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Block copolymer micelles for nanomedicine

"In comparison with traditional small molecule therapeutic agents, nanomedicine offers new hope for the detection, treatment and prevention of cancer and other fatal diseases such as cardiovascular disease, aging disease and AIDS due to its unique features engineered at molecular level (i.e., nanometer scale)."

KEYWORDS: antibody conjugation ■ anticancer drugs ■ biodegradable polymers ■ drug formulation ■ drug targeting

Almost 40% of newly discovered drugs have delivery problems due to their low solubility, permeability and stability. In comparison with traditional small molecule therapeutic agents, nanomedicine offers new hope for the detection, treatment and prevention of cancer and other fatal diseases, such as cardiovascular disease, aging disease and AIDS due to its unique features engineered at molecular level (i.e., nanometer scale). Nanomedicine can extensively improve the solubility of poorly water-soluble drugs, prolong the drug's half-life in systemic circulation, release the drug at a sustained and controlled rate, deliver the engineered drug in a targeted manner with little interference to the healthy cells, suppress drug resistance and reduce the immunogenicity. In short, desired pharmacokinetics and pharmacodynamics can be realized to increase the therapeutic effects and reduce the side effects of the drug to be delivered. With the rapid advances in nanotechnology many drug delivery systems, especially those for anticancer agents, have been developed, including polymer–drug conjugates (prodrugs), polymeric micelles, dendrimers, liposomes, other nano-polymeric compositions and inorganic particulates with a size range of 1–1000 nm. Some of these products have been introduced to the pharmaceutical market. For example, Doxil® (Janssen Biotech Inc., PA, USA) was the first liposomal drug formulation for the treatment of AIDS associated with Kaposi's sarcoma in 1995. The polymer–drug conjugate Abraxane® (Celgene Corporation, NJ, USA), an albumin nanoparticle formulation of paclitaxel, was approved by the US FDA in 2005 as a second-line treatment for breast cancer [1,2].

However, some major challenges have been raised in the clinical testing of numerous nanomedicine products. The obvious drawbacks are

the *in vivo* instability and the fast clearance from the blood by the reticuloendothelial system (RES). The most widely used strategy to overcome the instability is covering the nanocarriers with some hydrophilic or amphiphilic polymers such as poly(ethylene glycol) (PEG), poly(vinyl alcohol) or TPGS, a PEGylated vitamin E. Nanocarriers linked with highly hydrated flexible PEG successfully escaped from the RES [3]. The poly(vinyl alcohol) coating also improves the particle's stability. However, introducing too much adjuvant into the body resulted in undesirable toxicity. Moreover, the size, structure and surface electronic properties of the formulations were changed, resulting in an unfavorable therapy index. On the contrary, the micellar system, which mainly includes the polymeric micelle and phospholipid micelle, has successfully overcome the aforementioned drawbacks because these spherical nano-sized particles have a simple structure and no adjuvant is needed in their formulation. The lipid-based micelles showed a high potential for entrapping doxorubicin (Dox). However, the intrinsic structure of the phospholipid resulted in an untunable micellar structure with a diameter usually greater than 100 nm, which considerably limited the lesion's accumulation. Additionally, drug release from conventional liposomal formulations is quite limited once these nanoparticles reach the tumor [4].

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The nano-sized polymeric micelles (10–100 nm in diameter) are self-assembled from amphiphilic block copolymers, which possess a well-defined hydrophobic core and a hydrophilic corona structure in aqueous media. Block copolymer micelles can thus significantly improve the solubility of the hydrophobic drug formulated in the core via the similar-to-similar interaction. The densely packed corona, which consists of the hydrophilic end of the block copolymer, can protect the micellar system from the RES elimination by reducing the interaction with serum proteins and renal filtration [5]. In comparison with lipid-based micelles, block copolymer micelles provide a unique and powerful nanoplatform for anticancer drug delivery. The size of block copolymer micelles can be easily tuned by varying the block lengths of the amphiphilic copolymer. It is also easy to modify the micellar surface by functionalizing the hydrophilic shell. The tunable size range and the tailorable structure can both successfully reduce the renal filtration and obviously enhance tumor penetration. Some nano-size block copolymer micelles such as PEG-PLA/PCL or PEG-PPO-PEG have shown the ability to significantly improve their *in vitro/in vivo* performance. Several block copolymer micelles are currently undergoing Phase I/II clinical trials, which have shown significant antitumor efficacy and reduced systemic toxicity [1,5,6].

It is well-known that the endothelial cells of tumor blood vessels proliferate at a 30–40-fold higher rate than those in normal tissues, which results in larger endothelial cell gaps (200–700 nm, or sometimes even larger, up to 1.2 μm) compared with 7 nm in the normal tissue. Additionally, the high metabolism of tumor cells requires much more oxygen, nutrients, gas exchange and waste removal. But the heterogeneous structure and distribution of the tumor blood vessels may slow down the energy exchange inside and outside the tumor, and thus result in the unique characteristics of the circulation in the tumor, that is, the unnormal tumor blood vessels with gaps of 200–700 nm, the relatively high temperature inside the tumor ($T > 37^\circ\text{C}$) and the relatively low pH (5–6) [7,8]. In order to further improve the micellar delivery profile including the lesion's accumulating, cellular uptake and intracellular release, many new stimulus-responsive micelles have been extensively investigated with special focus on the tumor microenvironment. Utilizing the lower pH value in solid tumors and endosomes (pH 5.5), Kataoka's group explored novel multifunctional

pH-sensitive Dox-conjugated PEG-p(Asp-Hyd-Dox) block copolymer micelles. The pH linker broke at pH less than 6.0 and sustainable release followed. Micelle-forming PEO-PAsp(ADR) conjugates showed enhanced accumulation in tumors after 24 h (~10% dose per g tumor), which was much higher compared with the free ADR (~0.90% dose per g tumor). Later, they further investigated the pH triggered intracellular release profile of PEG-poly(aspartate hydrazone adriamycin) micelles, and observed that the micelles can stably circulate in physiological conditions (pH 7.4) and selectively release the drug by sensing the low intracellular pH (pH 5–6). *In vitro* and *in vivo* studies showed that the block copolymer micelles possess a good pH-triggered drug release capability, tumor-infiltrating permeability, and effective antitumor activity with extremely low toxicity [9,10]. Okano's group used the temperature-sensitive block copolymer poly(*N*-isopropylacrylamide) (PNIPAM) to investigate the cellular uptake of the micelles. At a temperature higher than the lower critical solution temperature (LCST) [$T > \text{LCST}$], the cell uptake of micelles was significantly enhanced. In addition, the LCST of such PNIPAM can be tuned to $T = 39^\circ\text{C}$ by introducing some hydrophilic monomer into the chain backbone. Thus, the system can circulate poorly at 37°C but disassociate as the temperature approaches 39°C . PNIPAM was also used to enhance the intracellular release as the cargo structure was disrupted at the phase transition [11,12].

The oxidative conditions in the extracellular medium and reductive conditions in the tumor were used to enhance intracellular drug release. For example, the bioreducible PEG-SS-P[Asp(DET)] micelles bearing the disulfide bridge showed one to three orders of magnitude higher gene transfection efficiency and a more rapid onset of gene expression than the micelles with no disulfide linkages [13]. Feng's group recently developed a micellar system containing a functional copolymer of D- α -tocopheryl polyethylene glycol succinate (Vitamin E TPGS or TPGS), which stabilized the micelle and further promoted synergistic effects with the encapsulated drug. This is a novel micellar system. The formulation formed by folic acid-conjugated D- α -tocopheryl polyethylene glycol succinate 2000 (Vitamin E TPGS2k) micelles successfully suppressed the tumor cell growth [14]. To improve the therapeutic effect, some other intelligent block copolymer micelle systems such as light-responsive poly(methacrylate)

and poly(acrylic acid) (PAzoMA-PAA) micelles were developed. This *trans*–*cis* photoisomerization of the azobenzene group improved drug release [15]. It has been shown that the Dox-loaded PEG-PCL block copolymer micelles conjugated with the tumor targeting avb3 ligand greatly enhanced cellular uptake of the micelles through receptor-mediated endocytosis [16].

“However, there is still a big gap between *in vitro* and *in vivo* experimental results due to lack of knowledge on the correlation between the tumor unique characteristics (needs) and the micellar physical and chemical properties (seeds).”

The advances of intelligent block copolymer micelles for drug delivery have dawned upon a new era of nanomedicine. However, there is still a big gap between *in vitro* and *in vivo* experimental results due to lack of knowledge on the correlation between the tumor unique characteristics (needs) and the micellar physical and chemical properties (seeds). It is helpful to know that the micellar *in vitro/vivo* performance is strongly affected by its physical and chemical properties, such as composition, dimension, microstructure and intelligent properties. The driving force for self-assembly is the strict solubility difference between the hydrophobic and hydrophilic blocks of the copolymer as described by the Flory-Huggins parameter (χ_{ps}) [17].

$$\chi_{\text{polymer, solvent}} = \frac{(\delta_{\text{polymer}} - \delta_{\text{solvent}})^2 V_s}{KT} + 0.34$$

Where δ_{polymer} and δ_{solvent} are the solubility parameters of the polymer and solvent, V_s is the molar volume of solvent, K is Boltzmann constant, T is the absolute temperature and the constant 0.34 is the entropic contribution. In fact, this force also determines the drug loading, that is, the interaction between drugs and the core-forming polymer segment (N_{segment}). The thermal translational energy per macromolecule is of the order of KT , whereas the interaction energy per macromolecule is proportional to its segment number N_{segment} , namely the product $N_{\text{segment}} \chi_{\text{polymer-drug}}$. This indicates that micellar self-assembly and drug loading is directly related to the corresponding block copolymer composition. In addition, in aqueous solutions, the condition for spherical micelle is the packing parameter $0 < \beta (=V_H/L_C A_0) < 1/3$, where V_H , L_C and A_0 are the volume occupied by the hydrophobic chain, the hydrophobic chain counter length and the surface area of hydrophilic chain, respectively.

Thus, the block copolymer composition further determines the micellar size and structure. In our studies, it was found that the overall size (D) is related to the length of the amphiphilic block lengths by a scaling relation as $D \propto N_{\text{hydrophobic}}^{0.16} N_{\text{hydrophilic}}^{0.6}$. The micellar core/corona size ($D_{\text{core}}/D_{\text{corona}}$) and the drug loading into micelle (determined by the volume of core V_{core}) were easily tuned by regulating core/corona forming block length [17]. On the other hand, as administered to the body, both the extremely diluting (5 ml in one intravenous injection to 3500 ml blood circulated in human body) and the high shearing stress in the viscoelastic blood stream (3.0–5.1 mPa·s for the whole blood, viscosity >1.0 mPa·s for the water) can deform the micelles. So its critical micelle concentration should be as low as possible to avoid *in vivo* disassociation. Additionally, the micelle should also be able to escape from the serum proteins' absorption and the RES. Experimentally, we can tune critical micelle concentration by changing the amphiphilic block lengths. Moreover, it was found that a decrease of the shell chain density (micellar surface area to aggregation number, $S_{\text{corona}}/N_{\text{agg}}$) strongly enhanced its stability. Increasing the hydrophobic/hydrophilic block length ratio resulted in an $S_{\text{corona}}/N_{\text{agg}}$ decrease. Such entropic loss dominates the noncharged micelle escaping *in vivo* [18].

“...to make more reliable block copolymer micelles for nanomedicine, we should firstly seek a solution to the major questions raised in clinical oncology. It is essential to revisit and disclose the fundamentals in block copolymer micelles for drug delivery, including the inherent mechanism of micelle formation, effects of micellar properties on drug loading efficiency and releasing, *in vivo* stability and tumor accumulation, when we optimize highly efficient next generation block copolymer micelles for cancer therapy.”

Based on the fundamental correlation as mentioned above, we further finely tailored T- and pH-sensitive poly(*N*-isopropylacrylamide-*co*-*N,N*-dimethylacrylamide-*b*-lactide) and poly(*N*-isopropylacrylamide-*co*-*N,N*-dimethylacrylamide-*b*- ϵ -caprolactone) (PID₁₁₈-*b*-PLA₅₉ and PID₁₁₈-*b*-PCL₆₀) block copolymer micelles for enhanced tumor uptake and intracellular drug release. The drug transported by these micelles was found to be around four-times higher than that by the commercial drug

formulation Taxotere® (Sanofi-aventis, NJ, USA). Both cytotoxicity assay against N-87 cancer cell and confocal laser scanning microscopy confirmed such promoting efficiency [17]. On the other hand, it is well known that specifically modifying the block copolymer micelles for targeting can promote tumor accumulation. The targeting ligands, such as antibodies, folic acid, transferrin and peptides (RGD) were used to decorate the micelle surface, which can specifically bind to the corresponding receptors over-expressed on the cancer cell surface. Practically, the antibody decoration represents a desirable fashion for the next generation block copolymer micellar system. Recently, some novel antibody conjugation strategies have been developed in our group for enhanced tumor accumulation by changing the binding site on the monoclonal antibody [19,20]. Overall, to make more reliable block copolymer micelles for nanomedicine, we should firstly seek a solution to the major questions raised in clinical oncology. It is essential

to revisit and disclose the fundamentals in block copolymer micelles for drug delivery, including the inherent mechanism of micelle formation, effects of micellar properties on drug loading efficiency and releasing, *in vivo* stability and tumor accumulation, when we optimize highly efficient next generation block copolymer micelles for cancer therapy.

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