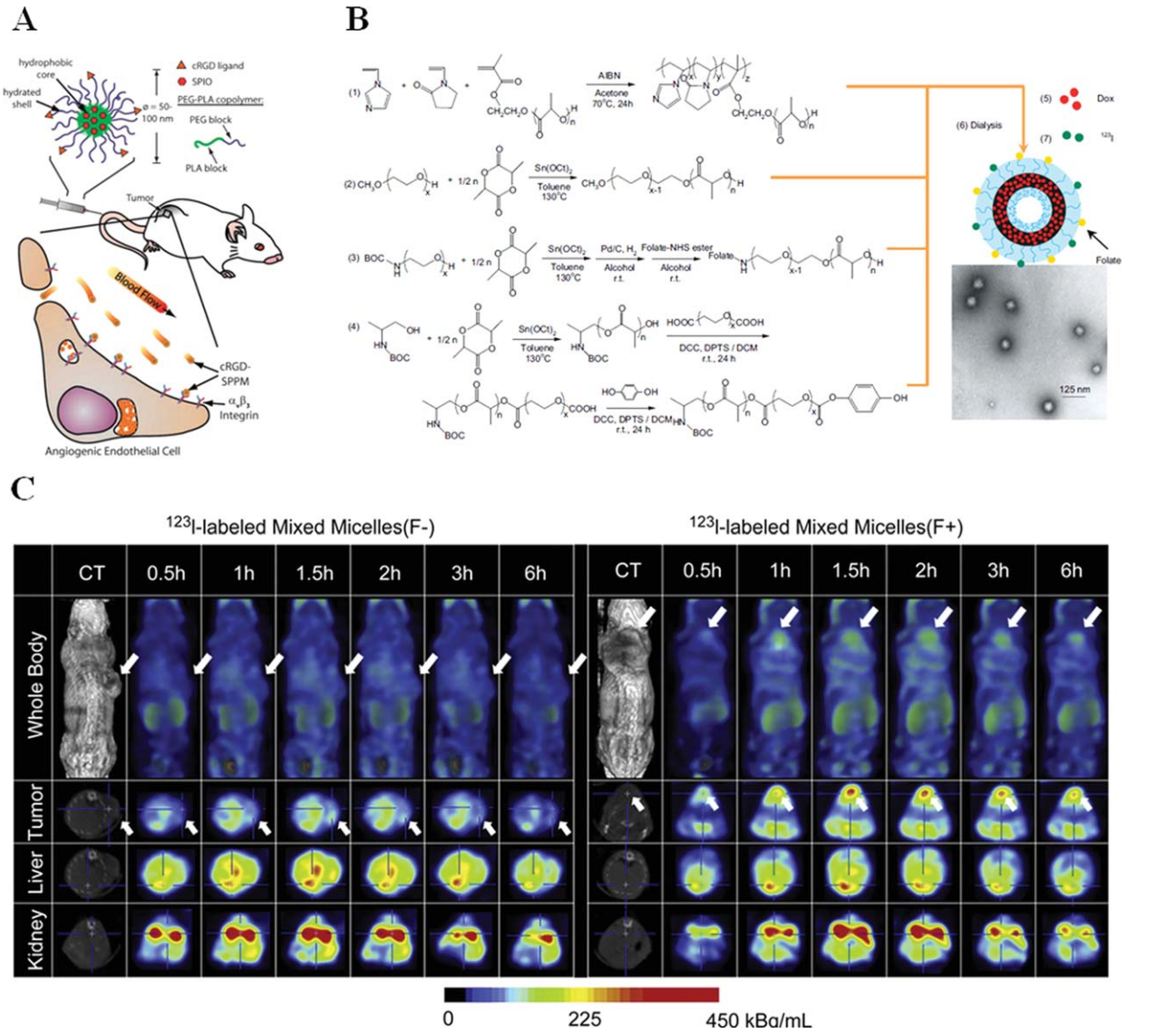

FIGURE 5. Multifunctional mixed micellar system for targeted drug delivery. A, Schematic illustration of a cRGD-encoded superparamagnetic polymeric micelles and its targeting to αvβ3-expressing endothelial cells in the tumor vasculature.[126] B, Representation of multifunctional micellar system that contains anticancer drug, a radiotracer and a targeting molecule and its TEM image.[45] C, SPECT images showing the biodistribution of ^{123}I-labeled nanoparticles (mixed micelles F−) and ^{123}I-labeled multifunctional hollow nanoparticles (mixed micelles F+) in Balb-c/nude mice bearing HeLa tumors. Arrows indicate areas of tumor cell deposit.[45]

CMC prevents dissociation of the micellar system.[11,76] The system can maintain the particles in the blood for a longer period, increasing their uptake in the peritumoral tissue by the EPR effect. As indicated above, targeting ligands can attach to the surface of micelles to support active targeting. In using anticancer drug micellar systems for diagnostic and/or imaging purposes in tumor therapy, the contrast and imaging moieties can be modified to multifunctionalized copolymers. Multifunctional mixed micellar systems with combined properties have recently been investigated extensively. For example, Bae and coworkers[79,124] prepared a mixed micellar system that was based on poly(ethylene glycol)-*b*-poly (L-histidine-*co*-L-phenylalanine) (PEG-*b*-His-*co*-Phe), PEG-*b*-PLLA and PEG-*b*-PLLA-folate. Mixing in more than 40% PEG-*b*-PLLA eliminated the pH dependence of the micellar system. Therefore, Bae and coworkers utilized L-phenylalanine (Phe), which was introduced into the histidine segment. The fluorescent probe Cy 5.5 was reacted with primary amines of poly(benzyl-His) and conjugated on the micelles for *in vivo* imaging. A noninvasive imaging and biodistribution study revealed the prolonged circulation of the drug carrier, tumor-selective accumulation, and intracellular drug delivery. Gao and coworkers[125,126] described the multifunctional micellar system that was based on PEG-PLA copolymers and functional PEG-PLA copolymers that target cancer via $\alpha_v\beta_3$ integrins [Fig. 5(A)]. They utilized the cyclic Arg-Gly-Asp (cRGD) ligand which can target $\alpha_v\beta_3$ integrins against tumor endothelial cells and then induce receptor-

TABLE I. Some Selected Multifunctional Nanomicellar Systems for Drug Delivery

Copolymers	Cargo	Stimuli Responsive	Targeting Ligands	Image Moiety	Ref.
PEG-*b*-PLA	Paclitaxel, etoposide, docetaxel, 17-AAG	Biodegradable	–	–	57–59,61
Acetal-PEG-*b*-PLA	Docetaxel	Biodegradable	Tyrosine, tyrosyl-glutmic acid	^{125}I	60
COOH-PEG-*b*-PLGA	Docetaxel, paclitaxel	Biodegradable	RNA aptamer DNA aptamer	–	63–66
PEG-*b*-PCL	Paclitaxel, rapamycin	Biodegradable	Folate	–	67–70
PEG-*b*-PLLA and P(HEMA)-*b*-p(His)	Doxorubicin	pH responsive	Folate	–	78,79
P(HEMA)-*b*-p(His)	Doxorubicin	pH responsive		–	80
PEG-*b*-PLA and HEMA-*co*-his)-g-PLA	Doxorubicin	pH responsive	Folate	Cy 5.5	30
PEG-*b*-PLA and P(NVI-*co*-NVP)-*g*-PLA	Doxorubicin	pH responsive	Folate	^{123}I	45
PEG-*b*-PLA and P(VCL-*co*-VIM)-g-PLA	Protoporphyrin IX	pH responsive	–	Cy 5.5	83
mPEG-*b*-P(HEMA-*co*-histidine-PLA	Porphyrin	pH responsive	–	^{99m}Tc	81
PEG-*b*-p(MEA-*co*-VIm)	Porphyrin	pH responsive	–	–	82
PEOz–PLLA	Doxorubicin	pH responsive	–	–	84
PLLA-PEOz-PLLA	Doxorubicin	pH responsive	–	–	85
PEG-p(Asp-Hyd-ADR)	Doxorubicin	pH responsive	–	–	86
PEO-PAGE	Doxorubicin	pH responsive	–	–	87
PEG-p(Asp-Hyd-LEV-PTX) and PEG-p(Asp-Hyd-4AB-PTX)	Paclitaxel	pH responsive	–	–	88
PEO-*hb*-PG	Doxorubicin	pH responsive	–	–	89
POEGMEMA-*b*-PHEMA	Platinum	pH responsive	–	–	90
P(NIPAAm-*co*-DMAAm)-*co*-PLA P(NIPAAm-*co*-DMAAm)-*co*-PCL P(NIPAAm-*co*-DMAAm)-*co*-Poly(LA-*co*-CL)	Doxorubicin	Temperature responsive	–	–	96,97
PEG-*b*-P(NIPAAm) PEG-*b*-poly(NIPAAm-*co*-HPMAm-lactate))	–	Temperature responsive	–	–z	98,99
PEG-*b*-pHPMAmDL	Paclitaxel, photosensitizers superparamagnetic iron oxide	Temperature responsive	–	–	22,31,102,103
PNIPAAm-*b*-PAA	–	pH responsive and temperature responsive	–	–	104
P(NIPAAm-*co*-DMAAm-*co*-UA)	Doxorubicin	pH responsive and temperature responsive	–	–	105
PNIPAM-*b*-P4VP	–	pH responsive and temperature responsive	–	–	106
P(NIPAAm-*co*-AA)-*b*-PCL	Paclitaxel	pH responsive and temperature responsive	–	–	107
P(tBA-*co*-AA)-*b*-PNIPAM	–	pH responsive and temperature responsive	–	–	108
mPEG-*b*-PLA and P(NIPAAm-*co*-MAAc)-g-PLA	Doxorubicin	pH responsive and temperature responsive	Galactosamine	FITC	26,32
mPEG-*b*-PLA and mPEG-*b*-P(NnPAAm-*co*-VIm)	Doxorubicin	pH responsive and temperature responsive	–	–	11
mPEG-*b*-PLA and mPEG-*b*-P(HPMA-Lac-*co*-His)	Doxorubicin, rapamycin	pH responsive and temperature responsive	–	Cy5.5	76,109

mediated endocytosis for the uptake of doxorubicin by cells. They loaded a cluster of superparamagnetic iron oxide nanoparticles inside the micellar core to ensure a favorable magnetic resonance imaging contrast. The integrated diagnostic and therapeutic design of the mixed micellar system supports the image-guided, target-specific treatment of lung cancer.

The authors' team has been developing a multifunctional mixed micelles system using diblock-graft[26,29,30,32,45,83] and diblock–diblock[11,76] copolymers. Our team has prepared multifunctional micellar systems for cancer cell targeting, distribution imaging, and anticancer drug delivery from an environmentally sensitive graft or diblock copolymer, a diblock copolymer mPEG-PLA and functionalized diblock copolymers. The mixed micelle has a multifunctional inner core that enables intracellular drug delivery, and an extended hydrophilic outer shell of mPEG to hide the inner core. Stability analysis of the mixed micelles in BSA solution demonstrated that the diblock copolymer mPEG efficiently protected the BSA that was adsorbed on the mixed micelles because the hydrophobic groups were efficiently screened by mPEG. The mixed micellar systems respond to the environmental in a way that makes them suitable for the intracellular drug delivery of anticancer drugs[11,26,29,30,45,76,109] and photosensitizers.[83] Micelle-conjugated galactosamine[26] and folate[30,45] as active targeting moieties ensure high intracellular and intratumoral accumulation. To diagnose the biodistribution of, the outer shell of micellar systems comprised modified functional moieties, such as fluorescein isothiocyanate,[26] Cy5.5,[30,76,82,83] or isotope ^{123}I.[45] Our group has prepared multicompartment micellar systems that contain (1) an anticancer drug, (2) an environmentally responsive core, (3) an image moiety, and (4) a targeting molecule[30,45] [Fig. 5(B,C)]. The mixed micellar strategy makes the multifunctional micellar system that contains both a drug-loaded core and a flexible shell an ideal platform for treating, imaging, and targeting tumors for anticancer drug delivery.

CONCULSIONS

In the past decades, advances in technologies and new functional materials allow scientist to exploit the increased understanding of drug delivery systems. Multifunctional nanomicellar systems are extensively studied using a single copolymer or multiple copolymers (Table I). Nanomicellar systems can be developed to be drug carriers because of their tumor targeting abilities, stimuli release of the entrapped drugs and high versatility. This review considered the role of biodegradable, pH and temperature responsive and other stimuli responsive micellar systems for targeted anticancer drug delivery. In addition, multifunctional micelles formed by mixing copolymers with distinctly different properties can be fine-tuned to meet the specific requirements of various applications. Mixing a copolymer PEG-*b*-PLA not only prevents dissociation, but also reduces the reduce cost of materials. This can maintain the particles in the blood longer, increasing their uptake in peritumoral tissue by the EPR effect. A number of investigates have shown that micellar systems can provide significant advantages for cancer therapy. This review presented that multifunctional nanomicellar systems have promising features as carriers for anticancer drugs owing to their superior performance in the treatment of cancer.

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