EXPERT OPINION

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Micelle-like nanoassemblies based on polymer–drug conjugates as an emerging platform for drug delivery

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Introduction: During the past decades, polymer–drug conjugates are one of the hottest topics in novel drug development fields. Amphiphilic polymer–drug conjugates in aqueous solution could form micelles or micelle-like nanoassemblies. Compared with polymer–drug conjugates and the micelles into which drugs are physically entrapped, micelles or micelle-like nanoassemblies based on polymer–drug conjugates bring several additional advantages, including increased drug-loading capacity, enhanced intracellular uptake, reduced systemic toxicity, and improved therapeutic efficacy.

Areas covered: This review focuses on recent progress achieved in the research field of micelles or micelle-like nanoassemblies based on polymer–drug conjugates. Firstly, properties of polymers, drugs, and linkers which could be used to build polymer–drug conjugate micelles or micelle-like nanoassemblies are summarized. Then, the characterization methods are described. Finally, the drug-targeting mechanisms are discussed. Micelles or micelle-like nanoassemblies based on polymer–drug conjugates as an emerging platform have the potential to achieve medical treatments with enhanced therapeutic effect.

Expert opinion: The application of micelles or micelle-like nanoassemblies based on polymer–drug conjugates may give new life to old active compounds abandoned due to their low solubility problems. For clinical application, there is a need to further optimize the properties of the polymer, drug, and linker.

Keywords: amphiphilic polymer–drug conjugates, drug delivery, micelle-like nanoassemblies, micelles, targeting mechanisms

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1. Introduction

Recently, nanotechnology receives a lot of attention because it is potentially altering the ways to treat diseases through advanced diagnostic and therapeutic methods [1]. Nanotechnology is a multidisciplinary field, and the concept of employing nanotechnology in medical research and clinical application is best known as nanomedicine. Drug delivery nanosystems constitute a significant portion of nanomedicine. Especially, various nanocarriers, such as liposomes, polymeric micelles, nanoparticles and nanosuspensions, hold great potential for drug delivery [2-4].

Interdisciplinary research at the interface of polymer chemistry, bioconjugation techniques, medicine, and cell biology/pharmaceutical sciences facilitates the development of polymer-based nanomedicines. The term "polymer therapeutics" is used to define a family of nanoscale entities, including polymeric prodrug such as polymer–protein conjugates and polymer–drug conjugates, and polymer-based drug delivery systems such as polymeric micelles, polymersomes, polyplexes, and polymer-based nanoparticles [5,6].

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Article highlights.

- Amphiphilic polymer–drug conjugates can self-assemble into micelle-like nanoassemblies in aqueous solution.
- The polymers contain water-soluble polymers such as poly(ethylene glycol) (PEG), poly(L-glutamic acid) (PGA), heparin, hyaluronic acid, and amphiphilic block copolymers like poly(ethylene glycol)-*block*-poly(lactide) (PEG-*b*-PLA). The drugs are limited to hydrophobic low molecular weight drugs such as paclitaxel and docetaxel.
- The characterizations of micelle-like nanoassemblies could be evaluated by a variety of techniques such as Fourier transform infrared (FT-IR) spectra, nuclear magnetic resonance (NMR) spectra, dynamic light scattering (DLS), atomic force microscopy (AFM).
- Multiple targeting strategies could be used to concentrate the conjugated drug at the site of action, mainly including passive and active targeting.
- For clinical application, it is necessary to improve the properties of the three factors of polymer–drug conjugates including polymer, drug and linker in the future.

This box summarized key points contained in the article.

Since the concept of "polymeric prodrug" was first proposed by Ringsdorf in 1975 [7], many polymer–protein and polymer–drug conjugates have entered the clinical use or the clinical trial stage (**Table 1**). In the 1970s, the concept of protein PEGylation was introduced by Davis and his coworkers [8,9]. The first polymer–drug conjugate systems entered clinical trials in 1994. It was an N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer-doxorubicin (DOX) conjugate (PK1) [10] which was a milestone for the development of polymer–drug conjugates. Kataoka and colleagues [11], Kopecek and coworkers [12], and Duncan *et al*. [13-15] also made a significant contribution to the advancements in this field. Exhaustive reviews on these conjugates can be found elsewhere [16-18]. In the present article, we focus on the hydrophobic low molecular weight drugs covalently bound to the polymers such as poly(ethylene glycol) (PEG), poly(L-glutamic acid) (PGA), heparin, hyaluronic acid, poly(ethylene glycol)-*block*-poly(lactide) (PEG-*b*-PLA), and the resultant polymer–drug conjugates can self-assemble into micelles or micelle-like nanoassemblies. The first polymer assembly drug carrier was HPMA-*p*-nitroaniline conjugates, which was prepared by Jindrich Kopecek and co-workers [19].

The vast majority of clinically used drugs have many disadvantages such as poor aqueous solubility, short plasma half-life, narrow therapeutic index, rapid clearance rate, non-specific biodistribution and serious side effects to normal tissues, limiting their clinical application. To overcome the above-mentioned drawbacks, researchers have tried to encapsulate the drugs into various drug delivery systems such as liposomes, polymeric micelles, nanocapsules, and microspheres. Among these drug delivery systems, the polymeric micelles with nanosized core-shell structure have received much attention as efficient drug delivery carriers. The micelles have several advantages [20]. The inner core made up of hydrophobic blocks which serves as the nanocontainer of hydrophobic drugs could effectively protect the drugs. The outer shell of hydrophilic blocks could avoid the phagocytic and renal clearance of the micelles and extend the blood circulation time. In addition, the nanoscale particle size (typically 10 – 100 nm) and prolonged circulation time facilitate the accumulation of drugs at the tumor sites through the enhanced permeability and retention (EPR) effect-mediated passive targeting.

Drugs can be physically encapsulated into and/or chemically conjugated to the polymeric micelles. However, the physical encapsulation has some disadvantages such as limited drug loading and limited control over drug release kinetics. When the hydrophobic low molecular weight drugs such as paclitaxel (PTX) and camptothecin (CPT) are attached to water-soluble polymers or amphiphilic block copolymers through biodegradable or cleavable bonds, polymer–drug conjugates are obtained. Due to the amphiphilicity, the polymer–drug conjugates can self-assemble into micelles or micelle-like nanoassemblies in aqueous media, which brings several additional advantages, including increased drug loading capacity, enhanced stability, prolonged *in vivo* circulation time, enhanced intracellular uptake, better controlled release, and improved therapeutic efficacy. Moreover, the linkers or spacers also can become active by triggering drug release under specific conditions, such as pH change.

This article aims to review recently available information regarding micelles or micelle-like nanoassemblies based on polymer–drug conjugates. Some natural and synthetic polymers which can be covalently conjugated with drugs to form amphiphilic polymer–drug conjugates are introduced. The techniques which are used to confirm the structures of the micelles or micelle-like nanoassemblies are also involved in the review. At the end of the article, we will discuss the mechanisms of the targeting property of these micelles or micelle-like nanoassemblies. Some micelles or micelle-like nanoassemblies were shown in **Table 2**.

2. Types of the polymers used

A lot of natural and synthetic polymers carrying multiple functional groups have been exploited for conjugation to drugs, including synthetic water-soluble polymers such as PEG, PGA and poly(L-γ-glutamylglutamine) (PGG), natural water-soluble polymers such as hyaluronic acid (HA), heparin and albumin, and some amphiphilic block copolymers such as poly(ethylene glycol)-*block*-poly(lactide) (PEG-*b*-PLA) and poly(ethylene glycol)-*block*-poly(ε-caprolactone) (PEG-*b*-PCL). The chemical structures of some polymers are shown in **Figure 1**.

2.1 Water-soluble polymers

The water-insoluble drugs directly conjugated to water-soluble polymers can be served as the hydrophobic core of micelles,

Table 1. Polymer–protein and polymer–drug conjugates in the market or clinical development [5,6,15,17,91-94].

Conjugates	Developer	Status	Indication
Polymer–protein conjugates			
PEG-adenosine deaminase (Adagen®)	Enzon	Market	Severe combined immunodeficiency disease (SCID)
SMANCS (Zinostatin Stimalmer®)	Yamanouchi	Market	Hepatocellular carcinoma
PEG-L-asparaginase (Oncaspar®)	Enzon	Market	Acute lymphoblastic leukemia
PEG-G-CSF (Neulasta™)	Amgen	Market	Neutropenia
PEG-interferon α 2a (PEG-Asys®)	Roche	Market	Hepatitis B and C
PEG-interferon α 2b (PEG-Intron™)	Schering-Plough	Market	Hepatitis C
PEG-HGH antagonist (Somavert®)	Pfizer	Market	Acromegaly
PEG-anti-TNF-α Fab (Cimzia®/CD870)	UCB Pharma	Market	Crohn's disease, Arthritis
PEG-human growth hormone (Pegvisomant)	Pfizer	Market	Acromegaly
PEG-hemoglobin (Hemospan®)	Sangart	Phase II	Delivery of CO and O_2 in trauma patients
Polymer–drug conjugates			
PGA-PTX (CT-2103/Xyotax™/Opaxio®)	Cell Therapeutics	Phase III	Various cancers, particularly non-small cell lung cancer and ovarian
PGA-CPT (CT-2106)	Cell Therapeutics	Phase I/II	Various cancers, particularly lung, ovarian and colorectal cancers
HPMA-DOX (PK1/FCE-28068)	Pfizer; Cancer Research	Phase II	Particularly lung and breast cancers
HPMA-DOX-galactosamine (PK2/FCE-28069)	Cancer Research	Phase I/II	Particularly hepatocellular carcinoma
HPMA-PTX (PNU-166945)	Pfizer; Cancer Research	Phase I (discontinued)	Various cancers
HPMA-DACH-platinate (AP5346/ProLindac™)	Access Pharmaceuticals	Phase II	Particularly ovarian and colorectal cancers
HPMA-CPT (MAG-CPT/PNU-166148)	Pfizer; Cancer Research	Phase I (discontinued)	Various cancers
HPMA-malonato-platinate (AP5280)	Access Pharmaceuticals	Phase I/II	Various cancers
PEG-CPT (Pegamotecan/Prothecan™)	Enzon	Phase II (discontinued)	Various cancers
PEG-DTX (NKTR-105)	Nektar	Phase I	Various cancers
PEG-SN38 (EZN-2208)	Enzon	Phase I	Various cancers
PEG-naloxone (NKTR-118/oral)	Nektar	Phase II	Opioid-induced constipation
PEG-irinotecan (NKTR-102)	Nektar	Phase II	Particularly ovarian and colorectal cancers
Polyacetal-CPT (XMT-1001/PHF-CPT)	Mersana Therapeutics	Phase I	Various cancers
Carboxymethyldextran-exatecan (DE-310)	Daiichi Pharmaceuticals	Phase I	Various cancers
β-Cyclodextrin-CPT (IT-101)	Insert Therapeutics	Phase I/II	Various cancers

CPT: Camptothecin; DACH: Diaminocyclohexane; DOX: Doxorubicin; DTX: Docetaxel; G-CSF: Granulocyte colony-stimulating factor; HPMA: *N*-(2-hydroxypropyl) methacrylamide; PEG: Poly(ethylene glycol); PGA: Poly(L-glutamic acid); PTX: Paclitaxel; SMANCS: Styrene maleic anhydride-neocarzinostatin; SN38: 7-ethyl-10-hydroxy-campothecin; TNF: Tumor necrosis factor.

Table 2. Overview of the micelles or micelle-like nanoassemblies based on polymer–drug conjugates.

Polymer	Drug	Linker/spacer	Comment	Ref.
PEG	DOX	Peptide	*In vivo*	[23]
PGA	PTX	Ester	*In vitro*	[31]
PGG	PTX	Ester	*In vivo*	[32-35,95]
	DTX	Ester	*In vivo*	[96]
Hyaluronic acid	PTX	Ester	*In vivo*	[43,97]
	PTX	Amino acid	*In vitro*	[98]
	Cur	Ester	*In vitro*	[99]
Heparin	PTX	Amino acid	*In vivo*	[100,50]
Albumin	MTX	Peptide	*In vitro*	[101]
	DOX/CPT	Peptide	*In vivo*	[102]
	DOX	Amide	*In vitro*	[103]
	DTX	Amide	*In vivo*	[54]
	DOX	Disulfide	*In vitro*	[104]
HPEE	PTX	Ester	*In vivo*	[70]
mPEG-*b*-PLA	PTX	Ester	*In vivo*	[58-60]
mPEG-*b*-PLLA	DOX	Hydrazone/*cis*-aconityl	*In vitro*	[90]
	DTX	Ester	*In vitro*	[105]
MPEG-*b*-P(LA-*co*-MCC)	DTX	Ester	*In vitro*	[106]
mPEG-*b*-P(LA-*co*-DHP)	DOX	Carbamate/ hydrazone	*In vitro*	[107]
PLA-*b*-PEG-*b*-PLA	PTX	Ester	*In vitro*	[108]
PEG-*b*-PLGA	DOX	Amide/carbamate	*In vivo*	[61-64]
PEG-*b*-PCL	DTX	Ester	*In vitro*	[65]
PEG-*b*-P(Asp)	DOX	Hydrazone	*In vivo*	[109-112]
mPEG-*graft*-PAA	CPT	Ester	*In vitro*	[69]
PBAsp	CPT	Ester	*In vitro*	[113]
PEO-*b*-PAGE	DOX	Hydrazone	*In vitro*	[114]
PEG-Pullulan	DOX	Hydrazone	*In vivo*	[89]
PEO-*b*-PPO-*b*-PEO	DOX	Hydrazone	*In vitro*	[115]
Pluronic F68	DOX	Amide	*In vitro*	[116]

Cur: Curcumin; HPEE: Hyperbranched poly(ether-ester); mPEG-*b*-P(LA-*co*-DHP): Methoxy poly(ethylene glycol)-*block*-poly(lactide-*co*-2,2-dihydroxymethylpropylene carbonate); MPEG-*b*-P(LA-co-MCC): Monomethoxy poly(ethylene glycol)-*block*-poly(L-lactide-*co*-2-methyl-2-carboxyl-propylene carbonate); mPEG-*b*-PLA: Methoxy poly(ethylene glycol)-*block*-poly(lactide); mPEG-*b*-PLLA: Methoxy poly(ethylene glycol)-*block*-poly(L-lactic acid); mPEG-*graft*-PAA: Methoxy poly(ethylene glycol)-*graft*-poly(L-aspartic acid); MTX: Methotrexate; PBAsp: α,β-Poly[(N-carboxybutyl)-L-aspartamide]; PEG-*b*-P(Asp): Poly(ethylene glycol)-*block*-poly(aspartic acid); PEG-*b*-PCL: Poly(ethylene glycol)-*block*-poly(ε-caprolactone); PEO-*b*-PAGE: Poly(ethylene oxide)-*block*-poly(allyl glycidyl ether); PEO-*b*-PPO-*b*-PEO: Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide); PGG: poly(L-γ-glutamylglutamine); PLGA: Poly(D,L-lactide-co-glycolide).

which is expected to improve the drug-loading capacity and reduce potential side effects.

2.1.1 Synthetic water-soluble polymers

The sophisticated polymer chemistry can provide an opportunity for the development of polymers with well-defined structures which makes polymers and their drug delivery systems controlled and more effective.

2.1.1.1 PEG

PEG, also known as poly(ethylene oxide) (PEO), is a kind of synthetic linear polyether and commercially available with different molecular weights and activation forms. PEG is soluble in water as well as in the organic media, and it has an extremely flexible main chain [21]. PEG possesses very low immunogenicity, antigenicity and toxicity and has been approved by the U.S. Food and Drug Administration (FDA) as excipients in a variety of pharmaceutical formulations. Over the last decades, PEG has been used to modify peptides and proteins in order to shield them from enzymatic degradation and immune response, and some conjugates have reached the market. Recently, PEG–drug conjugates have been widely studied [22].

PEG–DOX conjugates were synthesized using peptides GPLGV and GPLGVRG (P5D and P7D, respectively) as spacers which could be specifically cleaved by active matrix metalloproteinases (MMPs) [23]. Owing to the amphiphilicity, PEG–DOX could spontaneously assemble into micelles in water with a narrow particle size distribution from 73 to 121 nm. To increase the drug loading, free DOX was loaded into the MMPs-specific PEGylated peptide–DOX conjugate micelles using the O/W emulsion method and the mean particle size was increased slightly. The stability of the micelles was also improved after DOX was physically loaded into the micellar core. The *in vitro* degradation experiments confirmed that the PEG–DOX conjugates were degraded by active MMP-2 in a time-dependent manner. The cytotoxicity of the micelles against Lewis lung carcinoma (LLC) cells evaluated using MTT assay was significantly lower than that of free DOX. The *in vivo*

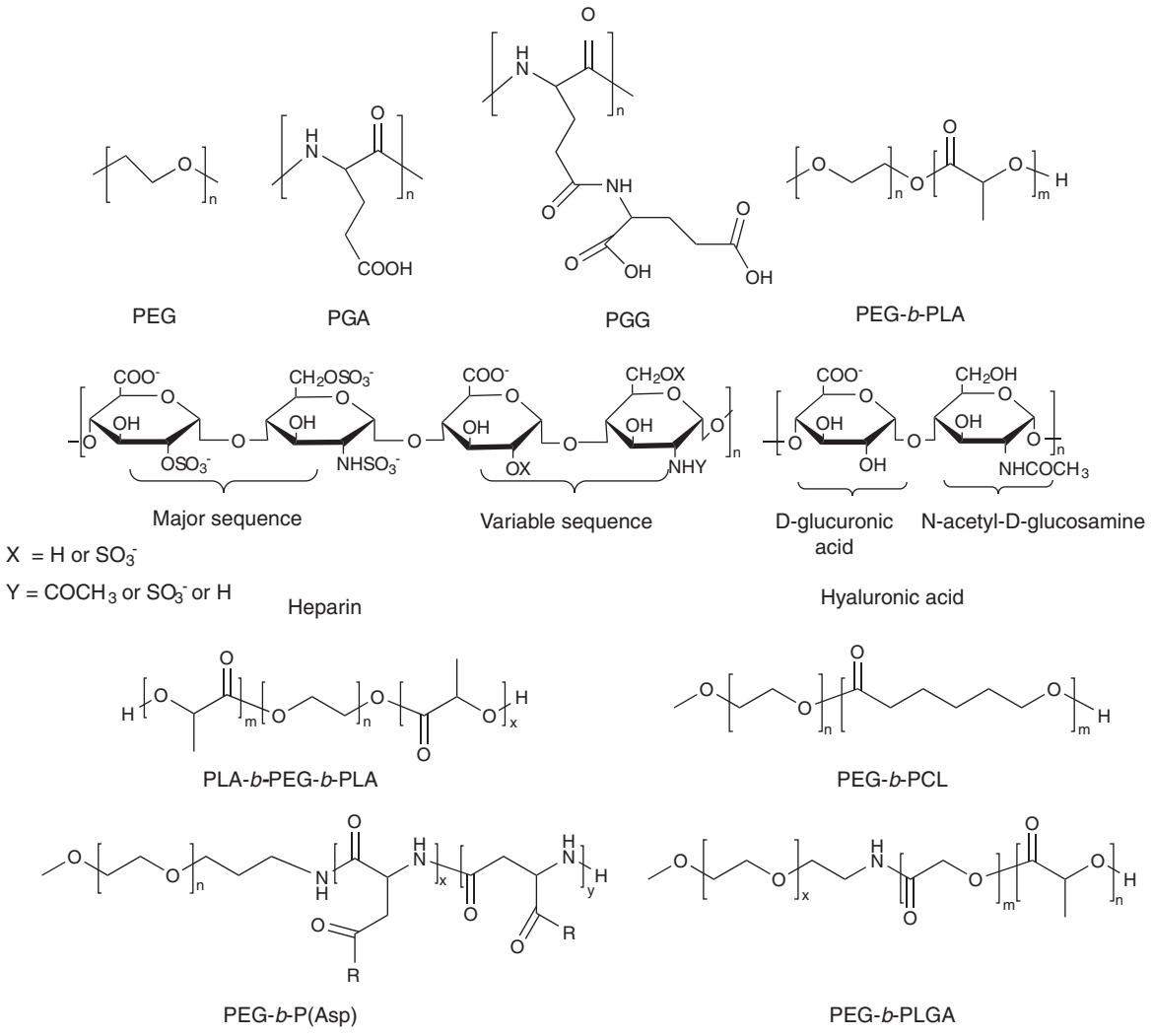

Figure 1. Chemical structures of some polymers used in the micelle-like nanoassemblies based on polymer–drug conjugates.

antitumor activity of the micelles in male C57BL/6J mice bearing LLC tumor was similar to that of the parent drug but with reduced systemic toxicity. The *in vitro* and *in vivo* activities of DOX-loaded conjugate micelles were higher than those of the conjugate micelles without free DOX. The pharmacokinetic studies in normal male C57BL/6J mice showed that the plasma levels of all conjugate micelles were higher than that of free DOX, indicating longer *in vivo* circulation time.

2.1.1.2 PGA

PGA is a synthetic polypeptide composed of naturally occurring L-glutamic acid (human essential amino acid) linked together via amide bonds. The side pendant γ carboxyl group of each L-glutamic acid which is negatively charged enhances the water solubility of PGA. The carboxyl groups of PGA provide more drug attachment sites than PEG which has only two sites at the two ends of the polymer chain. PGA is biodegradable while PEG is not readily biodegradable [24,25]. These properties make PGA a promising candidate for polymer–drug conjugation. The PGA–PTX (CT-2103) [26-28] and PGA–CPT (CT-2106) [29]conjugates have advanced to clinical trials. Up to now, PGA–PTX conjugate is probably the most successful polymer–drug conjugate and has advanced to Phase III clinical trials. It is perhaps the first product in this class to reach the market [30].

Lee studied the self-assembly behavior of folic acid (FA)–PGA–PTX [31]. The FA–PGA conjugate was synthesized by amide bonds between the activated carboxyl groups of PGA and the amine groups of aminated FA. PTX was attached to the PGA–FA conjugate via the ester bonds between the hydroxyl groups of PTX (see **Figure 2**) and the carboxyl groups of PGA. The solubility of FA–PGA–PTX in water was of 80 mg/mL with the content of PTX could reach 25.6% (wt.%). The transmission electron microscope (TEM) micrograph showed that the FA–PGA–PTX conjugate micelles had a regular spherical shape with the mean diameter of 300 nm. The ability of PTX

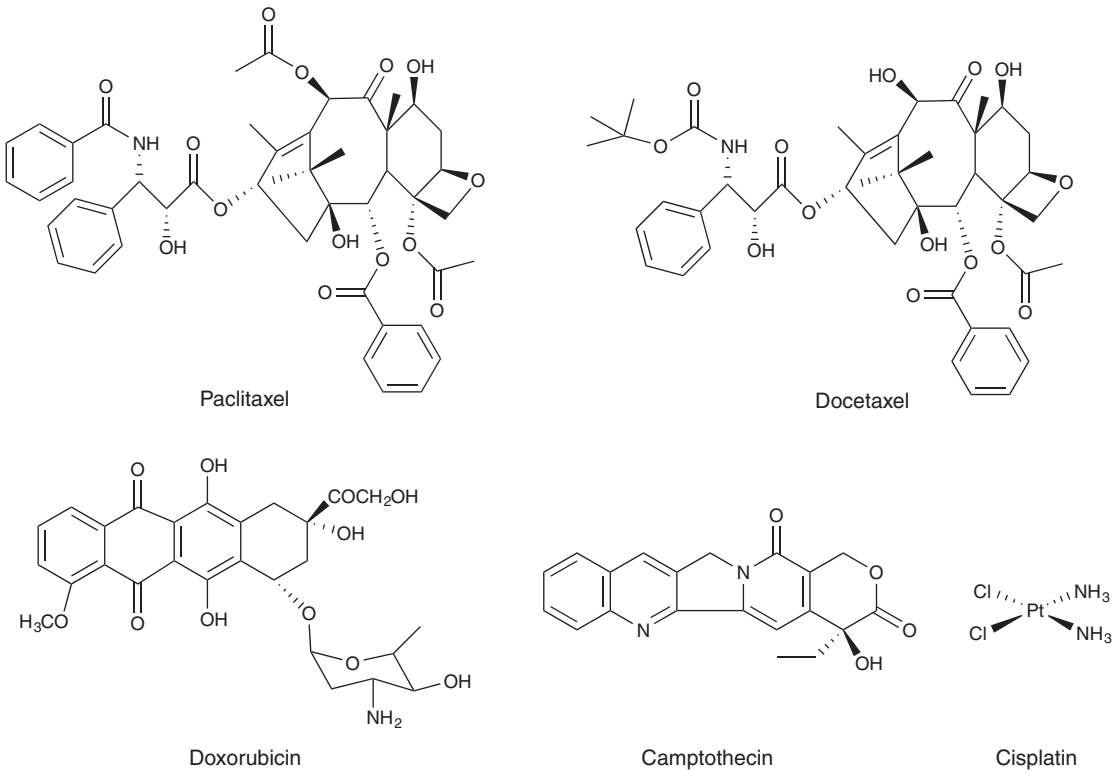

Figure 2. Chemical structures of some hydrophobic low molecular weight drugs.

to induce microtubule assembly *in vitro* markedly decreased after the conjugation to the FA–PGA. The results of the *in vitro* cytotoxicity test showed that FA–PGA–PTX micelles significantly decreased the cytotoxicity to normal cells compared with free PTX, but the cell viability of folate receptor (FR)-positive cancer cells MCF-7 treated with FA–PGA–PTX micelles markedly decreased, indicating that the FA–PGA–PTX micelles could selectively enter the FR-positive cancer cells by receptor-mediated endocytosis.

2.1.1.3 PGG

PGG, a modified polymer from PGA, is a novel and highly water-soluble polymer composed of a polyglutamate in which an additional glutamine side chain has been added to each glutamyl monomer in the backbone. PGG contains two carboxyl groups for drug attachment compared with one carboxyl group of PGA [32,33].

PTX was covalently bound to PGG via ester bond and the PGG–PTX conjugate with a macromolecular weight of about 130 kDa can spontaneously assemble to nanomicelles in aqueous environments with the PTX content about 36% (wt.%). The TEM image showed that the PGG–PTX micelles were spherical. The size determined by dynamic light scattering (DLS) was about 20 nm which was significantly smaller than that of Abraxane® (80 – 120 nm). The stability studies monitored by DLS and size exclusion high-pressure liquid chromatography showed that the PGG–PTX in lyophilized form could be stored for at least 3 months without any significant change in the drug content and particle size. Coumarin 6-labeled PGG–PTX micelles were used to study the cellular internalization in human non-small cell lung cancer cells (NCI-H460). After incubation for 30 min, the micelles were clearly uptaken by the NCI-H460 cells. The *in vitro* cytotoxicity of PGG–PTX micelles was higher with the incubation time extended, which was attributed to the gradual release of the active PTX from the conjugates. The hemolysis studies showed that no hemolytic phenomenon was observed up to 20 mg/mL PGG–PTX. The histology test suggested that all tissues were normal after 21 days treatment with PGG–PTX [34]. The *in vivo* toxicity in nu/nu mice was assessed by determining the maximum tolerated dose (MTD) defined on the basis of a 15% loss of body weight within 2 weeks. The MTD of PGG–PTX was 350 mg PTX/kg, which was 2.2-fold higher than that of PGA–PTX CT-2103 (160 mg/kg) and 4.4-fold higher than that of Taxol (80 mg/kg), indicating that PGG–PTX was significantly less toxic [35]. The pharmacokinetics of PGG–PTX micelles were studied in female nu/nu mice bearing NCI-H460 lung tumor. In plasma, the total taxanes C_{max} of PGG–PTX was 8.5-fold higher than that of free PTX. The $AUC_{0-340\ h}$ of total taxanes for PGG–PTX was 23.6-fold higher than that for PTX. The C_{max}, $AUC_{0-340\ h}$ of the extractable taxane and native PTX for PGG–PTX were also higher than those for PTX. These data suggested that PGG–PTX micelles significantly prolonged the *in vivo* circulation time [33].

2.1.2 Natural water-soluble polymers

The naturally occurring polymers have long been known to possess biological activities such as antiviral and antitumor activity. Owing to their biocompatibility, biodegradability, non-toxicity and non-immunogenicity, they have attracted intense attention as drug delivery carriers.

2.1.2.1 HA

HA, as shown in **Figure 1**, is a linear, anionic polysaccharide composed of N-acetyl-D-glucosamine and D-glucuronic acid linked via alternating β-1,4 and β-1,3 glycosidic bonds. It is biodegradable, biocompatible, non-toxic, and non-immunogenic. The biological function of HA has been investigated in deep. As a chief component of the extracellular matrix, HA plays a critical role in cell growth, differentiation, proliferation, migration, and even cancer metastasis [36,37]. Due to its strong affinity with cell-specific surface markers such as cluster determinant 44 (CD44) [38] and receptor for hyaluronate-mediated motility (RHAMM) [39,40] which are abundantly overexpressed on the surface of many types of tumors [41], HA has been popularly used as target ligand to design tumor-specific drug delivery carriers for anticancer drugs such as PTX [42]. The carboxylic groups and hydroxyl groups of HA can be used for chemical modification and conjugation.

HA–PTX conjugates were synthesized via the ester linkage between the activated carboxylic groups of HA and 2′-hydroxy of PTX with the drug content of 10.8% (wt.%) [43]. The atomic force microscopy (AFM), TEM, and DLS results showed that the conjugates could self-assemble into nanosized spherical micelle-like nanoassemblies in aqueous solution with about 200 nm in diameter. The critical aggregation concentration measured by the fluorescence spectroscopy was 7.8 μg/mL which was significantly lower than that of Pluronic copolymers (1 – 24 mg/mL). After 3 h incubation of the HA–PTX nanoassemblies in four different pH solutions at 37°C, the release of PTX was more rapid at more acidic pH, which might be due to the cleavage of acid-sensitive ester bond. In the *in vitro* cytotoxicity assay, compared with the Taxol formulation, the HA-PTX nanoassemblies were more cytotoxic to CD44 receptor overexpressed HCT-116 and MCF-7 cells, but less cytotoxic to NIH-3T3 cells which do not overexpress CD44 receptors. The HA–PTX nanoassemblies might be efficiently internalized to HCT-116 and MCF-7 cells via the HA receptor-mediated endocytosis.

2.1.2.2 Heparin

Heparin is a highly sulfated and linear polysaccharide with alternating units of sulfated glucuronic acid and structurally diverse glucosamine derivatives. Heparin has the highest negative charge density of any known biological molecule [44,45]. Heparin and its derivatives have been widely investigated in biomedical applications due to their biocompatibility, biodegradability, and biological activities. Heparin has been widely used as an injectable anticoagulant [46]. Furthermore, heparin is also able to interfere with the activity of growth factors such as bFGF and VEGF and inhibits cancer cell angiogenesis and tumor exacerbation, for example, heparin is capable of inhibiting the proliferation of arterial smooth muscle cells and hepatoma cells [47-49].

The amphiphilic heparin–PTX conjugates were synthesized by inserting a single amino acid spacer, either valine, leucine or phenylalanine [50]. The peptides or amino acids as spacers in the polymer–drug conjugates are most frequently used. The tetrapeptide spacer Gly-Phe-Leu-Gly is present in PK1, PK2, PNU-166945 and AP5280 which have entered clinical trials (**Table 1**). On the hand, they provide both a reactive carboxyl group and an amino group which can be easily reacted with the drugs and the polymers. On the other hand, the spacers ensure a controlled release of the drugs by the chemical or enzymatic hydrolysis. In the aqueous solution, the heparin–PTX conjugates self-assembled to form approximately spherical nanoassemblies with narrow size distribution (140 – 180 nm). Compared to the heparin, the anticoagulant activity of the nanoassemblies was significantly reduced, thereby decreasing the risk of excessive bleeding after systemic administration of the heparin–PTX nanoassemblies. In the *in vitro* cytotoxicity test, the heparin–PTX nanoassemblies exhibited higher cytotoxicity to MCF-7 cells than free PTX. Flow cytometric analyses showed that PTX released from the heparin–PTX nanoassemblies could arrest MCF-7 cells in the G_2/M phase of cell cycle. The *in vivo* antitumor activity of the heparin–PTX nanoassemblies was similar to that of free drug, but exhibited less systemic toxicity.

2.1.2.3 Albumin

Serum albumin is the most abundant plasma protein with the blood half-life of about 20 days and makes up half of total plasma protein. Human serum albumin (HSA) is one of the smallest proteins in blood plasma composed of 585 amino acid residues with a molecular weight of 66.5 kDa. HSA is the most versatile transport protein in serum for a lot of endogenous and exogenous compounds. Albumin is nontoxic, nonimmunogenic, biocompatible, and biodegradable. Albumin can accumulate in the tumor and inflamed tissues via the EPR effect. These unique features of HSA make it an attractive macromolecular carrier for drug delivery [51,52]. The development of albumin paclitaxel nanoparticles (Abraxane) that was approved in 2005 for treating metastatic breast cancer is considered as a landmark for nanomedicine [53].

To improve the solubility, docetaxel (DTX), as shown in **Figure 2**, was covalently bound to HSA via the amide bond after the 2′-hydroxy of DTX was activated by succinic anhydride [54]. The DTX–HSA conjugates could form the particles of 90 – 110 nm in diameter in aqueous media. The *in vitro* release studies showed that DTX–HSA nanoassemblies exhibited pH-dependent degradation and the release of DTX from the conjugates was faster at pH 5.5 than that of pH 7.4. In plasma, the DTX–HSA nanoassemblies showed faster degradation owing to the chemical and enzymatic mechanisms. The *in vitro* cytotoxicity against T47D and SKOV-3 cells determined by MTT assay showed that the IC_{50} values of the nanoassemblies

were lower than those of free DTX in both cell lines. Compared to Taxotere®, the plasma level of DTX was six- to seven-fold higher for DTX–HSA nanoassemblies, thereby increasing the chance of uptake by tumor cells.

2.2 Amphiphilic block copolymers

The aliphatic polyesters and their copolymers have been increasingly studied for drug delivery because of their excellent biodegradability and biocompatibility. However, they are generally hydrophobic, limiting their medical application. To improve the hydrophilicity, many efforts have been made. The most successful strategy was using PEG as a modifying polymer. The copolymers of hydrophilic PEG and hydrophobic aliphatic polyester such as poly(lactide) (PLA), poly(ε-caprolactone) (PCL), and poly(D,L-lactide-co-glycolide) (PLGA) have been investigated extensively as micellar carriers of drugs. The PEG hydrophilic corona located on the micelles surface can protect the drug from interacting with the serum proteins and decrease the inter-particle aggregation.

2.2.1 *PEG-b-PLA*

PLA is a linear aliphatic polyester (shown in **Figure 1**). The polymer is biodegradable and biocompatible. PLA has been approved by FDA for various commodities and medical applications. PLA can be synthesized either by condensation polymerization of lactic acid or by the ring-opening polymerization route of an intermediate called lactide (the ring-formed dimer of lactic acid). Due to the chiral nature of lactic acid, PLA exists in several distinct forms: poly(L-lactide) (PLLA), poly(D-lactide) (PDLA) and poly(D,L-lactide) (PDLLA) [55-57]. Due to the hydrophobicity, PLA is widely used as a core-forming block. Biodegradable amphiphilic block copolymer composed of PEG and PLA which can self-assemble into polymeric micelles has attracted considerable interest as drug carriers.

The di- or tri-block copolymers of PEG and PLA have been investigated by conjugation with various drugs. Jing and his team conjugated PTX to PEG–PLA [58-60]. They first prepared hydroxyl-terminated PEG–PLA. The carboxyl-terminated PEG–PLA was synthesized by reacting with diglycolic anhydride. PTX was conjugated to the copolymer via an ester linkage between the hydroxyl group of PTX and the carboxylic acid group in PEG–PLA–COOH. The PEG–PLA–PTX conjugate micelles were prepared by the dialysis method with both PLA block and PTX in the core and PEG block in the shell of the micelle. The critical micelle concentration (CMC) of the PEG–PLA–PTX conjugate was 6.31×10^{-4} g/L, which was lower than that of PEG–PLA with 1.78×10^{-3} g/L. The lower CMC was attributed to the enhanced hydrophobicity of the conjugate due to the addition of PTX. The PTX content in the conjugate was 10% (wt.%) (by ^{1}H NMR). The PEG–PLA–PTX conjugate micelles were spherical with the particle size about 130 nm. The *in vitro* cytotoxicity assay of the conjugate micelles against the human liver cancer H7402 cells by MTT method showed that at the same drug dose (20 ng/mL), the conjugate micelles showed almost the same activity as the free drug, indicating that the PTX could be released from the conjugate micelles without losing the antitumor activity. The *in vivo* antitumor studies in Wistar rats bearing intracranial C6 glioma tumor showed that, at the same PTX dose, the PEG–PLA–PTX conjugate micelles showed remarkable tumor growth inhibition with less toxicity due to the EPR effect.

2.2.2 *PEG-b-PLGA*

PLGA is biodegradable and biocompatible. PLGA is approved by the FDA as therapeutic devices. PLGA is a copolymer of lactic acid and glycolic acid linked by ester linkages. Different forms of PLGA can be obtained through adjusting the ratio of lactide to glycolide (e.g. PLGA 75:25 stands for 75% lactic acid and 25% glycolic acid). PLGA has been widely studied as delivery carriers for drugs, proteins, and other therapeutic agents. It can be hydrolyzed in the body into biodegradable original monomers lactic acid and glycolic acid which can be metabolized finally into carbon dioxide and water, resulting in minimal systemic toxicity.

The copolymers of PEG and PLGA with PEG as hydrophilic block and PLGA as hydrophobic block are amphiphilic and can self-assemble into polymeric micelles in the aqueous media. When DOX was conjugated to the di-block copolymer of PLGA–PEG via the carbamate linkage between the primary amino group of DOX and the terminal hydroxyl group of the PLGA chain which was pre-activated with *p*-nitrophenyl chloroformate, the DOX–PLGA–PEG conjugate was obtained [61]. The DOX–PLGA–PEG conjugate micelles were prepared from the DOX–PLGA–PEG conjugate with DOX and PLGA block as inner core and PEG as shell. The conjugation of DOX to PLGA–PEG was confirmed by gel permeation chromatography (GPC). The CMC of DOX–PLGA–PEG determined by fluorescence spectroscopy using pyrene as an extrinsic probe was similar to the CMC of PLGA–PEG. The size of DOX–PLGA–PEG micelles was almost equivalent to that of PLGA–PEG micelles. These results revealed that the conjugation of DOX to PLGA did not interfere the micelle-forming capability of PLGA–PEG. The loading and encapsulation efficiency of DOX in the DOX–PLGA–PEG micelles were 2.18% and 99.1% (wt.%), respectively, which were higher than those of the PLGA–PEG micelles (0.51% and 23.2% (wt.%), respectively) in which DOX was physically loaded. The chemically conjugation of DOX significantly increased the drug loading. The *in vitro* cytotoxicity assay against HepG2 cells showed that DOX–PLGA–PEG micelles were about 10-fold more cytotoxic than free DOX, which was ascribed to the enhanced uptake of DOX–PLGA–PEG micelles through their facilitated endocytotic transport relative to passive diffusion of free DOX. In the subsequent researches, to improve therapeutic effect of DOX–PLGA–PEG micelles, some active targeting ligands such as folate [62] and Hab18 F(ab′)$_2$ were decorated [63,64]. The results of *in vitro* and *in vivo* studies both demonstrated superior antitumor effects of the DOX–PLGA–PEG micelles depending on dual effects of passive and active targeting.

2.2.3 PEG-b-PCL

PCL is a biodegradable polyester and prepared by ring-opening polymerization of ε-caprolactone monomers in the presence of the catalyst. PCL has been approved by the FDA as a drug delivery device. It is miscible with a lot of polymers and the copolymers of PCL and PEG have been widely investigated in biomedical applications. Owing to their biocompatibility and biodegradability, PEG-*b*-PCL micelles with PEG as the micelle shell and PCL as the core are good candidates for the delivery of poor water-soluble drugs.

Mikhail *et al.* conjugated DTX to the hydrophobic PCL block of PEG-*b*-PCL and obtained the polymer–drug conjugate PEG-*b*–PCL–DTX [65]. Compared with the PEG-*b*-PCL, the CMC of the PEG-*b*–PCL–DTX was lower (14.0 mg/L vs 20.6 mg/L), owing to the enhanced hydrophobicity in the core after the conjugation of DTX. DTX was physically loaded into the PEG-*b*–PCL–DTX micelles using the dry-down method. The concentration of DTX could reach about 12 mg/mL which stranded for an 1840-fold increase in the water solubility of the drug. The *in vitro* release studies showed that, during the first 3 h, the release of DTX from PEG-*b*–PCL–DTX micelles was rapid and represented the release of physically encapsulated DTX, but was slower than that of DTX-loaded PEG-b-PCL micelles, which might be ascribed to the improved compatibility between free DTX and the core. Core-conjugated DTX was released in the subsequent 1 week and exhibited the sustained release behavior.

3. The physicochemical characterization of micelles or micelle-like nanoassemblies

To look into the possibility that the amphiphilic polymer–drug conjugates could form micelle-like nanoassemblies in aqueous media, their physicochemical characterizations are studied using Fourier transform infrared (FT-IR), ^{1}H nuclear magnetic resonance (^{1}H NMR), GPC, DLS, TEM, scanning electron microscopy (SEM), and others.

3.1 FT-IR, ^{1}H NMR, and GPC analysis

The chemical structures of polymer–drug conjugates could be usually identified by FT-IR, ^{1}H NMR, and GPC techniques. These three methods could obtain different information. The combination of these techniques would give us a detailed characterization of the structure of the polymer–drug conjugates.

FT-IR spectra which is considered as the molecular fingerprints is used to investigate the chemical interactions between the drug and the polymer matrix [66]. Potassium bromide (KBr) pellet method is often employed for better resolution and the samples are prepared by grinding the dry conjugates with solid KBr finely using mortar and pestle and then applying great mechanical pressure to the mixture to form a translucent pellet [67,68]. The characteristic bands of the bonds or spacers between the drug and the polymer would reveal the successful conjugation. In the FT-IR spectra of mPEG-graft-PAA-CPT conjugate [69], the appearance of absorption peaks at 1467 and 529 cm^{-1} was attributed to the stretching vibration of aromatic ring in CPT. The bands at 2887 and 1112 cm^{-1} corresponded to the stretching vibration of –CH- and –CO- in repeat units (-CH2CH2O-) of mPEG. In the FT-IR spectra of HPEE–PTX conjugates [70], the appearance of absorption peaks at 3000, 1600, 1585, 1500, 1450 and 730 cm^{-1} assigned to the vibration of the benzene ring in PTX. The band at 1050 cm^{-1} was attributed to the C–O–C stretching vibration of HPEE.

^{1}H NMR spectroscopy is one of the principal techniques used to obtain the physicochemical and structural information of organic compounds including small molecules and polymers [68]. The structure and composition of the polymer conjugates can be verified by ^{1}H NMR analysis. In suitable solvents, the characteristic proton peaks are identified. From the ^{1}H NMR results, we could roughly calculate the drug content of the polymer–drug conjugates and observe the formation of the nanoassemblies.

GPC is a type of size exclusion chromatography, in which analytes are separated based on the size or hydrodynamic volume of the analytes. GPC has been widely used to determine the molecular weights and polydispersity indexes of polymers and polymer–drug conjugates. The difference of the peak before and after drug conjugation in the GPC profiles could illustrate the successful conjugation.

3.2 Measurement of critical micelle concentration (CMC)

The self-assembly behavior of the polymer–drug conjugates in aqueous solution is characterized by the measure of critical micelle concentration (CMC). The solution concentration of polymers at which micelles appear is called the CMC. At the CMC, the physicochemical properties of the solution exhibit abrupt changes such as surface tension, interfacial tension, osmotic pressure, and electrical conductivity [71]. The CMC could be determined using a lot of methods such as surface tension [72], electrical conductivity [73], light scattering [74], and fluorescent spectroscopy [75]. The CMC of almost all reported micelle-like nanoassemblies were determined by fluorescent spectroscopy with pyrene as the fluorescent probe. The solubility of pyrene in water is very low and the fluorescence of the probe is sensitive to the polarity of the surrounding environment. Pyrene is preferentially solubilized into the hydrophobic core of the micelles, so pyrene fluorescence can be used to detect the formation of the hydrophobic regions of the micellar cores. The intensity ratio between the first and third bands in the pyrene fluorescence spectrum, called I_1/I_3 ratio, has been shown to correlate well with solvent polarity. Thus, the I_1/I_3 ratio serves as a measure of the polarity of the environment [71,75]. As the ratio of the hydrophobic block and hydrophilic block increases, CMC decreases and the micelles would be easier to form. Due to the addition of hydrophobic drugs, the CMC of the resultant polymer–drug copolymers decreases. The nanoassemblies would be also more stable correspondingly.

3.3 Morphological evaluation

The shape of a particle plays an important role in the *in vivo* biodistribution and cellular uptake of the nanoassemblies [76].

There are a variety of direct visualization techniques to characterize the morphology mainly including TEM, SEM, and AFM. Generally, the combination of these techniques would give us a detailed characterization of the morphology of the micelle-like nanoassemblies.

TEM can observe the internal structure of the nanoassemblies. It is necessary for negative staining of samples in the TEM technology. A dispersion of micelle-like nanoassemblies in water is placed onto a copper grid coated with carbon, negatively stained with phosphotungstic acid solution or uranyl acetate for 1 min and air-dried before observation.

The morphology and physical state of the particle surface are visualized by the SEM method, for which a drop of the nanoassemblies solution is deposited onto the silicon chip placed on the surface of the sample stub and air-dried overnight. The stub is coated with a fine gold under argon atmosphere by means of a sputter coater for 30s before observation.

AFM makes use of a cantilever with a sharp probe to scan the surface of the sample to observe the morphology and particle size. AFM is based on the nature of the probe–surface interaction and measures the forces between the probe tip and the sample. The AFM can provide a three-dimensional surface profile with great precision.

3.4 Determination of particle size and zeta potential

Particle size and zeta potential are the key parameters relevant to the physical stability and *in vivo* pharmacokinetic of the micelle-like nanoassemblies and an exact determination is highly important. Several techniques, including DLS, laser diffraction, hydrodynamic chromatography and coulter counter, can be used to measure the particle size and size distribution of the nanoassemblies. Among them, DLS, known as photon correlation spectroscopy, is the most commonly used technique to determine the size of small particles suspended in liquid medium. An important parameter of this method is polydispersity index (PDI, a value between 0 and 1). A PDI value of 0 demonstrates an ideal monodispersity, a PDI of 0.1 – 0.25 a narrow size distribution, while a PDI greater than 0.5 a broad distribution. The particle sizes of almost all of reported micelle-like nanoassemblies based on polymer–drug conjugates were lower than 200 nm. The nanoassemblies could reduce the drug uptake of liver and spleen and increase the drug accumulation in the tumor.

The particle surface charge or zeta potential is determined using laser Doppler electrophoresis which assesses the electrophoretic mobility of dispersed particles in the medium. The micelle-like nanoassemblies with an absolute value of zeta potential above 30 mV have been shown to be stable in aqueous solution [77,78].

4. Drug-targeting mechanisms of the micelle-like nanoassemblies

To improve the therapeutic effect, the targeting and localized delivery of the drug and site-specific drug release are the key challenges. Multiple targeting strategies could be used to concentrate the conjugated drug at the site of action. For example, drug targeting to the solid tumors can be achieved passively by the EPR effect which mainly makes use of the pathophysiological characteristics in the solid tumors. The conjugation of active targeting ligands to the surface of the micelles or a triggered release mechanism becomes also alternative approach.

4.1 Passive targeting via the EPR effect

The EPR effect provides the most probable path for passive drug targeting. The concept of EPR effect in solid tumors, which was first identified in the 1980s by Maeda and his colleague [79], is now regarded as a "gold standard" in the design of new nanocarrier drug delivery systems. The EPR effect plays a major role in the tumor-selective delivery of the micelle-like nanoassemblies based on polymer–drug conjugates as one of macromolecular drugs. Kataoka and his group first demonstrated directly the EPR effect-mediated targeting of the micelle-forming poly(ethylene oxide-aspartate)-adriamycin conjugates (PEO-PAsp(ADR)) in mice bearing murine colon adenocarcinoma 26 (C-26) tumor [80].

Solid tumors possess anatomical and pathophysiological differences from normal tissues or organs. The EPR effect can be mainly attributed to two factors of the solid tumors [81-83]: leaky tumor vessels and impaired lymphatic drainage system. The leaky tumor blood vessels provide an opportunity for extensive leakage of blood plasma components including macromolecular drugs into the tumor tissues. What's more, the impaired lymphatic drainage systems prevent the clearance of penetrative macromolecules and promote their accumulation in the tumor, thereby reducing adverse side effects to healthy tissues. The hydrophilic corona of the micelle-like nanoassemblies can provide a protective interface between the core and the blood components, stabilize the nanoassemblies against phagocytic clearance by the reticuloendothelial system or macrophages [84]. Therefore, the *in vivo* circulation time of drugs can be extended greatly, which facilitates the EPR-mediated targeted delivery.

Li *et al*. conjugated water-insoluble drug PTX to hydrophilic hyperbranched poly(ether-ester) (HPEE) via the ester bond [70]. The amphiphilic copolymer HPEE–PTX could self-assemble into the micelles with PTX as the inner core and HPEE as the shell in aqueous solution with the drug contents from 4.1% to 10.7% (wt.%). The TEM and DLS results showed that the micelles were spherical with mean particle size from 50 nm to 120 nm. The *in vitro* cytotoxicity against MCF-7 and Tca8114 cells exhibited that the cellular growth inhibition activities of the HPEE–PTX conjugate micelles could increase with cultural time prolonged, because the free PTX was gradually released from the HPEE–PTX micelles over time. The pharmacokinetic studies in SD rats showed that the plasma levels of PTX were significantly higher in the HPEE–PTX micelles than that of free PTX. The longer circulation time in the bloodstream of the HPEE–PTX micelles provided an opportunity for the accumulation of conjugated drugs at the tumor tissue via the

EPR effect. In the *in vivo* antitumor efficacy in nude mice bearing MCF-7 and Tca8113 tumors, the HPEE–PTX micelles exhibited higher tumor inhibition rates against both MCF-7 and Tca8113 tumors with lower toxic effect compared with free PTX formulation. The higher antitumor effect of the HPEE–PTX conjugate micelles *in vitro* and *in vivo* could be attributed to the EPR effect.

4.2 Ligand-based targeting

Active targeting is usually achieved by the conjugation of a targeting component called ligand to the surface of the micelle-like nanoassemblies. The ligands can specifically interact with receptors or antigens on the target cells, tissues or organs [85]. The ligands not only help the drug to reach the specific sites of action efficiently, but also enhance the cellular uptake of the drug by receptor-mediated endocytosis [86]. The characteristics of the ligands are important for the circulation time, the cellular uptake, the affinity of the nanoassemblies [87].

Folate, an anionic form of folic acid, has been widely utilized as targeting ligand for cancer therapy. Folate is a water-soluble vitamin with low molecular weight (MW = 441.4) and displays extremely high affinity to its receptor, folate-binding protein (FBP), which is vastly overexpressed on the surface of many human cancer cells [88]. Folate is non-toxic and non-immunogenic. Therefore, the drug–folate conjugates can promote the targeted delivery of the drug and the cellular uptake through folate receptor-mediated endocytosis. Scomparin *et al.* designed a novel folate modified pullulan bioconjugates for anticancer drug delivery [89]. The conjugates composed of pullulan derivatization with DOX or with DOX and FA and the detailed synthetic procedures were described in **Figure 3**. Pullulan activated by periodate oxidation was reacted with cysteamine (Cyst) and $PEG(NH_2)_2$ in turn and obtained NH_2-PEG-Pull-Cyst. DOX was conjugated to the pullulan through a pH-sensitive hydrazone bond between the cysteamine thiol groups of NH_2-PEG-Pull-Cyst and the maleimide groups of DOX intermediate containing a pH-sensitive hydrazone bond and the conjugate (NH_2-PEG)-Pull-(Cyst-DOX) was obtained. FA activated by N-hydroxysuccinimide was conjugated the amino groups of pendant PEG chains of (NH_2-PEG)-Pull-(Cyst-DOX) and the conjugate (FA-PEG)-Pull-(Cyst-DOX) was got. Photon correlation spectroscopy showed that (NH_2-PEG)-Pull-(Cyst-DOX) and (FA-PEG)-Pull-(Cyst-DOX) could form micelle-like nanoassemblies with the particle sizes of about 150 nm and 100 nm, respectively. The cell internalization kinetics studies with the folate receptor overexpressing HeLa cells showed that (FA-PEG)-Pull-(Cyst-DOX) was more rapidly uptake by cells than the non-folate conjugate. The cell toxicity of the conjugates against KB and MCF-7, which overexpress and do not overexpress the folate receptor, respectively, exhibited that both conjugates displayed higher IC_{50} values than free DOX. This result could be ascribed to the slow drug release from the polymer conjugate micellar particles.

Kataoka and his team studied the influence of folate on *in vitro* cytotoxicity, *in vivo* biodistribution, and *in vivo* anticancer activity of the micelle-like nanoassemblies. Two kinds of micelles were prepared from Fol-PEG-p(Asp-Hyd-ADR) and PEG-p(Asp-Hyd-ADR) which were abbreviated as FMA and MA, respectively. The *in vitro* cytotoxicity against KB cells, which overexpress FBP, exhibited that IC_{50} of FMA was similar to that of free ADR after 24 h incubation, which could be attributed to the enhanced cellular uptake of folate-conjugated micelles via folate receptor-mediated endocytosis. The flow cytometric analysis further confirmed the results of *in vitro* cytotoxicity. The surface plasmon resonance (SPR) analysis suggested that FMA with only 10% folate substitution ratio could recognize and interact with FBP due to the high affinity of folate for FBP (K_d < 1 nM). The biodistribution of FMA by changing folate contents was investigated in CD-1 nude mice bearing KB tumor. The AUC data for plasma showed the micelles possessed prolonged blood circulation time irrespective of folate content. The AUC values for each organ suggested that in comparison with MA, FMA with a higher folate content showed a higher accumulation in the liver and a lower tumor-targeting property. Therefore, the amount of folate should be carefully controlled to enhance tumor-targeting and to minimize nonspecific distribution of the micelles. Interestingly, the tumor accumulation of the micelles did not significantly differ before and after folate conjugation. Compared to free ADR and MA, the *in vivo* antitumor activity of FMA in CD-1 nude mice bearing KB tumor was more effective with lower toxicity.

4.3 Stimuli-triggered targeting

When drugs were linked to the stimuli-sensitive polymer or the bonds between the drug and the polymer were responsive to environmental or physical stimuli, such as the lower pH in tumor tissue, temperature or ultrasound, the resultant micelle-like nanoassemblies would be stimuli-response. These stimuli-response nanoassemblies can release the drug in a specially controlled manner and thereby encourage the drug selectively delivered to the site of action.

To improve the therapeutic efficacy of DOX, DOX was chemically conjugated to the di-block copolymer mPEG–PLLA via two acid cleavable bonds (shown in the **Figure 4**) [90]. Hydrazine was reacted with the activated mPEG–PLLA by *p*-nitrophenyl chloroformate and hydrazine-conjugated mPEG–PLLA was produced. The ketone group of DOX was reacted with hydrazine-conjugated mPEG–PLLA and the mPEG–PLLA–DOX conjugate containing the hydrazone was obtained. Similarly, the mPEG-PLLA–DOX conjugate containing *cis*-aconityl bond was also synthesized. Firstly, the activated mPEG–PLLA by *p*-nitrophenyl chloroformate was reacted with ethylene diamine and mPEG–PLLA–NH_2 was obtained. Then, DOX reacted with *cis*-aconitic anhydride and the product (CA-DOX) was activated by N-hydroxy succinimide. Finally, the activated CA-DOX was reacted with mPEG–PLLA–NH_2 and the *cis*-aconityl bond was formed. The successful

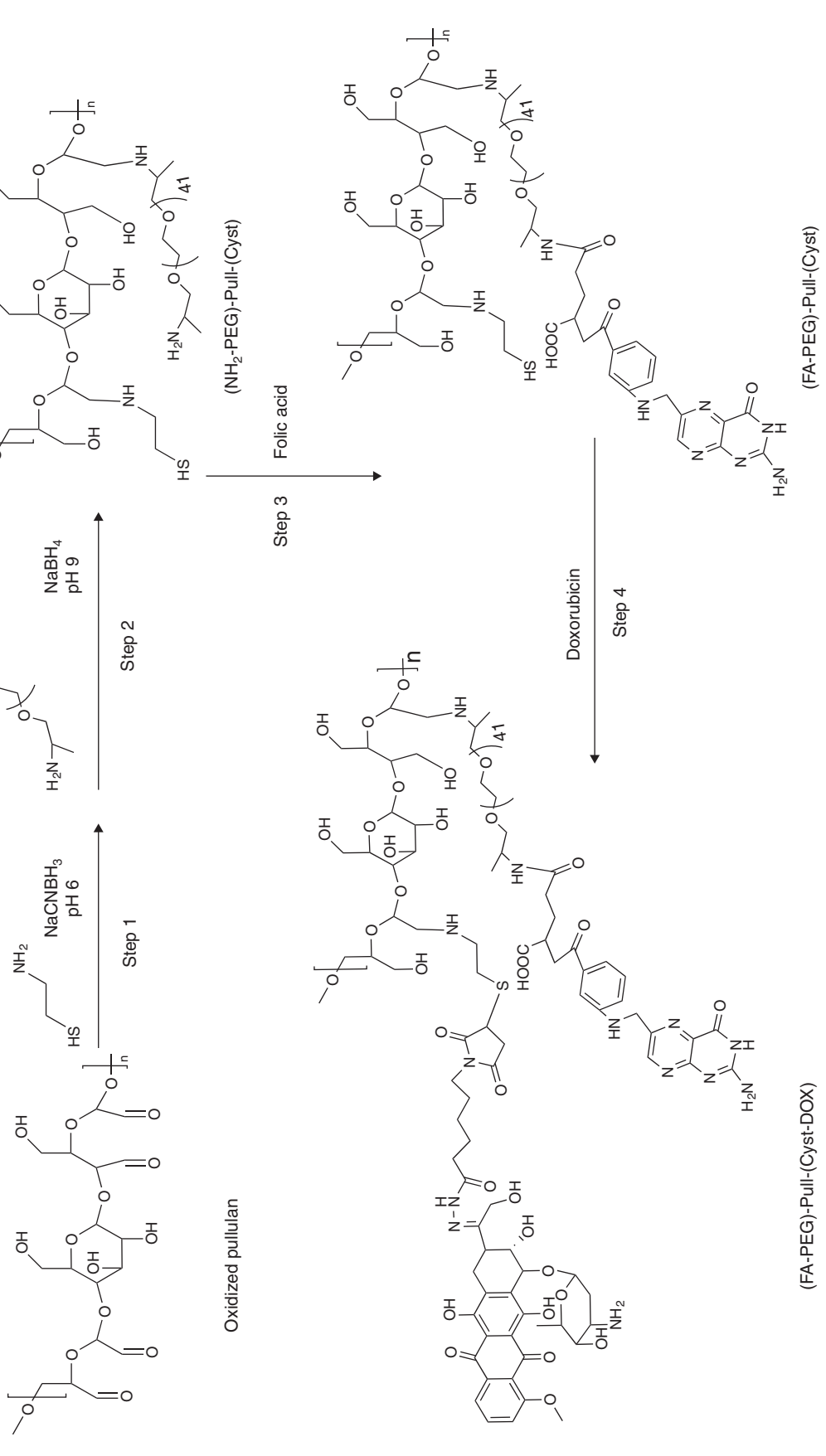

Figure 3. The synthetic procedures of (NH_2-PEG)-Pull-(Cyst-DOX) and (FA-PEG)-Pull-(Cyst-DOX).
Reproduced from [89] with permission from Elsevier.

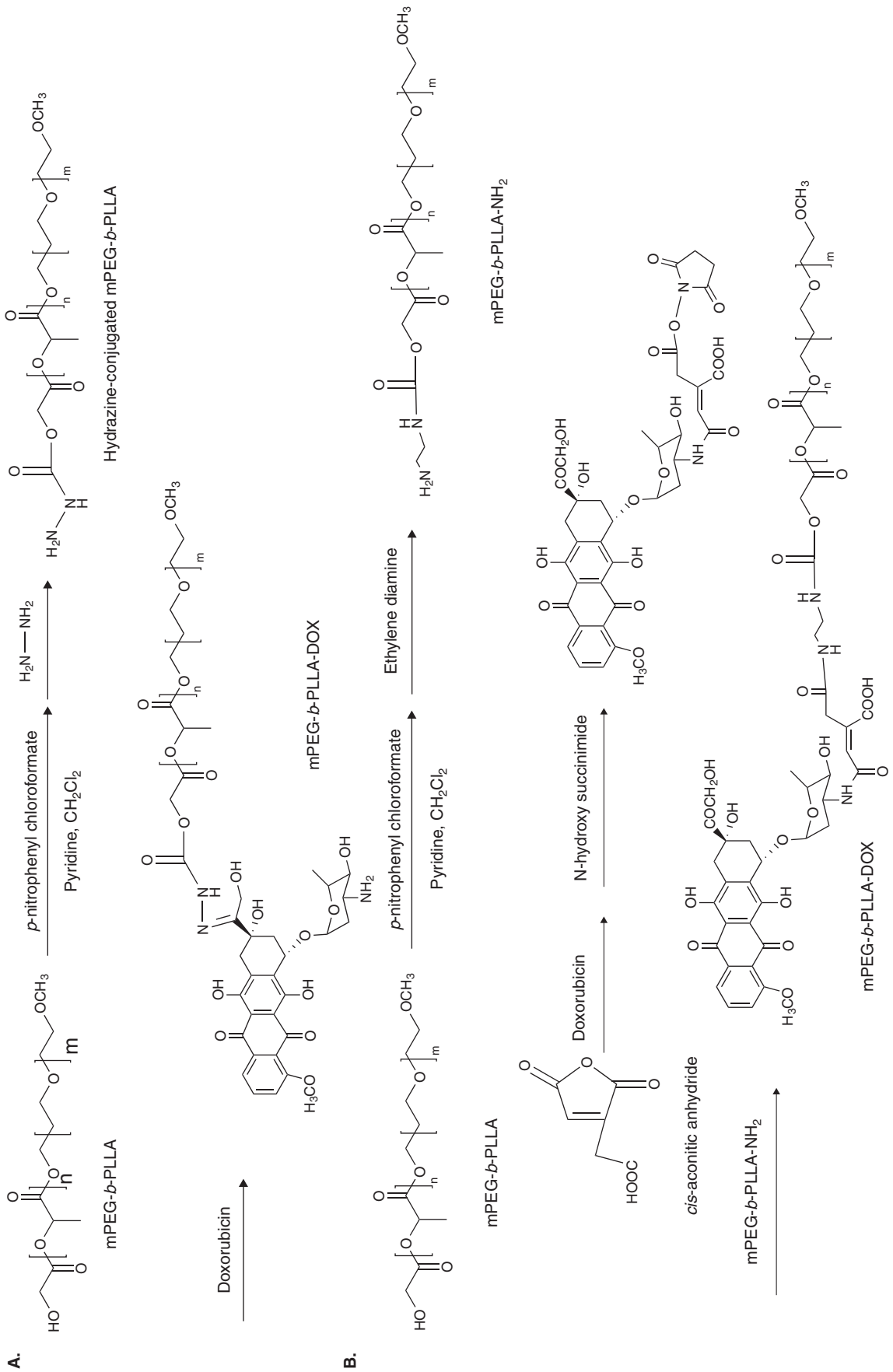

Figure 4. The synthetic route of mPEG-PLLA-DOX conjugates (A) via the hydrazone bond; (B) via the cis-aconityl bond.
Reproduced from [90] with permission from Elsevier.

conjugations were confirmed by GPC. The loading efficiency of DOX in mPEG–PLLA copolymer with the hydrazone bond was 34.5% (mol.%) and that with the *cis*-aconityl bond was 23.6% (mol.%). The CMC value and diameter of mPEG–PLLA–DOX were similar to those of mPEG–PLLA, indicating that the conjugation of DOX to mPEG–PLLA did not affect the micelle-forming ability of mPEG–PLLA. In the acidic condition, DOX was released in the intact structure from the mPEG–PLLA–DOX conjugate containing the hydrazone and the release of DOX was controlled by the acid-cleavable characteristic of the hydrazone bond. In the *in vitro* cytotoxicity test, the DOX conjugate micelles with the hydrazone bond were five-fold more cytotoxic than free DOX. This could be attributed to the fact that the DOX conjugate micelles were taken up by cells via the endocytotic transport and free DOX which can play the role of killing the tumor cells would be released depending on the cleavage of hydrazone bond in the relatively lower pH of endosome.

5. Expert opinion

A number of experimental results show that micelle-like nanoassemblies based on polymer–drug conjugates indeed can increase the solubility, prolong the *in vivo* circulation time, decrease the systemic toxicity and improve the therapeutic effects. Application of the micelle-like nanoassemblies may give new life to old active compounds abandoned due to their low solubility problems. The micelle-like nanoassemblies are different from the so-called new chemical entities (NCE) and can be considered to be a novel drug-loaded micellar formulation, which could avoid various additional development and regulatory hurdles. However, many problems need to be solved before they can reach clinical application.

Most polymers of clinically used polymer–drug conjugates are not biodegradable, such as PEG and N-(2-hydroxypropyl) methacrylamide (HPMA). For clinical application, there is a need to develop more biocompatible and biodegradable polymers which can be eventually eliminated from the body and decrease the systemic toxicity. As modern polymerization techniques such as reversible-addition fragmentation chain-transfer (RAFT) or atom transfer radical polymerization (ATRP) are developed, well-characterized, uniform polymers will be designed and synthesized. These uniform polymers have a homogeneous composition with a low degree of polydispersity, which can ensure a uniform pharmacokinetic profile for different batches of samples. In order to attach with drugs, the polymers should possess a functional group such as carboxyl groups or hydroxyl groups. The major drawback of PEG–drug conjugates is the limited drug-loading capacity of linear PEG which only contains two terminal hydroxyl groups for conjugation. Therefore, multiple reactive functional moieties for drug or active targeting ligand attachment should be introduced into the polymer chains including branched, hyperbranched or dendritic architectures.

Along with the polymers, the properties of the drugs are also very important. The drug has to contain at least a chemical group which is used to attach the polymer, limiting the scope of application. The site-specific conjugation is also important to retain the structural integrity and activity of the drugs. In addition, special attention should be paid to the nature of the linker, which conjugates the drug to the polymer. The ideal linkers or spacers need to be stable to prevent drug release prior to reaching the site of pharmacological action and be cleaved to trigger the drug release in a controllable manner in order to exert optimal therapeutic effect. The multidisciplinary close collaboration among polymer chemists, chemical engineers, molecular biologists and pharmaceutical and medical scientists will facilitate the design, development and clinical translation of micelle-like nanoassemblies based on polymer–drug conjugates. Thus, the micelle-like nanoassemblies hold great promise to benefit patients in the future.

Declaration of interest

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