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Review

Mechanisms of drug release in pH-sensitive micelles for tumour targeted drug delivery system: A review

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ABSTRACT

During the past decades, chemotherapy has been regarded as the most effective method for tumor therapy, but still faces significant challenges, such as poor tumor selectivity and multidrug resistance. The development of targeted drug delivery systems brings certain dramatic advantages for reducing the side effects and improving the therapeutic efficacy. Coupling a specific stimuli-triggered drug release mechanism with these delivery systems is one of the most prevalent approaches for targeted therapy. Among these approaches, pH-sensitive micelles are regarded as the most general strategy with advantages of increasing solubility of water-insoluble drugs, pH-sensitive release, high drug loading, etc.

This review will focus on the potential of pH-sensitive micelles in tumor therapy, analyze four types of drug-loaded micelles and mechanisms of drug release and give an exhaustive collection of recent investigations. Sufficient understanding of these mechanisms will help us to design more efficient pH-sensitive drug delivery system to address the challenges encountered in targeted drug delivery systems for tumor therapy.

1. Introduction

Compared with traditional chemotherapy, the development of nanotechnology-based targeting drug delivery system has led to a new strategy for cancer therapy (Chen et al., 2011; Lee et al., 2012). For example, nanoscale polymer micelles can be used for the delivery and controlled release of antineoplastic agents, thereby improving drug efficacy and reducing side effects (Duncan, 2003; Kanapathipillai et al., 2014; Zhang et al., 2012). The pathophysiological characteristics of most solid tumors are significantly different from those of normal tissues and organs, such as the rapid growth and poor structural integrity of tumor blood vessels, lack of tumor tissue lymphatic reflux system and the presence of a large number of tumor vascular permeability factor (VPF) (Maeda, 2001). It is noteworthy that the growing solid tumor shows an increase in vascular permeability with a pore size of 200 to 780 nm. However, the drug-loaded nanoparticles have a diameter in range of 60 to 500 nm, which are small enough to pass through these pores from the blood to the tumor interstitial space (Dreher et al., 2006; Yuan et al., 1995; Tavano and Muzzalupo, 2016). These physiological changes are conductive to obtain large amounts of nutrients and oxygen for the rapid growth of tumor tissue, while the nano-sized polymer micelles can deliver and accumulate anti-tumor drugs to tumor tissue via the enhanced permeability and retention (EPR) effect (Fang et al., 2011; Maeda et al., 2000). A variety of targeted tumor delivery system based on the EPR effect principle is regarded as the revolutionary improvement in the delivery of chemotherapeutic drugs to tumors. However, nano-sized carriers need to be effectively avoided removing from the blood circulation before reaching the tumor site or fulfilling their function (Gabizon et al., 1994). Thus, one of the most important properties of targeted drug delivery system is the long circulation of nanocarriers (Gabizon et al., 1994; Shen et al., 2012). The most common method in extending circulation is achieved by using a surface coating of a hydrophilic polymer, such as polyethylene glycol (PEG) (Allen et al., 1991; Jiang et al., 2013; Torchilin, 2009). The process is called PEGylation, so as to achieve the stealth effect and to help avoid the identification and destruction of the reticuloendothelial system (RES) (Allen et al., 1999; Moghimi et al., 2001; Torchilin, 2009). Therefore, the polymer micelles formed by the self-assemble of the hydrophilic segment (shell)-hydrophobic segment (nucleus) amphiphilic block copolymers have drawn a great deal of attention as potential candidate carrier vehicle due to their wide advantages in research of targeting drug delivery system (Gaucher et al., 2005; Kataoka et al., 2001; Rapoport, 2007). The hydrophobic drugs (e.g. doxorubicin (DOX) or paclitaxel) can be encapsulated into the micellar hydrophobic

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core in which the hydrophilic segment of the polymer is distributed around the hydrophobic core and extends outwardly in the aqueous medium to form a stable shell that allows the micelles to a stable dispersion (Kwon, 2003; Tong and Cheng, 2007; Torchilin, 2007). The polymer micelles have the following characteristics: excellent biocompatibility; suitable particle size (< 200nm); prolonged circulation time in the blood after intravenous injection; and can carry anti-tumor drugs passively targeted and accumulated in the tumor via the EPR effects (Cagel et al., 2017). Furthermore, the intake of the polymer micelles in target cells can be further enhanced through the ligand-receptor interaction after the nanocarriers arrive at target site via EPR effect during the systemic circulation. This phenomenon is termed as active targeting (Srinivasarao et al., 2015). Those smart polymer micelles can be prepared by conjugation of active targeting ligands on the PEG fragments of polymer micelles. The active targeting ligands include small-molecules, cell penetrating peptides, proteins, aptamer, sugar, monoclonal antibodies, their specific receptors are over-expressed on many cancer cells. Some common active targeting ligands such as folic acid (folate receptor), transferrin (transferrin receptor), RGD (integrin receptor), lactobionic acid (asialoglycoprotein receptor), AS1411 aptamer (nucleolus receptor), etc. have been extensively modified to the surface of the polymer micellar nanoparticles to achieve effective targeted drug delivery system (TDDS) (Bae et al., 2005a,b; Guo et al., 2011; Pan et al., 2016; Srinivasarao et al., 2015).

However, the drug release from polymer is affected by many factors. Too fast drug release before reaching the tumor site can cause the toxic side effects and reduce the concentration of drugs in the target area, while releasing too slowly can reduce the efficacy of drugs in the target site and increase the resistance of tumor cells. So the controlled release of the drug from the micelles becomes the key point of the drug delivery system. In recent years, in order to overcome these barriers, environmentally sensitive polymers which can respond and perceive on the exogenous stimuli (light, temperature, ultrasound, electrochemical triggers) or tumor microenvironment irritants (pH, enzyme activity, redox properties) to trigger drug release, have been extensively investigated (Felber et al., 2012; Fleige et al., 2012; Liu et al., 2014; Rapoport, 2007; Tavano and Muzzalupo, 2016; Thornton et al., 2005).

The rapid release of drugs at the tumor site is successfully achieved by designing and applying of these functional nanocarriers. Among these stimulus-responsive systems, pH-sensitive nanocarriers have been the most popular subjects for current researches in drug delivery system for the following reasons. One is that pH-sensitive nanocarries are incorporated via endocytosis develop markedly acidified lumens (pH 4.5–5.5) primarily through the activity of V-type H+ ATPase (Forgac, 2007). And then the acid-sensitive materials can capture the proton, and cause the chloride ion influx, resulting in increased lysosomal osmotic pressure, and finally lysosomal rupture and thus the endocytosis of drugs and carriers released to the cytoplasm (proton sponge mechanism). This ‘endosomal escape’ phenomenon can avoid the lysosomal degradation of palyload to improve the effective bioavailability (Xu et al., 2015; Zhang et al., 2014). The other reason is that the acidic environment of the tumor tissue relative to normal tissue can trigger the release of the encapsulated/conjugated drugs from the pH-sensitive nanocarriers at low pH. The energy of tumor cells is mainly derived from glycolysis. It has been known since the pioneering studies of Warburg that tumor cells can produce too much lactic acid even in normoxic condition (Warburg, 1956), however, the general characteristic of anoxia in solid tumor has been confirmed. Thus, the peculiarity of high glycolysis rate in tumor cells is the primary source of the low tumor pH (Gerweck and Seetharaman, 1996; Neri and Supuran, 2011). The interstitial cells of the tumor tissue were weakly acidic (pH < 7.0), while the normal tissue and the extracellular pH in the blood remains constant at pH 7.2–7.4 (Vaupel et al., 1989; Wike-Hooley et al., 1984). This essential discovery provides an important theoretical basis for the development and application of pH-sensitive nano-sized carriers. The pH-sensitive polymer micelles reach the tumor site via the EPR effect, and then through the endosomes (pH 5.5–6.0) or lysosomal (pH 5.0) pathway transport after intracellularization. In this process, the pH value decreases from the normal physiological condition (pH 7.4) to about pH 5.0 (You and Auguste, 2009). To date, a variety of pH-sensitive polymer micelles have been designed, and drugs could be physically encapsulated or chemically conjugated with the micelles (Danhier et al., 2010; Gao et al., 2013; Liu et al., 2013). These pH-sensitive drug-loaded micelles remain stable at physiological pH, and water-insoluble drugs encapsulated in the hydrophobic core are hardly leaked during the long systemic circulation, but they respond to low pH environment such as endosomes and become destabilise to release their drug cargo, to help achieve the desired anti-tumor effect.

In this review, a variety of strategies to design pH-sensitive polymer micelles and their mechanism of drug release are discussed. Particular attention is paid to the different release efficiencies caused by the different drug release mechanism, which provides a reference for the optimal design of pH-sensitive micelles.

2. pH-sensitive polymeric micelles

In order to response the acidic microenvironments in tumor, polymer micelles can be designed based on two main forms. One is that nano-micellar materials are composed of the polymers with ionizable chemical groups, which can accept or donate protons in response to an environmental pH change. Some representative molecular formulas are shown in Fig. 1. These ionizable polymers include cationic polymers and anionic polymers, at physiological pH, they remain deprotonated/deionized, resulting in protonation or charge reversal of the polymers under acidic pH causing structural damage of the nanocarriers and leads to specific release of the encapsulated drugs (Chang et al., 2009; Fleige et al., 2012; Ganta et al., 2008; Lee et al., 2008a). The second form is to use acid-labile bonds to increase intracellular drug release or endosomal escape. Hydrolysis of acid-labile bonds between drug and polymer, or within the polymer are regarded as a promising strategy to deliver drugs to tumor tissue and release drugs by breaking the acid-labile bonds at acidic pH (Xu et al., 2009). Typical acid-labile bonds used as linkages in the pH-sensitive polymers include hydrazone, imine, oxime, acetal, vinylether, and orthoester bonds (Bae et al., 2005b; Chen et al., 2009; Gillies and Frechet, 2003; Thambi et al., 2011; Wang et al., 2011) (Table 1).

3. Release mechanisms of pH-sensitive polymer micelles

There are many factors that can affect the release of drugs. The rate of drug release may be related to the rate of degradation of the polymer, the length of the micellar nucleus fragments, the physical state of the nucleus, the size of the drug molecule, the position of the drugs in micelles, and the drug-loading rate. Different forms of pH-sensitive micelles as drug carriers have different principles and ways of drug release. At present, according to different drug release mechanisms, pH-sensitive micellar drug delivery system can be summarized as the following four types (Fig. 2): 1) Protonation or deprotonation of polymers leads to the destruction of amphipathic structures that result in drug release, 2) The separation of the polymer micelle amphiphilic blocks leads to drug release, 3) The reduced hydrophobicity of the hydrophobic segment of the the polymer micelles causes the micelles to swell to release the drugs, and 4) The rupture of the acid-labile bond between the drug and polymer leads to drug release.

3.1. Protonation or deprotonation of polymers leads to the destruction of amphipathic structures that result in drug release

Amphiphilic block copolymers consist of hydrophilic and hydrophobic segments. In this strategy, the chemical properties of the polymer with ionizable chemical groups can be changed in response to an environmental pH change (Ganta et al., 2008; Gil and Hudson, 2004;

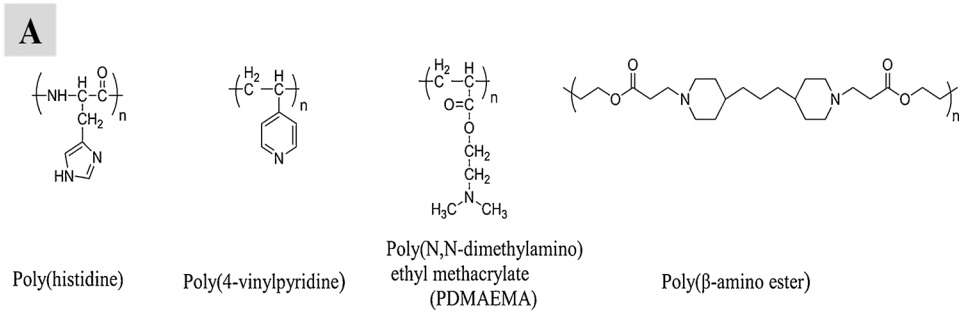
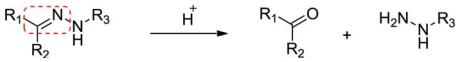
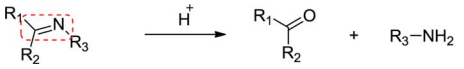
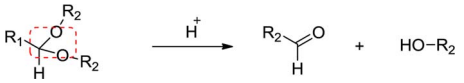
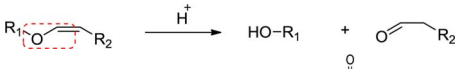
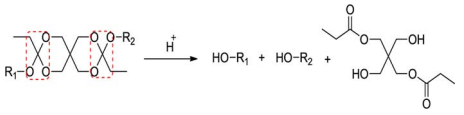


Fig. 1. Molecular formula of representative pH-sensitive cationic polymers (A) and anionic polymers (B).

Table 1
Examples of common acid-labile Linkers and their hydrolysis products.

Acid labile bond	Structure
Hydrazone	
Imine	
Oxime	
Vinylether	
Orthoester	

Lee et al., 2008a). In practical terms, acidic pH induced protonation leads to hydrophilic-hydrophobic phase transition for the anionic polymers, and hydrophobic- hydrophilic phase transition for cationic polymers, causing destruction of amphiphilic leading to drug release (Kanamala et al., 2016) (Fig. 2.1). Anionic polymers with carboxylic acid groups are commonly used as pH-sensitive polymers, such as poly (acrylic acid), poly (methacrylic acid) (PMAA), poly (2-ethyl acrylic acid) and poly (glutamic acid) (Bajaj and Singhal, 2011; Bersani et al., 2014; Ding et al., 2012; Kang et al., 2012; Yan and Gemeinhart, 2005). The carboxyl pendant groups are deprotonated to exhibit hydrophilicity at neutral pH, however, they become protonated and hydrophobic at acidic pH. For example, NK105 is a promising amphiphilic block copolymer consisting of PEG-poly (aspartic acid) polymers used to encapsulate paclitaxel for antitumor studies (Hamaguchi et al., 2005). The NK105 polymers were composed of PEG as the hydrophilic segment and the 4-phenyl-1-butanol modified poly(aspartate) as the hydrophobic segment. Paclitaxel was encapsulated into the hydrophobic core of the micelle system by physical entrapment though the self-association process. The unmodified carboxyl groups of poly (aspartate) polymer were deprotonated at physiological pH to maintain the stability of the micelle system, however, they were protonated and could cause the release of paclitaxel at acidic pH. Preclinical studies have shown that NK105 exhibits equivalent cytotoxic activity compared to free paclitaxel during the test on 12 human tumor cell lines derived from lung, gastric, oesophagus, colon, breast, and ovarian tumours. Remarkably, NK105 has been enrolled in Phase 3 trial in patients with metastatic breast cancer due to the excellent results of Phase 1 and Phase 2 clinical trials (Kato et al., 2012; Matsumura, 2014). In addition, another type of polymer with sulfonamide groups is also a common form of pH-sensitive anionic polymer (Sethuraman et al., 2008).

Furthermore, polymers with ionizable chemical groups such as tertiary amine groups, imidazole pendant groups and pyridine groups are commonly utilized as a segment to construct the amphiphilic block copolymers. These amino-rich hydrophobic segments can be deprotonated to maintain hydrophobic at physiological pH, but can be protonated to become hydrophilic at acidic pH. In this process, the amphiphilicity of the polymer micelles is destroyed, resulting in the disaggregation of the micelles leading to drug release. Histidine has been used as the pendant group for pH-sensitive micelles due to its ability to protonate at acidic tumor pH while remains neutral in physiological pH (Johnson et al., 2014). Li and coworkers synthesized PIA-PEG-FA-PHIS polymer as a carrier for tumor-targeted drug delivery (Sun et al., 2015). The imidazole (pK_a ~ 6.5) ring of PHIS has lone pair electrons on unsaturated nitrogen that endow it with pH-sensitive properties (Lee et al., 2003). When the polymer is delivered to the lysosomes, pH falls below 6.5, PHIS can accept protons and trigger hydrophobic to hydrophilic phase transitions, resulting in the instability of the micelles. Subsequently, the encapsulated drugs are released into the cytoplasm with the lysosomal membrane destruction to improve the anti-tumor effect (Lee et al., 2007; Lee et al., 2003; Oh and Lee, 2008; Park et al., 2012). Additionally, the micelles can be selectively taken up and internalized into the tumor cells by the active targeting of folic acid to enhance the drugs to tumor sites. The results of micelles being incubated at different pHs showed that they remained stable under physiological condition at pH 7.4 for over 48 h. Nevertheless, when the pH was decreased to 5.0, the PHIS chains of micelles were protonated, resulting in demicellization leading to rapid drug release. The intracellular drug release behavior was further evaluated by CLSM. In vitro cytotoxicity experiments showed that the DOX-loaded micelles exhibited more inhibition of proliferation of HeLa cells than free DOX with pH decreased from 7.4 to 5.0. Based on the experimental results, it can be concluded that PIA-PEG-FA-PHIS micelles are sensitive to acidic

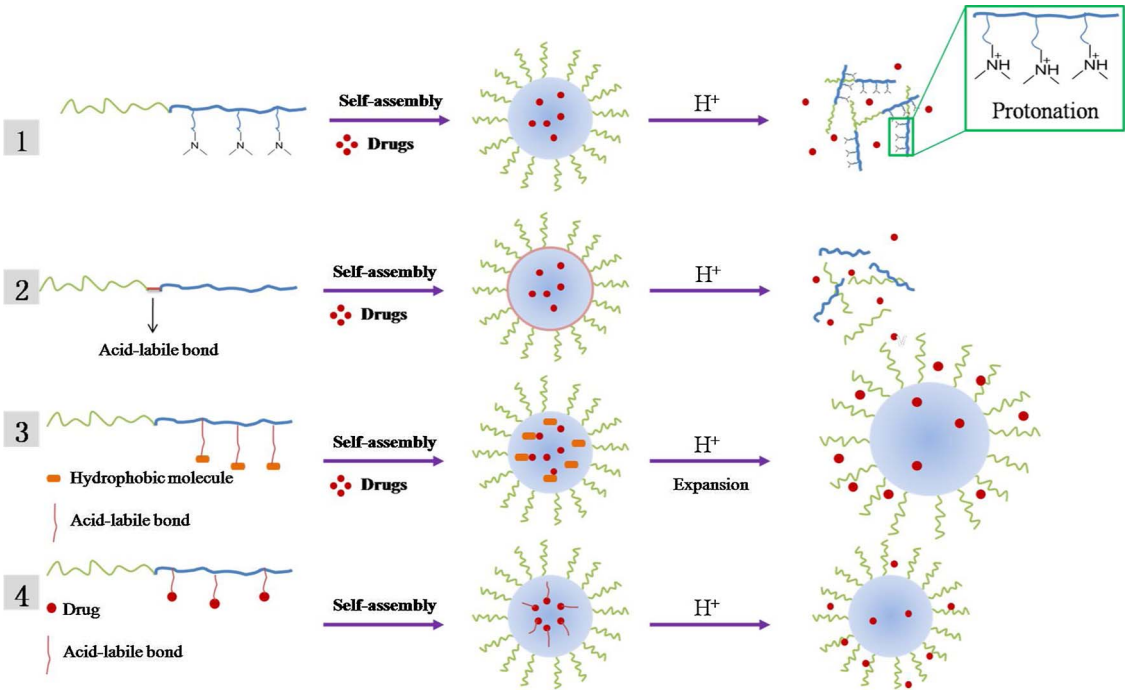


Fig. 2. Schematic representation of four types of pH-sensitive mechanisms for drug release: 1) Protonation or deprotonation of polymers leads to the destruction of amphipathic structures that result in drug release, 2) The separation of the polymer micelle amphiphilic blocks leads to drug release, 3) The reduced hydrophobicity of the hydrophobic segment of the the polymer micelles causes the micelles to swell to release the drugs, and 4) The rupture of the acid-labile bond between the drug and polymer leads to drug release.

tumor tissue and can reduce the leakage of drugs in normal circulation, but can effectively enhance the release of drugs in tumor cells, which can be beneficial to tumor targeted therapy.

Different protonated groups of pH-sensitive micelles result in a different pH response range for drug release. P123-PAE (Cai et al., 2016), DMAEMA-b-DEAEMA (Lee et al., 1999), DMAEMA-b-MEMA (Butun et al., 2001), PEO-DMAEMA (Lee et al., 2002) and other block copolymers are pH-sensitive micelles designed with the properties of protonation and deprotonation of tertiary amino groups under different pH conditions. Another cationic polymer, [poly (2-vinylpyridine)-block-poly (ethyl-ene oxide) (P_2VP-b-PEO)] (Posel et al., 2014), undergoes a phase transition upon deprotonation of the pyridine groups at pH 5.0.

3.2. The separation of the polymer micelle amphiphilic blocks leads to drug release

The hydrophobic and hydrophilic segments of the amphiphilic block copolymer are linked by pH-sensitive chemical bonds to construct pH-responsive tumor targeting drug delivery system with encapsulated anti-tumor drugs. The acid-sensitive bonds on these polymers are generally stable at pH 7.4, but hydrolyzed under acidic condition, whereby the polymer micelles are disaggregated to allow rapid drug release (Fig. 2.2).

Ma et al. developed amphiphilic dextran-retinal (DR) conjugates by linking all-trans retinoic acid and dextran via hydrazone bond, followed by self-assembly to generate pH-sensitive DR micelles (Zhang et al., 2016). The DR micelles were capable of encapsulating DOX into the hydrophobic core to generate DOX-loaded DR micelles. The release of DOX from DOX-loaded DR micelles was accelerated under acidic condition due to the cleavage of acid labile hydrazone bond. The in vitro release of DOX demonstrated that less than 10% of DOX was released in PBS at pH 7.4, but significantly elevated at pH 5.0 with about 100% of DOX release within 24 h. In vitro anti-tumor effects of DOX-loaded DR micelles, including apoptosis, cell cycle, and cellular senescence were evaluated in human breast tumor MCF-7 cells. Besides, bi-distribution and anti-tumor effects were investigated in MCF-7 xenografted mouse models. Compared with free DOX, DOX-loaded DR micelles enhanced tumor inhibition efficiency and reduced systemic toxicity. Furthermore, all-trans retinal, the precursor of ATRA, could be efficiently released in endsosomal/lysosomal compartments and converted into ATRA to inhibit tumor growth though RAR/RXR signaling pathway. Huang and researchers synthesized pH-sensitive amphiphilic poly (ethyleneglycol)-imine-benzoic-dipalmitate (PEG-I-dC_{16}) polymers for targeted delivery of DOX (Rongbin et al., 2016). Acid-sensitive Schiff base bonds were used to link PEG and double-stranded C_{16} to obtain the PEG-I-dC_{16} micelles, by comparison with the PEG-A-dC_{16} micelles linked by non-acid sensitive amide bonds, the release of DOX from the PEG-I-dC_{16} micelles was accelerated by changing the pH from 7.4 to 6.5. Confocal microscopy scan showed the acid-labile micelles had more accumulation in cellular than non-acid-labile micelles and free DOX, and the cytotoxicity of DOX-loaded acid-labile micelles was higher than that of DOX-loaded non-acid-labile micelles against A549 and $HepG_2$ cells in high concentration.

3.3. The reduced hydrophobicity of the hydrophobic segment of the the polymer micelles causes the micelles to swell to release the drugs

In this strategy, the most common method is to link 2,4,6-trimethoxybenzaldehyde to the hydrophobic segment via acetal bonds. At acidic pH condition, the acetal hydrolysis resulted in significant swelling of the micelles, as a result of reducing the hydrophobicity of hydrophobic segment (Fig. 2.3).

Zhong and coauthors reported the synthesis of pH-sensitive biodegradable micelles, which were prepared from the block copolymers PEG-PTMBPEC (Chen et al., 2010; Wei Chen et al., 2009). The size change of micelles in response to acetal hydrolysis was observed by dynamic light scattering (DLS). The results showed that placement of micelles into pH 4.0 acetate buffer (0.5M) resulted in rapid and remarkable swelling of micelles instead of micelle disruption. The micelle size increaesd from 150 nm to about 400 nm in 3 h, reaching over 1000 nm after 12 h. However, no change of micelle size was obaerved over 2

days at pH 7.4 at the same buffer concentration. The micelles were incubated with carboxy fluorescein (hydrophilic) and nile red (hydrophobic) after acetal hydrolysis and then observed clearly colocalization of carboxy fluorescein and nile red, confirming the amphiphilic nature of the micelles. Furthermore the ^{1}H NMR studies on micelles before and after acetal hydrolysis further corroborated swelling of the micelles. In vitro release studies showed a significantly faster release of paclitaxel and DOX at pH 4.0 and 5.0 than at pH 7.4. And then this group also developed core-crosslinked pH-sensitive degradable micelles based on poly(ethyleneglycol)-b-poly(mono-2,4,6-trimethoxy benzylidene-pentaerythritol carbonate-co-acryloyl carbonate) (PEG-b-P(TMBPEC-co-AC) diblock copolymer that contains acid-labile acetal and photo-crosslinkable acryloyl groups in the hydrophobic polycarbonate block for enhancing intracellular paclitaxel (PTX) release (Wu et al., 2012). Anti-tumor activity by MTT assays in MCF-7 and RAW 264.7 cells showed that PTX-loaded crosslinked micelles exhibited high anti-tumor activity compared to free PTX.

Gu and researchers developed PEG-PH-PLLA nanoparticles based on self-assembly of triblock copolymers poly(ethylene glycol)-poly (Lhistidine)-poly(L-lactide) (Liu et al., 2011). The anti-tumor drug DOX was encapsulated in the nanoparticles. Theoretically, the self-assembly nanoparticles were divided into three layers, including hydrophobic PLLA segment, pH-sensitive PH blocks and hydrophilic PEG chains. The PH layer swelled or shrank with the protonation/deprotonation at different pH to control the release of DOX. After 24.5 h, the accumulated release rate of the nanoparticles in pH 5.0 was nearly 80%, while that of nanoparticles in pH 7.4 was less than 40%. In vitro studies in HepG2 celles showed that the anti-tumor effect of DOX-loaded nanoparticles was preferable to free DOX.

3.4. The rupture of the acid-labile bond between the drug and polymer leads to drug release

Anti-tumor drugs are linked to the hydrophobic segment of the block copolymer via acid-sensitive chemically bonds to prepare prodrug micelles. In these delivery system, the polymers are generally stable in blood circulation and acid-sensitive chemically bonds are hydrolyzed after cellular internalization (Fig. 2.4). In this process, the micelles are not depolymerized and the drug release is relatively slow.

In this strategy, the modifiable groups of the model drug are the key to determining the form in which the drugs are linked to the polymer with acid sensitive bonds. For example, hydrazone bond has been successfully utilized to conjugate DOX to a wide variety of polymers. A new type of self-assembling amphiphilic block copolymer, poly(ethylene glycol)-poly(aspartate hydrazone adriamycin) (PEG-P(Asp-Hyd-ADR), was specially designed and synthesized by conjugating adriamycin (ADR) to the side chain of the core-forming PAsp segment via the pH-sensitive hydrazone bond (Bae et al., 2003). In vitro release of the experimental results showed that the micelles release ADR pH-dependently as the pH value decreases from pH 7.4 to 3.0. CLSM reveals that the micelles are trapped in lysosomes where they are programmed to function by responding to low pH, and the released ADR accumulates in the cell nuclei and effectively suppresses the synchronizing cell viability of cancer cells. Furthermore, Bae and group further studied the Fol-PEG-P(Asp-Hyd-ADR). The folate-bond micelles can be guided to the tumor cells in the body due to the selective overexpression of folate-binding proteins, and after the micelles enter the cells, pH-sensitive hydrazone bonds are hydrolyzed by intracellular acidic environment (pH 5–6) so that drug release profile of the micelles is controlled (Bae et al., 2005a). Similar forms of polymer micelles include DOX-PEG-PLLA (Yoo et al., 2002), mPEG-b-P (LA-co-DHP-hz-DOX) (Hu et al., 2010) and CF-PLU-DOX (Lee et al., 2008b).

Zhong et al. designed and prepared endosomal pH-sensitive paclitaxel (PTX) prodrug micellar nanoparticles by conjugating PTX onto PEG-PAA block copolymers via acid-labile acetal bond to the PAA block and investigated for growth inhibition of human cancer cells in vitro (Gu et al., 2013). The drug release profile in vitro showed that drug release from PTX prodrug nanoparticles was highly pH-dependent, in which 86.9%, 66.4% and 29.0% of PTX was released from PTX prodrug at 37 ˚C in 48 h at pH 5.0, 6.0, and pH 7.4, respectively. MTT assays showed that these pH-sensitive PTX prodrug nanoparticles exhibited high antitumor effect to KB and HeLa cells (IC_{50} = 0.18 and 0.9 μg PTX equiv/mL, respectively) as well as PTX-resistant A549 cells.

3.5. Analysis on advantages and disadvantages of four kinds of pH-response drug release

According to the four mechanisms of drug release of pH-sensitive micelles, the in vitro release profile of four forms of drug-loaded pH-sensitive micelles was compared and analyzed in this part.

Drug release from drug-loaded polymer micelles is controlled by the free diffusion of drugs and the rate of polymer depolymerization. The first two types of pH-sensitive polymeric micelles are able to physically encapsulate the water-insoluble anti-tumor drugs like DOX and paclitaxel. These drug-loaded micelles are stable under neutral conditions, but are protonated or depolymerized under acidic conditions. Resulting in the disappearance of the amphiphilic properties of these block copolymers to achieve the purpose of pH-responsive release of the encapsulated drugs. In this way, since the drugs are released rapidly under the action of micellar depolymerization and drug diffusion, the drug release effect is significant, about 60%–100% of drugs can be released in 5h at pH 5.0. The cumulative release rate is generally less than 30% in 24h at physiological conditions. The difference between the third and the first two is that pH triggering causes the hydrophobic small molecule to disappear from the side chain of the micellar hydrophobic block and the micelles are significantly amplified but do not depolymerize, resulting in relatively slow drug release. The fourth type of drug release is that the pH-sensitive drug binding linkers can be cleaved at acidic environment to control the drug release profile. However, since the micelles are not depolymerized during the process of releasing drugs, the effect of drug release is not superior to the forms mentioned earlier. These micelles are stable at pH 7.4 with less than 10% of drug released. And the drug release is slower than that of the forms mentioned earlier. Examples of drug-loaded pH-sensitive polymers and their mechanisms for triggered release are given in Table 2.

Ideally, there is little or no leakage of drugs under physiological conditions, but drugs are rapidly released at acidic conditions. The type of combining with the advantage of the release profile of the physically encapsulated drug-loaded micelles and the stability of the drug-conjugated micelles under physiological conditions is worth considering. Such as the pH-sensitive amphiphilic prodrug micelles consisting of the hydrophilic material and the hydrophobic chemotherapy drug through a pH-responsive linkage.

4. Summary and future prospects

The pH-sensitive micelles have unique advantages in tumor-targeted drug delivery system depending on their properties of pH-responsive drug release. Analysis of the mechanism of pH-responsive drug release provides valuable insights to the further study of pH-sensitive micelles. In order to further improve the pH-sensitive micellar drug delivery system, other factors of pH-sensitive micelles still require further systematic study, including compositional structure, particle size, Zeta potential, surface characteristics and properties of the encapsulated drug. In addition, the pH-sensitive active tumor targeting drug delivery system is also an important research strategy. Although there are still many issues to be resolved during the development of pH-sensitive micellar drug delivery systems, they have a non-negligible effect on overcoming possible adverse reactions of the chemotherapy and improving the therapeutic efficacy, so that they are still ideal choice for tumor targeting delivery system.

Table 2
Examples of drug-loaded pH-sensitive polymers and their mechanisms for triggered release.

pH-sensitive polymer/drug	Cumulative release under pH 7.4 conditions (24h)	Cumulative release under acidic conditions (24h)	Drug-loading method/release mechanism
P123-PAE/Cur (Cai et al., 2016)	16%	32% (pH 5.5)	Protonation or deprotonation of polymers leads to the destruction of amphipathic structures that result in drug release/physical encapsulation/ depolymerization
PIA-PEG-FA-PHIS/DOX (Sun et al., 2015)	10% <	85% (pH 5.5), 32% (pH 6.5)	
polyHis-PEG/DOX (Lee et al., 2003)	41%	83% (pH 5.0), 75% (pH 6.8)	
P123-PAA/DOX (Choo et al., 2011)	25%	65% (pH 5.0)	
MPEG-HPAE/DOX (Ko et al., 2007)	22%	70% (pH 6.4)(6h)	
DR/DOX (Zhang et al., 2016)	18%	98% (pH 5.0), 38% (pH 6.5)	The separation of the polymer micelle amphiphilic blocks leads to drug release/physical encapsulation/ depolymerization
PEG-b-C_{18}/DOX (Ding et al., 2009)	32% (8h)	45% (pH 6.8) (8h)	
PEG_{2k}-I-dC_{16} (Rongbin et al., 2016)	13%	50% (pH 6.5)	
PEG-OPCL-PEG/DOX (Jin et al., 2011)	18%	60% (pH 5.0)	
PEG-b-PtNEA/Nile Red (Huang et al., 2009)	10% <	100% (pH 5.0)	
PLH-PLGA-TPGS/DOX (Li et al., 2015)	52%	82% (pH 5.0)	The reduced hydrophobicity of the hydrophobic segment of the the polymer micelles causes the micelles to swell to release the drugs/ physical encapsulation/no depolymerization
$(PCL)_3$-[$(PDEMEMA)_{15}$-b-$(PPEGMA)_{12}]_3$/DOX (Lin et al., 2014)	28%	32% (pH 6.5), 55% (pH 5.0)	
PEG-PTMBPEC/DOX (Chen et al., 2009)	15%	50% (pH 5.0)	
PEG-PTMBPEC/PTX (Chen et al., 2009)	32%	58% (pH 5.0)	
PEG-PH-PLLA/DOX (Liu et al., 2011)	31%	75% (pH 5.0)	
PEG-b-$PMME_{40}$/Nile Red (Luo et al., 2014)	38%	90% (pH 5.0)	
H40-p(LA-DOX)-b-PEG-OH/FA/DOX (Prabaharan et al., 2009)	11%	62% (pH 6.6)	The rupture of the acid-labile bond between the drug and polymer leads to drug release/chemical conjugation/ no depolymerization
PEG-p(Asp-Hyd-LEV-PTX)/PTX (Alani et al., 2010)	30%	60% (pH 5.0)	
DTX-LEV-Hyd-PLA-PEG-POL/DTX (Hami et al., 2014)	10%	20% (pH 5.0)	
NC-6300/DOX (Harada et al., 2011)	23%	40% (pH 6.0), 58% (pH 5.0)	
PEG-p(Asp-Hyd-ADR) (Bae et al., 2005a,b)	5%	10% < (pH 6.5), 25% (pH 5.0)	
mPEG-b-p(LA-co-DHP-hz-DOX) (Hu et al., 2010)	5%	23% (pH 6.0), 38% (pH 5.0)	
PEO-PAGE/DOX (Hruby et al., 2005)	15%	42% (pH 5.0)	

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