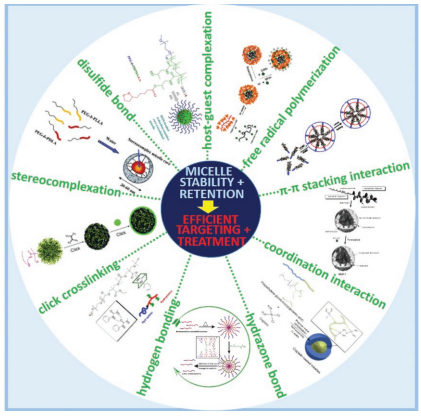
Physico-Chemical Strategies to Enhance Stability and Drug Retention of Polymeric Micelles for Tumor-Targeted Drug Delivery

Yang Shi, Twan Lammers, Gert Storm, Wim E. Hennink*

Polymeric micelles (PM) have been extensively used for tumor-targeted delivery of hydrophobic anti-cancer drugs. The lipophilic core of PM is naturally suitable for loading hydrophobic drugs and the hydrophilic shell endows them with colloidal stability and stealth properties. Decades of research on PM have resulted in tremendous numbers of PM-forming amphiphilic polymers, and approximately a dozen micellar nanomedicines have entered the clinic. The first generation of PM can be considered solubilizers of hydrophobic drugs, with short circulation times resulting from poor micelle stability and unstable drug entrapment. To more optimally exploit the potential of PM for targeted drug delivery, several physical (e.g., π–π stacking, stereocomplexation, hydrogen bonding, host–guest complexation, and coordination interaction) and chemical (e.g., free radical polymerization, click chemistry, disulfide and hydrazone bonding) strategies have been developed to improve micelle stability and drug retention. In this review, the most promising physico-chemical approaches to enhance micelle stability and drug retention are described, and how these strategies have resulted in systems with promising therapeutic efficacy in animal models, paving the way for clinical translation, is summarized.



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1. Introduction

Chemotherapy is an important modality to treat patients suffering from cancer. However, chemotherapeutic drugs are associated with severe side effects, while their therapeutic efficacy tends to be suboptimal. Targeted drug delivery using drug-loaded nanoparticles is considered a promising approach to overcome these problems by increasing the disposition of chemotherapeutic drugs in tumors, and by decreasing their unwanted localization in healthy organs.[1–3] The mechanism of nanoparticle-mediated tumor-targeted drug delivery relies on the prolonged circulation time of drug-loaded stealth nanoparticles after intravenous (i.v.) injection and their subsequent disposition in tumor tissues by the so-called enhanced permeation and penetration effect (EPR effect; passive targeting).[4] In addition, tumor cell-specific ligands can be coupled to the surface of these nanoparticles to promote receptor-mediated cellular uptake (active targeting).[5] To date, various nano-sized particulate systems including polymeric micelles (PM), liposomes, and inorganic nanoparticles have been used as tumor-targeted drug delivery systems, among which PM are the preferred carriers for hydrophobic chemotherapeutic drugs.[6–11]

PM are nanoparticles with a "core–shell" structure that are spontaneously formed (via "self-assembly") from amphiphilic block copolymers in aqueous media. The following characteristics of PM make them attractive systems for the tumor-targeted delivery of hydrophobic anticancer drugs: (1) PM have a hydrophobic core which is specifically suitable to load drugs with low water-solubility, and their hydrophilic shell ensures their colloidal stability resulting in long circulation kinetics; (2) the size of PM is normally in the 10–100 nm range which is beneficial to exploit the EPR effect; (3) the possibility of decorating the PM shell with ligands specific for cancer cells endows PM with active targeting capability; (4) the chemical composition, molecular weight, and architecture of the amphiphilic block copolymers can be adjusted to tailor drug loading capacity and stability, as well as drug release behavior.

Although PM are considered as one of the most promising tumor-targeted delivery systems, there are several challenges associated with their use, which have hampered their therapeutic potential in (pre)clinical studies. First of all, PM are dynamic systems and dilution of PM in the blood circulation after i.v. injection can lower polymer concentrations below the critical micelle concentration (CMC). Second, binding of micelle building blocks (unimers) to blood components such as albumin and apolipoproteins can result in dissociation of PM.[12,13] Third, premature drug release from PM before they reach target sites also leads to short circulation half-lives of payloads.[14] It has been shown that the extent of tumor disposition of drug-loaded nanoparticles is positively correlated with their half-life in the blood circulation.[15] Therefore, poor micelle stability and premature drug release are main reasons for low targeting efficiency of many of the developed PM formulations.

In order to overcome these drawbacks associated with the use of PM, various physico-chemical strategies have been explored to develop drug-loaded PM with high stability and drug retention in the circulation. These include the exploitation of physical interactions between the (hydrophobic segment of) polymer chains and loaded molecules, the chemical cross-linking of either the shell or the core of PM, and the conjugation of drugs to PM. In the present article, the aforementioned physico-chemical strategies are reviewed.

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Wim Hennink obtained his Ph.D. degree in 1985 at the Twente University of Technology (The Netherlands) with a thesis on biomaterials research. From 1985 until 1992 he had different positions in industry. In 1992, he was appointed as Professor at the Faculty of Pharmacy of the University of Utrecht. Currently, he is Head of the Department of Pharmaceutics, a position he took in 1996. From 1997 he is editor of the Journal of Controlled Release. His main research interests are in the field of polymeric drug delivery systems. He has published ≈600 papers and book chapters and holds 25 patents.

2. Physico-Chemical Strategies to Enhance the Stability of PM

PM are preferred drug delivery systems compared to classical micelles based on low molecular weight surfactants for hydrophobic drugs. Due to the much higher molecular weight of micelle-forming polymers, they have a much lower CMC and PM are consequently more stable than small molecule micelles. However, standard PM formed with, e.g., poly(ethylene oxide)-*b*-poly(caprolactone),[16] have shown to be rapidly dissociated in the blood circulation and eliminated, and therefore, strategies to enhance the stability of PM have been developed, which are discussed in this section.

2.1. Physical Strategies

2.1.1. π–π Stacking

π–π stacking in aqueous media occurs between aromatic groups with relatively strong strength because of the special electron configuration of aromatic conjugated π systems.[17] Kataoka and co-workers designed PM based on PEG-*b*-poly(α,β-aspartic acid) with the poly(α,β-aspartic acid) block modified with doxorubicin via amide bonds (Figure 1).[18] Free doxorubicin was physically loaded in the PM, and π–π stacking, apart from hydrophobic interactions, was expected to occur between the aromatic groups of free and polymer-bound doxorubicin. This resulted in significantly enhanced micelle stability, in a 29-fold increase in the area under the curve (AUC) in blood and in a 3.4-fold increase in the tumor disposition of doxorubicin for the micellar formulation as compared to free doxorubicin.[19] This formulation, NK911, was the first micellar nanomedicine evaluated in clinical trials and is currently being tested in the phase II stage against metastatic pancreatic cancer.[18] The same strategy has thereafter been applied for three other PM systems, which are in different stages of clinical trials against various cancers.[18] NK105 is a paclitaxel-loaded PM formulation based on PEG-*b*-poly(α,β-aspartic acid) modified with 4-phenyl-1-butanol.[20] In the formulation NK102, 7-ethyl-10-hydroxy-camptothecin (SN-38) is covalently conjugated to PEG-*b*-poly(L-glutamic acid) by esterification of the phenol groups of the drug and the carboxylic acid groups of the polymer.[21] NC-6300 is prepared from PEG-*b*-poly(aspartate) partially modified with hydrophobic benzyl groups and hydrazine groups. The benzyl groups provide π–π stacking and hydrophobic interactions to stabilize the PM whereas the hydrazine groups are used for chemical conjugation of epirubicin to the polymer.[22,23]

The strategy of combining aromatic π–π stacking with hydrophobic interactions to enhance micelle stability has also been applied by Hennink and colleagues. Their first approach was modification of the hydroxyl end group

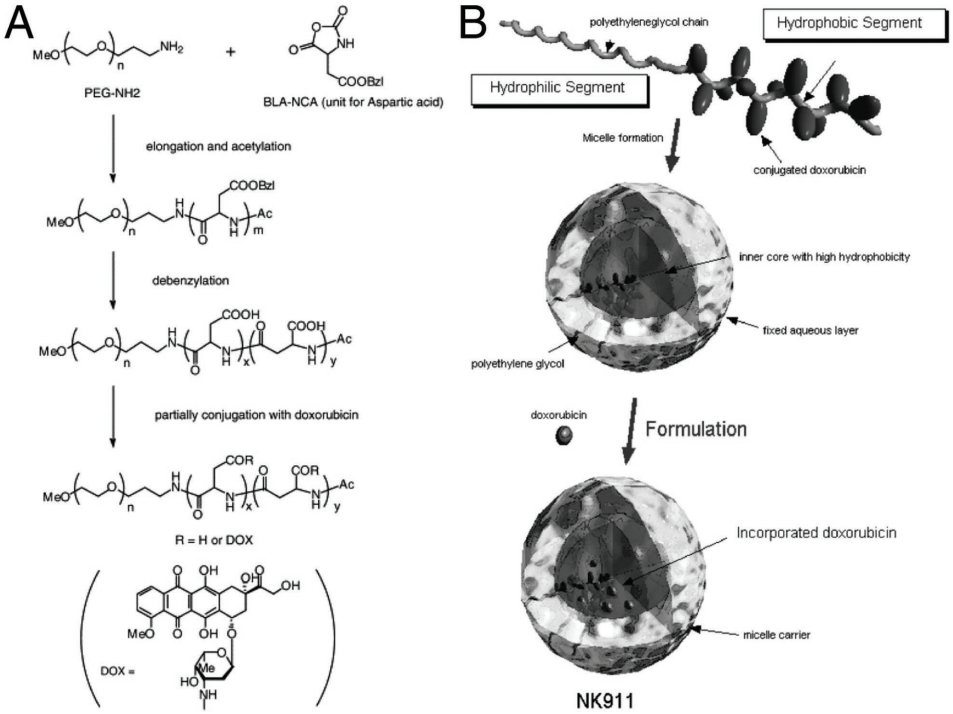

Figure 1. Schematic illustration of preparation of NK911 formulation. A) Doxorubicin was conjugated to the carboxylic acid groups of PEG-*b*-poly(α,β-aspartic acid) via amide bonds, and B) the amphiphilic polymer self-assembled into PM, in which doxorubicin was physically loaded. Reprinted with permission.[18] Copyright 2014, Elsevier.

of mPEG-oligocaprolactone with benzoyl or naphthoyl groups.[24] As expected, the critical aggregation concentration (CAC) of the polymers decreased by aromatic modification. In another study, methoxy poly(ethylene glycol)-*b*-(*N*-(2-benzoyloxy/naphthoyloxypropyl)ethacrylamide)-*co*-(*N*-(2-lactoyloxypropyl) methacrylamide) were synthesized by copolymerization of benzoyled or naphthoyled hydroxypropyl methacrylamide (HPMAm) monomers with HPMAm-Lac via a macroinitiator approach.[25] It was shown that by increasing the amount of aromatic repeating units in the polymers, their critical micelle temperature (CMT) and CMC decreased, which suggests better stability of the PM. Notably, the occurrence of π–π stacking in the PM was experimentally confirmed by solid-state NMR. In a recent study,[26] PM composed of methoxy poly(ethylene glycol)-*b*-(*N*-(2-benzoyloxypropyl) methacrylamide) (mPEG-*b*-p(HPMAm-Bz)) showed a good stability and long circulation kinetics, comparable to those of chemically cross-linked PM.[27] These findings clearly show that aromatic π–π stacking combined with hydrophobic interactions enhances the stability of PM to a similar extent as chemical cross-linking.

2.1.2. Stereocomplexation

Stereoselective association of polymers with different tacticities or configurations is described as stereocomplex formation (stereocomplexation), and is stronger than the interaction between polymer chains with the same tacticity or configuration. The most well-known examples of polymers with stereocomplexation are isotactic and syndiotactic poly(methyl methacrylate) (PMMA)[28] and enantiomeric poly(L-lactide) (poly(L-lactic acid) (PLLA)) and poly(D-lactide) (poly(D-lactic acid)(PDLA)).[29,30] The first demonstration of PM stabilized by stereocomplexation was reported by Leroux and colleagues.[31] PM with crystallized cores were prepared with an equimolar mixture of PEG-*b*-PDLA and PEG-*b*-PLLA, which showed enhanced kinetic stability compared to PM composed of isotactic or racemic polymers alone. Chen and colleagues reported stereocomplex PM formed with dextran-*b*-PLLA/PDLA[32] and Hedrick and colleagues prepared stereocomplex PM from block copolymers with poly(*N*-isopropylacrylamide) as the hydrophilic block and PLLA/PDLA as the hydrophobic block.[33]

2.1.3. Hydrogen Bonding

Urea derivatives can have intermolecular interactions via hydrogen bonding.[34] Hedrick, Yang, and colleagues designed and synthesized a series of amphiphilic block copolymers, PEG-*b*-poly(ethyl-random-urea carbonate) (PEG-*b*-p(E-U)C) or PEG-*b*-poly(ethyl-random-benzyl carbonate) (PEG-*b*-p(E-B)C)), with increased fractions of urea- or benzyl-modified repeating units. The CMC of PEG-*b*-p(E-U)C decreased with increasing molar ratio of urea modified repeating units which resulted in enhanced micelle stability. Furthermore, in the presence of the micelle-destabilizing agent sodium dodecyl sulfate (SDS), PM based on PEG-*b*-p($E_{0.6}$-$U_{0.4}$)C showed better colloidal stability than those based on PEG-*b*-p($E_{0.6}$-$B_{0.4}$)C.[35] Overall, the better stability of PM containing urea moieties can be explained by extra hydrogen bonding formation apart from hydrophobic interactions in the micellar core.

2.1.4. Host–Guest Complexation

Macrocyclic host–guest complexation-mediated binding of two chemical entities is another strategy for the construction of PM.[36,37] β-cyclodextrin (β-CD) and adamantyl (ADA) are a pair of compounds with high inclusion affinity, which was utilized by Jiang and colleagues to prepare non-covalently connected micelles (NCCM). Homopolymers modified with β-CD or ADA groups associated into PM or vesicles depending on association conditions.[38] In another example, chlorin e6 was included in water-soluble derivative of calix[4]arene modified with PEG, and the inclusion complex formed PM in aqueous solution which were used for photodynamic therapy.[39]

2.2. Chemical Strategies

2.2.1. Free Radical Cross-Linking

Free radical polymerization of suitable monomers can be carried out in different solvents, including water of different pH's and temperatures. Although conventionally used for the synthesis of linear vinyl polymers as well as networks, free radical polymerization has also been utilized for chemical cross-linking of PM. Kataoka and colleagues modified the end group of the hydrophobic block of PEG-*b*-PLA with methacrylic acid anhydride and upon self-assembly, the formed PM were cross-linked via free radical polymerization and shown to be stable in the presence of SDS.[40] Triblock copolymers of mPEG-PCL-mPEG were synthesized by Kissel and colleagues with a maleic linker in the middle and the formed PM were cross-linked via free radical polymerization initiated by $K_2S_2O_8$. The formed core-cross-linked PM (CCPM) were colloidally stable against dilution in various solvents.[41] Hennink and colleagues used free radical polymerization for chemical cross-linking of thermosensitive PM.[27] The first step of their approach was modification of the hydroxyl side groups of the thermosensitive block of mPEG-*b*-p((HEMAm-Lac_1)-*co*-(HEMAm-Lac_2))

with methacryloyl chloride. The PM were prepared by a fast-heating method[42] and cross-linking of the methcrylate side groups was initiated with Irgacure 2595 under UV illumination. Because ester bonds were present in the cross-links, the CCPM exhibited pH-dependent hydrolysis at 37 °C.[27] The CCPM showed substantially prolonged circulation time after i.v. injection, with around 50% of injected dose still present in the blood 6 h post-injection. The long circulation time and relatively small size of the CCPM (68 nm) resulted in significantly higher tumor accumulation, i.e., around 6% injected dose (ID) of the CCPM whereas far below 1% ID of the non-cross-linked counterpart accumulated in the tumor.[27]

2.2.2. Click Cross-Linking

Click reactions are characterized by high reaction efficiency under mild conditions. After its invention by Sharpless and colleagues,[43] copper(I)-catalyzed azide alkyne cycloaddition (CuAAC), as one of the most popular click reactions, has been extensively employed for the construction of nanostructures.[44,45] Wooley, Hawker, and colleagues designed two methods to functionalize the shell or core of PM based on poly(acrylic acid)-*b*-poly(styrene) with azido or alkynyl groups, and PM based on the polymers were readily cross-linked with multivalent cross-linkers with alkynyl or azido groups, respectively.[46,47] The remaining clickable groups on the polymer chains were used to covalently immobilize functional moieties such as fluorophores. In another study, Lavasanifar and colleagues synthesized PEG-*b*-PCL with alkynyl substituted caprolactone, and PM based on this polymer were cross-linked using (bis)azide reagent in the presence of copper catalyst at room temperature.[48] Due to hydrolysis of the polyester backbone, the obtained CCPM are biodegradable. Besides CuAAC, several other click reactions including [4+4] cycloaddition of anthracene,[49] thiol–ene,[50] and isocyanates–amine reaction[51] have also been utilized to prepare CCPM. Recently, copper-free click chemistry has been developed as a bioorthogonal tool to avoid the use of toxic copper catalysts,[52] providing an attractive option for chemical cross-linking of PM.

2.2.3. Disulfide Cross-Linking

Disulfide bonds are normally formed by oxidation of compounds with thiol groups, and they are important for the structural stabilization of many proteins.[53] This reaction has been extensively employed in drug delivery research, including for the cross-linking of polyion complex (PIC) micelles by Kataoka and colleagues.[54] A major advantage of disulfide cross-linking is that these bonds are cleavable under reducing condition. Therefore, this strategy enables controlled micelle destabilization and drug release from CCPM inside cells in which a low redox potential exists (intracellular glutathione concentration of 0.5–10 mm vs 2–20 μm in extracellular fluids).[55,56] Zhong and co-workers modified dextran,[57] PEG-*b*-p(HPMAm),[58] and PEG-*b*-PCL[59] with lipoic acid which contains a disulfide bond, and these modified polymers formed PM that were reversibly cross-linked via disulfide bonds. The results showed that the CCPM were colloidally stable against dilution in aqueous[58] and organic solutions.[59] Interfacial cross-linking of PM via disulfide bonds was realized by Shuai and colleagues and Wang and colleagues, respectively, using two different types of triblock copolymers with thiolated middle blocks.[60,61] In the study performed by Shuai and colleagues, the antitumor efficacy of the doxorubicin-loaded interfacially cross-linked PM was significantly enhanced than that of free doxorubicin or PEG-PCL micelles. Lam and colleagues synthesized thiolated telodendrimers containing cysteine side groups, which self-assembled into PM that were subsequently cross-linked via oxidation of the thiol groups.[62] An in vivo study showed that the blood concentration of the CCPM was eight times higher than that of the non-cross-linked PM at 8 h post i.v. injection, demonstrating that PM stability was significantly enhanced by disulfide cross-linking. In line with this, the CCPM loaded with paclitaxel showed a significant tumor accumulation and better therapeutic efficacy in xenograft ovarian cancer model than paclitaxel-loaded non-cross-linked PM or free paclitaxel.

2.2.4. Hydrazone Cross-Linking

Hydrazone bonds are relatively stable at neutral pH but quickly cleaved at slightly acidic pH. Therefore, they are considered an efficient tool for triggered drug release in tumoral extra/intracellular environments with pH values below 6.5.[63,64] Hydrazone bonds have been extensively utilized for reversible conjugation of drug molecules with ketone or aldehyde groups,[65] but only rarely for chemical cross-linking of PM. In a recent study by Hennink and colleagues, a thermosensitive triblock copolymer fully based on a methacrylamide backbone was synthesized by sequential reversible addition fragmentation chain transfer (RAFT) polymerizations and ketone groups were present in the middle block of the polymer.[66] PM were prepared by fast heating of an ice-cold aqueous solution of the polymer and adipic acid dihydrazide was added to the micellar dispersion to cross-link the PM via hydrazone bonds (Figure 2). As expected, the cross-linked PM showed good stability at pH 7.4 and 37 °C, and were completely de-cross-linked in 10 h at pH 5.0 and 37 °C which represents the physiological conditions in late endosomes and lysosomes.

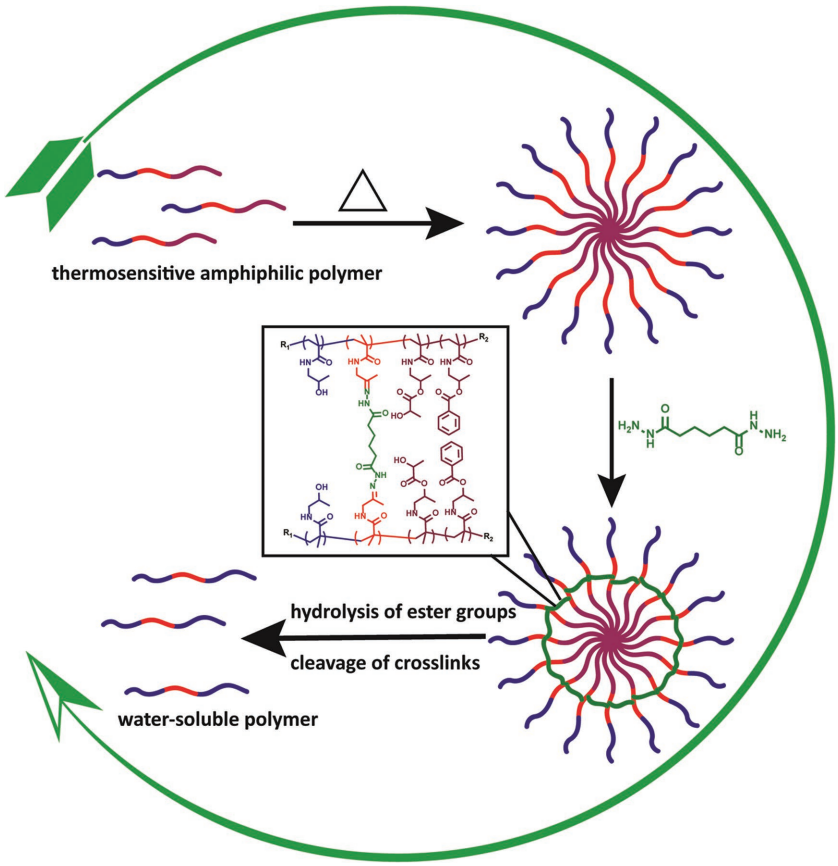

Figure 2. Hydrazone cross-linking of thermosensitive PM. The triblock copolymer self-assembled into PM in aqueous media which were cross-linked via hydrazone bonds formed between the ketone groups of the polymer and hydrazide groups of the cross-linker. The cross-linked PM were de-cross-linked by cleavage of the hydrazone bonds under acidic conditions. Adapted with permission.[66] Copyright 2015, American Chemical Society.

3. Physico-Chemical Strategies to Increase Drug Retention in PM

Compared to micelle stability, less attention has been paid to drug retention in PM, in spite of the fact that this is at least equally important. Previous research has shown that payloads can be rapidly released in the circulation, even if the PM themselves exhibit good stability, which can be ascribed to the transfer of the payloads to plasma components such as albumin.[3,13,14,67,68] For instance, core-cross-linked mPEG-*b*-p((HEMAm-Lac$_1$)-*co*-(HEMAm-Lac$_2$)) PM exhibited a substantially prolonged circulation kinetics similar to that of PEGylated stealth liposomes (50% of injected dose was still present at 6 h post-injection). However, paclitaxel loaded in these CCPM was very quickly released and eliminated.[69] Another study on paclitaxel-loaded PEG-*b*-PCL PM showed a similar result.[70] Therefore, it should be pointed out that drug retention is equally important as micelle stability in order to achieve efficient tumor-targeted drug delivery. Various physico-chemical strategies to enhance drug retention in PM have been exploited and are reviewed below.

3.1. Physical Strategies

3.1.1. π–π Stacking

NK911 is based on PM composed of PEG-*b*-poly(α,β-aspartic acid), in which doxorubicin is both conjugated and physically entrapped. Doxorubicin molecules can have intermolecular π–π stacking interactions, which contribute substantially to micelle stability and doxorubicin retention in the PM. Results showed that the circulation kinetics and tumor disposition of doxorubicin administered as the NK911 formulation were significantly improved compared to those of free doxorubicin (29-fold and 3.4-fold increase in the AUC in blood and in tumor disposition, respectively).[19] Furthermore, the antitumor potency of the NK911 formulation was stronger in various tumor models including Colon 26, M5076, P388 (i.v. implanted), Lu-24, and MX-1 compared to free DOX.[19] In the NK105 formulation, the carboxylic groups of PEG-*b*-poly(aspartate) were reacted with 4-phenyl-1-butanol and paclitaxel was physically loaded in the PM. It was shown in mice that pharmacokinetics (PK) parameters of paclitaxel as NK105 formulation were significantly better compared to those of Taxol ($t_{1/2}$ of 5.99 vs 0.98 h for NK105 and Taxol at a dose of 50 mg kg^{-1}, respectively), and the tumor AUC of paclitaxel in NK105 was 25-fold higher than that of Taxol. As a result, the therapeutic efficacy of NK105 was substantially better than free paclitaxel in HT-29 xenograft model.[20] Hennink and colleagues synthesized micelle-forming polymers by copolymerizing benzoyl- or naphthoyl-modified HPMAm with HPMAm-Lac using a PEG macroinitiator. The formed aromatic PM showed slower release rates of paclitaxel and docetaxel in buffer than non-aromatic PM, which suggested a better drug retention in the aromatic PM due to π–π stacking interactions between the drug molecules and the aromatic pendant groups of the polymer chains.[25] PEG-*b*-p(HPMAm-Bz) self-assembled in aqueous solution into PM and paclitaxel was loaded in the PM with high retention due to π–π stacking interactions (Figure 3). In mice, it was shown that these micelles exhibited an excellent stability, and that paclitaxel loaded in these PM had a significantly prolonged presence in the circulation (with a

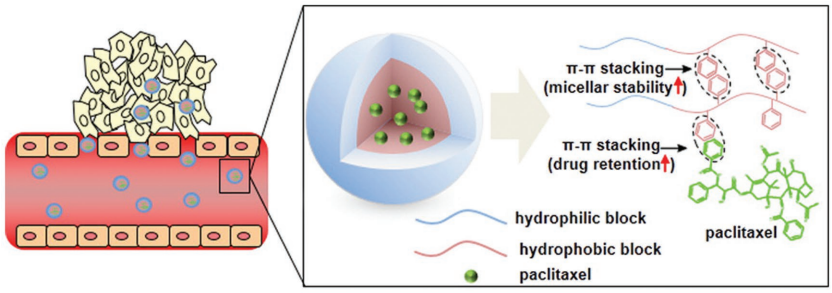

Figure 3. Schematic representation of PM stabilized by π–π stacking for tumor-targeted drug delivery. π–π stacking interactions between paclitaxel and aromatic pendant groups of the polymer contributed to the high retention of paclitaxel in the PM and therefore high tumor targeting efficiency. Reprinted with permission.[26] Copyright 2015, American Chemical Society.

$t_{1/2}$ of ≈8 h), leading to a 20-fold increase in tumor accumulation of paclitaxel and to superior antitumor efficacy.[26]

3.1.2. Coordination Interaction

Platinum metallo-organic compounds such as cisplatin and oxaliplatin are extensively used chemotherapeutic agents. However, their suboptimal biodistribution and the resulting side effects including nephrotoxicity, ototoxicity, neurotoxicity, nausea, vomiting, and myelosuppression, severely limit their clinical application.[71,72] In order to increase the tumor disposition and minimize the off-target effects of platinates, several different tumor-targeted drug delivery systems have been evaluated. Because platinum ions can complex with carboxylate groups via coordination interactions, block copolymers with carboxylic acid side groups were synthesized by Kataoka and colleagues, enabling the incorporation of platinates in PM. The first generation of cisplatin-loaded PM was based on PEG-*b*-poly(α,β-aspartic acid). Although these cisplatin-loaded PM showed five- and fourfold increases in plasma and tumor AUC compared to free cisplatin, rapid and high accumulation in liver and spleen was observed.[73] The same group used PEG-*b*-poly(L-glutamic acid) instead of PEG-*b*-poly(α,β-aspartic acid) to prepare the second generation of cisplatin-loaded PM (Figure 4), which showed significantly improved PK properties, as exemplified by 65-fold higher plasma AUC and 20-fold higher tumor concentration compared to free cisplatin, as well as better therapeutic efficacy in tumor models including colon carcinoma (C26), human gastric cancer (MKN-45), and pancreatic adenocarcinoma (BxPC3).[18,74] The improved stability was explained by the formation of ordered α-helical bundles of poly(L-glutamic acid-cisplatin) in the core of the PM.[75] The cisplatin-loaded PEG-*b*-poly(L-glutamic acid) micelles, which are referred to as NC-6004, were evaluated in a phase I clinical trial in the United Kingdom against advanced cancers. NC-6004 showed mild adverse effects of nausea and vomiting, which are typically associated with cisplatin, and induced grade 2 nephrotoxicity at 90 and 120 mg m^{-2}. Hypersensitivity reactions were more severe than in patients treated with free cisplatin, which could be explained by the prolonged circulation and strongly enhanced $AUC_{0\text{-inf}}$ of NC-6004 as compared to free cisplatin.[76] From this study, the maximum-tolerated dose

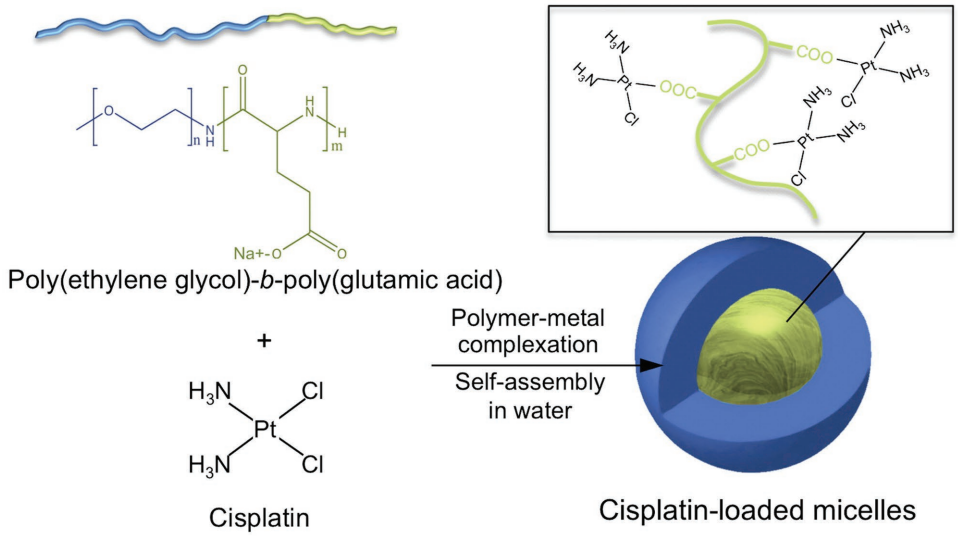

Figure 4. Cisplatin loaded in PM via coordination interaction. Coordination interactions occurred between the carboxylic acid groups of PEG-*b*-poly(L-glutamic acid) and cisplatin, and cisplatin was efficiently loaded in the PM with a high retention. Reprinted with permission.[18] Copyright 2014, Elsevier.

(MTD) and recommended dose of NC-6004 were concluded to be 120 and 90 mg m^{-2}, respectively.[76] Another platinum drug, (*trans*-l-1,2-diaminocyclohexane)platinum(II) (oxaliplatin), has also been loaded in PEG-*b*-poly(L-glutamic acid) PM and this formulation, which is termed NC-4016, exhibited 1000-fold higher plasma $AUC_{0-72\,h}$ as compared to free oxaliplatin.[77] A phase I clinical trial of NC-4016 has been initiated in the United States in 2013 (NCT01999491).[18]

Bronich, Kabanov, and colleagues synthesized PEG-*p*(glutamic acid)-*p*(phenylalanine) to prepare PM for combination chemotherapy. The polymer self-assembled into PM via hydrophobic interactions and π–π stacking between the phenyl groups, and cisplatin was loaded in the PM via coordination interaction with the carboxylic acid groups of the polymer and paclitaxel was entrapped in the hydrophobic core of the PM. In vitro and in vivo synergistic cytotoxicity of the drug combination against human ovarian A2780 cancer cells was observed.[78]

3.1.3. Hydrogen Bonding

Following the approach using urea modified block copolymers to prepare PM with a better stability,[35] doxorubicin was loaded in these PM by Hedrick, Yang, and colleagues.[79] The authors argued that the carbonyl, hydroxyl, and amine groups of doxorubicin have intermolecular hydrogen bonding with the urea carbonates of the polymer. Their results showed that by introducing urea modified repeating units in the polymers, the release of doxorubicin was significantly sustained, which is a strong evidence of enhanced drug retention exploiting hydrogen bonding. The doxorubicin-loaded PM were internalized by HepG2 cells via an endocytotic pathway, while free doxorubicin was taken up by a passive diffusion mechanism. The cytotoxicity of doxorubicin loaded in the PM was lower than that of the free drug, which might be due to the slow doxorubicin release from the PM.

3.2. Chemical Strategies

3.2.1. Hydrazone Bond Formation

Drugs with ketone or aldehyde groups can be coupled to PM with hydrazine functionalities to yield hydrazone bonds, which are rapidly cleaved at intracellular acidic pH of late endosomes and lysosomes. In a formulation termed NC-6300, PEG-*b*-poly(aspartate) was functionalized with hydrazine groups and epirubicin was conjugated to the polymer via hydrazone bonds, which showed significantly better therapeutic efficacy in subcutaneous C26 tumor model.[22,23] In another study of Kataoka and colleagues, a similar approach was applied to covalently entrap doxorubicin in PM, and prolonged blood circulation half-life and improved therapeutic efficacy of the doxorubicin-loaded PM were reported.[80,81] Taxanes have also been covalently conjugated to PM via hydrazone bonds. Kwon and colleagues reported modification of paclitaxel with a ketone containing linker, and this paclitaxel prodrug was conjugated to PEG-*b*-*p*(aspartate-hydrazide) via a hydrazone bond.[82] The release of paclitaxel from the PM was accelerated at pH 5.0 as compared to that at pH 7.4. In a study performed by Zhang and colleagues, docetaxel was conjugated to Pluronic P123 via a hydrazone linkage. The 2-hydroxyl group of docetaxel was esterified with a levulinic acid and the new compound bearing a ketone group was modified with adipic acid dihydrazide to yield a docetaxel prodrug with a hydrazine group. The terminal hydroxyl group of P123 was also modified with a levulinic acid to generate a ketone group, to which the prodrug was conjugated via a hydrazone linkage. The conjugated docetaxel was slowly released from the PM at pH 7.4, but the release rate substantially increased in buffer of pH 5.0.[83]

3.2.2. Free Radical Polymerization

Free radical polymerization has been applied for conjugation of drug molecules to PM. Since common chemotherapeutic drugs do not have polymerizable vinyl groups in their chemical structures, modification of the drugs is necessary before conjugation. Although free radical polymerization generates non-degradable carbon–carbon bonds, linkers with desired degradation kinetics can be introduced between the modified drugs and vinyl groups which ensure tunable release of the conjugated drugs from the PM. Dexamethasone was modified with methacrylate groups via sulfide, sulfoxide, and sulfone ester linkers which exhibited different hydrolysis rates at physiological conditions.[84] These prodrugs were chemically loaded in the core of CCPM via a one-pot free radical polymerization. The three types of PM loaded with different dexamethasone prodrugs showed different dexamethasone release profiles and efficient targeting to inflamed joints in mouse and rat models of arthritis.[84] Following the same strategy, docetaxel was methacrylated via an ester bond and the prodrug was covalently loaded in CCPM via free radical polymerization. The half-life of docetaxel as CCPM formulation in the blood circulation in rats was significantly extended ($t_{1/2}$ = 16.2[85] vs 0.88 h of free docetaxel[86]). Moreover, the tumor concentration of docetaxel delivered via the CCPM was 20- and 59-fold (2 and 4 d, respectively) as compared to that of Taxotere (30 mg kg^{-1}). The docetaxel-loaded CCPM exhibited superior therapeutic efficacy in MDA-MB-231 xenograft tumor model as compared to free docetaxel.[85] A phase I clinical trial of PM which are core-cross-linked and chemically loaded with docetaxel via free radical polymerization (CriPec) has begun in Europe in 2015 (NCT02442531).[87] In another study, doxorubicin was modified with a methacrylate group via a hydrazone bond

and covalently entrapped in CCPM surface modified with a therapeutically active nanobody. The conjugated doxorubicin exhibited prolonged circulation times in blood, good target site accumulation, and rapid release under acidic condition, together resulting in promising antitumor efficacy.[88,89] Besides anticancer drugs, a therapeutic peptide has also been covalently loaded in CCPM via free radical polymerization. Leuprolide is a therapeutic peptide with a fast blood clearance rate after i.v. injection. In a recent study, leuprolide was methacrylated via a hydrolytically sensitive ester bond and incorporated in CCPM via free radical polymerization. It was shown that the blood circulation kinetics of leuprolide covalently entrapped in the CCPM was increased substantially compared to the free peptide (AUC of leuprolide as CCPM formulation was >100-fold higher than that of the free peptide), and leuprolide released from the CCPM was biologically active and induced long-lasting plasma testosterone levels.[90]

3.2.3. Disulfide Bonds

Disulfide bonds have been used to cross-link PM because of their ease of formation and intracellular cleavage (see Section 2.2.3). This chemistry has also been exploited to link drugs to PM in order to enhance drug retention. Kataoka and colleagues modified the hydroxyl group of camptothecin with 3-(2-pyrinyldithio)propionic acid and the obtained prodrug was subsequently coupled to PM based on PEG-*b*-poly(glutamic acid) modified with pyridyl disulfide groups which were reduced using dithiothreitol (DTT) to generate free thiol groups. Camptothecin coupled to the PM was slowly released in phosphate buffer pH 7.4, which was highly enhanced in the presence of 3×10^{-3} M of DTT.[91] In another study, camptothecin modified with a pyridyl disulfide group was conjugated to an amphiphilic diblock copolymer based on 2-methacryloyloxyethyl phosphorylcholine and lipoic acid modified 2-hydroxyethyl methacrylate synthesized by sequential RAFT polymerizations. The block copolymer self-assembled in water and the formed PM were cross-linked making use of the residual disulfide groups on the polymer backbone. The release rate of camptothecin conjugated to the PM was significantly slower than that of camptothecin physically encapsulated in the PM, but on the other hand the release of conjugated camptothecin was triggered under a reducing condition.[92]

4. Conclusions and Future Perspectives

Various physico-chemical strategies have been evaluated to improve the stability of PM and drug retention in PM. It has been demonstrated that upon enhancing micelle stability and drug retention, pharmacokinetic profiles can be significantly improved, resulting in increased tumor accumulation and more potent antitumor effects. At the same time, off-target effects can be reduced. Currently, seven (out of approximately a dozen in total) micellar nanomedicines in clinical trials are based on such strategies, showing the importance of the stability and retention enhancing methods for clinical translation of PM.

To further facilitate the clinical translation and performance of PM, several aspects that need to be addressed in future studies are: (i) particle size: nano-sized drug delivery systems accumulate in tumors, however, the therapeutic efficacy is highly dependent on the tumor penetration of the loaded drugs. Several studies showed that nanoparticles with small size (down to 20–30 nm) can penetrate more deeply into tumor tissues and better therapeutic efficacy can be gained.[93–97] Therefore, PM with small size are essential for better therapeutic efficacy; (ii) release behavior: lessons learned from liposomal nanomedicines have shown that efficient release of drugs from nanocarriers is essential for the therapeutic efficacy, since the drugs have no pharmacological activity when they are entrapped. Therefore, PM with optimal drug release behavior, triggered by endogenous and/or exogenous stimuli, may increase the drug's efficacy; (iii) theranostics: it is increasingly recognized that theranostic nanoparticles, which besides drug molecules also carry imaging agents, can be employed to visualize and quantify biodistribution and target site accumulation.[98–101] By incorporating such imaging markers, it would be possible to assess whether PM are able to target tumors (and metastases) efficiently. This may further improve the therapeutic efficacy of micellar nanomedicines, by selecting the "right" patients for this treatment modality; and (iv) combination therapy: proper drug combinations have shown improved effect on tumors in (pre-)clinical studies.[102,103] Currently, all the clinically tested PM only contain a single drug. PM/nanomedicines that can load multiple drugs with sufficient stability and drug retention are promising for better clinical performance.[104–106] Overall, PM have already shown great promise in improving anticancer treatment by increasing the anti-tumor potency and decreasing the side effects of chemotherapeutic drugs, and the four considerations above can contribute to the development of the next generation of micellar nanomedicines with better therapeutic index.

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