Expert Opinion

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Biodegradable amphiphilic polymer–drug conjugate micelles

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The coupling of drugs to macromolecular carriers received an important impetus from Ringsdorf's notion of polymer–drug conjugates. Several water-soluble polymers, poly(ethylene glycol), poly[*N*-(2-hydroxypropyl) methacrylamide], poly(L-glutamic acid) and dextran, are studied intensively and have been utilized successfully in clinical research. The promising results arising from clinical trials with polymer–drug conjugates (e.g., paclitaxel, doxorubicin, camptothecins) have provided a firm foundation for other synthetic polymers, especially biodegradable polymers, used as drug delivery vehicles. This review discusses biodegradable polymeric micelles as an alternative drug–conjugate system. Particular focus is on A-B or B-A-B type biodegradable amphiphilic block copolymer such as polylactide, morpholine-2,5-dione derivatives and cyclic carbonates, which can form a core–shell micellar structure, with the hydrophobic drug-binding segment forming the hydrophobic core and the hydrophilic segment as a hydrated outer shell. Polymeric micelles can be designed to avoid uptake by cells of reticuloendothelial system and thus enhance their blood lifetime via the enhanced permeability and retention effect. Active tumor-targeting may be achieved by modifying the micelle surface with specific ligands. The potential application areas are discussed and future challenges are highlighted.

Keywords: biodegradable, nanomedicine, polycarbonate, polylactide, polymer–drug conjugate, polymeric micelles

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1. Introduction

Cancer is a major cause of mortality and > 11 million people are diagnosed with cancer every year [1]. The worldwide incidence of cancer continues to increase, largely owing to the ageing population. It has been estimated that there will be 16 million new cancer cases every year by 2020 [2]. Most clinically used chemotherapeutic drugs are low-molecular-mass compounds and they are limited by a relatively low therapeutic index, owing to toxic side effects [3]. For example, paclitaxel is one of the significant antineoplastic agents, derived from the bark of the Pacific yew tree *Taxus brevifolin* [4]. It has been shown to have a significant activity against various solid tumors, including ovarian, breast, non-small-cell lung cancer, head and neck carcinomas, and so on; but its low water solubility (0.25 μg/ml) [5] requires coinjection in a vehicle composed of 1:1 blends of polyethoxylated castor oil and ethanol, which has been proved to cause hypersensitivity reactions [6].

Several macromolecular delivery systems are under investigation to circumvent these limitations and improve the potential of anticancer drugs. The most attractive concept is the 'magic bullet' proposed by Paul Ehrlich, recipient of the Nobel Prize for Physiology or Medicine in 1908, in the late nineteenth century, which suggested a drug that selectively destroys diseased cells but is not harmful to healthy cells. Over the past few decades, numerous scientists have attempted to

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discover or develop such ideal drugs for the treatment of cancers. However, the real targeted drug delivery has not been realized.

During the last decade, nanomedicine has emerged as a new field of medicine that uses nanoscale materials to deliver drugs, genes and imaging agents. The nanoscale materials used for drug delivery include liposomes, polymeric micelles, polymer/drug conjugates and polymer–DNA complexes. Polymeric micelles were first introduced as drug delivery vehicles in the 1980s by Helmut Ringsdorf and co-workers [7,8]. Various morphologies of polymer micelles, including spherical, rod and lamellar structures, were obtained by Eisenberg and co-workers [9-11]. Letchford and Burt discussed the major features of nanoparticles that are formed from amphiphilic copolymers and examined the factors that influence the physicochemical properties of the formed nanoparticles [12]. The advancements in this field are also associated with the work by Kazunori Kataoka and co-workers [13-16], Le Garrec *et al.* [17] and Jean-Christoph Leroux and co-workers [18].

As drug carriers, micelles have manifested several attractive features and advantages over other types of carrier, such as core–shell structure with the drug in the core and thus effectively protected, low toxicity in the human body, and prolonged circulation time in the blood owing to its high water solubility (avoiding phagocytic and renal clearance) [19]. In addition, the passive accumulation of the micelles in a solid tumor is achieved by the enhanced permeability and retention (EPR) effect of the vascular endothelia at the tumor, proposed by Matsumura and Maeda [20,21] and approved by many other researchers [22-24]. It is also worth mentioning that micelles as an invasive body are generally taken up by the cell through endocytosis, and transferred into endosomes, and then fused with lysosomes in which the proton concentration is 100 times higher (pH 4.0 – 5.0) than the physiological condition (pH 7.4) and there are diversiform acid hydrolases. It is such an acidic environment and such hydrolases that trigger the release of drug from the micelles [25].

It is believed that polymer micelles are much more stable and have stronger mechanical strength than liposomes, but burst drug release cannot be avoided for them because of their dynamic instability. To overcome burst release, it was proposed that drug molecules be chemically combined to an amphiphilic copolymer chain to form a polymer–drug conjugate. As the drug may lose its medical activity in its conjugated state and can restore its medicine activity after being released in its pristine form from the conjugate, this polymer–drug conjugate may be considered as a 'polymeric prodrug'. In fact, the concept of 'polymeric prodrug' was first proposed by Ringsdorf in 1975 [26]. In Ringsdorf's original model, water-soluble macromolecular carriers with functional groups along the backbone were chosen for attaching drugs or spacers. Drug molecules are bound to a macromolecule through a spacer, which can incorporate a predetermined breaking point to ensure release of the drug at the site of interest. Since then, polymer–drug conjugates have become a fast-growing field. Several water-soluble polymers, for example, poly(ethylene glycol) (PEG) [27,28], poly[*N*-(2-hydroxypropyl)methacrylamide] (PHPMA) [29,30], poly(L-glutamic acid) (PGA) [31,32] and dextran [33], are being studied intensively and have been successfully used in clinical research. Results from early clinical trials of these polymer–drug conjugates have demonstrated several advantages over corresponding pristine drugs, including fewer side effects, enhanced therapeutic efficacy, ease of drug administration and improved patient compliance; but disadvantages of these polymer–drug conjugates are obvious. First, most of the clinical polymer–drug conjugates are not biodegradable. Their molecular masses are limited to several thousands to ensure renal elimination. As the polymer backbone is non-biodegradable, the polymer–drug linker is critical for drug release and needs a special design to ensure cleavage at a pointed site and in a desired manner. Second, water-soluble polymers are chosen as drug carriers, because solubility or solubilization is often the first consideration for hydrophobic drugs. By polymer–drug conjugation, water base formulation becomes easy. On the other hand, controlled release of drugs is difficult because the conjugate is dissolved and distributed in blood and fluids all over the body; because of the limited molecular mass and high molecular fluidity and flexibility, the EPR effect is limited for these polymer–drug conjugates.

Therefore, historically, polymer micelles and polymeric prodrugs (polymer–drug conjugates) are different concepts. Polymer micelles take the shape of nanoparticles but physically encapsulate drug molecules, whereas polymeric prodrugs chemically combine drug molecules and exist in individual molecules. If a biodegradable amphiphilic copolymer is used for drug conjugation, the features of polymer micelles and polymeric prodrugs may be combined to form a brand new drug delivery system, and may lead to a new era of polymer therapeutics.

2. Biodegradable polymer–drug conjugate micelles

2.1 General considerations

An ideal polymer–drug conjugate should display the following features: i) a biodegradable, non-toxic and non-immunogenic backbone that can be degraded or eliminated from the body; ii) suitable functional groups for attaching drug molecules and targeting moieties; iii) an ability to protect the drug against premature metabolism in transit; and iv) passive (EPR effect) or active tumor targeting.

Usually, amphiphilic block copolymers of the A-B or B-A-B type, where A represents a hydrophilic block and B represents a hydrophobic block, are used to construct polymeric micelles. After conjugation with drug, they can form a core–shell micellar structure with the hydrophobic drug-bound B segments in the hydrophobic core and the hydrophilic A segments as a hydrated outer shell, as shown in **Figure 1**. Other examples,

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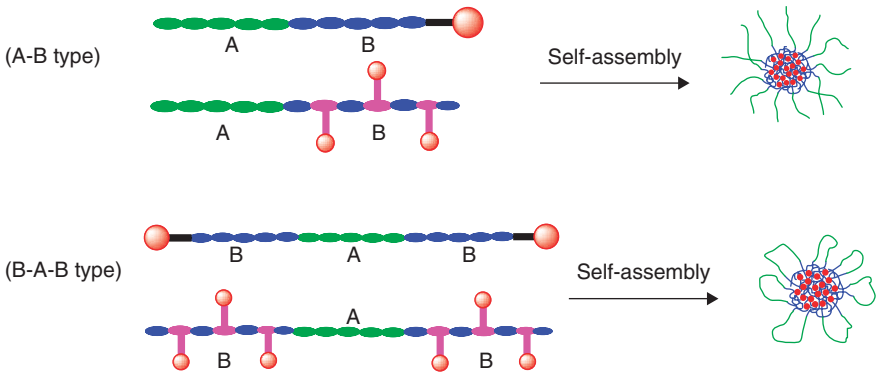

Figure 1. Selected structural and architectural types of polymer–drug conjugate, where A represents a hydrophilic block and B represents a hydrophobic block.

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including star-shaped copolymers [34], graft copolymers [35,36] and dendrimers [37-39], have also been reported.

2.2 The hydrophilic blocks

A variety of hydrophilic polymers with a flexible nature can be selected as shell-forming segments. Among them, poly(ethylene glycol) that has a repeat unit of $–CH_2–CH_2–O–$, also known as poly(ethylene oxide) (PEO), is the most commonly used. PEG presents unique properties such as: i) low immunogenicity and toxicity; ii) high solubility in water and in many organic solvents; iii) high hydration and flexibility of the chain, potentially decreasing the interactions of the polymeric micelles with constituents of biological fluids and providing good 'stealth' properties, allowing prolonged circulation in the bloodstream [40-42]; and iv) approval by FDA for human use [43]. In addition, the PEG molecules in the outer layer of the polymeric micelles can inhibit hydrophobic interactions between the inner cores of different micelles, thus blocking interparticle aggregation. When amphiphilic block copolymers are prepared with heterobifunctional PEGs having different functional groups, the polymeric micelles can be modified with targeting moieties for drug delivery to specific cells and/or tissues [44].

2.3 The hydrophobic blocks

A large variety of polymers have been studied as the hydrophobic segments in polymeric micelles: poly(propylene glycol) (PPO) [45], poly(amino acid) with functional groups [46-50], and poly(ester)s such as poly(lactic acid) (PLA) [51,52], poly(ε-caprolactone) (PCL) [53,54], poly(trimethylene carbonate) (PTMC) [55,56] and poly(morpholine-2,5-dione) [57-59]. The choice of the core-forming segment is the major determinant for important properties of polymeric micelles, such as micelle stability, drug loading capacity and drug release profile.

Biocompatible polyesters have been widely used for drug delivery. Among them, poly(glycolic acid), poly(L-lactic acid), poly(ε-caprolactone), poly(DL-lactic acid), and copolymers of lactic acid, glycolic acid and/or ε-caprolactone, are often used as the hydrophobic segments of the copolymers.

2.4 Polymer–drug conjugation

Most drug molecules have reactive or derivable groups. The coupling group on the polymer chains and the co-monomer functionality were chosen to satisfy the reactive group on the drug molecule. For example, as shown in **Figure 2**, paclitaxel has two OHs at positions 7 and 2′; a COOH group is needed to form an ester linkage, therefore the OH end group(s) of PEG-*b*-PLA or PLA-*b*-PEG-*b*-PLA is converted into COOH group(s) [60,61]. Doxorubicin has three reactive groups, that is, OH, C=O and NH_2 (**Figure 2**); COOH or $C(=O)\text{-}NH_2NH_2$ is needed to couple with them [62-66]. Other drugs referenced in this paper, such as docetaxel, indomethacin (IND), haloperidol and TNP-470, which can be modified, are also shown in **Figure 2**.

To attach the drug molecules to the polymer chains as pendant groups, reactive groups are introduced into the polymer chains by copolymerizing functionalized cyclic monomers with cyclic ester monomers (L-lactide, glycolide and/or ε-caprolactone). Amino acid *N*-carboxy anhydrides (NCA) [46,47] and morpholine-2,5-diones with protected carboxyl or amino groups [57-59], and substituted ε-caprolactones [67-69] or cyclic aliphatic carbonates carrying OH, COOH, NH_2, $CH{=}CH_2$, C≡CH or N_3 groups [70-75] are examples of these co-monomers. Among them, amino acid NCA is unique in introducing polypeptide segments into the polymer chain and possibly resulting in special features related to polypeptides [76,77]. Six-membered cyclic carbonates are usually synthesized with higher efficacy and higher purity, and have comparable copolymerization activity with cyclic esters so that high-molecular-mass and random copolymers are easy to obtain. Of course, in the functional copolymer synthesis, necessary protection and deprotection are needed for the coupling reactions.

Some spacers may be used to link drug molecules to ensure desired distances between drug molecules and polymer backbone and therefore to reduce the crowding effect and steric hindrance. The ideal linker between polymer chain and drug should be stable in physiological condition and destroyable at an appropriate site of action. For example, amino acid spacers such as glycine, alanine and small

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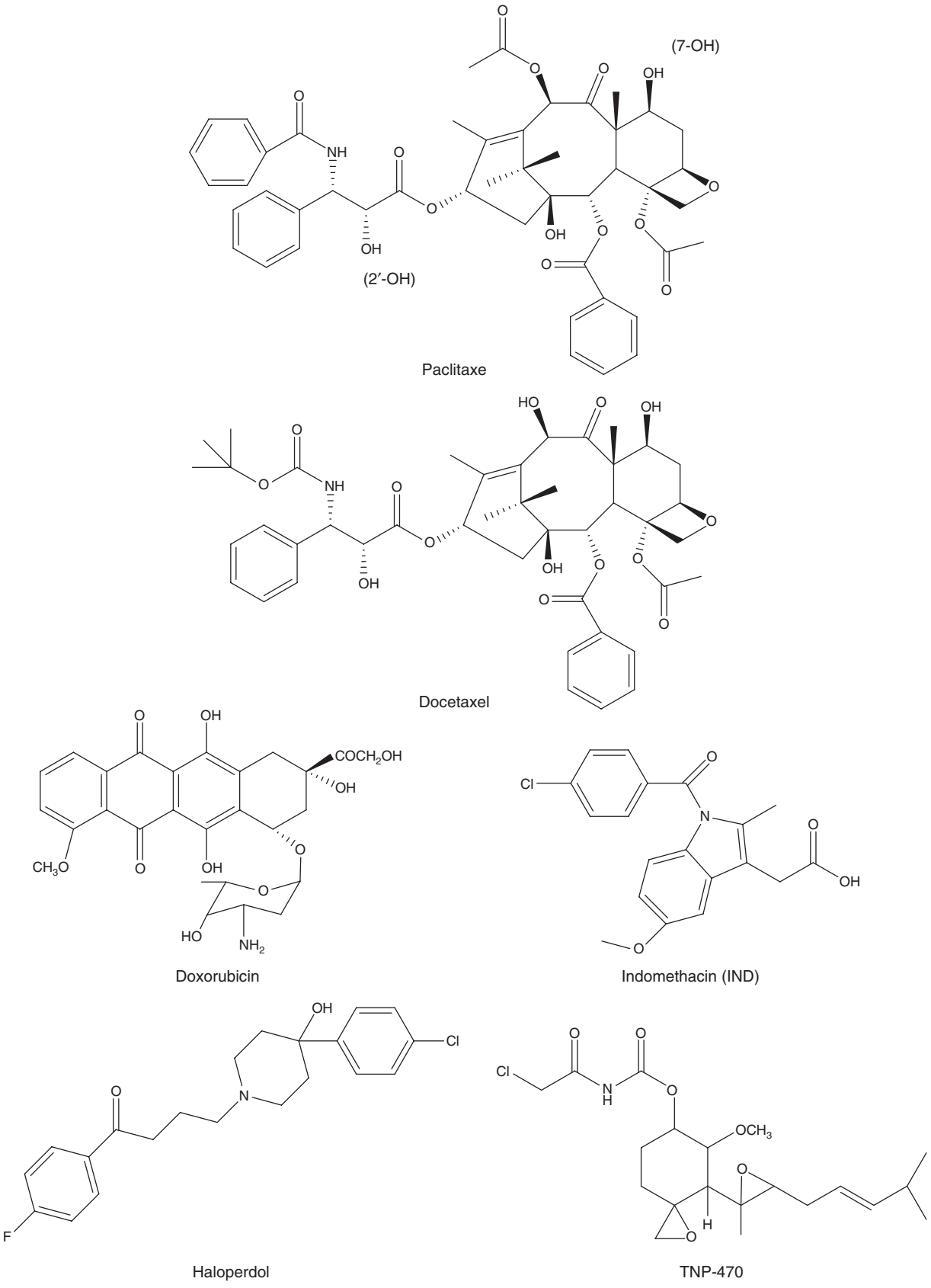

Figure 2. Chemical structures of drugs referenced in the text.

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peptides are used extensively in preparing poly[*N*-(2-hydroxypropyl)methacrylamide]-based polymer–drug conjugate because of their chemical versatility for covalent conjugation and biodegradability [62,63]. Other heterobifunctional coupling agents have also been reported [78,79].

2.5 Micelle preparation

Because the drugs used are usually hydrophobic and are linked to the hydrophobic segments, the polymer–drug conjugates remain amphiphilic. Therefore, they can self-assemble into nanomicelles with the drugs in the micellar core and PEG segments in the micellar shell. Any technologies often used in diblock copolymer self-assembling may be used to manufacture the polymer–drug conjugate micelles. These technologies include the emulsion method [80], emulsion plus solvent evaporation [81,82], solvent replacement (dialysis) [83], emulsion plus freeze-drying [84,85], film rehydration [86] and direct heating of thermosensitive polymer [87]. Other special methods such as sonication [88], extrusion [89,90], freeze–thaw cycles [91] and vortexing [92] have also been reported.

2.6 Typical examples

All polyesters only have ester groups in their backbones and do not contain reactive groups, except the terminal hydroxyl (OH) groups. This OH group is used to combine drug molecules. For example, by converting the terminal hydroxyl group of amphiphilic diblock copolymer mPEG-*b*-PLA to carboxyl through directly reacting with malic anhydride [61], or by first reacting with mono-*t*-butyl ester of diglycolic acid and subsequently deprotecting the *t*-butyl group with TFA [60], the authors prepared carboxyl-terminated diblock copolymers mPEG-*b*-PLA or triblock copolymers PLA-*b*-PEG-*b*-PLA and attached paclitaxel (PTX) to their molecular ends. By changing the block lengths of PEG and PLA, the paclitaxel content in the conjugates can be adjusted in a wide range up to 8 – 15 wt%. The polymer micelles were prepared by an emulsion plus solvent evaporation method, followed by freeze-drying. The micelles obtained were of spherical shape and had an average size of ~ 60 nm. The antitumor activity of paclitaxel conjugate against human liver cancer H7402 cells and C6 glioma cells was evaluated using the methyl thiazolyl tetrazolium (MTT) method. By incubating with human liver cancer H7402 cells, at the same drug content (20 ng/ml), the conjugate shows almost the same cytotoxicity as pure paclitaxel, which implies that the paclitaxel is released from mPEG-PLA-paclitaxel without losing cytotoxicity. Fluorescently labeled mPEG-*b*-PLA was incubated with C6 glioma cells, and confocal laser scanning microscope (CLSM) images demonstrated cellular internalization into cells, as shown in **Figure 3** [93].

B-A-B type triblock copolymer of poly(lactic acid)-*b*-poly(ethylene glycol)-*b*-poly(lactic acid) (PLA-*b*-PEG-*b*-PLA) was also investigated for conjugation of PTX [61]. By fully using the two terminal hydroxyl groups of the copolymer, PTX content in the conjugate could double that for the diblock analogue, as reported in [60]. The antitumor activity of the conjugate against woman HeLa cancer cells, evaluated by the MTT method, showed similar antitumor activity to that of free PTX.

This kind of di- or triblock copolymer has been investigated by conjugation with various drugs. Benny *et al.* conjugated TNP-470, one of the most broad-spectrum angiogenesis inhibitors, to monomethoxy-polyethylene glycol-poly(lactic acid) [94]. The amphiphilic nature of this polymeric drug enables self-assembly of micelles in an aqueous medium with the TNP-470 located in the core. Their results indicated that, by administering them orally, the conjugate micelles can be effectively absorbed in the intestine and accumulate in tumor tissue. Hans and co-workers conjugated haloperidol, an antipsychotic drug commonly used to treat schizophrenia, to the terminal end of mPEG-PLA [95]. They first prepared succinylated mPEG by reacting mPEG-OH with succinic anhydride in anhydrous pyridine. Diblock copolymer with carboxyl acid end group mPEG-PLA-COOH was synthesized by ring-opening polymerization with LA using synthesized succinylated mPEG as macroinitiator. Haloperidol was conjugated to -COOH via an ester linkage between the hydroxyl group in haloperidol and the carboxylic acid end group in mPEG-PLA-COOH.

Amphiphilic diblock or triblock copolymers with pendant functional groups were investigated extensively because more than one drug molecule can be attached to the polymer chain. The polymeric micelles consisting of a PEG-poly(aspartic acid) block copolymer conjugated with doxorubicin (NK911) are undergoing Phase II trials at present [96]. PEG is believed to form the outer shell of the micelle, producing a 'stealth' effect that prevents NK911 from being captured by the liver and spleen. The doxorubicin-conjugated poly(aspartic acid) chain is hydrophobic and is believed to form the hydrophobic inner core of the micelles in aqueous media. The authors have synthesized versatile pendant functionalized PEG-*b*-PLA amphiphilic block copolymers by introducing functionalized carbonate, *N*-carboxyanhydride and morpholine-2,5-dione derivatives, as mentioned above. These polymers can be used as new drug delivery vehicles. In one example, paclitaxel was conjugated to the pendant carboxyl groups of triblock copolymer poly{(lactic acid)-*co*-[(glycolic acid)-alt-(L-glutamic acid)]}-*b*-poly(ethylene glycol)-*b*-poly{(lactic acid)-*co*-[(glycolic acid)- alt-(L-glutamic acid)]}via its 2′-hydroxyl. The polymer–drug conjugate self-assembles into core–shell micelles in aqueous system with drug-binding segment encapsulated in the core [59,97]. *In vitro* release of paclitaxel from the conjugate micelles showed that its release rate depends on pH value. *In vitro* cytotoxicity of the paclitaxel conjugate against rat brain glioma C6 cells was evaluated by the MTT method and the results showed that the paclitaxel can be released from the conjugate without losing cytotoxicity. Mahmud *et al.* [98] synthesized poly(ethylene oxide)-*b*-poly(ε-caprolactone) (PEO-*b*-PCL) block copolymers bearing doxorubicin (DOX) side groups (PEO-*b*-P(CL-DOX)) on the PCL block. They

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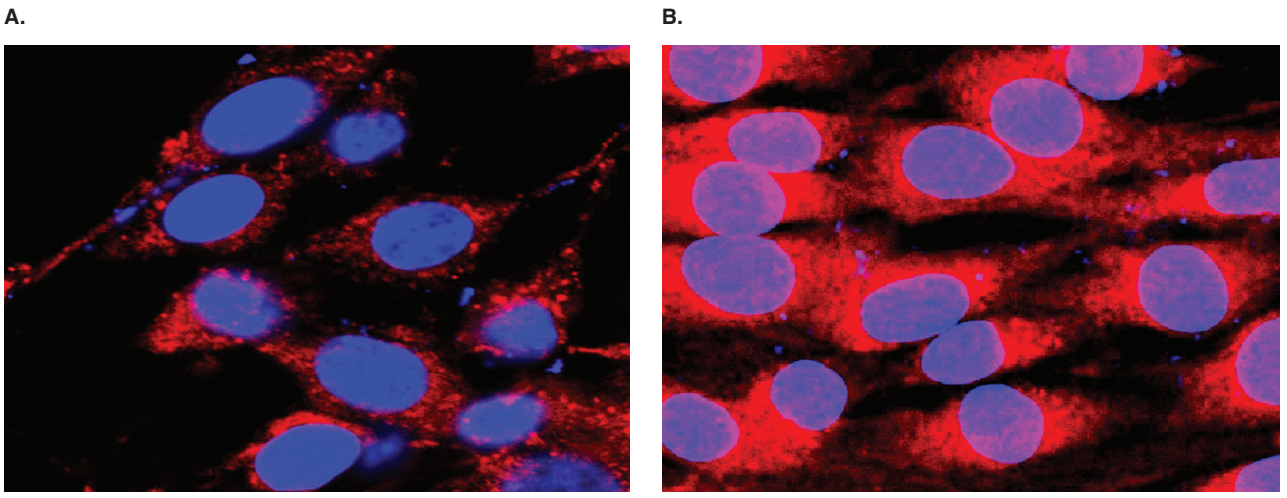

Figure 3. Fluorescence microscopy images of (A) Rhodamine B-labeled PTX and (B) mPEG-*b*-PLA micelles in C6 cells.
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investigated the release of DOX from the conjugate at different pH values and compared it with encapsulated DOX micelles. Their results confirmed that conjugation can afford efficient control over the release rate in physical pH and maintains cytotoxicity in cancer cells.

For non-degradable polymers, drug molecules are commonly conjugated to the copolymer through biodegradable spacers, which have been investigated extensively by Ulbrich and other groups [62,64,99]. For example, Giacomelli *et al.* [99] prepared double hydrophilic copolymer poly(ethylene oxide)-*b*-poly-(glycerol monomethacrylate) (PEO-*b*-PG2MA) by atom transfer radical polymerization (ATRP), using a PEO-based macroinitiator. Hydrophobic non-steroidal anti-inflammatory agent IND was conjugated to the pendant hydroxyl groups through its carboxylic acid moiety. The IND weight contents in the conjugate were in the range 15 – 49%. The release of IND from the conjugate PEO-*b*-(PG2MA-IND) micelles is a pH-dependent process. At approximately neutral pH, the ester bond linkages are stable and sustained release takes place under acidic condition.

2.7 Passive targeting

Passive targeting is one of the approaches to reduce the side effects in intravenous chemotherapeutic administration. Tumor blood vessels are generally characterized to be abnormalities such as a relatively high proportion of proliferating endothelial cells, increased tortuosity, pericyte deficiency and aberrant basement membrane formation [1]. Differences in the biochemical and physiological characteristics of healthy and malignant tissues are responsible for the passive accumulation of macromolecules in tumors. In general, low-molecular-mass compounds diffuse into normal and tumor tissue through the endothelia cell layer of blood capillaries. By contrast, the blood vessels surrounding tumors are highly permeable, and less subject to drainage. The macromolecules can enter into tumor tissue and subsequently accumulate in solid tumors. This feature has been termed 'enhanced permeability and retention' (EPR effect), and this phenomenon was first identified by Matsumura and Maeda [20,21]. Subsequently, numerous studies have confirmed the passive accumulation of macromolecules and nano-sized particulates in solid cancers [22-24]. Based on the research, nanoparticles have been mostly designed from amphiphilic block copolymers, with PEG as the hydrophilic block to minimize reticuloendothelial system (RES) interactions, whereas the hydrophobic core acts as the drug reservoir. The biodistribution and uptake of nanoparticles are influenced by many factors, such as molecular mass, surface charge, conformation and hydrophobicity [100]. The pathophysiological mechanisms of the EPR effect, architectural difference of tumor blood vessel, various factors involved and artificial augmentation of the EPR effect with respect to tumor-selective delivery have been reviewed by Maeda *et al.* in recent papers [101,102]. However, the influence of the different factors on the EPR-mediated uptake of the polymer in solid tumors is not yet completely understood.

2.8 Active targeting

Active targeting is usually achieved by chemically attaching a targeting moiety that strongly interacts with antigen or receptor existing on the target organ, tissue, or cells to a drug molecule, leading to preferential accumulation of the drug in the corresponding organ, tissue, or cells [103]. The direct advantages of drug-targeting delivery are the enrichment of the drug in the desired organ, tissue or cells, and thus enhancement of curing the disease and reduction of adverse side effects. Cell-specific targeting moieties such as monoclonal antibodies, oligosaccharides and peptides have been investigated by many researchers. It is known that specific interaction and recognition between antibody and

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antigen, or between ligand and receptor, and cellular uptake of the drug by receptor- or antigen-mediated endocytosis [104] were the main mechanisms of the targeting effects; but up to now, few targeting drugs have been used clinically, because direct coupling of targeting moieties to drug molecules is difficult and incorporation of targeting moieties brings great changes in the physical and chemical status of the drugs, and thus has a bad impact on the medical effects of the drugs.

For example, Gao and his group [105] synthesized RGD-poly(ethylene glycol)-*b*-poly(DL-lactide) diblock copolymer. Doxorubicin and a cluster of superparamagnetic iron oxide (SPIO) nanoparticles were loaded inside the micelle core. Their *in vitro* MRI and cytotoxicity studies demonstrated the ultrasensitive MRI imaging and $\alpha_v\beta_3$-specific cytotoxicity of these micelles. Wakebayashi *et al*. synthesized α-lactosyl-poly(ethylene glycol)-poly(2-(dimethylamino)ethyl methacrylate) block copolymer (lactose-PEG-PAMA) and it was used to construct a polyion complex micellar-type gene vector for selective transfection of hepatic cells [106]. Choi *et al*. prepared lactose-poly(ethylene glycol)-grafted poly-L-lysine (Lac-PEG-PLL) polymers and used them as polymeric gene carriers [107]. Transfection experiments showed that Lac-PEG-PLL delivers DNA efficiently to a hepatoma cell line *in vitro*. *N*-(2-hydroxypropyl)methacrylamide (HPMA)-based copolymers have been shown to be efficient carriers for anticancer drugs. To improve the targeting efficacy of anticancer drugs, Julyan *et al*. designed galactosamine targeted DOX-conjugated HPMA copolymers using lysosomally degradable tetrapeptide sequence as the spacer [108]; and they studied the effect of the type of sugar moiety and its three-dimensional cluster arrangement on biorecognition using three human colon-adenocarcinoma cell lines and reached the conclusion that targeting of the anticancer agent, doxorubicin, using HPMA copolymer conjugates bearing multivalent galactoside residues can improve their cytotoxicity. Monoclonal antibodies, which were first shown to be capable of binding specific tumor antigens in 1975 [26], and several monoclonal antibody-based therapeutic agents have been approved by the FDA. Numerous other ligands have also been used for active targeting, such as folate and transferrin [108-110].

In the authors' research, targeting moiety is not attached to the drug molecule itself. Instead, it is conjugated to an amphiphilic block copolymer that contains or does not contain a drug molecule, as shown in **Figure** 4. The block copolymers that carry both drug and targeting moieties can self-assemble into micelles, with the drugs in the core and the targeting moieties on the surface of the micelles. The copolymers that contain only the targeting moieties are allowed to co-assemble with drug-bearing block copolymers of a similar composition to achieve targeting micelles, as shown in **Figure** 4. Obviously, although two polymers are synthesized for the latter case, the synthesis of the two polymers themselves is relatively simple compared with the one bearing both drug and targeting moieties. Take folate-targeting as an example. Folates (FA) are low-molecular-mass vitamins required by eukaryotic cells. Elevated levels of folate receptors (FRs) are expressed on epithelial tumors of various organs, such as colon, lung, prostate, ovaries, mammary glands and brain, and its conjugates with many drugs have the ability to deliver the drugs to pathological cells without causing harm to normal tissues [1,111]. The authors conjugated FA to the lysine residues of copolymer poly(ethylene glycol)-*b*-poly(L-lactide)-*b*-poly(L-lysine) (PEG-PLA-PLL) and mixed the conjugate with pure PEG-PLA-PLL and a fluorescent model drug (mFITC) to prepare folate-conjugated micelles (folate(+)micelles) [112]. The pure PEG-PLA-PLL was used to prepare folate-free micelles (folate(-)micelles) as a control. HeLa cells as a folate-receptor overexpressing cell model and CHO cells as an FR-free cell model are used to test endocytosis of both folate(+) and folate(-)micelles. Fluorescent microscope results indicated that the folate(+)micelles show an active target endocytosis by HeLa cells, whereas the folate(-)micelles can be internalized by both HeLa and CHO cells without selectivity because of the positive surface charges on folate(-)micelles. The distribution of micelles was also investigated *ex vivo* on H22 cancer-bearing mice by means of frozen slicing. The results also indicated that the folate(+) micelles accumulate in tumors much more than in the liver and other organs at 24 h, in the order H22 cancer >> liver, kidneys >> spleen, whereas the order of folate(-)micelles was liver > spleen >> kidneys > H22 cancer.

3. Conclusions

Polymer–drug conjugates have demonstrated several advantages over their parent drugs, including fewer side effects, ease of drug administration and enhanced therapeutic efficacy. Compared with simple polymer–drug micelles based on physical encapsulation of drugs in polymer substrates, polymer–drug conjugate micelles show improved release kinetics, especially elimination of burst release at the early stage. Biodegradable copolymers are used as the drug carrier to overcome the shortcomings of non-biodegradable polymers. Similar conjugation of targeting moieties to the carrier polymer endows polymer–drug conjugate micelles' targeting capability. Therefore, polymer–drug conjugate micelles are a new generation of drug delivery system, especially for anticancer drug delivery. Great progress has been made and further rapid developments are expected.

Construction of these polymer–drug conjugate micelles includes the following strategies.

1. A biodegradable and amphiphilic block copolymer is used as the carrier of the desired drug; poly(ethylene glycol) is one of the best candidates for the hydrophilic block(s) because of its 'stealth' effect to the RES and prolongation of the blood circulation time of the micelles; polylactide or copolymers of lactide with other cyclic comonomers are the candidates for the hydrophobic block(s) because of their biodegradability under biophysical conditions.

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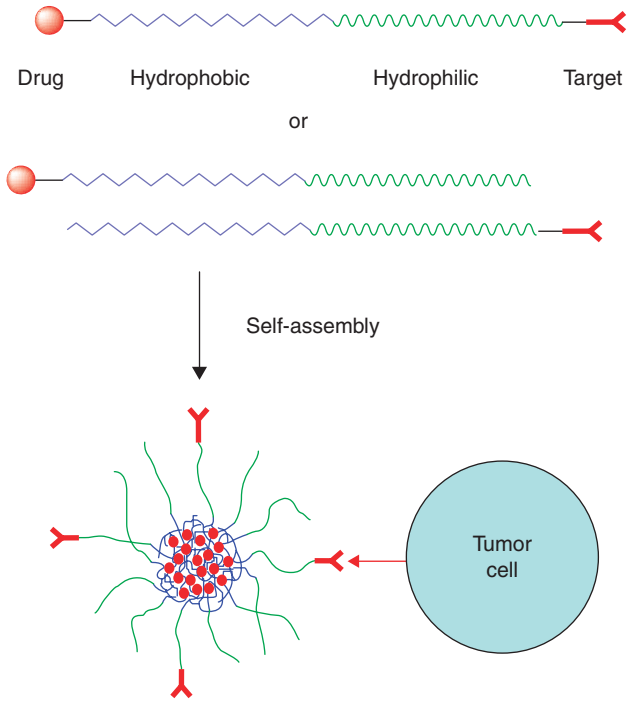

Figure 4. Schematic diagram of targeting strategies.

2. Drug molecules are chemically combined to the copolymer, either at the chain ends or as pendant groups along the polymer chain.
3. Copolymer chains are functionalized to react with the drug molecules directly or by means of a spacer; copolymerization of functionalized amino acid *N*-hydroxyanhydrides, morpholine-2,5-diones, or aliphatic cyclic carbonates with lactide is an effective approach to functionalized polylactide.
4. The polymer–drug conjugates remain amphiphilic so that self-assembling of the conjugates leads to the formation of nanoscale micelles.
5. There are two approaches to targeting polymer–drug conjugate micelles, one is self-assembling of an amphiphilic copolymer carrying both drug and targeting moieties, and the other is to synthesize a block copolymer–drug conjugate and a block copolymer-targeting moiety conjugate, both with similar backbone structure, and to co-assemble both of them into a mixed micelle.
6. All targeting moieties for low-molecular-mass drugs or liposomes, such as folate, lactose, antibody or other ligands, may be used to make copolymer-targeting moiety conjugates.

4. Expert opinion

Traditionally, polymers have been considered as auxiliary materials in medicine formulations. For polymer–drug conjugates or polymeric prodrugs, polymers are chemically combined to drug molecules. According to the definition and classification in medicine science, they belong to Class I chemical drugs and have to pass a series of preclinical and clinical evaluations and trials before clinical utilization. In this sense, polymer–drug conjugates or polymeric prodrugs are 'polymer medicines'; all administrations or treatments related to polymer–drug conjugates or polymeric prodrugs are 'polymer therapies'. Of course, protein or gene drugs are polymeric drugs in structure; but they are usually products of bioprocesses, and therefore they are not classed into chemical drugs and do not belong to the 'polymer medicine' defined above. As reviewed above, amphiphilic polymer–drug conjugates take the form of nanomicelles because of their self-assembling ability. In this sense, they are drug-loaded polymer micelles, which are defined in the literature as micelles based on physical encapsulation of drugs in a polymer matrix; but polymer–drug conjugate micelles are different from traditional drug-loaded polymer micelles in that the drug molecules and matrix polymers have been covalently bound together.

In short, biodegradable polymer–drug conjugate micelles are a new generation of drug formulation. They are products of multidiscipline intersection, such as polymer materials science, medicine science, and nanoscience and nanotechnology. The developments so far are encouraging. Their advantages over the traditional chemical drugs or polymersomes have been demonstrated, such as enhanced water solubility, prolonged blood circulation time, higher curing efficacy, reduced side reaction and toxicity, and possibility of passive and active targeting – but they are still in their infancy. Many scientific and engineering problems should be solved before they can be used clinically. First, from the chemical point of view, great efforts should be made in the design, synthesis and optimization of amphiphilic block copolymers or graft copolymers, in the choice of the polymer–drug linkage and the coupling reactions, in micellization of the polymer–drug conjugates, and in the targeting strategy of the polymer–drug conjugate micelles for each clinically significant drug. Second, special medicine issues for polymer–drug conjugate micelles should be studied. For example, for a polymer–drug conjugate micelle system, a drug may exist in three forms: conjugate micelles, individual conjugate molecules, or released drug molecules. In a medicine kinetic study or a metabologic study, these three forms should all be considered. It is a tough task to distinguish and to determine separately the relative contents of the three forms both in blood and in various organs. It is known that endocytosis is the main mechanism for polymer–drug conjugate micelles, and this is especially important for cell-targeting conjugate micelles. Therefore, new methodology is needed to study the internalization process of the conjugate micelles and to determine the distribution of the conjugate micelles inside and outside cells. Molecular imaging is a promising technology for this purpose, including fluorescent probing of the conjugate micelles, individual conjugate molecules, and free drug molecules, and multichannel measurements of these fluorescent probes, in

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the levels of cells, tissues, organs, or even living animals. In short, further developments need great efforts from scientists in different disciplines. There coexist both challenges and opportunities. Biodegradable and amphiphilic polymer–drug conjugate micelles are expected to enter clinic practice in the near future.

Declaration of interest

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