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PEG based anti-cancer drug conjugated prodrug micelles for the delivery of anti-cancer agents

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Due to the high cost and uncertain success of new drug development, tremendous effort is devoted to increasing the efficacy of established anti-cancer drugs. Development of polymer prodrug conjugates has evolved recently in the nano-medicine field for cancer diagnosis and treatment. The major advantage of using polymer drug conjugates is that the chemical and physical properties of polymers can be tuned to increase the efficacy and to reduce the toxicity of the drug. The stimuli responsiveness provides the release of the prodrug in a controlled manner which avoids undesired side effects, organ damage, and toxicity caused by the fluctuations associated with periodic administration. A large number of anti-cancer drug polymer conjugates have been studied for cancer therapy due to their promising clinical applications in chemotherapy. In this paper, poly(ethylene glycol)(PEG) based anti-cancer drug conjugates will be discussed followed by a review of different types of PEG-*b*-poly(ϵ -caprolactone)(PEG-*b*-PCL) copolymer drug conjugates and histone deacetylase inhibitor-polymer conjugates as novel therapeutics. The pH sensitive release of prodrugs will be discussed for polymer prodrug conjugates that are currently under investigation.

1. Drug polymer conjugates

Due to the high cost and uncertain success of new drug development,¹ tremendous effort is devoted to developing novel formulations to increase the bioavailability of established hydrophobic drugs.²⁻⁵ Polymer drug conjugates emerged recently in the nano-medicine field for cancer diagnosis and treatment. Polymeric micellar drug delivery systems have faced many barriers such as poor drug loading and poor blood stability.⁶ These barriers have made drug polymer conjugates more attractive as drug delivery systems. The polymer helps to hold the drug and hence act as a carrier medium for the drug. More importantly the chemical and physical properties of polymers used in polymer-drug conjugates are tuned to increase the efficacy and to reduce the toxicity of the drug.⁷⁻¹⁰

The polymer drug conjugate concept was first proposed by Helmut Ringsdorf in 1975.¹¹ The Ringsdorf model consists of three components: 1) a solubilizer or the hydrophilic segment to ensure the water solubility, 2) a drug, usually bound to the polymeric backbone via a linker, and 3) a targeting moiety that functions to provide transport to a particular biological target. These polymer-drug conjugates offer several advantages over traditional small molecule therapeutics. The aqueous solubility of the water insoluble drug can be dramatically increased by the conjugation. If a drug is highly water soluble it will be difficult to deliver in a

controlled manner because they generally have low permeability through lipophilic tissue. As a result, they are unlikely to penetrate some target tissues in effective concentrations. On the other hand, poorly water soluble drugs will have low tissue permeability as they are difficult to dissolve in the biological environment.¹² The polymer drug conjugates self-assemble in aqueous media so the hydrophobic drug can be incorporated in the core of the micelles. The lack of degradation and/or deactivation of the drug increases circulation time in the blood stream.¹³ The drug can be delivered in a controlled manner. Thus it is possible to avoid undesired side effects, organ damage, and toxicity caused by the fluctuations associated with periodic administration. Polymer drug conjugation also provides an opportunity to alter drug pharmacokinetics and biodistribution.¹⁴ This is particularly useful to avoid the rapid metabolism or clearance of the drugs. Another major advantage is that the targeting moiety functions to carry the drug to the site of pharmacological action. The widely studied targeting moieties include sugars, hormones, growth factors, antibodies, antibody fragments, and peptides.¹⁵⁻²² A substantial amount of effort is currently directed toward developing anti-cancer drug polymer conjugates.

Several drug delivery approaches have been developed to enhance the efficacy of established anti-cancer drugs. The "prodrug" strategy was introduced to overcome the physiological barriers.^{23, 24} In this method the drug is covalently attached to a macromolecule to form a derivative of a drug and that derivative is metabolized or activated *in vivo* into active drug. This strategy is important to overcome the low solubility of the drug, reduce adverse effects, and

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prolong blood circulation. A large number of anti-cancer polymer conjugates have been studied in cancer therapy due to their promising clinical applications in chemotherapy.^{13, 24-30}

Prodrugs can be used to improve the pharmacokinetics of the drugs. Small molecule drugs are chemically modified by attachment to pharmacologically inactive functional groups. Once they have reached their intended target they are metabolically activated *in vivo* into active drugs. Drugs can be incorporated into nano-drug carrier systems like lysosomes, polymeric micelles, polymeric nanoparticles, polymer drug conjugates, etc., which have been shown to be efficient to deliver drugs and genes.³¹

2. Amphiphilic block copolymers for drug delivery

Recently, a large number of anti-cancer drugs have been approved by the FDA. However, many of them have limited clinical applications due to unsuccessful delivery systems and numerous anti-cancer drugs exhibit poor water solubility.³²⁻³⁴ Standard formulation techniques are needed for delivering these drugs to a target. Micelles based on amphiphilic diblock copolymers provide an ideal encapsulation for the hydrophobic drugs. Amphiphilic diblock copolymers self-assemble in aqueous environment to form micelles such that the hydrophobic part is placed inside (micellar core) and the hydrophilic part is on the outside (shell). Micellar encapsulation helps to solubilize, stabilize, and deliver the hydrophobic drug to the target.³⁵ The hydrophobic core is stabilized by the hydrophilic shell interactions with the surrounding environment which positively influences prolonged blood circulation time.

The driving force behind the micelle formation is the decrease in the free energy of the system due to the removal of hydrophobic segments from the aqueous environment to form the core and the hydrophilic blocks exposed to aqueous environment to form the shell. The thermodynamic stability can be explained in terms of critical micelle concentration (CMC). The lower the CMC of the polymer, the more stable the micelles are in the medium.³⁶ This is important in terms of biomedical applications because micelles with lower CMC values prevent the dissociation into unimers upon dilution with large volume of blood. Amphiphilic diblock copolymers of PEG-*b*-PCL have shown CMC values as low as 10^{-4} g/L.^{29, 37} The hydrophobic core influences the CMC value the most. An increase in the length of a hydrophobic block causes a significant decrease in the CMC.³⁸ However, the increase in the length of a hydrophilic block results in a small increase in the CMC. An increase in the molecular weight of the polymer results in a lower CMC.³⁶ It has been reported that the diblock copolymers have lower CMC values than that of the triblock copolymers.³⁹ The typical pharmaceutical micelles should possess a size from 10 to 100 nm in order to establish high stability both *in vivo* and *in vitro*. The micelles should have a long circulation time in the blood and should be able to collapse into non-toxic unimers and eventually cleared from the body by releasing the loaded cargo to the target in a controlled manner. The compatibility between the

hydrophobic block and the incorporated drug increases the loading capacity of the drug.³⁹ Nanoparticles have been shown to accumulate in tumor tissues that have leaky vasculature and poor lymphatic drainage.⁴⁰⁻⁴⁴ Therefore, the micelles with 10-100 nm size tend to accumulate due to the enhanced permeability and retention (EPR) effect.^{43, 45-48} Nanoparticles with hydrodynamic diameter of 5-6 nm represent the threshold for renal clearance.⁴⁹

Numerous polymer compositions have been studied to conjugate different drugs. Among the hydrophilic polymers poly(vinyl pyrrolidone) (PVP),^{50, 51} poly(vinyl alcohol) (PVA)⁵², polyglutamic acid (PGA),⁵³ poly(malic acid) (PMA),⁵³ poly(ethylene glycol) (PEG)⁵⁴ and N-(2-hydroxypropyl)methacrylamide (HPMA) copolymers^{55, 56} have been used (Figure 1). Among them, FDA approved PEG is the most commonly employed hydrophilic polymer for drug delivery. The hydrated PEG shell reduces aggregations which results in increased stability and prolonged blood circulation times of drug that are shielded by or bound to PEG. PEG is used in different applications based on its molar mass. PEG with molar masses of 20 kDa to 50 kDa is used for the conjugation of low-molar-mass drugs which slow down the renal clearance. PEG with molar masses of 1 kDa to 5 kDa is often used for the conjugation of larger drugs. In the later case, PEG reduces the nonspecific interactions with the blood.⁵⁷

The hydrophobic core forming polymers such as poly(propylene oxide) (PPO), polycaprolactone (PCL), poly(lactide) (PLA), polystyrene (PS), poly(aspartic acid) (PASP), and poly(glutamate) PGLU have been reported (Figure 2).⁵⁸ Among them, the most common materials are hydrophobic polyesters such as polycaprolactones. Their mechanical properties, hydrophilicity, biocompatibility, and biodegradability can be tuned by functionalizing the polymer backbone making them advantageous for biomedical and pharmaceutical applications.⁵⁹⁻⁶³

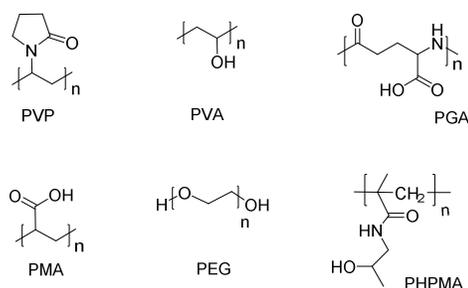


Figure 1 Hydrophilic shell forming polymers.

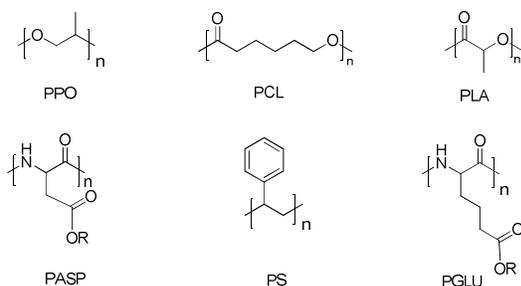


Figure 2 Hydrophobic core forming polymers.

3. PEG based micellar prodrugs for delivery of anti-cancer agents

Many studies have been carried out to attach anti-cancer drugs to the hydrophobic polymer backbone.^{14, 64-71} The polymer then self-assembles to form micelles where the anti-cancer conjugated side chains form the hydrophobic micellar core, while the hydrophilic segment forms the micellar shell. Many cleavable linkages such as carbamate⁷², amide⁷³⁻⁷⁵, cis-acotiny⁷⁶, benzoic imine⁷⁷ and hydrazone^{65, 78-81} have been employed. Among them the hydrazone linkage is the most versatile and can be selectively cleaved under acidic conditions.

3.1 DOX conjugated amphiphilic copolymers prodrugs

In 2005, the Kataoka group reported folate (FA) conjugated amphiphilic block copolymers, FA-poly(ethylene glycol)-poly(aspartate hydrazone doxorubicin) (FA-PEG-PASP-DOX) where the anti-cancer drug doxorubicin (DOX) was conjugated through an acid-sensitive hydrazone bond to the side chains of the core-forming PBLA block (Figure 3a). FA was end-functionalized to conjugate PEG with aldehyde groups which allowed the active FA ligands to conjugate onto the surface of the micelles. FA promotes the intracellular transport by directing the FA-bound micelles to the cancer cells. The drug has been released from the micelles upon the acidic cleavage of the hydrazone bonds after entering the cells.⁸² Surface plasmon resonance measurements have proved that FA-bearing micelles could interact with the FA receptor.

Similar FA-conjugated amphiphilic hyperbranched block copolymers have been used to conjugate DOX onto the hydrophobic segments of the block copolymer via an acid-labile hydrazone linkage (Figure 3b). The micelle sizes were in the range of 17–76 nm when measured by DLS. It has been found that the release of DOX depended on the pH values and the rapid release of DOX at acidic pH due to the hydrolysis of hydrazone linkage was observed. The slow rate of degradation during the first two weeks proved it was an excellent drug

carrier and the extensive degradation allowed for renal excretion.⁸³

FA-receptor-targeted delivery of DOX-PEG-FA conjugate was reported in 2005 (Figure 3c). DOX and FA were conjugated to the α - and ω -terminal end groups of PEG, respectively. Hydrophobically deprotonated DOX molecules were aggregated within the core by forming nano-aggregates with the average size of 200 nm. These nano-aggregates showed enhanced cellular uptake, increased targeting capacity, and increased cytotoxicity in cells with overexpressed FA receptors.⁸⁴

A diblock copolymer composed of poly(L-lactic acid)(PLLA) and methoxy PEG has also been reported, where DOX was chemically conjugated to the polymer.⁷⁶ An acid cleavable hydrazone bond (Figure 3d) and a *cis*-aconityl bond (Figure 3e) were formed between DOX and the PLLA. The micelle size and the CMC values were shown to be comparable with those of unconjugated micelles. The micelles with hydrazone linkages released about 40% of the loaded DOX within the first day in acidic medium. It has been found that the intact DOX was regenerated by hydrolysis of hydrazone linkages.

Polymer, PEG-*b*-PASP-DOX conjugation has been achieved through acid cleavable hydrazone linkages.⁶⁷ The drug loading content of 42.5 wt % was reported with respect to single block copolymer chain. The micelles had a 65 nm hydrodynamic diameter. The micelles were stable at physiological conditions. However 100 % of the drug was released at pH 3.0 (Figure 3f).

The same polymeric system has been modified to obtain both reduction and pH sensitive DOX conjugated disulphide cross linked poly(ethylene glycol)-*b*-poly(aspartate) (PEG-*b*-P[ASP(Hyd-DOX)]) (Figure 3g).⁶⁴ Compared with non-cross-linked PEG-*b*-P[ASP(Hyd-DOX)] micelles, disulfide cross-linked micelles demonstrated excellent stability and slower drug release kinetics under nonreducing conditions. Moreover, the disulphide cross-links in the micellar core have reduced the systemic toxicity caused by DOX.⁶⁴

Anti-cancer drugs can be conjugated to the polymer via different types of bonds. The DOX covalently attached to biodegradable block copolymer methoxy-poly(ethylene glycol)-*b*-poly(lactide-co-2,2-dihydroxymethylpropylene carbonate) was reported.⁷² Pendant hydroxyl groups of the polymer were linked to the DOX via carbamate linkage (Figure 4a) and hydrazone linkage (Figure 4b). These amphiphilic polymers self-assembled to form micelles with sizes ranging from 70 to 100 nm. Both carbamate and hydrazone linkages have shown pH- dependent behavior. However, the micelles formed from hydrazone linkages have shown increased sensitivity towards acidic cleavages compared to carbamate linkages.⁷²

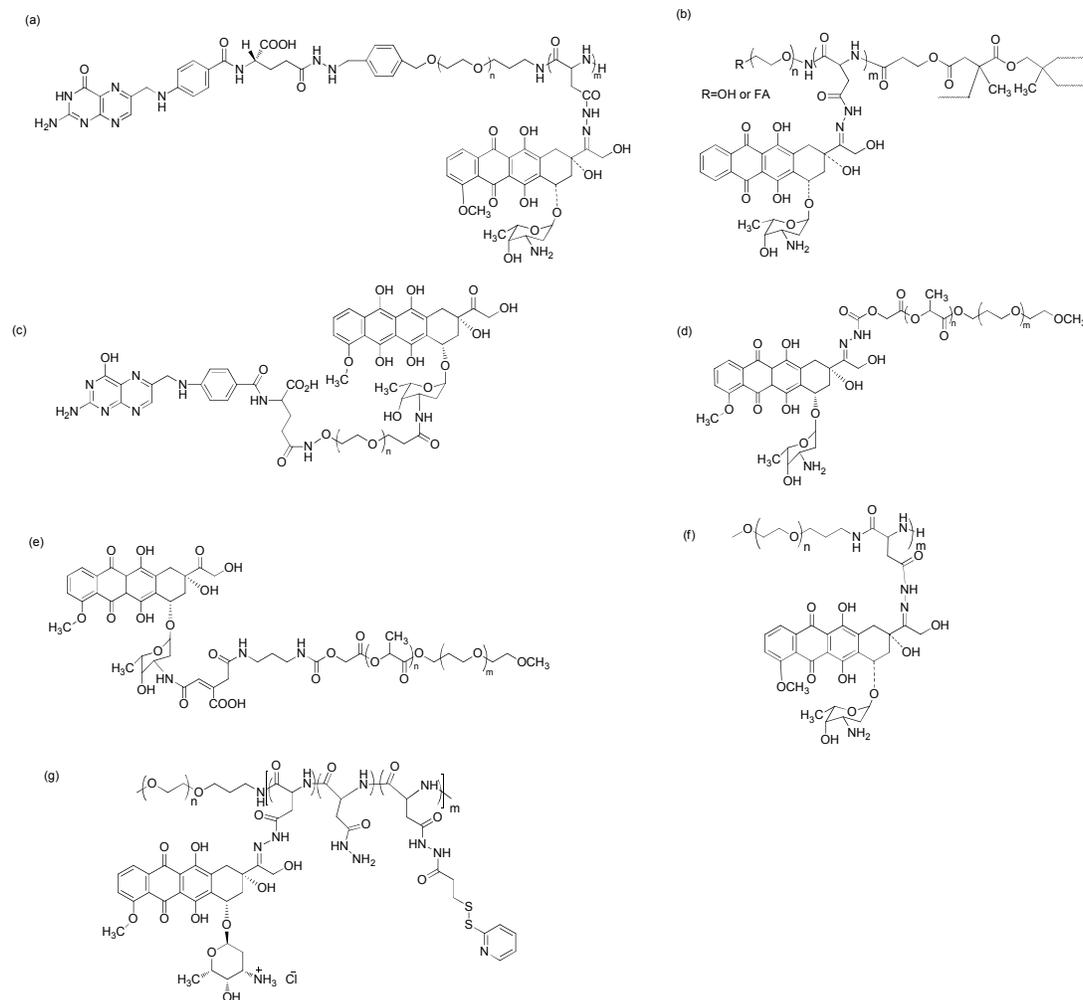


Figure 3 (a) FA-PEG-PASP-DOX amphiphilic block copolymers via a hydrazone linkage (b) FA-conjugated amphiphilic hyperbranched block copolymers via a hydrazone linkage (c) FA-receptor-targeted FA-PEG-DOX conjugate (d) DOX- PLLA-PEG conjugate via a hydrazone linkage (e) DOX- PLLA-PEG conjugate via a cis-aconyl linkage (f) PEG-*b*-PASP-DOX conjugation via a hydrazone linkage (g) dual stimuli responsive PEG-*b*-P[ASP(Hyd-DOX)] via a hydrazone linkage

A dual pH sensitive DOX conjugated polymer has been designed from parental monomethoxyl poly(ethylene glycol)-*b*-poly(allyl ethylene phosphate). The copolymer had unique

properties such as sensitivity towards extracellular and intracellular pH environments to simultaneously enhance cellular uptake and promote acid-triggered intracellular drug

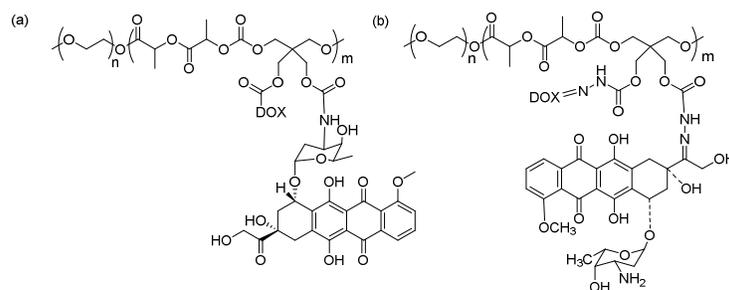


Figure 4 (a) DOX-PLA-PEG conjugate via carbamate linkage (b) DOX-PLA-PEG conjugate via hydrazone linkage .

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release (Figure 5a). The DOX has been linked to the polymer via acid labile hydrazone bonds.⁷⁹

A hydrolytically cleavable hydrazone bond has been used to conjugate DOX to poly(allylglycidyl ether)-*b*-poly(ethylene oxide) as shown in Figure 5b. The DOX loading content increased up to 17 % using acetic acid–sodium acetate pH-buffering system under anhydrous conditions which supplies the mildly acetic conditions needed for the reaction. Furthermore, it eliminated the electrostatic repulsive forces between polymer and DOX. The prodrug micellar drug delivery system was shown to have a long circulation time in the bloodstream.⁸⁵

Mixed micelles were reported by Yang, et al in 2015. Co-assembly of cyclic (Arg-Gly-ASP-D-Phe-Lys) c(RGDfK) functionalized poly(ethylene oxide)-*b*-poly(ϵ -caprolactone) (cRGD-PEO-*b*-PCL) (Figure 5c) and benzoic-imine linked PEGylated DOX (PEG-DOX)(Figure 5d) promoted the interaction with the receptors on the cancer cell membrane at neutral pH and the hydrolysis at tumor's pH respectively.⁷⁷ Micelles of cRGD-PEO-*b*-PCL displayed lower CMC than the PEG-DOX assemblies which indicates the better thermodynamic stability of cRGD-PEO-*b*-PCL micelles. However, PEG-DOX has been co-assembled with cRGD-PEO-*b*-PCL at physiological pH to form targeting mixed micelles (cRGD-PEO-*b*-PCL/PEG-DOX (TM micelles)). No significant difference in drug loading was observed for TM micelles and the parent PEO-*b*-PCL micelles. It was also shown that when paclitaxel (PTX) was loaded into TM micelles that there was improved cytotoxicity on U87MG cell lines.

Polymeric micelles of DOX conjugated poly(ethylene glycol)-*b*-poly(aspartate) (PEG-*b*-PASP) block copolymers have been gained much attention over the past years due to their ease of forming micelles in water⁷³⁻⁷⁵ (Figure 5e). The micelle formation behaviour has been shown to be dependent on the composition and media.^{68, 86} The DOX conjugation provided sufficient hydrophobicity in the PASP segment, thus forming stable micelles. The micelles remain in the circulation for prolonged periods of time without degradation. DOX conjugated micelles remained in the blood for 24 hours, while free DOX disappeared immediately from the blood in a few minutes.⁸⁷ The *in vivo* anti-cancer activity and application against several solid tumors were evaluated.⁸⁸ DOX can be incorporated into the polymers by chemical conjugation and physical entrapment.^{78, 89} The physically entrapped DOX employs the major cytotoxic function, while conjugated DOX mainly increases the micelle stability.⁸⁹

DOX-conjugated Y-shaped mPEG-*b*-poly(glutamate-hydrazone-DOX)₂ and linear copolymers of mPEG-*b*-poly(glutamate-hydrazone-DOX) were reported.⁸⁰ The DOX has been conjugated to the polymer through an acid cleavable hydrazone bond (Figure 5f). The drug loading

content of the Y-shaped polymeric micelles were twice that of linear polymers as the Y-shaped polymers provided more sites for conjugation. Furthermore DOX conjugated Y-shaped PEG-(polypeptide)₂ copolymers showed several advantages over linear copolymers, such as assembling into smaller nanoparticles and faster drug release in acid, thus providing higher cellular uptake and enhanced extracellular/intracellular drug release.

Recently, DOX conjugated poly(ethylene glycol)-*b*-poly(N-(2-hydroxypropyl) methacrylamide-co-N-methacryloyl glycerylglycine) (PEG-*b*-P(HPMA-co-MAGG)) copolymers have been reported.⁹⁰ The introduction of the MAGG group causes the polymer to become more negative and it has been found that PEG-*b*-PMAGG copolymers are rapidly cleared from blood circulation and accumulate in the liver. However, longer circulation time and lower liver uptake, when compared to the DOX-free polymer, have been observed with DOX conjugation. Furthermore, drug conjugates with lower nonspecific uptake and enhanced tumor accumulation in the kidney have been achieved with the appropriate decrease of the negative charge. DOX conjugated pH sensitive phospholipid prodrug based phosphorylcholine have shown prolonged circulation, high accumulation in tumors, fast cellular uptake and burst drug release in cancer cells.⁹¹ Interestingly, these novel prodrug micelles have shown better ability to be internalized by cancer cells than that of the PEG prodrug micelles.

3.2 PTX conjugated PEG based amphiphilic copolymers prodrugs

PTX loaded and PTX conjugated monomethoxy poly(ethylene glycol)-*b*-poly(lactide)(mPEG-PLA) block copolymers were compared by means of physicochemical characteristics, drug release and anti-cancer activities (Figure 6c).⁹² The hydroxyl end-group of the block copolymer PEG-PLA was converted into a carboxylic acid and then esterified with PTX.

The CMC of PEG-PLA–PTX micelles was around 6.31×10^{-4} g/L which is one order of magnitude less than PEG-PLA, which is attributed to the enhanced hydrophobicity with the PTX conjugation.⁶⁶ The loading efficiency of the PEG-PLA was 16.7 % whereas the PTX content in the conjugate was 10%. Conjugate micelles have shown less initial burst release than that of encapsulated micelles at pH 7.4. The prolonged release makes the polymer conjugate more suitable for tumor therapy by reducing the required drug dose and reducing toxic side effects in humans.⁶⁶

3.3 Selected PEG based anti-cancer drugs conjugated amphiphilic copolymers prodrugs

The anti-cancer drug chlorambucil, which is used against chronic lymphatic leukemia, lymphomas, and advanced ovarian and breast carcinomas, has limited clinical applications due to its side effects such as nausea, myelotoxicity, and neurotoxicity.⁹³ In 2015, the Chilkoti group designed a prodrug monomer in which polymerizable cyclic carbonate was linked to an ethylene glycol linker and chlorambucil and it was polymerized with 1,5,7-triazabicyclo-[4.4.0]dec-5-ene (TBD) as the organocatalyst and mPEG as macroinitiator (Figure 6b).⁷¹ The amphiphilic polymer prodrug self-assembled and their relatively low CMC was an indication of good thermodynamic stability of micelles. The method has been generalized for hydroxyl and amine functionalized hydrophobic anti-cancer drugs. Camptothecin, a hydroxy-functionalized anti-cancer drug,^{70, 94} has also been selected to synthesize the polymer prodrug (Figure 6c).⁷¹

Several attempts toward prodrug based micellar drug delivery systems with different antitumor agents, such as indomethacin⁹⁵ (Figure 7a), curcumin⁹⁶ (Figure 7b) 5-fluorouracil⁹⁷ (Figure 7c), and methotrexate⁹⁸ (Figure 7d) and daunorubicin⁹⁹ (Figure 7e) have been achieved successfully. 9-Fluorenylmethoxycarbonyl conjugated, PEG5000-lysyl-(α -Fmoc- ϵ -t-Boc-lysine)₂¹⁰⁰ and PEG5000-lysyl-(α -Fmoc- ϵ -Cbz-lysine)₂(PLFCL)¹⁰¹ polymers have also been reported for the PTX and DOX delivery respectively.

3.4 Poly(ethylene glycol)-b-poly(ϵ -caprolactone) copolymer anti-cancer drug conjugates

Biocompatible biodegradable PCL has been extensively studied due to controlled drug delivery applications. In 2012 Chang et al. reported methoxy poly(ethylene glycol)-b-poly(ϵ -

caprolactone-co- γ -hydroxyl- ϵ -caprolactone) (mPEG-*b*-P(CL-co-HCL)) bearing pendant hydroxyl groups on the PCL block. The hydroxyl groups were formed through the reduction of ketones. The CMC of the mPEG-*b*-P(CL-co-HCL) polymers were $6.3 \times 10^{-4} \sim 8.1 \times 10^{-4}$ mg/mL and the micelle sizes were in the range 100 to 140 nm. Higher loading capacity and slower *in vitro* release of DOX were observed, which was due to the hydrogen-bonding formation between DOX and hydroxyl groups of the hydrophobic core.³⁷

Docetaxel, anti-cancer drug for breast cancers, belongs to the Class IV of the biopharmaceutical classification system with poor water-solubility and low permeability which is not suitable for an oral drug.¹⁰² To overcome this problem, docetaxel was recently encapsulated in pH sensitive amphiphilic poly(ϵ -caprolactone)-poly(ethylene glycol)-poly(ϵ -caprolactone) copolymer (PCEC) based micelles to improve the solubility and the permeability of docetaxel.¹⁰³

Mikhail and Allen reported chemotherapeutic docetaxel (DTX) conjugated to the hydrophobic block of poly(ethylene glycol)-*b*-poly(ϵ -caprolactone) (PEG-*b*-PCL) copolymers in 2010¹⁰⁴ (Figure 8a). PEG-*b*-PCL block copolymers were modified to generate carboxyl-terminated PEG-*b*-PCL (PEG-*b*-PCL-COOH) copolymers and finally conjugated with DTX. Physical encapsulation of DTX in PEG-*b*-PCL-DTX micelles has resulted in significantly higher drug loading than that of DTX loaded PEG-*b*-PCL micelles.

Self-associating PTX conjugated PEO-*b*-PCL copolymers have been achieved through formation of an ester bond between a hydroxyl group in PTX and free side carboxyl groups on poly(ethylene oxide)-*b*-poly(α -carboxyl- ϵ -caprolactone) (PEO-*b*-PCCL).⁶⁵ The micelles of PEO-*b*-P(CL-PTX) significantly improved the solubilization of PTX (Figure 8b).

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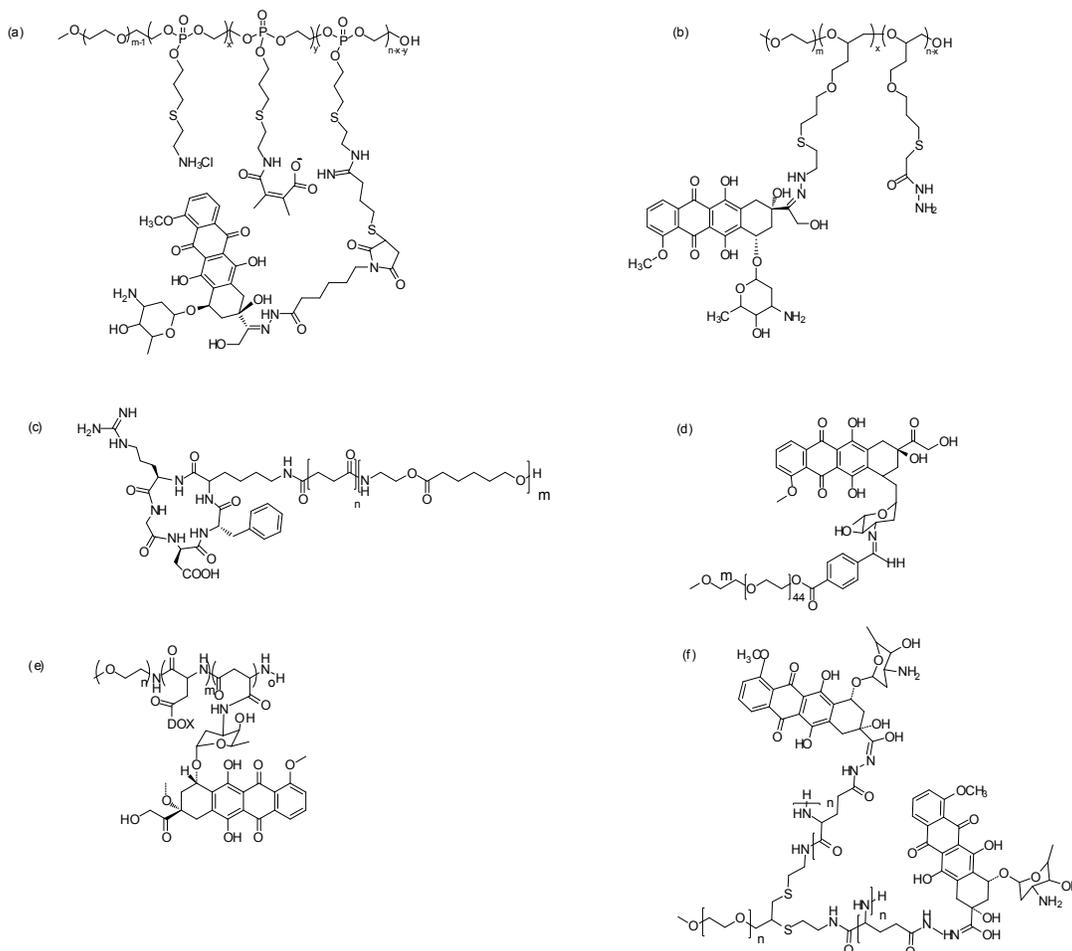


Figure 5 (a) Monomethoxyl poly(ethylene glycol)-*b*-poly(allyl ethylene phosphate)-DOX conjugate via a hydrazone linkage (b) poly(allyl glycidyl ether)-*b*-poly(ethylene oxide)-DOX conjugate via a hydrazone linkage (c) cyclic (Arg-Gly-ASP-D-Phe-Lys) functionalized poly(ethylene oxide)-*b*-poly(ϵ -caprolactone) (d) benzoic-imine linked PEGylated DOX (e) DOX conjugated PEG-*b*-PASP block copolymers via amide linkage (f) Y-shaped mPEG-*b*-poly(glutamate-hydrazone-DOX)₂ via hydrazone linkage.

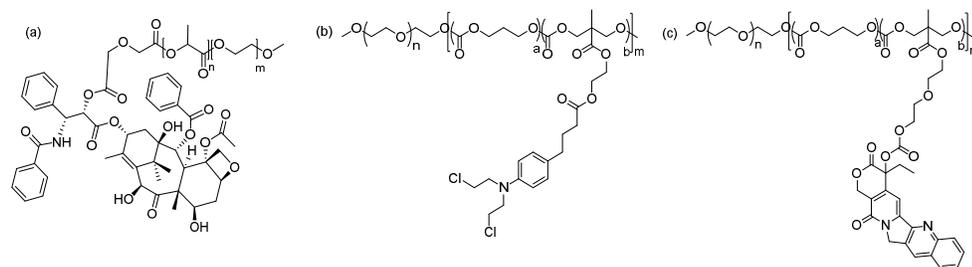


Figure 6 (a) PTX conjugated PEG-*b*-PLA, Anti-cancer drugs (b) Chlorambucil (c) Camptothecin conjugated prodrugs.

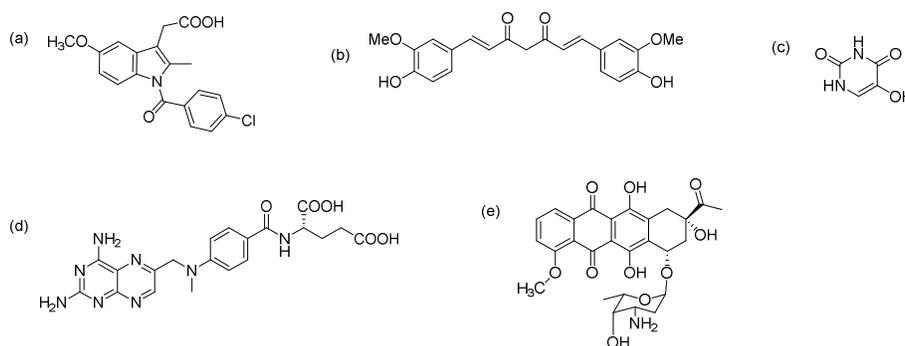


Figure 7 Structures of antitumor agents linked to PEG containing polymers (a) indomethacin (b) curcumin (c) fluorouracil (d) methotrexate (e) daunorubicin.

In 2008, Forrest et al. developed a solvent free formulation of PTX using amphiphilic block co-polymer micelles of poly(ethylene glycol)-*b*-poly(ϵ -caprolactone) (PEG-*b*-PCL). The prodrug in PEG-*b*-PCL micelles caused a significant increase in circulation in the serum and provided more sustained release allowing longer circulation in the body.⁶⁹

A similar study has been reported by Mahmud et al., in 2008.¹⁰⁵ PEO-*b*-PCL block copolymers having functional pendant α -benzyl carboxylate or carboxyl group (Figure 8c) formed more thermodynamically stable micelles than the parent PEO-*b*-PCL micelles. Pendant benzyl carboxylate and carboxyl groups in the micellar core allow formation of block copolymer conjugates of anti-cancer drugs. Synthesis of PEO-*b*-P(CL-DOX) was accomplished via reduction of PEO-*b*-PBCL to PEO-*b*-PCCL. An improvement in the DOX solubility was obtained due to the increased hydrophobic or electrostatic interactions between DOX and the PBCL (poly(α -benzylcarboxylate- ϵ -caprolactone) or PCCL (poly(α -carboxyl- ϵ -caprolactone) micellar cores.

The carboxyl groups of the micellar core may increase the DOX encapsulation due to the possibility of hydrogen-bonding and/or electrostatic interaction between DOX and carboxylic

acid functional groups in the micellar cores. However, the mole % of loaded DOX to monomer was ranked as PBCL > P(CL-DOX) > PCCL > PCL and the π - π interaction between the aromatic rings of PBCL or conjugated DOX and physically encapsulated DOX may account for higher DOX solubilization in PBCL and P(CL-DOX) cores.¹⁰⁵

Recently, methoxy poly(ethylene oxide)-*b*-poly(α -carboxylate- ϵ -caprolactone) (PEO-*b*-PCCL) micelles were complexed with cisplatin to develop pH-responsive polymeric micelles for the delivery of cisplatin [*cis*-dichloro-diammine platinum(II), (CDDP)] (Figure 9a). The results indicated a great potential for the developed formulation in platinum therapy of breast cancer as the micelles slowly released CDDP at physiological pH.¹⁰⁶

Micelles with hyperbranched amphiphilic diblock copolymers have also been synthesized.^{81, 107} The hydrophobic block forming random copolymer was composed of PCL and poly(malic acid). The polymer H40-((poly(β -malic acid)-hydrazone-DOX)-co-poly(ϵ -caprolactone))-methoxy-poly(ethylene glycol)/poly(ethylene glycol)-FA was prepared by the random copolymerization of benzyl malolactonate and CL (Figure 9b). Boltorn H40, a fourth generation hyperbranched globular polyester used for drug delivery applications due to its biodegradability, bioavailability

and high number of chain end functionalities, was used as the macroinitiator in the presence of $\text{Sn}(\text{Oct})_2$ catalyst. DOX was conjugated onto the poly(malic acid) by acid-sensitive hydrazone bonds. The micelles provided excellent *in vivo* stability with a size of 20 nm. A higher drug loading content of

14.2 % was obtained due to the highly branched micellar core. Higher cellular uptake was observed for the FA-conjugated micelles due to the active tumor-targeting ligand FA, thereby leading to a higher cytotoxicity.⁸¹

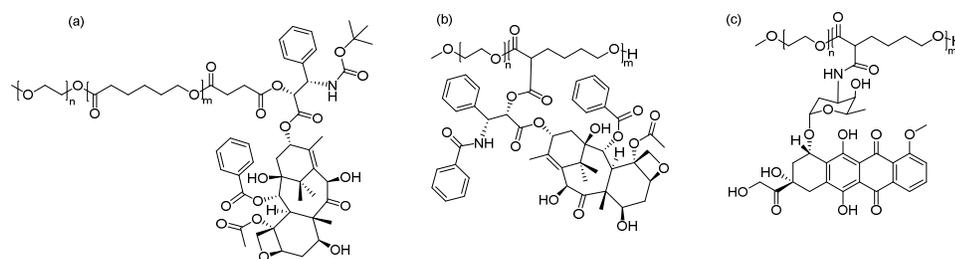


Figure 8 PEG-*b*-PCL drug conjugates (a) docetaxel conjugate (b) PTX conjugate (c) DOX conjugate.

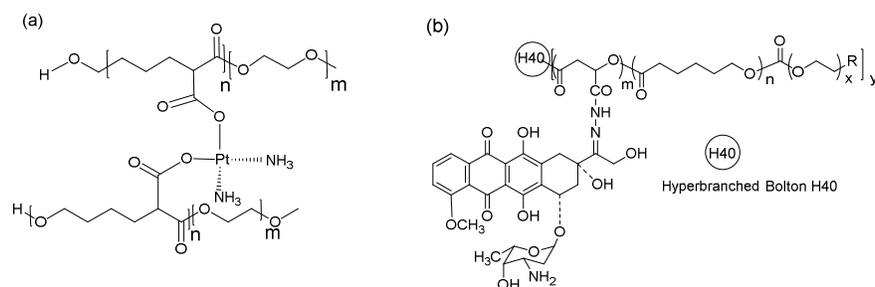


Figure 9 (a) PEG-*b*-PCL-cisplatin conjugates through ester linkage (b) H40-((poly(β -malic acid)-hydrazone-DOX)-co-poly(ϵ -caprolactone))-methoxy-poly(ethylene glycol)/poly(ethylene glycol)-FA.

4. Polymer-histone deacetylase inhibitor conjugates

In recent years, histone deacetylase inhibitors (HDACi) have gained much attention due to their promising anti-cancer activity.¹⁰⁸ Among them HDACi that belong to class I, II, and IV are currently being tested in phase I/II clinical trials.¹⁰⁹⁻¹¹² However, there are not many micellar drug delivery vehicles of polymer-HDACi conjugation reported.

Tacedinaline(CI-994), HDACi which belongs to the benzamide-related group, possesses anti-tumor effects on cancer cells in culture. CI-994 inhibits the stimulation of TSG expression in cancer cell lines. CI-994 also has a higher half life than other HDAC inhibitors.

Recently, Denis et al. reported norbornene (NB)-polyethylene oxide (PEO) macromonomer polymerized by ring-opening metathesis polymerization (ROMP) and functionalization through azide-alkyne click chemistry (Figure 10a). The NB and PEO parts of the macromonomer allowed

the formation of spherical nanoparticles with 300 nm sizes. They demonstrated that the release of the HDAC inhibitor correlated with cell viability and apoptosis.¹¹³

Vorinostat (suberoyl anilide hydroxamic acid) is approved by FDA for the treatment of cutaneous T-cell lymphoma.¹¹⁴ In 2014, Denis et al. reported vorinostat-norbornene (NB)-polyethylene oxide (PEO) conjugate nanoparticles for acid-responsive delivery and passive tumor targeting. They hypothesized that a pH-responsive drug delivery system for vorinostat could improve its delivery by passive targeting and endocytosis. This nontoxic delivery system allows the selective distribution of vorinostat in mesothelioma tumors *in vivo* and subsequent histone deacetylation, hence improving the activity of this HDAC inhibitor *in vivo* (Figure 10b).¹¹⁵

Recently a valproate ester substituted poly(ethylene glycol)-*b*-poly(γ -2-propylpentanoate- ϵ -caprolactone) diblock copolymers were reported by Stefan group (Figure 10c). Valproic acid is known to have anti-cancer properties.¹¹⁶⁻¹¹⁸ The valproic acid, HDACi has been linked to the caprolactone ring with the aid of DCC/ DMAP coupling. The block copolymers self-assembled into micelles and degraded at pH

6. After 3 days the valproate content has been decreased by 7 mol% which demonstrated the capability of delivering valproic acid in a sustained manner by the cleavage of the valproate ester groups.²⁹²⁹

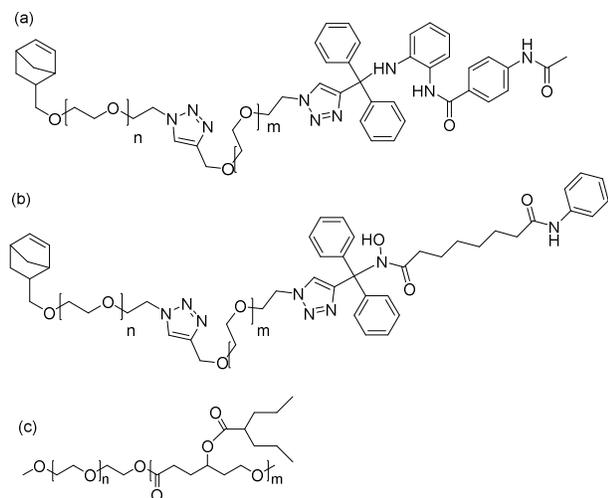


Figure 10 Polymer-histone deacetylase inhibitor conjugates (a) Tacedinaline conjugate (b) Vorinostat conjugate (c) valproic acid conjugate.

4. Conclusions

Recently, rapid advances in the study of prodrug micelles for the delivery of anti-cancer agents have been reported. Covalently attached drugs to the biodegradable polymers offer attractive alternatives by improving the pharmacokinetics and biodistribution of the drugs. The prodrug approach reduces the adverse effects and prolongs blood circulation. In addition, targeting moieties linked to the polymers direct the drug to the site of the pharmacological action. Chemical attachment and the physical encapsulation of anti-cancer drugs results in increased drug loading and formation of stable micelles. Acid cleavable hydrazone linkages have been used in many prodrug micelles to release the conjugated drug upon hydrolysis. Most of the polymeric prodrug micelles demonstrated the sustained drug release from the degradation of the micelles and *in vitro* cytotoxicity on various cell lines. In summary, prodrug micelles have demonstrated great potential in the delivery of anti-cancer agents in a controlled manner.

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