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REVIEW

Advance of Amphiphilic Block Copolymeric Micelles as Drug Delivery

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The poor water-solubility of the anticancer drugs cause significant problems during clinical application. The amphiphilic block copolymeric micelles are the most promising system to solve this problem. This manuscript introduce the formation of these polymers as well as the preparation of the polymers and the micelles. The newly developments which include the micelles prepared methods based on supercritical carbon dioxide (ScCO₂) and the tumor targeting drug-loaded micelles are also discussed in this paper.

Key Words: Drug delivery, Copolymeric micelle, Preparation method, Supercritical carbon dioxide, Folic acid, pH-Sensitive.

INTRODUCTION

Many therapeutic anticancer drugs cause significant problems during formulation for clinical application because of its' poor water-solubility. The well-known anticancer drugs like paclitaxel (PTX), doxorubicin(DOX) and 5-fluorouracil (5-FU) also face this problem and are limited in their clinical application. Therefore, a lot of efforts have been made to enhance the water-solubility of these drugs. Among them, polymeric micelles systems are one of the most promising methods.

Polymeric micelles usually can be obtained by self-assembly of amphiphilic block copolymers which are composed by the hydrophobic blocks and the hydrophilic ones. A unique feature of polymeric micelles is their core-shell structures. The lipophilic core could solubilize hydrophobic drugs and the hydrophilic shell could make the entire micelle assembly water-soluble. Therefore, the systems could solubilize the hydrophobic drugs and expand the pharmaceutical potential of lipophilic drug molecules.

Comparison with the other methods, there are many advantages of polymeric micelles for drug delivery usage. The low critical micelle concentration (CMC) of the polymer can make the micelles has a strong anti-dilution ability and be more stable in the blood circulation. The nanosize (10-100 nm) and hydrophilic outer shells of polymeric micelles prevent their uptake by reticuloendothelial system (RES), thus can prolong their circulation time in blood^{1,2}. The small size can also lead to passive accumulation of polymeric micelles in solid tumor

sites due to the enhanced permeability and retention (EPR) effect of the vascular endothelia at the tumor³⁻⁵, which can reduce the side effects of the entrapped drug to healthy/normal tissues.

Due to the excellent property, the polymeric micelles drug delivery system is the most promising one for clinical application. This paper introduces the progress of the polymer micelles systems based on amphiphilic block copolymers. The formation of the polymer as well as the preparation methods of the drug-loading micelles are discussed and the physical stimuli-responsive polymeric micelles, which are the recent highlight of the field, will be also introduced by this review.

Formation and the preparation of the polymer: The micelles acted as drug carrier are prepared by amphiphilic block copolymers, which are composed by the hydrophobic blocks and the hydrophilic ones. Obviously, the materials of the polymer should be harmless and biodegradable. The performance of the polymer determine the properties of the micelles.

Materials of the hydrophilic blocks: The hydrophilic blocks will be the shell of the micelles and will directly contact with cells during clinical application. Therefore, they appeal the good water solubility and the excellent biocompatibility. To our best of knowledge, the polyethylene glycol (PEG) is the most widely used material as hydrophilic blocks. The PEG has been proved the low toxicity on the human body. Besides that, the excellent hydrophilic block could make the micelles composed by PEG to be stabilized in the circulatory system. Furthermore, the molecular structure of PEG could avoid

uptake by reticuloendothelial system (RES) and prolong the micelle's circulation time in blood. Despite the continued development of the hydrophobic blocks, PEG is still widely used as hydrophilic blocks by researchers in their recent works⁶⁻⁸.

Except PEG, poly(2-methacryloyloxyethyl phosphorycholine) (PMPC), a bioinspired polymer, is another ideal candidate for hydrophilic shell-forming blocks. It's well known that phospholipid is an important component of lipid bilayer which plays a vital role to the biocompatibility of biomembranes. In order to mimic the bilayer surface structure of biomembranes, a great deal of effort has been made to synthesize phospholipid analogues polymers during the past four decades. Among these polymers, 2-methacryloyloxyethyl phosphorycholine (MPC) is the most famous one and has been proven that it can inhibit protein absorption and platelet adhesion remarkably⁹⁻¹¹. Due to its excellent biocompatibility, MPC has been widely used for biomedical applications including drug delivery. Salvage and his coworkers prepared nanoparticles using PMPC-b-PDPA diblock copolymers. *In vitro* assays showed that these MPC-DPA diblock copolymers had negligible cytotoxicity¹². Hsiue *et al.* prepared PMPC-b-PLA polymeric nanoparticles, low cytotoxicity of the polymer and nanoparticles was confirmed by cytotoxicity assay and growth inhibition assays with HFW (human fibroblasts) cell which indicates the potential use in biomaterials of these nanoparticles¹³.

Anyway, compared to the materials of the hydrophobic blocks, the potential hydrophilic ones are limited. Although there are some efforts on modifying the hydrophilic materials in order to obtain the better performance, most of the attentions are paying to look for the better hydrophobic materials.

Materials of the hydrophobic blocks: Although the hydrophobic blocks are not directly contract with cells before the micelles decomposed, they still appeal friendly to human body. To date, the materials of the hydrophobic blocks are focused on the polyester and polyamides which benefit for their harmless, biodegradable and good biocompatibility. For example, the polylactide (PLA)¹⁴, [poly(lactic-co-glycolic acid)] (PLGA)¹⁵, polycaprolactone (PCL)¹⁶, poly aspartamide (PAsn)¹⁷, [poly(L-histidine)](PHis)¹⁸ and [poly(α -benzyl-L-glutamic acid)] (PBLG)¹⁹ are all used by different researchers. Due to the core-shell structure, the hydrophobic blocks are directly connected with the drug loaded by the micelles. The amount of the drug loading and the drug release property of the polymer micelles are mainly determined by the hydrophobic blocks. Therefore, the methods that enhanced the drug loading and controlled the drug release encouraged the researcher to develop the hydrophobic materials. On one side, the drug loading method based on the physical and/or chemical interaction between drug and hydrophobic blocks are helped to obtain more drugs in the micelles. On another side, the designed hydrophobic materials could achieve the physical stimuli-responsive drug delivery. These newly developments will be discussed at the following parts of this review.

Preparation of the polymer: Beside the materials, the molecular weight and their segment ratio of the hydrophilic and hydrophobic blocks also determine the micelles' property. Obviously, the prepared methods of well-defined block

copolymers with controlled molecular weight and narrow molecular distribution are welcomed. Fortunately, some suitable methods which named living free radical polymerizations have been developed in the recent years. The most famous ones are nitro-oxide-mediated polymerization (NMP)²⁰, atom transfer radical polymerization (ATRP)²¹ and reversible addition-fragmentation chain transfer (RAFT)²² radical polymerization. Among them, reversible addition-fragmentation chain transfer (RAFT) radical polymerization is more versatile compared to other methods since it is metal free²³ and can be carried out with a wider range of monomers in various solvents²⁴⁻²⁶. The block copolymers of pBMA-b-pMPC with controlled molecular weight and narrow molecular distribution have been successfully synthesized in our laboratory. The detailed synthesis method could be found in previous paper²⁷.

Preparation of the drug-loaded micelles: The ways of preparation of drug-loaded polymer micelles could be divided as chemical methods and physical ones. The former is based on the reversible chemical bond of the hydrophobic block and the drug which is in the micelles' core and wrapped by the polymers. The encapsulation efficiency of the drug will be approaching to 100 % by this method and it was much higher than the physical methods. Unfortunately, even excluding the additional chemical reactions needed by this method, the blocks and the drugs can form reversible chemical bond are scared. There are just a few micelles prepared by the methods. Adriamycin (ADR) incorporated into polymeric micelles forming from poly(ethylene glycol)-poly(aspartic acid) block copolymer is the typical system²⁸. Actually, the most commonly used methods for preparation drug-loaded micelles are physical processes. The principle of such methods is that the amphiphilic block copolymer could be self-assembly in solution to form shell-core structure micelles and drugs could be automatically collected in the core with the interaction and/or the hydrogen bond strength between the micelles' hydrophobic core and the insoluble drugs. Although the drugs' encapsulation efficiency prepared by the methods would be lower than the chemical ones, the simple process (the micelles forming and the drug loading processes are both achieved by one step) as well as the much broad range of applications (it seems that nearly all of the insoluble drugs could be packed by the micelles) encouraged a lot of developments on physical methods. Among them, the most famous ones are organic solvent evaporation method and dialysis method.

Solvent evaporation method: Drug and amphiphilic block copolymer are both dissolved in volatile organic solvents, such as acetone, methanol or acetonitrile. Then the solution is mixed with water and opened to air followed by agitating or rotary evaporating in order to evaporate the organic solvents. After getting rid of the organic solvents, the solution is centrifuged and followed by filtration to remove the unloaded drug. Finally, the micelle solution is freeze dried to obtain the drug loading micelles or diluted with distilled water to get desired concentrations. A typical prepared process for MPC loading in PMPC-b-PBMA by our laboratory is as follows²⁷. 50 mg of the PMPC-b-PBMA copolymer and 10 mg MPC were dissolved in 5 mL mixture solvent of chloroform and ethanol (3:2 v/v) and then this solution was slowly added to 30 mL distilled

water under vigorous stirring to induce the micelle formation. The solution was open to air and stirred overnight to remove the organic solvent by evaporation. After the evaporation of organic solvent, the micelle solution was centrifuged (2500 rpm) for 0.5 h followed by filtration using 0.45 μm membrane filters to remove the unloaded drug. Finally, the micelle solution was diluted with distilled water to obtain desired concentrations.

Dialysis method: Drug and amphiphilic block copolymer are both dissolved in the suitable organic solvents. Then the solution is loaded into the dialysis bag whose cut-off molecular weight is less than the molecular of polymer but larger than the one of organic solvent. The dialysis bag filled with solution is then immersed into plenty of water and began to dialysis. Fresh water is continued added to replace the ongoing dialysis water until the organic solvent has been removed cleanly and the micelles loaded drugs are formed. After that, the micelle solution is treated by freeze drying to obtain the drug loading micelles or diluted with distilled water to get desired concentrations. The biggest problem of the method maybe is that it will take several days to get rid of the organic solvent by dialysis progress and will produce a lot of waste water.

Methods based on carbon dioxide: Supercritical carbon dioxide (ScCO_2) has been believed as an environment-friendly alternative solvent and widely used because of nontoxic, inflammable, low-cost and so on. In the field of drug loading micelles, ScCO_2 has been used as: a solvent, in the rapid expansion of supercritical solutions (RESS) method²⁹; an antisolvent, in the supercritical antisolvent precipitation (SAS) method³⁰; a solute, in the particle from gas saturated solution (PGSS) method⁵. Although many manuscripts have reported the drug loading nanoparticles preparation by using ScCO_2 , there are comparatively few reports using it to prepare the shell-core structure micelles. Recently, our group has created a new method which named supercritical fluid evaporation (SFE) method to prepare the drug-loaded micelles with shell-core structure by amphiphilic block copolymer. The principle of this method is as the same as the solvent evaporation method. Therefore, the drug and the hydrophobic blocks of polymer must be soluble in ScCO_2 , obviously this will limit the use of this method. Fortunately, some drugs and the hydrophobic blocks could be solubilized by ScCO_2 (although it needs much higher pressure than the critical pressure of CO_2). For these systems, the SFE method can be an effective method to replace the organic solution evaporation method.

A schematic representation of the supercritical fluid evaporation apparatus is shown in Fig. 1. A typical process of preparation paclitaxel loaded PBMA-b-PMPC micelles by SFE method is as follows. Firstly, PBMA-b-PMPC, paclitaxel and water were mixed under ultrasound for 40 minutes and the mixture was added into the high-pressure cell which was placed into ice-water bath. After that, nitrogen from a cylinder was introduced into the cell and the pressure was kept at 3 MPa which was used to limit the introduced volume of carbon dioxide subsequently. Then carbon dioxide from another cylinder with saturation pressure of 6.2 ± 0.2 MPa at room temperature (*ca.* 28 $^\circ\text{C}$) was led into the high pressure cell, where it was condensed into liquid because the cell was kept in the ice-water bath. After 1.5 h, the valve contracted the CO_2

cylinder was closed and the nitrogen was again introduced and the pressure enhanced to 13 ± 0.2 MPa in order to help paclitaxel solve into ScCO_2 . Subsequently, the bath was raised to 45 $^\circ\text{C}$, the pressure inside the high-pressure cell could reach 20 ± 1 MPa. After stirring for 36 h, the system was connected to the atmosphere and the CO_2 was released in *ca.* 5 min, then the drug-loaded micelles were formed. The micelle solution was centrifuged (4000 rpm) for 0.5 h followed by filtration using 0.45 μm membrane filters to remove the unloaded drug. Finally, the micelle solution was diluted with distilled water to obtain desired concentrations. The micelles prepared by this method is without any organic solution and show a small diameter and high loading content.

Fig. 1. A schematic representation of the supercritical fluid evaporation apparatus A, B-nitrogen and carbon dioxide cylinder, respectively; C-high pressure cell; V, P-control valves and pressure gauges; 1,2,3-three direction link

Targeting drug-loaded micelles: Although shell-core structure micelles could increase the solubility of hydrophobic drugs and achieved the slow-release rate of the drug in the body. The uniform rate of drug release would reduce the drug utilization and produce a certain side effects. In the recent years, a lot of attention has been paid on the development of the micelles for tumor targeting. The most effective ones are based on folic acid as a targeting agent and pH-sensitive micelles.

Folic acid as targeting agent: Folic acid, an essential vitamin for the biosynthesis of nucleotide bases and one-carbon metabolism, has high binding affinity for the folate receptors (K_d - 10^{-10} M). The two pathways for entry of folate derivatives into cells are through facilitated transport by a membrane transport protein and endocytosis. Endocytosis is a natural mechanism by which cells transport essential molecules, such as vitamins and hormones, across the cell membrane and into the cell interior. This process involves specific binding of a ligand to a membrane-bound receptor, with subsequent inward folding of the cell membrane to form and release an intracellular vesicle termed an endosome³¹. Folate receptor (sometimes referred to as the membrane folate binding protein (FBP)) is a 38 kDa glycosyl-phosphatidylinositol (GPI)-anchored cell surface glycoprotein that exists in three isoforms, α , β and γ . Folate receptor expression is normally low in adult tissues; high levels of expression have been found only in a few tissues such as placenta, kidney, lung and choroids plexus. Folate receptors are also highly

overexpressed in cancers of epithelial origin, including cervical, ovarian, renal cell, breast, colorectal carcinomas and choroids plexus³². It is reported that the expression level of the folate receptor in tumors is 100-300 times higher than that observed in normal tissues³³. Thus, the selective overexpression of folate receptors in tumors and its high affinity for folic acid provide a unique opportunity for folic acid to be used as a targeting ligand to deliver therapeutic agents to cancer cells.

A typical successful work was reported by Park *et al.*³⁴. They linked folic acid to amphiphilic block copolymers composed of methoxy poly(ethylene glycol) (MPEG) and poly(ϵ -caprolactone) (PCL). Then Paclitaxel-loaded folate-conjugated MPEG/PCL micelles were synthesized. The size of the micelles was about 50-130 nm depending on the molecular weight of the block copolymers. *In vitro* release kinetics tests showed sustained release of *ca.* 60 % of initial loading amount. Folate-conjugated MPEG/PCL micelles containing paclitaxel resulted in high cytotoxicity for cancer cells.

pH Target: Clinical studies indicated that the tumor cells show weak acid compared to normal ones (pH 7.4)³⁵. The drug-loaded micelles which stabilize in the neutral condition and dissociate in the weak acid dissociation could achieve the drug targeted released in the tumor cells. Recently, pioneers have successfully prepared such targeted drug-loaded micelles based on the different of pH.

The Bae's group has achieved a lot of excellent work on the pH sensitive micelles. At the manuscript reported in 2003, the adriamycin (ADR)-loaded and pH-sensitive micelles was made by pullulan acetate (PA) and sulfonamide (sulfadimethoxine; SDM) (PA/SDM). The *in vitro* study indicated that the pH-sensitivity of PA/SDM can apply for targeting tumor extracellular pH (pHe). At tumor pHe, the PA/SDM nanoparticles showed a drastically enhanced cytotoxicity compared to that at normal pH³⁶. Another pH-sensitivity polymeric micelle is also developed by Bae's group³⁷. The micelle was composed by block copolymers of poly(L-lactide) (PLLA)/poly(ethylene glycol) (PEG) which end was modified with a novel pH sensitive element of a sulfonamide, sulfadimethoxine (SD; pKa 6.1), or a polymer (PSD; Mw: 3000) of its derivative. Due to deionized SDM on the surface, the PLLA/PEG/PSD micelle aggregated below pH 7.0 and achieved the target to tumor cells. Similarly, the micelles of poly(vinyl sulfadimethoxine) (PSDM)-deoxycholic acid (DOCA) conjugates target also obtained the targeted property³⁸. The pH-sensitivity property of the three micelles was based on the different stability of sulfadimethoxine between neutral and acidic state. In fact, some poly amino acids have the same property. For example, the polymeric micellar system composed of poly(L-Histidine)-b-poly(ethylene glycol) and poly(L-Lactide)-b-poly(ethyleneglycol) block copolymers showed wonderful pH-sensitivity⁶. The experimental results indicated that the micelles were quite stable from pH 7.4-7.0 but underwent destabilization as pH decreased further. Besides that, flower-like micelles of poly(L-lactic acid)-b-poly(ethylene glycol)-b-poly(L-histidine) and PEG-poly(β -amino ester) block copolymer micelles were also showed pH-sensitivity⁸. The detailed discussion about the pH-sensitive micelles could be found in Bae's recent review³⁹.

Conclusion

The drug-loaded micelles formed by amphiphilic block copolymers could successfully enhance the water solubility of the hydrophobic drugs. The system has a strong antidilution ability and be stable in the blood circulation for a long time. Furthermore, it could reduce the side effects of the entrapped drug to healthy/normal tissues. Therefore, it will be the most promising drug delivery system for clinical application. The amphiphilic block copolymers are composed by the hydrophobic blocks and the hydrophilic ones. At present, the most current materials of the former are polyethylene glycol(PEG) and poly(2-methacryloyloxyethyl phosphorycholine) (PMPC), the latter are the polyester and polyamides. The most famous prepared methods of forming the drug-loaded micelles are solvent evaporation method and dialysis method.

With the development of the supercritical technology, the green prepared method which use supercritical carbon dioxide to replace organic solvent has become a reality. On the other side, the successful synthesis of targeting polymer materials for tumor cells give a possibility for further improve the drugs efficacy as well as reduce their side effects.

In recent years, targeting drug-loaded micelles are the highlight of the drug delivery systems. The most effective ones are based on folic acid as a targeting agent and pH-sensitive micelles. However, there is still a long distance from the laboratory to practical application.

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