Synthesis and Application of Responsive Polymersomes

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Abstract. Polymersomes as drug carriers make up for the shortcomings of traditional chemotherapeutic drugs, such as easy to synthesize, short circulation time, early release, and continuous cytotoxicity. Responsive polymersomes can alter their properties such as composition, size, shape, and surface functional groups according to different stimuli, enabling the delivery of cargo at hydrophilic, hydrophobic or other specific sites, and the control of drug release. This article provides an overview of what is known about different types of responsive polymeric vesicles and describes the main design choices. In this review, the mechanism and advantages of four responsive polymersomes are discussed. Furthermore, the article focuses on the synthesis methods and applications of representative examples of each stimulus. In particular, the latest progress of four type of responsive polymersomes are emphasized. Finally, based on the great progress in the current research on responsive polymersomes, this paper discusses the potential for future development and selection of more biosafety responsive polymersomes directions.

Keywords: Responsive Polymersomes, Synthesis, Application.

1. Introduction

In recent decades, because of the rapid development of polymer material science and nanotechnology, nano-drug delivery has become a vital research field. Among the carrier materials used in nano-drug delivery systems, polymersomes, also named polymer micelles, are usually assembled from amphiphilic block copolymers or graft copolymers. Because of their excellent properties, such as good mechanical stability, low biological toxicity, and good capacity to encapsulate drugs, polymersomes have gradually become one of the most promising nanocarrier materials. In most cases, polymersomes are used with targeted drug synthesis and diagnostic imaging in medical applications. Additionally, polymersomes are applied to mimic eukaryotic organelles or bacterial microcompartments, construct cell-like systems artificially and be nanoreactors for enzymatic reactions in the biosynthesis. Typical polymeric vesicles surrounded by polymeric bilayer membranes, complex interlaced or amphiphilic membrane structures, possess hollow structures resembling cells and liposomes with sufficient hydrophilic polymer spread inside and outside the membrane. Hydrophobic drugs can be loaded through hydrophobic-hydrophobic interactions in hydrophobic bilayer membranes, and water-soluble small-molecule drugs including proteins, DNA, and RNA can be encapsulated in internal hydrophilic cavities [1].

But in practical applications, it is necessary to consider the impact of the unique microenvironment around the tumor tissue and some physiological barriers to the nano-drug delivery systems during the delivery process. For example, enzymatic degradation in the blood, protein adsorption, and ultrafiltration in the kidney have the potential to induce a reticuloendothelial system that leads to the recognition, capture, and clearance of nanoparticles. Besides, while the intricate blood-brain barrier in the human body protects the central nervous system, it hinders the delivery of the nano-drug particles to brain tumor tissue to a certain extent [2]. The microenvironment around tumor tissue is quite complex, which imposes more stringent requirements on the performance of various aspects of the carrier used for nano-drug delivery. Since scientists discovered that polymeric micelles have different structures and can be engineered using flexible synthetic methods and diverse modification methods, their research on stimuli-responsive polymersomes has grown in depth. Stimuli-responsive polymeric micelles will occur changes in chemical structure or physical properties such as membrane fusion, disassembly, and bond cleavage. The changes will result in the breakup of the polymers and

drugs can release. Standard stimuli involve temperature, light, magnetism, pH, enzyme activity, redox potential and so on [1]. Stimuli-responsive polymersomes can choose the time, site, and dose to release drugs, improve drug use efficiency and reduce the toxicity. Through the control of the molecular design and some appropriate polymerization methods, various responsive covalent bonds, groups, or fragments can be selectively introduced into the polymer chain, and it is the most convenient strategy to prepare polymer nanocarriers with single or multiple stimuli responsiveness.

In the late 1970s, Blumenthal et al. successfully achieved the release of neomycin with temperature-responsive liposomes as a carrier in vitro using topical thermal therapy [2]. They first proposed the concept of stimulus-responsive drug delivery. Since the pH around tumor tissues differs from that in normal physical condition, the natural physical gradient of pH makes pH-responsive micelles good carriers. Therefore, pH-responsive polymeric micelles are also the first type of carriers to be studied. The assembly behavior of triblock polymer PAA2-b-PS890-b-P4VP40 in DMF/THF/H_2O mixed solvent at different pH was reported. When the pH changed from 1 to 14, the vesicle structure gradually transformed into a nanoparticle or an elliptical configuration. When the pH increased to 14, it converted back into a vesicle structure [3]. Except for pH, light is also a familiar stimulus. As a green, clean, and controllable stimulation method, light, including UV, visible, and IR/NIR, has many advantages such as none invasiveness, low toxicity, easy, and precise control. After reaching the target site, the drug molecules can be released via adjusting the light frequency, wavelength, action time, and active site to release precisely. For example, the amphiphilic diblock copolymer poly(ε-caprolactone)-ONB-SS-polymethacrylic acid) by double emulsion method was synthesized, which was assembled into photoresponsive polymers. The hydrophobic core-shell UCNPs induced photocleavage of ONB chains under near-infrared light irradiation at 980 nm. And the 980 nm diode laser irradiation produced a significant inhibitory effect on tumor growth in mice transplanted with A549 lung tumors [4]. What's more, shown as an effective method, ultrasound has been widely used in diagnosis and therapy due to its advantages, such as convenient administration, low cost, and deep penetration.

A growing number of typical markers are able to differentiate between normal and tumor tissues. With the in-depth study of responsive polymer micelles, advanced drug delivery vehicles have greatly improved the therapeutic efficiency and reduced the possibility of damaging normal cells during treatment. More and more synthetic pathways of novel polymersomes and their mechanism of interaction with pathological tissues have been reported. This article reviews the synthesis and applications of five common stimuli-responsive polymersomes. At the same time, it discussed the synthetic routes and principles of polymeric micelles from three aspects: synthesis of responsive scaffolds, modification by responsive functional groups, and synthesis of polymer-drug conjugates with responsive covalent bonds, involve the principle and typical examples. The future development and recent research progress in stimuli-responsive polymersomes also included.

2. Responsive polymersomes

2.1. pH responsive polymersomes

In the course of early research, pH-responsive polymers were the most extensively studied. To date, pH-responsive polymeric micelles and vesicles have been the most promising and sought-after research topics in cancer-controlled drug delivery systems. This polymer has been deeply studied to deliver drugs to specific sites in an organ, inside a cell, and so on that are associated with certain pathological conditions. Apart from the extracellular pH of tumor and inflammatory tissues is lower than its of normal physical condition, the pH in endosomes and lysosomes declined as well [3]. Because of the natural pH gradient, pH-responsive polymersomes can be good drug delivery vehicles. Such pH-responsive polymers are commonly synthesized by introducing acid-cleavable bonds or ionizable groups into block copolymers or directly forming multimeric complexes through electrostatic interactions.

Some pH-responsive weak bonds, such as acylhydrazone bonds, hydrazide and kesterone structures are also widely used to design pH-responsive polymer. The methotrexate (MTX) anticancer drug from poly(monomethoxyethylene glycol)-b-polydiblock copolymer (ε-hexanoic acid tone) (mPEG- PCL) that were targeted for loading and release in polymeric micelles was obtained and the drug release was pH-dependent (Figure 1). The MTX/mPEG-PCL system showed that large amounts of drug were more likely to be released at lower pH, while the pH of the physiological environment would inhibit the release of MTX. The diblock copolymer is prepared by ring-opening polymerization using hydrochloric acid as a catalyst, so that an amphiphilic chain (DP of 1.09) can be obtained. The copolymer spontaneously assembles in aqueous solution to form homogeneous nanosized polymer micelles. MTX was captured in micellar cores by dialysis with an efficiency of 70%. Experiments show that the effect of MTX/mPEG-PCL on MCF-7 breast cancer cells is stronger than that of free TMX [5]. Therefore, the system can effectively maintain the capsule drug until it is subject to acid stimulation.

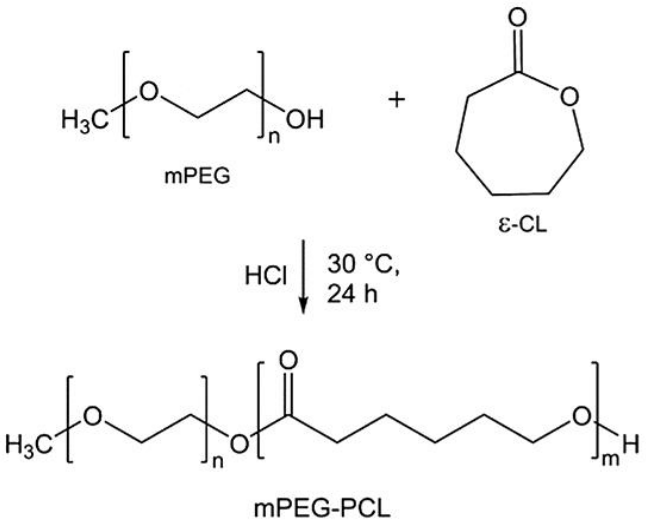

Figure 1. Structure of mPEG-PCL and its synthesis route [5]

Also, the protonation or deprotonation process of carboxyl group and tertiary amine is used to construct pH-responsive polymer nanocapherers [3]. These polymers include polyacrylamide (PAAm), polyacrylic acid (PAA), polymethacrylic acid (PMAA), polyN, N-diethylamine ethyl methacrylate (PDEAEMA), and so on. These polymers will swell and collapse due to charge interaction when pH changes, so as to realize hydrophilic and hydrophobic transformation and the change of assembly structure. Liu et al. developed a novel DOX-loadable pH-responsive drug delivery system which can reach tumor site accurately [6]. The prepared spherical polymer vesicles can moderate the release of doxorubicin (DOX) hydrochloride and promote the rapid release of chemotherapeutic drugs in an acidic pathological environment. In this system, three polymers of amino-terminated polyethylene glycol-folate (NH_2-PEG-FA), polyacrylamide hydrochloride (PAH) and quaternary ammonium salt chitosan (QCS) were assembled into polymer micelles. DOX and PLGA were made into microspheres inside the micelles by double emulsion method and solvent evaporation method. And the coated DOX was uniformly dispersed in the core-shell nanostructure by electrostatic self-assembly method. Experiments show that this drug-loading system also has excellent acid-base responsive drug release properties in vitro. It is found that core-shell polymers such as DOX@PLGA@PEG-FA can achieve intelligent and sustained drug release by forming a pH-sensitive system [6]. Since FA can be exposed on the polymer surface as a targeting agent, it has the potential to interact with folate receptors on the membrane of many cancer cells.

In addition to their significant role in drug loading, pH-responsive polymers have also received extensive attention as intelligent artificial nanoreactors. Japir et al. developed a class of polymeric vesicles that can selectively adjust the crosslinking density according to tumor pH to control membrane permeability [7]. By synthesizing diblock copolymers of polyethylene glycol (PEG) and methacrylate monomers containing piperidine or coumarin groups, they can self-assemble into small

molecules that can encapsulate DOX and macromolecular glucose oxidase (GOD) (Figure 2). After photocrosslinking optimization, the polymer exhibits strong stability at pH from 6.5 to 7.4 due to crosslinking density and pH-responsive fragments, and membrane-selective permeability due to pH-sensitive formation. Over a wide pH range, MCL-PSs are highly tolerant to environmental changes that protect their vesicular morphology. Changing the pH value can enhance the membrane permeability of DOX- and GOD-loaded polymers to initiate drug release and regulate enzymatic reactions. When small molecules such as DOX, glucose, and O_2 in the polymer diffuse or release, the increased concentration of GOD leads to the generation of a large amount of hydrogen peroxide to generate and release DOX, thereby killing cancer cells [7].

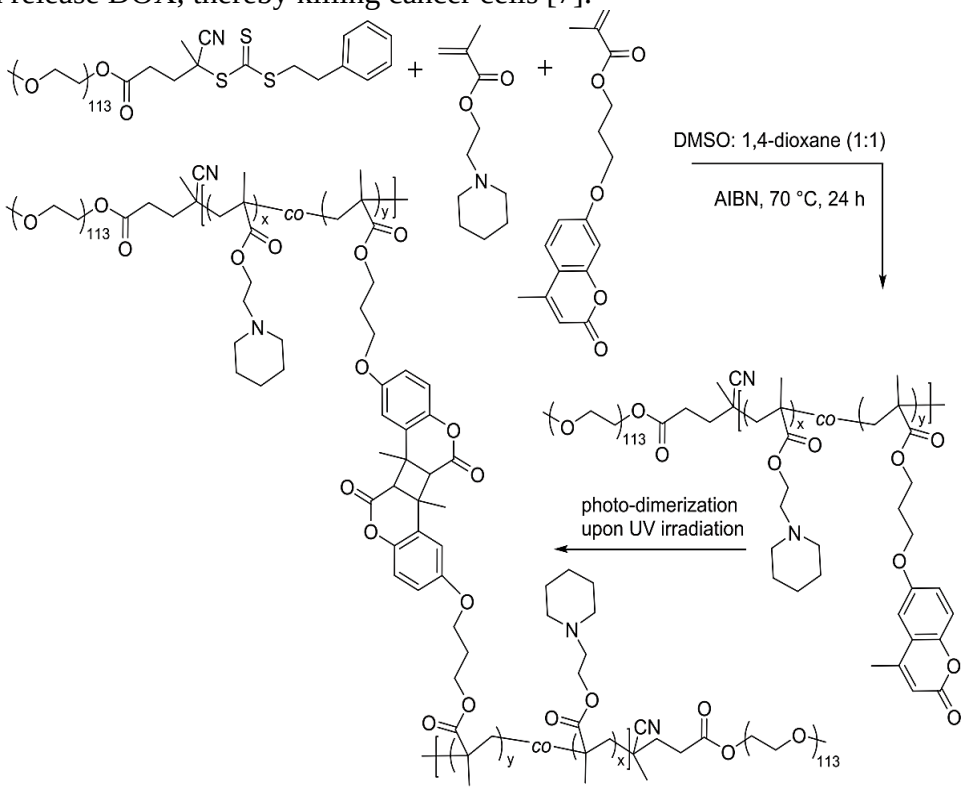

Figure 2. Schematic illustration of the synthesis of block copolymer PEG-b-P (PEMAx-co-CMAy) and the coupling reaction of photocrosslinking conditionse [7]

2.2. Temperature-responsive polymersomes

Temperature is a widely used and studied polymer triggering factor. It was found that the morphology and polymerization kinetics of block copolymer vesicles were significantly affected by the reaction temperature. For example, He et al. reported the photometric measurement at 37 °C of NIPAM seeded with plate-like polymersomes with PHPMA as the main component [8]. Incorporating PNIPAM into the vesicle membrane, a thermoresponsive enzymatic nanoreactor can be constructed, and the activity of the enzyme can be artificially controlled by changing the reaction temperature. In the study, DMA mediated with DDMAT was polymerized in RAFT solution to produce a macro RAFT agent, which is denoted as PDMAn–DDMAT (n=32, 48) (Figure 3). In this experiment, DMA and DDMAT were first synthesized by RAFT solution polymerization method to synthesize PDMA-DDMAT and then synthesized by aqueous photocuring method to prepare temperature-responsive polymers [8]. The results showed that the micellization rate of PDMA-PNIPAM in water at 70 °C was much faster at 37 °C. They constructed a phase diagram of PDMA-P(NIPAM-co-ALAM) at these two widely different temperatures, which further confirmed that the higher the temperature, the faster the micellization rate of the copolymer. They also found that only spheres were obtained at 37 °C, and this result was independent of the DP and solids content of PNIPAM.

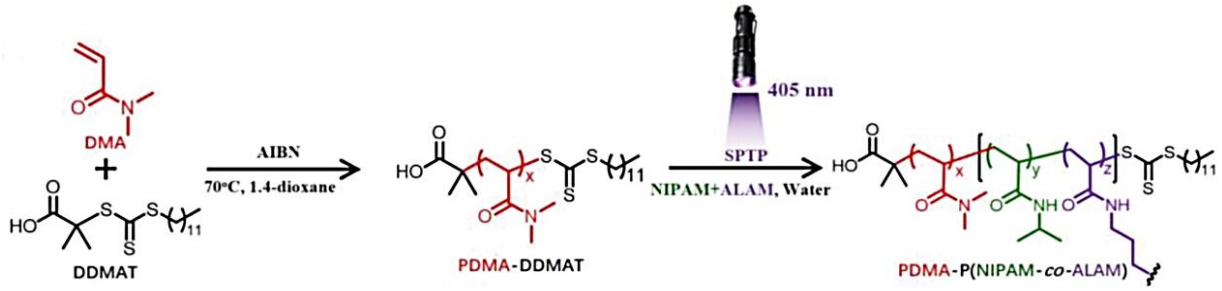

Figure 3. Synthesis of PDMA-P (NIPAM-co-ALAM) temperature-responsive vesicles by DMA and DDMAT [8]

Peng et al. reported vesicles composed of block copolymer PEO-b-P (NIPAM-r-TMPM) with thermally responsive properties. Vesicles cross-linked by TMPM do not normally disintegrate at low temperature [9]. However, the gelled porous bubbles can undergo reversible size transitions with increasing temperature. As PTMPM content in the polymersomes increased, the cell size changes decreased. Experiments showed that the gelled polymersomes formed by PEO45-b-(PNIPAM66-r-PTMPM9) doubled in size from 22 ℃ to 36 °C, whereas the size transition of gelled porous vesicles formed by PEO45-b-(PNIPAM66-r-PTMPM17) is much smaller. In addition, the cross-linked network of gelled single vesicles can keep the vesicles intact at different temperatures, so the size changes are not obvious [9]. The comparisons illustrate that differences between vesicle structures lead to different manifestations of thermally responsive properties.

Hybrid polymersomes composed of two or more types of amphiphilic molecules such as phospholipid polymers or small molecules have also been gradually developed. Kim and colleagues showed that polymeric vesicles functionalized with cycloarginine-glycine-aspartate (cRGD) ligands demonstrated highly efficient cytotoxicity under heat [10]. They were taken up 8-fold and 10-fold by αvβ3 integrin in highly expressing U87MG and HUVEC cells, respectively. At the same time, it was found that the vesicles formed by lipids and elastin-like polypeptides undergo phase transitions according to temperature changes. The change of structure is shown in Figure 4.

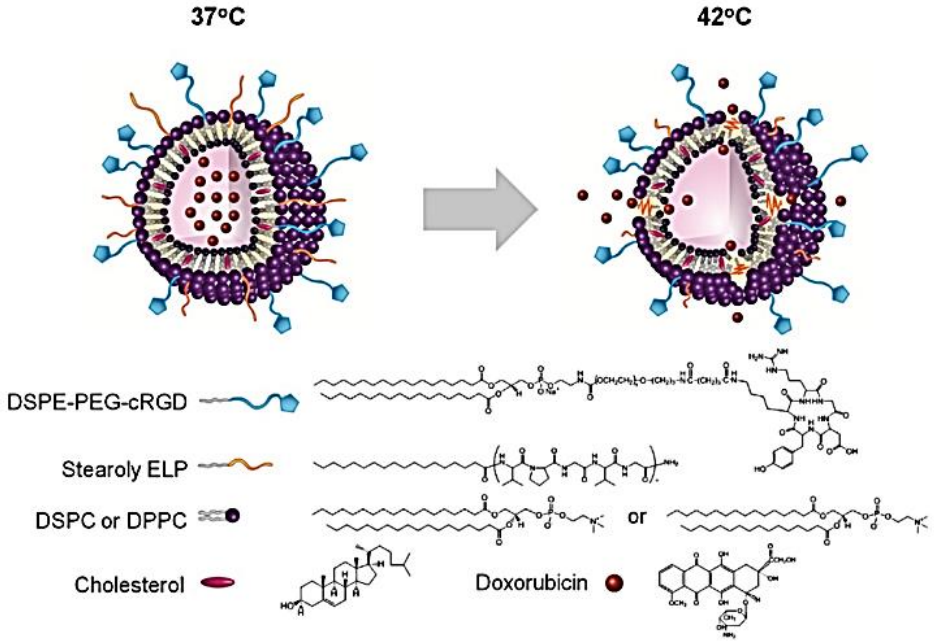

Figure 4. The constituents of hybrid polymersomes in which lipids, a target unit and an elastin-like peptide contribute to the thermal response to doxorubicin [10]

2.3. Enzyme-responsive polymersomes

In drug delivery, the advantages of enzyme-reactive drug carriers are emerging as enzymes at the diseased tissue exhibit more activity. The enzymes around the pathological tissue are usually

abnormally active. Therefore, designing controllable responsive polymeric vesicles for overexpressed enzymes has applications in many fields, such as diagnostics, drug delivery, and sensing. Kumar et al. designed an enzyme-responsive polymersome which cleaves with phosphatase (ALP) at the phosphate end of the polymer [11]. The study divided ALP into three types: ALP-responsive polysome P0, and hydrophilic modification into it forms P1 and hydrophobic modification into it forms P2. Their structures and mechanisms are shown in Figure 5. Phenyl ether derivatives are usually used to cap the ends of the P0 backbone because it protects the phosphate trigger. The synthesis of ALP-responsive P0 requires deprotection of the benzyl ether group and the tert-butyl group on the polymer side chain. When they form polymeric vesicles, an enzyme-responsive depolymerization reaction releases the guest molecules non-covalently linked to the polymersomes [11].

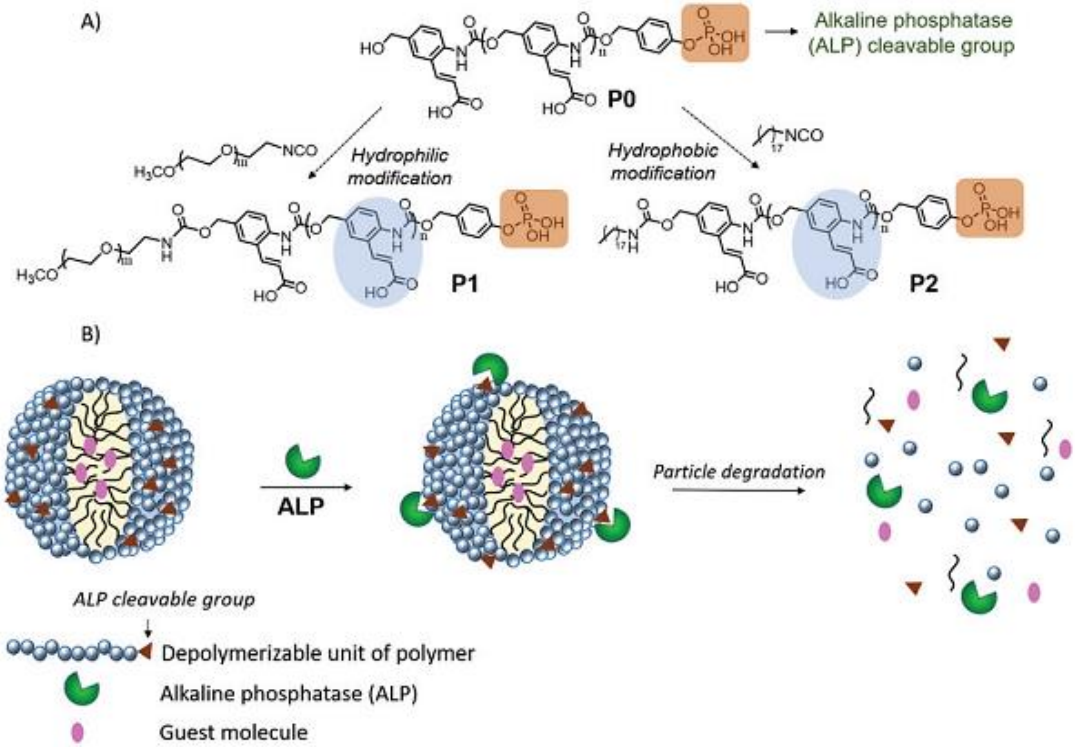

Figure 5. (A) Structure of the ALP-responsive polymer, P0; hydrophilicand modified P1 and hydrophobic modified P2. (B) Diagram of the composition of P1 and P2 and the process of their interaction with ALP [11]

The standard enzymatic action is the cleavage of chemical bonds, which can be at the interface of the hydrophobic monolayer of the polymer, the interface of the hydrophilic monolayer of the polymer, or the junction between the hydrophobic layer and the hydrophilic layer. This enzyme can react in the main chain or side chain of the polymer. The study by Ramezani et al. demonstrated that the enzymatic hydrolysis of the junction region can lead to the deformation of the polymersome and the rapid release of the drug [12]. In the study, polyethylene glycol (PEG) and polylactic acid (PLA) can be linked via the synthetic peptide PVGLIG, which is specifically recognized and cleaved by metalloprotease 2 (MMP-2) enzymes. And the synthesized PEG-b-PVGLIG-PLA chimeric triblock polymer can form vesicles loaded with hydrophobic SN38 (Figure 6). The prepared chimeric polymer formulation releases SN38 under physiological conditions, and when it is exposed to MMP-2 enzyme, the release rate can increase 7-fold.

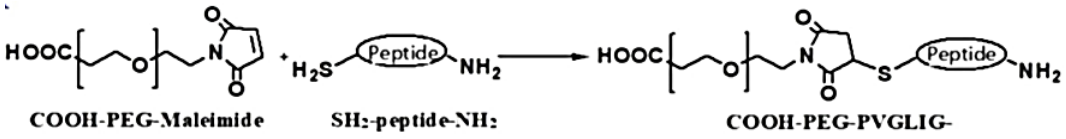

Figure 6. Synthesis of PEG-peptide conjugate [12]

Since the diffusion of enzymes in the hydrophobic membrane is restricted, the enzymes usually degrade the outer surface of the hydrophilic brush first rather than the inner surface. Bacinello combined the hydrophilic peptide PVGLIG with the hydrophobic synthetic polymer PTMC to synthesize PVGLG-PTMC hybrid amphiphiles assembled into polymersomes. In the presence of MMP-2, these vesicles are broken down. Photon correlation spectroscopy showed that the intensity of scattered light dropped by nearly half over two days. For the reason that MMP-2 was more accessible to the sites of the hydrophilic brush, PVGLIG degraded faster on the hydrophilic brush than as a linker, and an analogous reduction in light intensity was observed with five days [13].

2.4. Redox-responsive polymersomes

The concentration of redox molecules in the environment of cancer cells is quite different from that under normal physiological conditions, and their activities are disturbed after stimulation. Considering that cellular activities at tumor tissue can affect the role of redox molecules, redox-responsive polymeric micelles can be good carriers for targeted drug delivery. Among the disordered redox molecules, reactive oxygen species (ROS), glutathione (GSH), and hydrogen sulfide (H_2S) are commonly used as anchors for the accurate arrival of drug-carrying systems. Current studies usually assemble redox-responsive fragments on the basis of polymer micelles, such as thioethers, thioketones, and arylboronic esters, which are all responsive components of ROS-responsive polymers [3].

ROS is a class of ions and free radicals with high oxidative activity in cells, mainly including superoxide, hydroxyl radicals, hypochlorite ions, peroxynitrite, hydrogen peroxide, singlet oxygen, and so on. The strategies of ROS-responsive polymersomes can generally be divided into the following three types: First, introduce the responsive functional groups into the side chains of the hydrophobic part of the polymer. After high-concentration ROS oxidation, the side chain is broken, and the hydrophobic segment is converted into a hydrophilic segment. The hydrophilic and hydrophobic balance in the polymer chain is broken, and the micellar core disintegrates, thereby releasing the drug. Second, the responsive functional group is used as the linking group between the hydrophilic segment and the hydrophobic segment. Under the stimulation of ROS, complete oxidative cleavage occurs between the hydrophilic and hydrophobic segments in the polymer. The micellar structure is destroyed, and the embedded drug is released. Third, the drug molecule and the polymer are connected by responsive groups. Under the oxidation of ROS, the linker is induced to break and the drug molecule is activated, so as to achieve the anti-tumor effect [14]. Jia et al. proposed a CD-based ROS-responsive micelle to deliver antitumor drugs. It is the first time that the researchers used mono(6-amino-6-deoxy)-β-cyclodextrin and the hydrophilic polymPEG-NHS to synthesize MPEG-CD. Afterwards, MPEG-CD reacted with PHB-CDI, a compound containing boronic acid bonds, to synthesize ROS-responsive MPEG-CD-PHB (PCP), which was used to encapsulate DOX and purine-18. PDP micelles can enter tumor cells through enhanced permeability and retention (EPR). Because of the high molecular weight of H_2O_2 around cancer cells, when the drug enters the pathological tissue,the high concentration of ROS stimulates the increase of DOX and P18 from the polymersomes. Cytotoxic ROS are then generated under near-infrared laser light, enabling chemical photodynamic therapy [15].

Because of the shortage of ROS scavenging system in tumor tissues, the inherent redox balance in cells is broken, and cells are in a state of oxidative stress for a long time. In order to adapt to changes in the environment and continue to survive, cancer cells can counteract the excessive accumulation of ROS by upregulating GSH levels. Disulfide bonds can remain stable under normal physiological conditions. However, due to the increased molecular level of glutathione in tumor tissue, once the vesicles enter cancer cells, GSH in the cytoplasm will act as a reducing agent to break the disulfide bond and generate oxidized glutathione by itself. The glycerol (GSSG), and reduces disulfide bonds to sulfhydryls. Therefore, polymersomes containing disulfide bonds have great research value in the treatment of cancer. In 2020, Lin et al. reported that glutathione sensitivity could be adjusted via modulating the hydrophility of the polymersomes, which can be modulated by the coupling degree

of CPT. CPT is linked to the amphiphilic poly(ethylene glycol)-b-poly(glucose carbonate) (PEG-bPGC) via disulfide bonds. In order to prepare the amphiphilic polymer, the first step is to prepare the polymer by ring-opening polymerization with methoxy polyethylene glycol as the initiator. In the second step, when the photoinitiator 2,2-dimethoxy-2-phenylacetophenone (DMPA) provides reaction conditions, the polymer reacts with mercaptopropionic acid with thioacetylene. In the third step, the hydroxyl group of CPT-SS-OH and the carboxylic acid group on the polymer undergo an esterification reaction to conjugate CPT with the side chain of the polymer (Figure 7). CPU with a higher degree of coupling can make the drug release milder and reach the target cells more precisely, which is beneficial to achieve the best therapeutic effect [16].

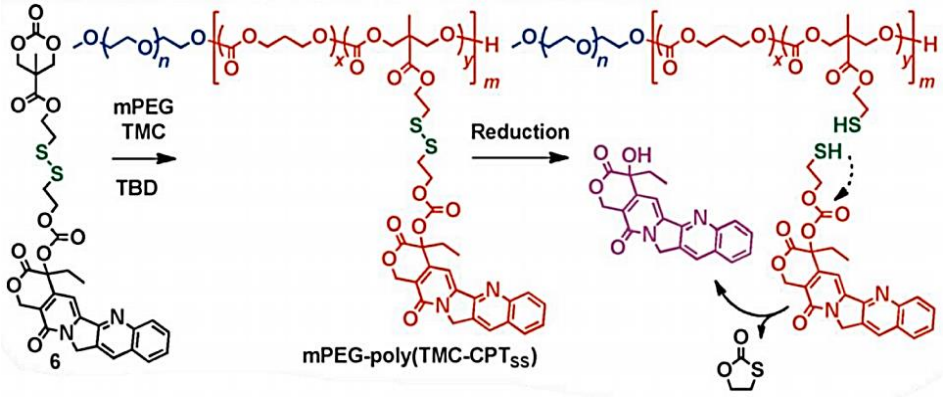

Figure 7. Schematic diagram of the synthesis process of amphiphilic block copolymers [16]

Shi et al. designed diselenene-containing nonionic gemini redox-responsive polymersomes to deliver of indomethacin (IND). The polymersome carrier with nanoscale size has high drug loading capacity and delivery efficiency, good stability in simulated circulating liquid, and good redox responsiveness [17]. The results show that it has a larger radius and weaker electronegativity than S, and the Se-Se bond energy (172 kJ/mol) is lower than the S-S bond energy (240 kJ/mol). This results in the diselenene-containing polymer micelles being more prone to bond cleavage under redox conditions. Therefore, the non-ionic gemini polymer micellar carrier responds preferentially to ROS over 1,4-dithiotriol, which is beneficial to the controllable release of IND in the tumor environment. On the other hand, due to the existence of the redox potential gradient, the polymer carrier containing diselenene can react with extracellular ROS instead of intracellular GSH. This also ensures that the drug can be delivered more precisely at the tumor site.

3. Conclusions

According to the examples reviewed in the previous section, four types of responsive polymersomes have been studied and applied continuously. The pH-responsive polymeric vesicles that were first investigated have been extensively developed due to the pH gradient of pathological and normal physiological environments. There are generally three preparation methods for pH-responsive polymers. The pH-responsive polymers have shown excellent application value both in drug loading and as intelligent artificial nanoreactors, people have also tried to develop multi stimulus-responsive polymers based on it. Scientists found that block copolymer vesicles whose morphology and polymerization kinetics are significantly affected by reaction temperature and developed many excellent functions such as drug carrier, nanoreactors, hydrogels, and more. Therefore, temperature-responsive polymers are also promising materials. Enzyme-responsive polymers have an advantage in delivering drugs to lesions where the enzyme is overexpressed, and the site of the enzymatic reaction can be precisely controlled. Similarly, tissue lesions will also lead to changes in the concentration of redox molecules, and related ROS, GSH, H_2S responsive vectors can effectively achieve drug accumulation in target cancer cells, improve the therapeutic efficiency and reduce the toxicity. In addition to improve the efficiency and selectivity of drug delivery, the

biosafety of the polymersoms should be fully considered. Future research on responsive polymersomes may focus on reducing toxic side effects.

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