Expert Opinion

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Overcoming the barriers in micellar drug delivery: loading efficiency, *in vivo* stability, and micelle–cell interaction

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Importance of the field: Spontaneously constructed from block copolymers in aqueous media, the polymer micelle has been extensively studied as a potential carrier of poorly water-soluble drugs, but cellular uptake pathways and stability of micelles in blood have not yet been clearly understood. An in-depth insight into the physical and biological behaviors of polymer micelles is necessitated for designing next-generation micelles.
Areas covered in this review: This review suggests possible solutions to improve micellar drug loading capacity, scrutinizes the parameters influencing the micelle stability in blood, and also discusses the fate of micelles in cellular and *in vivo* environment, respectively. Direct and indirect evidences from the literatures mostly published after 90's were collected, analyzed and summarized.
What the reader will gain: A critical analysis of micelle's stability *in vivo* and micelle-cell interaction is provided to highlight the key issues to be addressed to affirm that micelle can properly work as a drug carrier in clinical settings.
Take home message: With a clear understanding of its behaviors in biological environment, the polymer micelle is a promising nanocarrier for chemotherapy.

Keywords: block copolymers, cellular uptake, drug delivery, molecular imaging, polymer micelles, stability

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1. Introduction

A polymer micelle is a nanoparticle structured by one hydrophilic shell and one hydrophobic core. It can be divided into two main categories: hydrophobically assembled micelles and polyion-complex micelles [1]. The former ones usually consist of amphiphilic copolymers with a hydrophobic block and a hydrophilic block. Balance between those two blocks in an aqueous medium induces spontaneous formation of nano-sized particulates. For most block copolymers, poly (ethylene glycol) (PEG) is used as a hydrophilic block. Different micelle properties originate from the nature of hydrophobic core-forming materials, which include biodegradable polyesters such as poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL), and poly(glycolic acid) (PGA) [2]. The polyion-complex micelles have used charged polymer blocks, such as poly(ethyleneimine) (PEI), poly(aspartic acid) and poly(L-lysine) (PLL), to deliver either therapeutic nucleic acids (e.g., oligodeoxynucleotide, plasmid DNA) or oppositely charged protein drugs [3,4]. In this review, efforts are made to scrutinize the current state of the art of the hydrophobically assembled micelle, which is referred as 'polymer micelle' from now.

Almost one-third of newly discovered drugs are highly insoluble in water, but there is no standard method to solubilize such drugs [5]. As a result of the capability to load lipophilic molecules into the hydrophobic core, polymer micelles have been

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Article highlights.

- Use of conventional polymer micelles in clinic has been dampened by inherent problems of micelles including limited capacity of drug loading, poor stability in blood and lack of understanding on interactions with cells.
- The drug loading capacity of polymer micelles can be significantly enhanced only when the hydrophobic effect between polymers and drugs, which has been considered as the only mechanism to load poorly soluble drugs, is combined with other interactions such as hydrogen bonding, electrostatic interaction and dipole-dipole interaction.
- The stability of physically assembled polymer micelles in blood has significance, in that even if targeting ligands are conjugated, micelles may not be able to transport sufficient amount of drugs to the targeted tissue simply by drug desertion during systemic circulation.
- The micelle-cell interaction, which lays one of the most fundamental and significant bases for micellar chemotherapy but remains poorly understood, should be continuously investigated to develop next-generation micelle systems to provide better therapeutic efficacy.
- In addition to understanding the current problems, it is important not only to utilize the physical properties of micelles (e.g. stimuli-sensitive micelles), but also to overcome thehurdles (e.g. crosslinked micelles, unimolecular micelles).

This box summarizes key points contained in the article.

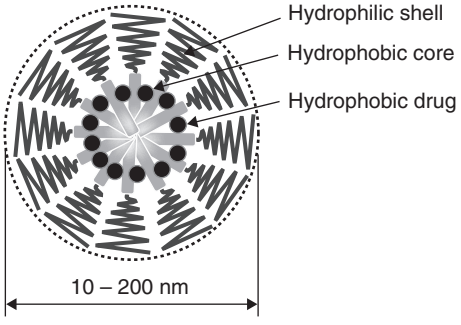

Figure 1. Schematic of a hydrophobically assembled polymer micelle. The hydrophobic core loading lipophilic drugs is protected from the environment by the hydrophilic shell.

widely used to solubilize and deliver poorly water-soluble drugs (**Figure 1**). Besides the solubilizing power, the micellar drug carriers have several important properties. First, the hydrophilic corona creates a highly water-bound barrier, which blocks the adhesion of opsonins [6]. Second, owing to the nanoscale size (10 – 200 nm in diameter), micelles retard the rate of body clearance by renal filtration and the reticuloendothelial system (RES). These properties significantly increase the blood residence time of micelles and allow them to permeate through the leaky inflamed blood vessels [7]. Third, imaging contrast agents and multiple drugs can be integrated into a single vehicle, allowing both diagnosis and therapy [8]. Finally, surface modification of the vehicles by specific ligands enhances the targeting efficiency and reduces the toxicity to normal cells in chemotherapy [9].

Table 1 contains the polymer micelles that have been examined in clinical phases. In spite of seductive advantages, only four micelle systems have been clinically examined [10-16]. To make micelles a reliable carrier for cancer therapy, it is important to find out the major challenges for translating polymer micelles from academic research to clinical application. In this regard, it is essential to revisit the inherent problems of polymer micelles. Polymer micelles should overcome three major hurdles to achieve a maximal therapeutic effect: the low drug loading efficiency, the poor blood stability after injection, and the difficulty in transporting through cell membranes (**Figure 2**). This review aims to discuss executive strategies being developed to overcome such problems.

2. Drug loading capacity of polymer micelles

2.1 Theories of drug solubilization by micelles

The first-generation polymer micelle served as an excipient to solubilize (or load) highly lipophilic drugs. One representative polymer micelle is composed of PEG-*b*-poly(D,L-lactic acid) (PDLLA or PLA) for paclitaxel (PTX) solubilization (see **Table 1**). The loading capacity is ~ 10 – 20% (wt/wt) [17]. Such conventional polymer micelles have only a passive role, the enhancement of drug solubility in water, which is derived from hydrophobic interaction between hydrophobic polymer blocks and drugs [2]. The hydrophobic interaction, more exactly hydrophobic effect, is a phenomenon induced by the London dispersive force that exists between any kinds of molecule. The hydrophobic effect is provoked when hydrophobic molecules are mixed with water, because the London dispersive force between lipophilic drugs and hydrophobic blocks is much stronger than that between the lipophilic drug and water.

Although the hydrophobic effect is a major driving force, drug loading capacity and efficiency also depend on the miscibility between polymers and drugs. To explain the mechanism of drug loading into a polymer micelle, the Hildebrand–Scatchard solubility parameter (δ), $\delta = \sqrt{(\Delta E_{vap}/V)}$, is often used, where ΔE_{vap} is the energy of vaporization and V is the molar volume of the solvent [18]. As loading drugs into a polymer micelle means mixing of the polymer with drugs, the loading capacity can be described by the Flory–Huggins theory, expressed by $\chi_{drug\text{-}polymer} = (V_{drug}/RT)(\delta_{drug} - \delta_{polymer})^2$, where $\chi_{drug\text{-}polymer}$ is the Flory–Huggins interaction parameter between the drug and the polymer, V_{drug} is the volume of the drug, R is the ideal gas constant, T is the temperature, and δ_{drug} and $\delta_{polymer}$ are the Hildebrand–Scatchard solubility parameters of the drug and the polymer, respectively [19,20]. The above equation describes the miscibility between polymers and drugs.

Letchford and colleagues investigated the miscibility of PEG-*b*-PCL with five different drugs [21] and observed that etoposide, paclitaxel, plumbagin, curcumin and indomethacin

Table 1. Polymer micelles in clinical studies.

Polymer (excipient)	Trade name	Clinical phase	Patients	Pharmacokinetic parameters					Ref.
Block ratio	**Drug**		**Dose**	**C_{max} (ng/ml)**	**AUC (ng*h/ml)**	**CL_T (l/(h m^2))**	**$t_{1/2}$ (h)**	**Vd (l/kg)**	
PEG-*b*-PDLLA	Genexol®-PM	Phase II	Patients with solid tumors refractory to conventional chemotherapy and for whom no effective therapy existed (n = 3)	3107 ± 1476*	11,580 ± 4277	29.3 ± 13.8	11.4 ± 2.4	-	[10-12]
2000–1750	PTX		Intravenous infusion of 300 mg/m^2 in 500 ml 5% dextrose for 3 h every 3 weeks						
PEG-*b*-PAsp	NK105	Phase I	Patients with solid tumors refractory to conventional chemotherapy and for whom no effective therapy was available (n = 7)	40,170 ± 5533	369,800 ± 35,200	0.4086 ± 0.0373	10.6 ± 1.3	4.5 ± 1.6	[15,16]
12,000–8000	PTX		Intravenous infusion of 150 mg/m^2 in 5% glucose solution at a speed of 250 ml/h every 3 weeks						
PEG-*b*-PAsp-DOX	NK911	Phase I	Patients with metastatic or recurrent solid tumors refractory to conventional chemotherapy and for whom no effective therapy was available (n = 11)	-	3262.7 ± 425.2	6.7 ± 1.1	2.8 ± 0.3	14.9 ± 3.6	[14]
5000–4000–543	DOX		Intravenous infusion of 50 mg/m^2 for 1 – 6 min						
Pluronic® L67/F127	SP1049C	Phase I	Patients with histologically proven cancer refractory to conventional treatment or for which no suitable conventional therapy existed	-	1772 ± 265	-	2.79 ± 2.16	-	[13]
~ 2000/~ 12,600	DOX		Intravenous infusion of 50 mg/m^2 in 0.9% sodium chloride solution containing 0.25% (wt/vol.) L61 and 2% (wt/vol.) F127 at a rate of 2 ml/min once every 3 weeks						
(Cremophor EL/ethanol)	Taxol®	-	Patients with a diagnosis of recurrent or metastatic advanced solid tumor who had failed standard therapy (n = 12)	3543 (57)‡	12,603 (21)	20.5	14.8 (32)	443 (31)	[77]
	PTX		Intravenous infusion of 175 mg/m^2 for 3 h once every 3 weeks						
(Free DOX)	Adriamycin®	-	Patients with metastatic or recurrent solid tumors refractory to conventional chemotherapy and for whom no effective therapy was available (n = 7)	-	1620.3 ± 1062.9	14.4 ± 5.6	0.8 ± 1.1	-	[14]
-	DOX		Intravenous infusion of 50 mg/m^2 for 1 – 6 min						

*Standard error.

‡Per cent coefficient of variation.

AUC: Area under the curve; CL: Clearance; DOX: Doxorubicin; PAsp: poly(aspartic acid); PDLLA: Poly(DL-lactic acid); PEG: Poly(ethylene glycol); PTX: Paclitaxel; Vd: Volume of distribution.

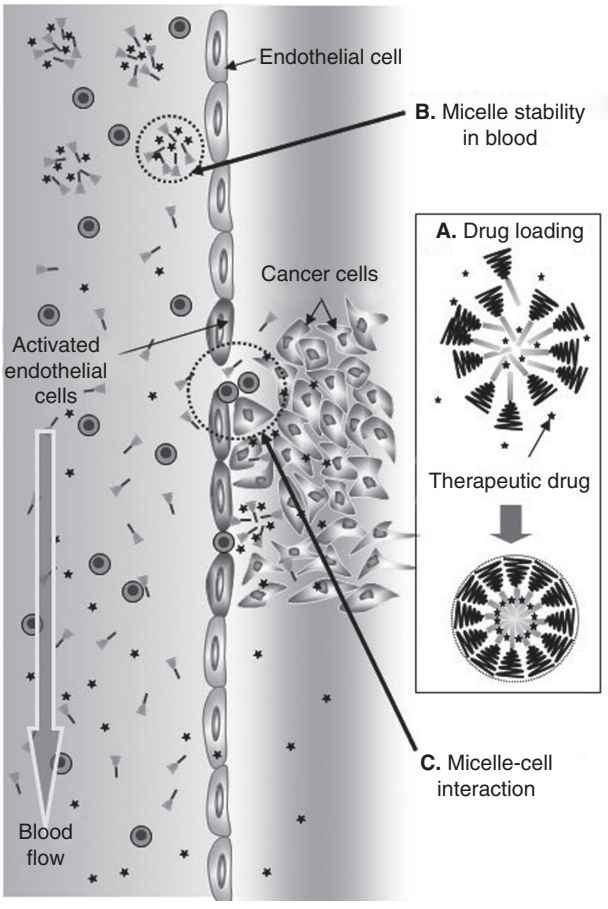

Figure 2. Three major problems in developing an effective micellar drug delivery system. A. Low drug loading content and efficiency. **B.** Poor stability in bloodstream. **C.** Cell membrane as a barrier for the intracellular drug delivery.

followed the ascending order of $\chi_{drug\text{-}polymer}$. As a lower value (< 0.5) of the Flory–Huggins parameter means a better solubility, indomethacin is the drug best solubilized in PEG-*b*-PCL micelle among the five. It should be noted that the hydrophobicity of each drug does not follow the order of the Flory–Huggins parameter, indicating that a hydrophobic effect is not the only mechanism to explain the efficiency of drug loading into polymer micelles. Similarly, Liu *et al.* examined the heat produced during mixing between 15 homopolymers and ellipticine, an anticancer drug [22]. The heat of mixing is another parameter to describe the miscibility between polymers and drugs. The drug loading efficiency was found to be highly dependent on the heat of mixing and the order was poly(benzyl-L-aspartate) (PBLA) > PCL > PDLLA > PGA. It is therefore obvious that the drug loading into a polymer micelle is not only forced by the hydrophobic effect, but also facilitated by other interactions between polymers and drugs to increase the miscibility.

Although the polymer–drug miscibility is apparently one of the most important parameters to govern the drug loading capacity of polymer micelles, the hydrophilic–lipophilic balance (HLB) of block copolymers is worth noting. For example, block copolymers with longer hydrophobic block showed better drug loading property, which was confirmed by determining the partition coefficient of drugs into PEG-*b*-PCL micelles [21]. On the contrary, a longer PEG chain deteriorates the partition coefficient. However, it may be very difficult and time-consuming to determine the miscibility of a given drug with various polymer micelles with different HLBs and molecular masses. If the miscibility of most drugs with one polymer micelle is significantly increased, the limitation of low drug loading capacity can be overcome. The hydrotropy described below may be one of the possible solutions.

2.2 Hydrotropy

The hydrotropy is a collective molecular phenomenon describing a solubilization process whereby the presence of large amounts of a second solute, called hydrotrope, significantly enhances the aqueous solubility of poorly soluble compounds [23,24]. Although the mechanism of hydrotropic solubilization has not been fully clarified, it has been explained in several ways, such as hydrophobic effect, hydrogen bonding and stacking interaction [23]. The hydrotropes form non-covalent aggregates only above a certain concentration, which is called the minimal hydrotrope concentration (MHC) [25]. As hydrotropes are small molecules containing both hydrophobic and hydrophilic moieties, the hydrophobic effect can be a driving force to generate aggregates such as surfactants. In addition, polar groups of the hydrophilic moieties can interact with drugs by means of hydrogen bonding [26]. Hypothetically, hydrotropes may break the hydrogen bonding between drugs that is considered as one of the drug crystallization mechanisms. In addition, most hydrotropes have an aromatic ring substituted by heteroatoms. Depending on the substituted atom species, the benzene rings can interact with each other and be stacked (π–π stacking) [27,28]. For example, nicotinamide, a representative hydrotrope, has a pyridine ring of a nitrogen-substituted aromatic ring and an amide group. The benzene ring is basically hydrophobic and the pyridine ring redistributes the π electrons over the ring. The amide group and nitrogen in pyridine ring may act as the hydrogen bonding donor and acceptor, respectively. As most poorly soluble drugs consist of one or multiple benzene rings and polar groups, the self-aggregation property of hydrotropes can be expanded to complexation between hydrotropes and lipophilic drugs [29,30].

The significance of multiple interaction parameters in describing the solubilization phenomenon is indicated in the linear solvent free energy relationship (LSER) equation [28],

$$\log SP = c + rR_2 + s\pi_2 + a\sum a_2 + b\sum \beta_2 + vV_x$$

where *SP* is the property of interest for a drug (i.e., partition coefficient), R_2 is the excess molar refraction of the solution

derived from the London dispersion force, π_2 is the drug dipolarity/polarizability, $\Sigma\alpha_2$ is the hydrogen bonding acidity of the drug, $\Sigma\beta_2$ is the hydrogen bonding basicity of the drug, and V_x is the McGowan's characteristic volume calculated from molecular structure. The *c*, *r*, *s*, *a*, *b* and *v* are regression coefficients. Based on the LSER theory, drug partition in two immiscible phases of water and micelle core-forming polymer is explained by transferring the free energy of drugs in water to that in polymer. This free energy is proportional to the sum of multiple independent interactions. Therefore, the LSER equation suggests that the important parameters to maximize the miscibility are hydrophobicity, electrostatic interaction, dipole–dipole interaction, hydrogen bonding and size of the drug.

When the miscibility between polymers and drugs is optimized, it is expected that much improved drug loading capacity of polymer micelles will be obtained. Introduction of the hydrotropy into polymer micelles is one solution to accomplish the optimized miscibility. As reported by Lee *et al.*, nicotinamide derivatives are excellent hydrotropes for PTX solubilization [31]. More than 60 candidates of hydrotrope were studied and the degree of solubility enhancement was examined. The poor solubility of PTX (0.3 μg/ml) was significantly enhanced up to 39 and 29 mg/ml by 3.5 M aqueous solutions of *N*,*N*-diethylnicotinamide (DENA) and *N*-picolylnicotinamide (PNA), respectively. A PEG-containing polymer micelle based on a polymerizable derivative of DENA, 4-(2-vinylbenzyloxy)-*N*,*N*-DENA (VBODENA), could load PTX up to 37% (wt/wt) with > 90% (wt/wt) of loading efficiency, while a conventional polymer micelle of PEG-*b*-PDLLA loaded ~ 20% (wt/wt) PTX [17]. Another polymer micelle consisting of PEG and poly[4-(2-vinylbezyloxy)-*N*-PNA] (PEG-*b*-PVBOPNA) also presented high drug loading capacity and efficiency with PTX [32]. Interestingly, the PEG-*b*-PVBOPNA spontaneously generates polymer micelles by simply adding PTX into the aqueous polymer solution, which provides an excellent opportunity for micellar drug formulation without using any solvent system. The chemical structures of the hydrotropes and the hydrotropic block copolymers are shown in **Figure 3**. However, there are no more reports about the effect of the hydrotropic polymer micelle on loading capacity and efficiency with other drugs. Further studies may lead to a substantial solution to overcome the low drug loading capacity of polymer micelles.

3. Micelle stability

3.1 Micelle stability in water

The second obstacle retarding the development of effective micellar drug carriers is the poor stability of micelles in an aqueous environment. Even though multiple interactions may coexist to improve the drug loading capacity, the polymer micelle is still a physically assembled structure. In aqueous medium, the micelle stability is influenced by many factors, such as polymer concentration, molecular mass of the core-forming block, and drug incorporation. It is well known that the micelle stability depends on the polymer concentration. A polymer micelle has a critical micelle concentration (CMC) that is the lowest concentration limit for polymers to produce a micelle structure [20]. When diluted below CMC, polymer micelles are gradually disintegrated into unimers. The value of CMC is determined primarily by the molecular mass (size) and the hydrophobicity of the core-forming block of copolymers. For example, poloxamer block copolymers decrease their CMC values by increasing the molecular mass of hydrophobic poly(propylene oxide) block [33]. Also, Attwood *et al.* found that different species of core-forming block led to different CMC values as a function of the degree of polymerization [2]. They showed that the CMC of block copolymer consisting of ε-caprolactone exceeds that of lactic acid, with that of polypropylene oxide being the least, in accordance with the hydrophobicity of the repeating units.

Polymer micelle is a dynamic structure. In addition to the thermodynamic aspect, the kinetic stability stems from the unimer exchange between micelles. The Aniansson–Wall equation describes the exchange rate between unimers and micelles [34]. The exchange rate could be experimentally determined by non-radiative energy transfer (NET), which is now known as the Förster resonance energy transfer (FRET). The FRET is a physical property of energy transfer from a donor dye to an acceptor dye. If both dyes exist within a range of Förster distance, non-radiative fluorescence from the excited donor dye can be effectively used as the excitation energy of the acceptor dye, resulting in emission of acceptor fluorescence [35]. The unimer exchange is determined not only by the explusion/insertion of unimers, but also by the fusion/split of micelles. Halioğlu and co-workers identified that, whereas the explusion/insertion of unimers occurs at a lower concentration of polymer, the fusion/split of micelles is the major mechanism of unimer exchange at higher concentration [36].

It should be noted that those studies on the kinetic stability were conducted without loading hydrophobic drugs. It is believed that incorporation of drugs makes polymer micelles more stable. As the CMC is proportional to the standard free energy and also the free energy is important to the miscibility between polymers and drugs (i.e., LSER equation), polymer micelles loading lipophilic drugs can be stabilized much more than blank micelles. Therefore, it is necessary to identify the unimer exchange rate in the presence of hydrophobic drug for clear understanding of the kinetic stability.

3.2 Micelle stability in biological environments

After systemic injections, polymer micelles become much diluted by blood. Furthermore, the polymer micelles are confronted with numerous blood components, such as proteins and cells. Savić *et al.* showed that a conventional polymer micelle of PEG-*b*-PCL is unstable in serum-containing culture media with or without cells, but stable in phosphate-buffered saline [37]. The polymer micelle that conjugated with a fluorophore at the end of the PCL segment was slowly disintegrated by incubation in the cell culture media, and the disintegration could be quantified by

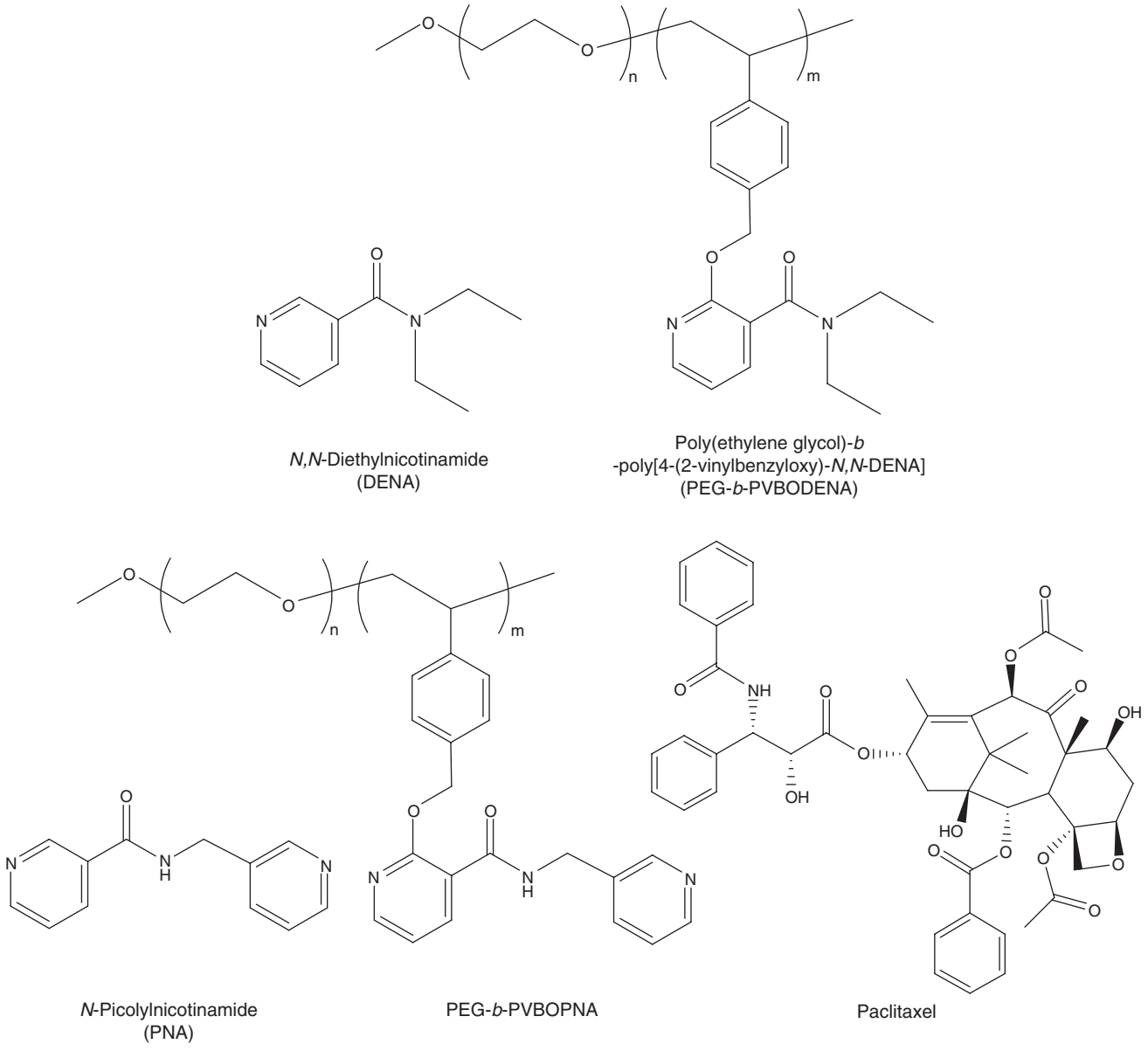

Figure 3. Chemical structures of hydrotropes and hydrotropic block copolymers that have been used to solubilize paclitaxel.

measuring free dye content out of hydrolytic degradation. Recently, Chen *et al.* demonstrated that another conventional polymer micelle made of PEG-*b*-PDLLA is not stable in blood [38]. The authors used the FRET technique to monitor the stability of polymer micelles in real time after intravenous administration. The micelle was loaded with a pair of FRET dyes. The FRET ratio detected in the blood vessels of the mice ears was reduced to half of the initial value at 15 min post intravenous injection, indicating that the dyes were released from the core of the micelles. It was further revealed that the main components responsible for the release of lipophilic dyes from micelles into the blood are α- and β-globulins rather than γ-globulin or serum albumin.

In polymer micelles, PEG is commonly used to construct the hydrophilic shell. Although PEG is known to be biocompatible, many researchers have reported interactions between PEG and proteins. According to Xia *et al.* PEG interacts with pepsin in buffer solutions, which was observed by the quasi-elastic and electrophoretic light scattering methods [39]. The interaction was mediated by hydrogen bonding between carboxyl groups of the protein and oxygen atoms in the PEG backbone. Interactions between PEG with other proteins such as α-chymotrypsin [40] and hen-egg-white lysozyme [41] were also reported. Indeed, it is not surprising that PEGs interact with serum proteins because PEG was used to detect soluble immune complexes caused by inflammatory diseases

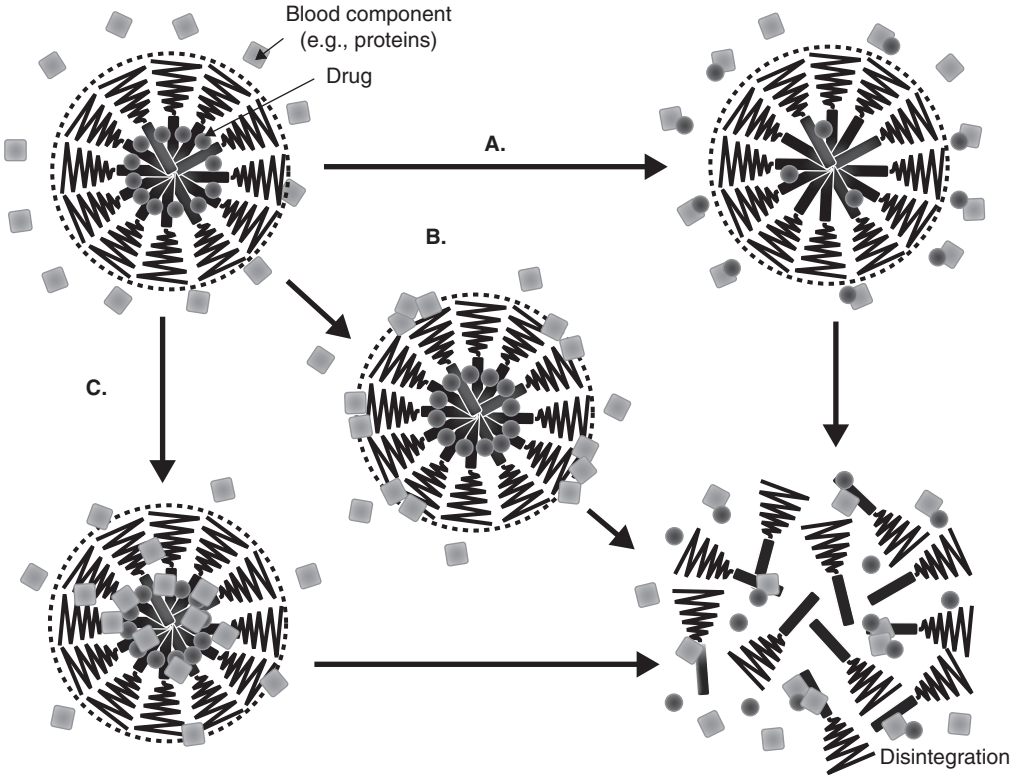

Figure 4. Three possible mechanisms of micelle disintegration in the presence of serum proteins. A. Drug extraction. **B.** Protein adsorption. **C.** Protein penetration.

such as systemic lupus erythrematosus (SLE) and rheumatoid arthritis (RA) [42]. The immune complexes are precipitated in the presence of PEG and the agglomeration is mediated by fibronectin. Serum albumin has also showed direct interaction with PEG [43]. The hydrogen bonding between PEG and albumin was revealed by Fourier transform infrared (FTIR) spectroscopy [44], and the van der Waals interactions between PEG and albumin were detected using binding force measurements [45]. Furthermore, crystal structure analysis of PEG–protein complexes showed that multiple coordination between PEG backbone and positively charged amino acids, that is, Lys, Arg, His, serves as another interaction force [46]. The formation of complex between PEG and proteins possibly induces micelle aggregation as well as unimer extraction, which may significantly affect the micelle stability *in vivo*.

There exists some evidence that proteins possibly penetrate the hydrophilic shell of the micelles. For example, it was observed that a micelle consisting of PEG-*b*-PCL block copolymer was slowly degraded in the presence of lipase K [47]. To hydrolyze ester bonds of PCL, the lipase should directly contact with PCL molecules. Therefore, the micelle degradation by lipase K implies that serum proteins seem to overcome the hydrophilic corona and reach the micelle core. Similarly, Chen *et al.* showed that enzymatic degradation of a polymer micelle composed of PEG-*b*-poly(3-hydroxybutyrate)-*b*-PEG (PEG-*b*-PHB-*b*-PEG) depended on enzyme (PHB depolymerase) concentration, polymer concentration and PHB block length [48]. In addition to the enzyme penetration, micelles can be degraded by enzymatic hydrolysis of unimers that had dissociated from micelles, as suggested by Carstens *et al.* [49].

Figure 4 summarizes the possible mechanisms accounting for the instability of micelle induced by serum proteins, including protein adsorption, protein penetration and drug extraction. Although the effect of drug extraction on micelle stability has not been investigated yet, the loss of drug from a micelle may inevitably provoke micelle disassembly because drug-containing polymer micelles are more stable than blank micelles.

3.3 Stimuli-sensitive micelles

In addition to the understanding of micelle stability under biological environments, manipulating the physical stability of polymer micelles provides an exciting opportunity to enhance the therapeutic effect. Polymer micelles advanced from conventional ones can actively respond to environmental signals, provoking changes of their physical stability [50]. A representative example is the pH-sensitive polymer micelle, which becomes destabilized and liberates drugs depending on the environmental pH. Cancer or inflammation makes the extracellular pH at the disease site acidic by means of hypoxia or over-producing metabolic wastes [51]. Also, intracellular

lysosome maintains an acidic condition to facilitate enzymatic degradation of entrapped molecules [52]. A therapeutic strategy aiming at such a low pH has utilized pH-sensitive polymers specifically to unload drugs at a diseased tissue/cell. Lee and colleagues developed a pH-sensitive polymer micelle consisting of PEG-*b*-poly(L-histidine) (PEG-*b*-PHis) [53]. The micelle was stable at physiological pH (7.4), but was rapidly destabilized by decreasing the pH to < 7. The PEG-*b*-PHis micelle containing doxorubicin (DOX) showed excellent cytotoxicity to MCF-7, a human breast cancer cell, with multi-drug resistance (MDR). Another target naturally presenting pH variation is the gastrointestinal tract [54]. Oral drug formulation must pass through acidic stomach (pH 1 – 2) and basic intestine (pH 8 – 9) along the tract; because nutrients as well as drugs are absorbed mostly in intestine, in this case it is desirable that a micellar formulation is stable at lower pH and becomes disintegrated at higher pH. As a result, the drug release can be completed within the gastrointestinal transition time (< 12 h). For this purpose, Kim *et al.* introduced acrylic acid into a hydrotropic polymer micelle [55]. The acrylic acid moiety is protonated and hydrophobic below pH 4.5, whereas it becomes ionized and hydrophilic at higher pH. Hydrotropic polymer micelles are considered to be stable because the hydrotropic block copolymer has multiple interactions with the loaded drug. Kim and colleagues showed that the acrylic acid moiety successfully promoted drug release (e.g., PTX) from PEG-*b*-PVBODENA micelles in simulated intestinal fluid (higher pH) rather than in simulated gastric fluid (lower pH) [55]. There exist many other forms of polymer micelles possessing sensitivity responsive to temperature, redox state, magnetic force, or ultrasound [50]. These polymer micelles suggest an alternative solution to overcome the stability problem of micelles under biological condition.

4. Micelle–cell interaction

If the hydrophilic corona is biologically inert, a polymer micelle hardly interacts with cell membrane. However, as discussed above, the hydrophilic shell of polymer micelles is not totally inert. It has been observed that polymer micelles enter cells by means of endocytosis. Allen *et al.* found that a polymer micelle of PEG-*b*-PCL was internalized, possibly by endocytosis [56]. They demonstrated that fluorescent dyes loaded in micelles are located inside cells after incubation. Cellular uptake of a tritiated drug, FK506, was also enhanced by using the micellar carrier. However, intracellular accumulation of dyes and labeled drugs is not direct evidence for the endocytosis of polymer micelle. By labeling the PEG-*b*-PCL polymer with a fluorescent probe, tetramethyl rhodamine B isothiocyanate (TRITC), it was reported that polymer micelles consisting of the dye-labeled polymers were successfully internalized with [57] or without loading drug [58].

The mechanism explaining endocytosis of polymer micelles has not been fully clarified. One possibility focuses on the role of the labeled dye. In fluorescence imaging studies, one should be cautious about the change of polymer property by fluorophore labeling. As the TRITC is positively charged, TRITC-labeled PEG-*b*-PCL also has positive charge [59]. It is known that positively charged macromolecules can be effectively internalized by electrostatic interaction with heparan sulfate on cell surface [60]. To revisit the endocytosis of micelles, a recent study by Chen *et al.* revealed that PEG-*b*-PDLLA micelles consisting of fluorescein isothiocyanate (FITC)-labeled PEG-*b*-PDLLA could not be endocytosed into cultured HeLa cells [61]. The authors performed a dual-color imaging experiment in which the copolymer was conjugated with FITC, and another dye, DiI, was physically incorporated into the micelles. As a result, the DiI was found inside cells within 30 min, whereas the FITC remained outside the cells even after 24 h. This experiment indicates that DiI may be released from the micelle and enter tumor cells separately. To monitor directly the intactness of the micelle in real time, Chen *et al.* loaded a FRET pair (DiI/DiO) into the micelle. By monitoring the FRET efficiency, they demonstrated that core-loaded probes are released to the cell plasma membrane during incubation, indicating that plasma membrane mediates the cellular uptake of the hydrophobic molecules loaded in polymer micelles [61].

On the other hand, polymer micelles are shown to increase the drug accumulation inside cells without endocytosis. For example, polymer micelle made of Pluronic® (Poloxamer; BASF Corp., NJ, USA) P-85 or P-105 enabled effective accumulation of a hydrophobic dye inside cells by inhibiting P-glycoprotein (P-gp) [62,63]. The P-gp is an important protein that endows the MDR phenotype to cancer cells. A polymer micelle consisting of PEG-*b*-PCL also showed a similar effect on blocking the P-gp function [64]. It is, however, noticed that the P-gp inhibition can be facilitated only at low polymer concentration, usually under CMC [65]. Hence, the MDR inhibition seems to be caused by an action of unimers rather than polymer micelles. As the P-gp pump is effectively blocked by block copolymers with relatively long hydrophobic block (HLB < 20) [66] and owing to the fact that P-105 increases the membrane permeability [63], it is highly suspected that unimers dissociated from micelles may perturb the plasma membrane, resulting in acceleration of drug penetration through cell membranes. Notably, there is no evidence showing that polymer micelle or unimer directly binds to P-gp molecules, implying that the MDR inhibition may be due to a downstream effect of suppressing the phenotype. In summary, the micelle–cell interaction as well as the cellular uptake of polymer micelles could be mediated by means of complicated molecular and cellular events, which should be clarified by continuous research.

Expression of targeting moieties onto micelle surface provides one solution to deal with the cellular uptake problem. In parallel to or in combination with the stimuli sensitivity, tagging the targeting molecule has been a major strategy to enhance the therapeutic effect of micellar drug carriers. Micelles conjugated with different targeting moieties such

as biotin, folate, antibodies, growth factors, or homing peptides have been developed [67]. Those micelles are designed especially for intracellular delivery of anticancer drugs. However, most of the micelles are based on the physical assembly of block copolymers so their stability in blood is not guaranteed. If a polymer micelle having a highly specific homing molecule on its surface is rapidly disintegrated in the bloodstream, the therapeutic effect cannot be maximized. Therefore, improving the micelle stability in blood should be considered in order to optimize the active targeting strategy using targeting moieties.

5. *In vivo* fate of polymer micelles

Pharmacokinetics and pharmacodynamics of drugs formulated using polymer micelles have been widely studied, and have been excellently summarized by Aliabadi and colleagues [68]. *In vivo* fate of polymer micelles has also been investigated. The radioisotope has been a chief tool to monitor the biodistribution of polymer micelles. **Table 2** lists polymer micelles and radioisotopes used to determine their *in vivo* distribution. Without loading any therapeutic drug, polymer micelles are mostly located in liver, kidney, spleen and blood. This means that micelles seem to prolong the circulation time in blood, which has been an important rationale to develop micellar formulation of lipophilic drugs. However, it is not obvious whether the trace of isotope in blood indicates unimersor micelles. To define clearly the biodistribution of polymer micelles, other methods that can inform the structural integrity of the polymer micelles in blood should be considered. On the other hand, **Table 2** also demonstrates that micelles (or unimers) are highly distributed to organs that have excretion and metabolism functions. Assuming that micelles circulate stably along the bloodstream for a certain period of time, a high toxicity of the loaded drug would be expected owing to slow drug release at those organs. Therefore, although micelles can be used as a drug carrier of high loading efficiency, one should be cautious to avoid increasing the toxicity to healthy organs. To clarify the *in vivo* toxicity–biodistribution relationship of drug-containing polymer micelles, their structural integrity and fate in the body should be visualized by many means. Often, pharmacokinetic data are translated to the biodistribution of polymer micelles, as shown in **Table 2**. However, if the *in vivo* stability of micelles is not guaranteed, the biodistribution data cannot represent the real location of the polymer micelles. Recent advances in imaging technology may provide useful tools to monitor the micelle stability and biodistribution of drugs at the same time.

6. Conclusions

Although holding great promise as a nanocarrier of various drugs, polymer micelles face several challenges in translation from lab research to clinical practice. Three obstacles to advance the polymer micelle to an effective drug delivery system are: low drug loading capacity, low blood stability and yet-to-be-clarified interactions with cell membranes. Conventional polymer micelles have utilized the hydrophobic effect to load poorly soluble drugs. To maximize the drug loading content in a polymer micelle, it is essential to study the miscibility between polymers and drugs. The hydrotropic polymer micelles present a solution to increase the drug loading capacity by introducing extra attractive forces other than the hydrophobic effect, which include hydrogen bonding, electrostatic interaction and π–π stacking into block copolymers. For *in vivo* administration, the micelle stability in blood should be guaranteed and clarified to deliver the drug successfully to target tissues. Conventional polymer micelles are not stable in blood primarily because they are physically assembled. Three possible scenarios of micelle disintegration are drug extraction, protein adsorption and protein penetration. The final quest is to clarify the interactions between micelles and cell membranes. There is evidence that both electrostatic and hydrophobic interactions play important roles in micelle-mediated drug delivery into cells and perturbation of the plasma membrane.

7. Expert opinion

In parallel with the mechanism study, attempts have been made to improve drug loading content/efficiency, stability in blood and cellular uptake of polymer micelles. The stimuli-sensitive polymer micelle showed an alternative solution to figure out the inherent problems facing polymer micelles. Controllability of micelle disintegration using environmental stimuli can enhance the therapeutic effect. Also, the ligand-conjugated polymer micelle increased the probability of cellular uptake of polymer micelles, which is useful for the intracellular drug delivery. A combination of stimuli sensitivity and targeting moiety has been frequently used to maximize the specificity and selectivity of therapeutic effect at disease sites.

Crosslinked micelles have given a clue to increasing the stability of physically assembled polymer micelles in blood [69]. Chemically or electrostatically crosslinked polymer micelles prevent micelle disintegration in the bloodstream. However, crosslinking between polymer chains may result in another problem of drug controlled release owing to the lack of a biodegradation mechanism. To figure out this problem, one pilot study used disulfide-mediated crosslinking, which can be reversely disintegrated by reducing agents [70]. Further effort on combination of targeting moiety, stimuli sensitivity and crosslinking strategy promises a substantial form of the polymer micelle as an effective drug delivery carrier.

Another approach to improve the *in vivo* stability is to generate unimolecular micelles. Recently, Prabaharan and co-workers introduced a new strategy to prepare multi-arm unimolecular micelles based on hyperbranced copolymers. These polymer micelles were constructed by polymerization of L-lactide from Boltorn® H40, a dendritic polymer containing 64 primary hydroxyl groups, and then coupled with MPEG, thus forming a highly stable unimolecular micelle

Table 2. Studies on biodistribution of polymer micelles after intravenous administration.

Polymer	Block ratio	Drug	Micelle size (nm)	Label	Model	Biodistribution	Ref.
PEG-*b*-PCL	6000–1000	N/A	60	^{125}I-PEG	Mice	Blood > bone > kidney > liver > lung > brain	[78]
	5000–5000	N/A	56	^{3}H-PCL	Mice	Liver > kidney > spleen, lung > heart	[79]
	21,000–10,000	IND	190	Drug	Rats	Liver, spleen > lung, heart	[80]
	5000–13,000	CsA	99 – 118	Drug	Rats	Blood > heart > plasma > liver > kidney > spleen	[81]
	2000–2400	N/A	~ 25	F-5-CADA	Mice	Too bright background fluorescence from mice	[37]
PEG-*b*-PLA	5000–7000	N/A	300	^{125}I-PLA	Rats	Blood > bowel (inflamed site) > liver > kidney > spleen	[82]
	14,000–6000	N/A	72				
	2000–1750	PTX	< 220	Drug	B16-bearing mice	Tumor > liver > kidney, spleen > lung, heart > plasma	[83]
	2000–3240	PTX	-	Drug	Mice	Liver > kidney > blood > heart > lung, spleen	
	5000–5000	N/A	300	^{125}I-PLA	Rats	Blood > liver > kidney > lung, heart, spleen	[82]
	5100–5300	N/A	37 – 38	^{125}I-Tyr(-Glu)	Mice	Liver > kidney > lung, spleen	[6]
	33,000	PTX	128	^{125}I	MDA-MB-435-bearing mice	Kidney > liver > lung > spleen > heart > tumor > muscle > blood	[84]
PEG-*b*-PAsp-DOX	4300–1900	DOX	50	^{125}I-PEG	Mice	Blood > spleen > kidney > liver > lung > heart	[85]
PEG-*b*-P(Asp(Bz-70))	5000–4700	CPT	192	Drug	C26-bearing mice	Spleen, tumor, plasma > kidney, lung > heart	[86]
PEG-*b*-P(Asp(Bz-75))	-	ATRA	19	^{3}H-ATRA	Mice	Liver > kidney > lung > spleen	[87]
PEO-*b*-PPO-*b*-PEO	1150–2300–1150 (P85)	N/A	15	^{3}H	Mice	Liver > spleen > kidney > lung > brain	[88]
	1125–3250–1125 (P105)	PTX	24	Drug	Rats	Lung > spleen > kidney > liver > ovary > heart > blood	[89]
PVA-*b*-PLA	2480–1820	PTX	16 – 17	Drug	C26-bearing mice	Liver > kidney, lung > spleen > heart > muscle	[90]
Micelles with targeting ligands							
RGD-PEG-*b*-PLA	33,000	PTX	128	^{125}I	MDA-MB-435-bearing mice	Kidney > liver > tumor > lung, spleen > heart > muscle > blood	[84]
FA-PEG-*b*-P(Asp-Hyd-DOX)	-	DOX	62 – 96	Drug	KB-bearing mice	Lower FA content: blood > liver, spleen > tumor > kidney, heart; Higher FA content: liver > blood, spleen > tumor > kidney, heart	[91]
Crosslinked micelles							
PAA-*b*-PMAA	4800–18,600	N/A	24	^{64}Cu (PET)	Mice	Spleen > liver > kidney > blood > lung	[4]
	6000–7000	N/A	19 – 37			Lower PEG density: spleen > liver > kidney > lung > blood; Higher PEG density: spleen > liver, blood > lung > kidney	
PEG-*b*-P(HEMAm-Lac)	-	N/A	53	^{3}H	14C-bearing mice	Non-crosslinked micelle: liver > tumor > skin > spleen > blood; Crosslinked micelle: liver > blood > skin, tumor > spleen	[92]

ATRA: All-*trans* retinoic acid; Bz: Benzyl; CsA: Cyclosporine A; CPT: Camptothecin; DOX: Doxorubicin; F-5-CADA: Fluorecein-5-carbonyl azide diacetate; FA: Folate; Glu: Glutamic acid; HEMAm: *N*-(2-hydroxyethyl) methacrylamide; Hyd: Hydrazone; Lac: Lactate; N/A: Not applied; PAsp: Poly(aspartic acid); PCL: Poly(ε-caprolactone); PEG: Poly(ethylene glycol); PEO: Poly(ethylene oxide); PET: Positron emission tomography; PLA: Poly(DL-lactide); PMMA: Poly(methacrylate); PPO: Poly(propylene oxide); PTX: Paclitaxel; PVA: Poly(vinyl alcohol); Tyr: Tyrosine.

with high molecular mass (~ 109 kDa) [71]. The H40-PLA-*b*-MPEG micelle showed an initial burst of 5-fluorouracil (5-FU), an anticancer drug, followed by a sustained release. Micelles were slowly degraded within 6 weeks. Moreover, introduction of folate by PEG tethers showed improved tumor-targeting ability of the unimolecular micelle [72]. The micelle degradation was accelerated under acidic environment provided by solid tumors and endosomal vesicles, which might provide more efficient therapeutic effect. Unimolecular micelle conjugating DOX by means of hydrolysable hydrozone bond presented a high sensitivity to environmental pH and significantly enhanced the cellular uptake as well as cytotoxicity of the drug micelle [73].

Recent advances in molecular imaging have provided in-depth insight into the micelle stability and its interaction with cells. As described earlier, the FRET technique, as one of the optical imaging methods, suggested the possibility of clarifying the mechanisms of micelle disintegration under physiological conditions and simultaneously elucidating the fate of micelles and drugs. Optical imaging techniques have now been expanded to visualize the *in vivo* fate of polymer micelles, which was typically examined using radioisotopes [74]. In particular, the development of fluorescent probes with a long wavelength (e.g., near infrared light) is desirable for small animal imaging due to minimized autofluorescence from living tissues [75,76]. The multifunctional polymer micelle includes not only multiple targeting strategies (i.e., stimuli sensitivity and targeting ligand), but also imaging probes to visualize *in vivo* behavior. In addition, the use of imaging modalities makes it possible to follow up the therapeutic effect after drug treatment. Therefore, diagnosis, therapy and prognosis of a certain disease can be accomplished using a single micellar drug delivery system. For this reason, the multifunctional polymer micelle is a good candidate to realize personalized medicine. Pharmacogenomics, high-throughput screening, biopanning and combinational chemistry have continuously discovered small molecular therapeutic drugs, ligands highly selective to a specific disease, and pathophysiological microenvironment distinguished from normal condition. Therefore, polymer micelles will evolve further by actively combining and using those findings.

Declaration of interest

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