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Review

pH-sensitive polymeric micelles triggered drug release for extracellular and intracellular drug targeting delivery

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ABSTRACT

Most of the conventional chemotherapeutic agents used for cancer chemotherapy suffer from multidrug resistance of tumor cells and poor antitumor efficacy. Based on physiological differences between the normal tissue and the tumor tissue, one effective approach to improve the efficacy of cancer chemotherapy is to develop pH-sensitive polymeric micellar delivery systems. The copolymers with reversible protonation–deprotonation core units or acid-liable bonds between the therapeutic agents and the micelle-forming copolymers can be used to form pH-sensitive polymeric micelles for extracellular and intracellular drug smart release. These systems can be triggered to release drug in response to the slightly acidic extracellular fluids of tumor tissue after accumulation in tumor tissues via the enhanced permeability and retention effect, or they can be triggered to release drug in endosomes or lysosomes by pH-controlled micelle hydrolysis or dissociation after uptake by cells via the endocytic pathway. The pH-sensitive micelles have been proved the specific tumor cell targeting, enhanced cellular internalization, rapid drug release, and multidrug resistance reversal. The multifunctional polymeric micelles combining extracellular pH-sensitivity with receptor-mediated active targeting strategies are of great interest for enhanced tumor targeting. The micelles with receptor-mediated and intracellular pH targeting functions are internalized via receptor-mediated endocytosis followed by endosomal-pH triggered drug release inside the cells, which reverses multidrug resistance. The pH sensitivity strategy of the polymeric micelles facilitates the specific drug delivery with reduced systemic side effects and improved chemotherapeutical efficacy, and is a novel promising platform for tumor-targeting drug delivery.

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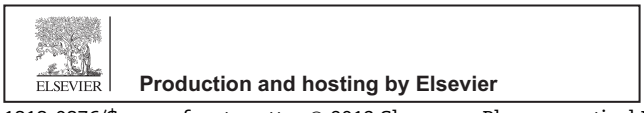

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1. Introduction

Cancer is a leading cause of death around the world. The most common cancer treatments are chemotherapy, radiation and surgery, with chemotherapy being the major treatment modality. However, the conventional chemotherapeutic agents are limited by their undesirable properties, such as poor solubility, low tumor targeting, insufficient cellular drug uptake, low therapeutic efficacy, and cytotoxicity to normal tissues, which may be the cause of treatment failure in cancer. Currently, nanotechnology has been extensively studied for their potential applications in cancer diagnosis and treatment for effective cancer therapy over the years. Nano drug delivery systems are being trialed for specific targeting-delivery of drugs to cancer sites in order to improve the therapeutic efficacy due to improved distribution specificity, increased internalization and intracellular drug delivery while minimizing undesirable side effects [1–3].

Among the various nanotechnology approaches, the polymeric micelles have received considerable scientific attention as a versatile nanomedicine platform with improved pharmaceutical and efficacious response in the field of drug delivery. Polymeric micelles have been utilized as a novel promising colloidal carriers for the targeted delivery and controlled release of drugs, proteins and genes in the cancer diagnosis and therapy. It is attributable to such appealing properties as small particle size and narrow size distribution, distinctive core-shell structure, high solubilization capacity and structural stability, and tumor passive localization by enhanced permeability and retention (EPR) effect for passive targeting. Moreover, polymeric micelles can also be modified with ligands or antibodies for active targeting to increase the selectivity for tumor cells and enhance intracellular drug delivery. PEGylation of polymeric micelles can avoid recognition and uptake by reticuloendothelial system, decrease plasma opsonin adhesion and increase the circulation time of micelles in the blood. All the above properties of polymeric micelles lead to overcome the problems associated with the conventional chemotherapy, such as nonspecific toxicity, lack of tumor selectivity, and the development of multidrug resistance (MDR) in various tumor cells. Therefore, the polymeric micellar systems have been considered as promising anticancer drug carriers in nanotechnology [4–8].

Nowadays, although polymeric micelles have been reported to accumulate preferably in tumor due to passive targeting and/or receptor-mediated active targeting, the inefficient drug release in the tumor cells can be another barrier that may significantly lower drug's efficacy. In order to overcome this barrier, the polymeric micellar systems with controlled micellar dissociation and triggered drug release mechanism are developed. The use of stimuli-responsive polymeric micelles offers an interesting opportunity for drug delivery as programmable systems in the optimization of cancer therapy. The stimuli systems enable the polymeric micelles to release drug in response to specific external or internal stimuli, such as temperature, pH, ultrasound or enzymes, by including thermo- or pH-sensitive components or by attaching specific targeting moieties to the outer hydrophilic surface of polymeric micelles [9,10]. Compared to the conventional drug delivery systems, the stimulus-sensitive nanopreparations show a superior ability for control and adjustment of the location and time of drug release and internalization in the tumor cells and their microenvironment by responding to local stimuli. Among these stimuli, change in acidity as an internal signal is particularly crucial for the development of micellar drug carriers that facilitate tumor targeting. The external conditions as well as internal pH stimuli can be used to modify the behavior of the polymeric micelles that control drug release, improve drug internalization, control the intracellular drug fate and even allow for certain physical interactions, resulting in an enhanced tumor targeting and antitumor effect [11].

Table 1 summarizes some investigations of polymeric micelles used to deliver various drugs via pH-sensitive targeting in the recent years. The purpose of this review is to provide a detailed description of the application of pH-sensitive polymeric micelles for tumor chemotherapy. The emphasis is placed on more recent developments in the area of pH-sensitive nanotechnology, particularly for those responding to tumor extracellular pH and intracellular pH for drug targeted delivery and the MDR treatment, including the multifunctional polymeric micelles designed for tumor targeting and MDR reversing.

2. pH-sensitive polymeric micellar delivery system

2.1. *Tumor pH microenvironment*

For polymeric micellar drug delivery systems, drug release from polymeric micelles is governed by the rate of drug diffusion, partition coefficient, micelle stability and rate of biodegradation of the copolymers. Triggered release of a drug from the long circulating micelle vehicles at tumor sites while maintaining a minimal release rate during circulation, is a very desirable property for tumor chemotherapy [25].

In cancer therapy, the tumor microenvironment is one of the many areas which are studied to design new therapy modality. In the stimuli-responsive delivery systems, the anticancer agent can be released by an appropriate stimulus of tumor area, such as pH, glucose and temperature. Among all applied stimuli, acidic pH as an internal stimulus has been considered as an ideal trigger for the selective release of anticancer drugs due to the fact that the pH at both primary and metastasized tumors is lower than the pH of normal tissue. In addition, the pH targeting approach is regarded as a more general strategy than the conventional specific tumor cell surface targeting approaches, because the acidic tumor microenvironment is common in solid tumors [26–28].

The existing pH of tumor tissue has been considered as an ideal trigger for the selective release of anticancer drugs in tumor tissues and/or within tumor cells. Compared to the extracellular pH of normal tissues and blood constant at pH 7.4, the measured tumor extracellular pH (pHe) values of most solid tumors range from pH 6.5 to 7.2, which is lower than the normal tissues. Moreover, changes in pH are also encountered once the micelles enter cells via endocytosis where pH can

Table 1 – Applications of pH-sensitive polymeric micelles for extracellular and intracellular drug targeted delivery.

Polymeric micelles	Drug incorporated	Properties	References
N-Boc-histidine-poly[(D,L-lactide)]-co-glycolide]-poly(ethylene glycol)-poly[(D,L-lactide)]-co-glycolide] micelles	Doxorubicin	The polymeric micelles can trigger drug release under tumor extracellular microenvironments and exhibit excellent cellular uptake.	[12]
Poly(ethylene glycol)-cis-aconityl-chitosan-stearic acid polymeric micelles	Doxorubicin	PEGylation significantly reduced cytotoxicity of the conjugate, and acid-triggered PEG degradation combined with DOX release was feasible in efficient internalization to tumor cells.	[13]
Poly(histidine)-poly(ethylene glycol) and poly(D-lactic acid)-poly(ethylene glycol)-folate mixed micelles	Doxorubicin	The cytotoxicity of micelles was approximately 7.5-fold higher than free doxorubicin.	[14]
Galactosylated chitosan-graft-poly (N-isopropylacrylamide) micelles	Oridonin	The micelles could enhance uptake through galactose receptor-mediated endocytosis, and the cytotoxicity enhanced due to the tumor extracellular pH triggered drug release.	[15]
Poly(ethylene glycol)-poly(aspartate-hydrazone-adriamycin) [PEG-p(Asp-Hyd-Adr)] micelles	Adriamycin	The micelles performed an intracellular pH-triggered drug release capability, tumor-infiltrating permeability, and effective antitumor activity with extremely low toxicity.	[16]
AP peptide-poly(ethylene glycol)-poly(D,L-lactic acid) (AP-PEG-PLA) and poly(ethylene glycol)-poly(β-amino ester) (PEG-PAE) micelles	Doxorubicin	The micelles exhibited excellent cellular accumulation with AP peptide targeting ability and triggered drug release under tumor pHe.	[17]
Poly[(D,L-lactide)]-co-glycolide]-poly(ethylene glycol)-folate (PLGA-PEG-FOL) and poly (β-amino ester)-poly(ethylene glycol)-folate (PAE-PEG-FOL) mixed micelles	Doxorubicin	The drug release can be triggered at endosomal pH, resulting in an enhanced nucleus drug concentration and improved the cytotoxicity.	[18]
Monoclonal antibody 2C5-DSPE-poly(ethylene glycol) and poly (histidine)-poly(ethylene glycol) mixed micelles	Paclitaxel	The micelles could enhance the tumor cell-specific internalization by 2C5-mediated endocytosis and trigger drug release, resulting in the improved anticancer efficacy.	[19]
Poly(ethylene glycol)-poly(mono-2,4,6-trimethoxy benzylidene-pentaerythritol carbonate) [PEG-b-P(TMBPEC-co-AC)] micelles	Paclitaxel	The micelles showed high anti-tumor activity with superior extracellular stability and rapid intracellular drug release	[20]
Poly(HEMA-co-histidine)-poly(D, L-lactic acid) and folate-poly(ethylene glycol)-poly(D, L-lactic acid) mixed micelles	Doxorubicin	The micelles exhibited great anti-tumor efficacy with folate mediated cancer targeting and pH triggered intracellular drug delivery	[21]
Poly(ethylene glycol)-poly(L-histidine)/poly(ethylene glycol)-poly(L-lactic acid) [PEG-PHis/PEG-PLA] mixed micelles	Doxorubicin	The micelles exhibited MDR reversing via receptor-mediated endocytosis followed by drug release inside the cells.	[22,23]
Poly(ethylene glycol)-poly(D,L-lactic acid)-poly (β-amino ester) [PEG-(PLA-PAE)] micelles	Doxorubicin	The release of doxorubicin from the micelles was accelerated by decreasing pH from 7.4 to 5.0.	[24]

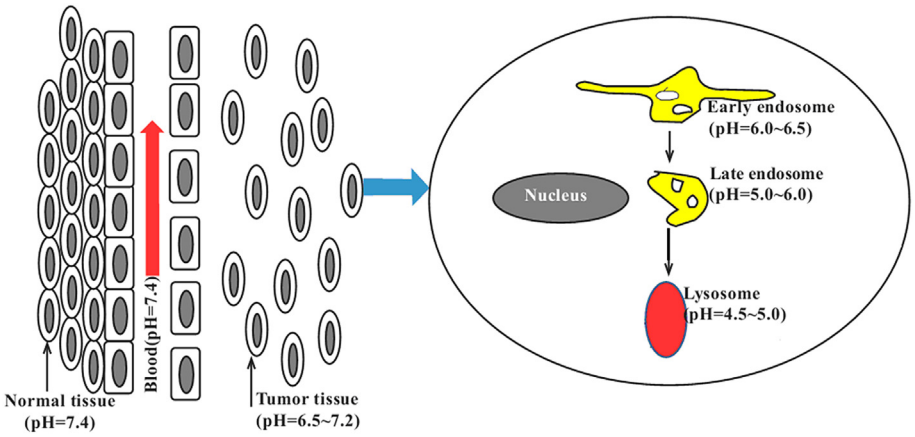

Fig. 1 – Schematic illustration of various pH gradients of normal tissue, blood and tumor tissue as well as endosome–lysosome process [26].

drop as low as 5.0–6.0 in endosomes and 4.0–5.0 in lysosomes (Fig. 1). The pH gradient is caused by hypoxia that upregulates glycolysis, followed by the production of lactate and protons in extracellular microenvironments. The low pH in tumor extracellular space or in various subcellular organelles is a significant signal for targeting. Therefore, the acidity in the tumor microenvironment and endosomes has been the most utilized stimulus for the design of the stimulus-sensitive nanopreparations. Differences in pH between the normal tissues and the tumor tissues, as well as the acidic environment in endosomal and lysosomal compartments, can be exploited as an internal stimulus for triggered drug release, and pH-sensitive drug release is competitive and favorable for chemotherapy [29,30].

Therefore, the pH-sensitive polymeric micelles for targeting drug delivery to tumors are stable at physiological pH but deformed to facilitate release of the drug under mild acidic conditions outside or inside tumor cells, which may result in significantly enhanced therapeutic efficacy and minimal side effects. Polymeric micelles may be designed to display tunable pH sensitivity and controlled release by introduction of ionizable amino groups into their backbones. The pH-sensitive polymeric micelles can be designed to carry, deliver, and control the release of hydrophobic agents in tumor tissue by relying on the EPR effects and the low pH in the vicinity of tumor tissue. Drug release from micelles at the targeted tumor area can be enhanced by applying an internal or external trigger. These systems increase drug accumulation at tumor sites or at intracellular compartments in tumor cells with less drug distribution and thereby decreased damage to the normal tissues [31].

2.2. *The pH-sensitive strategies*

The pH-sensitive nanocarriers can be constructed from stimuli-responsive polymers that are able to sense small changes in microenvironmental pH and the pH triggers a corresponding change in the polymer's physical properties such as size, shape or hydrophobicity. The pH-sensitive polymeric micellar systems developed by two main strategies are used to fabricate pH-sensitive drug delivery systems. One approach is utilization of pH-labile chemical bonds (pH-sensitive polymer-drug conjugates), such as hydrazone, cis-acotinyl and acetal bonds. Presence of acid-labile linkages between drug and polymer enables drug release either in relatively acidic extracellular fluids in a tumor, or after endocytosis in endosomes or lysosomes in tumor cells. In

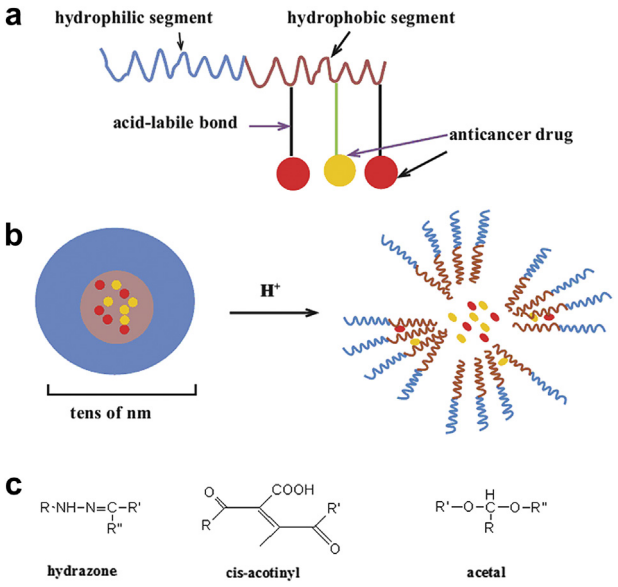

Fig. 2 – (a) Acid-sensitive amphiphilic block copolymers in which the anticancer drugs are conjugated to the side chain of the core-forming segments through the acid-liable bonds; (b) pH-sensitive polymeric micelles are stable under physiological condition (pH 7.4) but gradually release drugs under acidic environment such as solid tumors, endosomes and lysosomes through the cleavage of the acid-labile bonds; (c) chemical structures of acid liable chemical bonds [29].

addition to direct conjugation of drugs, these linkages are inserted into the main chain, side chain or at the terminal of the core-forming blocks (Fig. 2). In general, these bonds are stable at neutral or alkaline pH but occur hydrolytic cleavage at acid pH. In other words, the micelles are relatively stable at blood pH 7.4, but start to release the drugs under acidic conditions [32–34].

The hydrazone linkers have been used extensively in polymeric conjugates of anticancer drugs for acid-triggered drug release. Bae et al. prepared micelles based on mPEG-b-poly(aspartate hydrazone doxorubicin) where doxorubicin (DOX) was conjugated to the hydrophobic segments through acid-sensitive hydrazone linkers. Selective release of the drug at endosomal pH suppressed tumor growth in mice with enhanced therapeutic efficacy and decreased systemic toxicity compared to free DOX [19]. These results indicated that the hydrazone linkages could be stable at physiological pH 7.4 but degraded effectively at the lower pH of endosomes and lysosomes (pH 5–6) to release drug. In addition, conjugates of doxorubicin and alginate using the cis-aconityl bond, palitaxel and PEG using the hydrazone bond have been used for pH-triggered carrier systems [35,36].

The other strategy is attached "titratable" groups in the copolymers, such as amines or carboxylic acids, to control the micelle formation by inducing physical dissociation or interior structural change via variation in pH level. The pH-triggered release of drugs can be established by protonation of pH-sensitive polymers that form the hydrophobic core of polymeric micelles at physiological pH. Destabilization occurs when the protonatable groups become charged below the pKa, leading to repulsion between the polymer chains and micellar dissociation. Many examples of protonation of polymers that trigger micelle destabilization have been reported, including poly(histidine) (polyHis), poly(acrylic acid) and polysulfonamides (Fig. 3). Among of them, polyHis is the most commonly used pH-sensitive component in micelle-based pH-triggered release systems since this polymer contains an imidazole ring endowing it with pH-dependent amphoteric properties [14,15,17]. In addition, the polyHis presents a strong endosomolytic property by its proton sponge effect and/or its interaction with anionic phospholipids comprising the endosomal compartments. These properties of polyHis contribute to the development of a smart micelle system responsive to tumor extracellular pH (pHe) or more acidic endosomal pH. Lee et al. prepared micelles based on a triblock copolymer PLA-b-PEG-b-polyHis, which showed triggered release of doxorubicin when the pH was lowered from 7.4 to 6.0. After 5 h, 40–50% of doxorubicin was released at pH 6.8–6.0, while 60–70% was released after 24 h. Cytotoxicity was 60% at pH 6.8 and 74% at pH 6.0, while only minimal release and cytotoxicity were observed at pH 7.4. These evidences illustrated that polyHis could triggered drug release in tumor pHe and endosomal pH, which resulted in higher cytotoxicity of anticancer drugs.

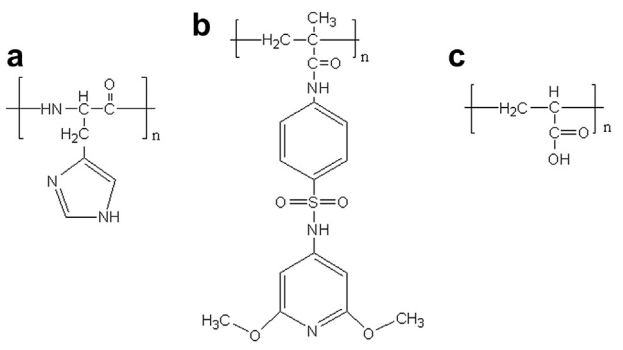

Fig. 3 – Chemical structures of representative pH-sensitive polymers: (a) poly(histidine); (b) polysulfonamides; (c) poly(acrylic acid).

3. Tumor extracellular pH targeting

The tumor pHe is a consistently distinguished phenotype of most solid tumors from the surrounding normal tissues. The mean pH of tumor extracellular fluid is around 7.06 with a range of 5.7–7.8, lower than physiological pH 7.4, due to the increased production of glycolysis at tumor sites under either aerobic or anaerobic conditions. Therefore, this acidic pHe can be exploited as a drug-release trigger. A tumor pHe-targeting polymeric micelles with sufficient sensitivity to the weak acidic pHe encapsulates drug, and the drug should be released outside tumor cells and then diffuse into the cells, which can significantly increase the drug accumulation in the tumor tissue [37–39].

Recent studies have highlighted the development of some promising carriers with pHe-sensitive and therefore tumor-selective delivery. In the previous studies, poly(lactide-co-glycolide) (PLGA)-b-PEG-b-PLGA copolymer is capped by N-Boc-histidine in each end. The modification did not impact the biodegradability and biocompatibility of the polymer. DOX was incorporated in the micelles. The accumulation DOX release at 12 h at pH 6.2 was 2-fold than that at pH 7.4. Cellular uptake of DOX was studied on human breast cancer cell. The higher uptake under pH 6.2 was attributed to DOX rapid release at pHe. Therefore, the pH-induced micelle destabilization and triggered release of drug by tumor pHe, after the accumulation of the micelles in the tumor sites via enhanced permeability, presented a more effective modality of chemotherapy by providing higher local concentration of the drug at tumor sites and minimal release of the drug from micelles during blood circulation (pH 7.4) [13]. The above investigations illustrated that tumor pHe is a more universal way of tumor targeting.

However, although the drug release from the pH-sensitive micelles triggered by the tumor pHe can substantially reduce the systemic toxicity and enhance in vitro and in vivo anticancer activity, it can not solve the problem of MDR of tumor cells to anticancer drugs.

4. Tumor intracellular pH targeting

4.1. *Tumor intracellular pH targeting for escaping lysosome degradation and improving therapeutic efficacy*

The endocytic pathway is one of the uptake mechanisms available to cells. Following micelles internalization, the

endocytosed micelle fuses with endosomes. The ATPase in the endosomal membrane causes an influx of protons which results in a continuous pH drop as endosomes mature from early endosomes to late endosomes. The pH decreases even further when late endosomes fuse with lysosomes.

For the endocytosed polymeric micelles, the fate of these polymeric micelles relies on the efficiency of lysosomal escape to a significant extent. Anti-cancer drugs usually loaded in micelles can be released from micelles in endosomes or lysosomes by pH-controlled hydrolysis after they are taken up by the cell via the endocytic pathway. If the drugs can not promptly escape from endosomes, the acidic environment of lysosomes might activate the lysosomal enzymes for the degradation of the released drug. This caused a limited delivery of therapeutic agents to the intracellular targets. The inferior drug release inside the tumor cells is one of the key reasons for low therapeutic efficacy of drug-loaded micelles. Therefore, the task to facilitate drug escapement from the endosomal/lysosomal vehicles is very important.

To avoid premature degradation by late endosomes or lysosomes and attain maximum pharmacological effect, a polymeric micellar delivery system must be developed in which the drug is released from the endosomes into the cytosol before degradation in lysosomes. Therefore, the intracellular pH-sensitive moieties with the ability to destabilize the endosomal membrane or rupture the endosomes can be used to modify polymeric micelles that facilitate endosomal escapement. In order to improve the therapeutic effect of anticancer drugs that target the nucleus or cytosol or other organelles after cellular uptake, the antitumor drugs should be released rapidly from the micelles in the acidic microenvironment of endosomes/lysosomes. In addition, the use of drug-loaded micelles that destabilizes at an early endosomal pH of 6.0 should maximize intracellular drug delivery and minimize drug release at the extracellular pH and at the lysosomal pH [40,41].

4.2. *Tumor intracellular pH targeting for MDR reversing*

Most of the anticancer agents are not very effective in tumor chemotherapy, due to MDR in various tumor cells. MDR is to say that cancer cells become resistant to the cytotoxic effects of various structurally and mechanistically unrelated chemotherapeutic agents [42]. There are a variety of mechanisms associated with MDR cells that need to be circumvented for a successful tumor treatment. P-glycoprotein (P-gp) and other drug efflux transporters are considered to be critical in pumping anticancer drugs out of cells and causing chemotherapy failure [43].

Nanotechnology provides an innovative and promising alternative strategy compared to conventional chemotherapeutics to circumvent MDR by encapsulating, attaching, and conjugating drugs to the nanocarriers. One strategy for overcoming drug resistance is based on the development of new drug delivery systems to achieve drug accumulation in tumor tissues, tumor cells, or even subcellular organelles of tumor cells, such as polymeric micelles [44]. Polymeric micelles have been shown to bypass the P-gp-based MDR, probably because cells take up drugs loaded in the carriers via endocytosis rather than diffusion through the cell membrane where P-gp is located. However, the plain micelles have the relative slow drug release, and the lower intracellular drug concentration could fail to reach the optimum therapeutic threshold promptly, which may contribute to the development of MDR in tumor cells.

The rapid drug release could provide the sufficient drug concentration and kill the tumor cells before they acquire the ability to against the drug. Therefore, the quick drug release is critical for the chemotherapeutic agents to efficiently kill the cancer cells without the acquired drug resistance. The dramatically increased drug concentration in the tumor microenvironment or cytoplasm may overcome the efflux receptor (P-gp) or other mechanism-driven drug resistance. In order to overcome this barrier, to develop micellar systems with a triggered release mechanisms is able to release drugs in response to internal pH stimuli. When polymeric micellar nanosystems are combined with triggered release mechanisms by endosomal or lysosomal acidity plus endosomolytic capability, the micellar nanocarriers demonstrate to overcome MDR of various tumors [16,45] (Fig. 4).

5. Multifunctional polymeric micellar delivery system

5.1. *Multifunctional polymeric micelles for drug targeting*

To fully exploit the application of polymeric micellar delivery systems, development is required regarding drug loading, long circulation and retention, tumor accumulation, targeting cellular uptake and intracellular drug release. Although there are benefits to active targeting, such as enhanced efficacy, active targeting strategy can result in a high accumulation of the polymeric micelles, but ineffective drug release lowered the antitumor efficacy of drugs. Currently, increasing attention in micelle-based anticancer therapy is given to multifunctional micelles containing at least two out of three features regarding targeting ligand and extracellular or intracellular triggered drug release [46–49]. The combined use of these approaches will further improve specificity and

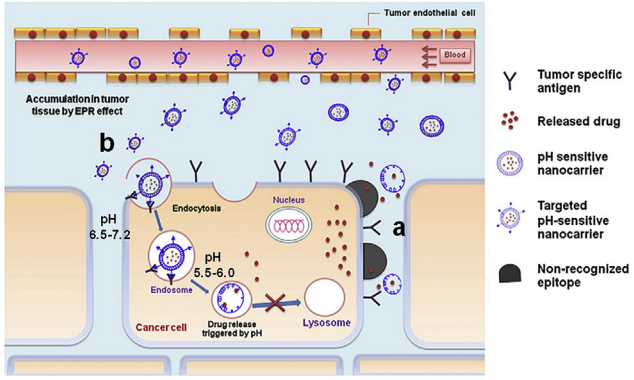

Fig. 4 – Schematic representation of pH-responsive polymeric micelles triggered drug release for extracellular (a) and intracellular (b) drug targeting delivery [26].

efficacy of micelle-based drug delivery. Among of multifunctional polymeric micelles, the combination of multiple mechanisms for micelle-based drug delivery in anticancer therapy, characterized by active targeting and pH triggered release offers challenging opportunities to improve therapeutic efficacy. Additionally, the combining active targeting and pH sensitivity as triggered drug release mechanism results in more efficient tumor targeting and drug delivery.

Bae et al. designed a pHe-sensitive multifunctional micelles with a ligand that can bind its receptors only if exposed to an acidic tumor extracellular environment ($6.5 < pH < 7.4$). The ligand biotin was anchored to the surface of the micelles made from the mixed PHis-PEG and PLLA-PEG-PHis-biotin. At neutral pH, the PHis was insoluble and collapsed on the hydrophobic core surface. Thus, the biotin was buried in the PEG corona and hence not available for binding (Fig. 5). Once the micelles were exposed to the acidic extracellular of tumors, the PHis became soluble, and exposed biotin for binding to the tumor receptors [22].

Torchilin et al. developed a pH-sensitive poly(histidine)-PEG/DSPE-PEG mixed micelles for achieving the quick intracellular drug release in response to the acidity by an early endosomal pH of 6.0. In this way, higher intracellular drug concentrations are obtained. As shown in these studies, when the ligands conjugated to the micelles bind to their specific receptors on the cell membrane, the micelles are be internalized by endocytosis, and the combination of active targeting and triggered release resulted in superior cytotoxicity and antitumor activity as compared to the non-multifunctional micelles [14]. Therefore, multifunctional drug carriers combining the targeting ability and the stimuli sensitivity prove the concepts of specific tumor cell targeting, enhanced cellular internalization, and intracellular rapid drug release.

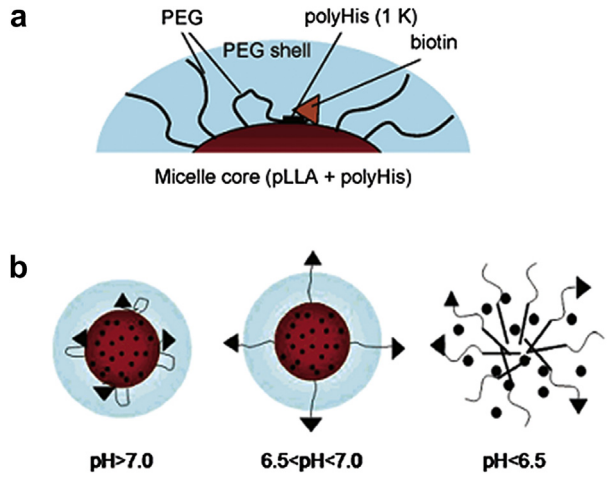

Fig. 5 – (a) Above pH 7.0, biotin anchored on the micelle core via pH-sensitive PHis is shielded by PEG shell of the micelle. (b) Biotin is exposed on the surface at acidic conditions ($6.5 < pH < 7.0$) and can interact with cells, which facilitates biotin-receptor-mediated endocytosis. When pH is further lowered ($pH < 6.5$), the micelle destabilizes, resulting in enhanced drug release and disrupted endosomal membrane [30,47].

5.2. *Multifunctional polymeric micelles for MDR reversing*

Tumor targeting approaches have been developed for improved antitumor efficacy and reduced toxicity by increasing cellular accumulation and altering biodistribution of anticancer drugs through specific cell surface interactions. Adding a targeting moiety to the surface of polymeric micelles, such as a ligand or an antibody, helps the efficient cellular uptake and improves drug retention in MDR tumor cells via receptor-mediated endocytosis, while avoiding P-gp efflux and being able to enhance the reversal of MDR. Additional, the endosomal pH triggering anticancer drug release and the endosomal escaping activity of tumor intracellular pH-sensitive polymeric micelles allowed cytosolic delivery of anticancer drug, by avoiding drug sequestration mechanism in MDR cells and through bypassing MDR proteins expression on cellular membrane.

When the intracellular pH-triggered drug release is combined with receptor-mediated active targeting, the multifunctional polymeric micelles can not only recognize the cancer cells via receptor-mediated binding, but also release the drug at tumor sites. This technology may help trigger the activation of nanosystems to inhibit tumor growth and reverse MDR. Therefore, designing an advanced multifunctional micellar delivery system should be a priority to reverse MDR in cancer chemotherapy. For example, the protonation of poly(histidine) in the hydrophobic core of the PEG-poly(histidine)/PEG-poly(L-lactic acid) mixed micelles in the tumor cells resulted in destabilization of micelle cores and triggered drug release [30]. When these micelles were targeted to multidrug-resistant MCF-7/Adr cells using folate labeling, more cells were killed by the drug. The internalization of micelles proceeds via folate receptor-mediated endocytosis followed by the triggered drug release inside the cells, which circumvent drug efflux by P-glycoprotein pumps located in the cell membrane and reverse MDR [31].

Bae et al. successfully prepared DOX-loaded pH-sensitive poly(histidine(His)-co-phenylalanine(Phe))-b-poly(ethylene glycol) (PEG) and folate conjugated-poly(L-lactic acid) (PLLA)-b-PEG mixed-micelles in order to challenge MDR in cancers. This mixed-micelle system composed of poly(His-co-Phe)-b-PEG (80wt%) and PLLA-b-PEG-folate (20%) released minimal drug above pH 6.0 and demonstrated the triggered release at pH 6.0, indicating tuned micelle destabilization at early endosomal pH. In addition, the research group of Bae successfully prepares DOX-loaded poly(histidine)-b-PEG-b-PLLA pH-sensitive micelles conjugated with folic acid for active targeting to overcome multidrug resistance. All the above results showed that when the micelles were internalized into tumor cells via folate receptor-mediated active endocytosis, the poly(histidine) block became protonated at endosomal pH, resulting in micelle destabilization, and subsequent release of the drug into the cytosol by disruption of the endosomal membrane, which could effectively kill both DOX-sensitive and resistant tumor cells through a high dose of DOX in the cytosol. In this way, drug interaction with the P-gp transporter, present in the cellular membrane and the major cause of MDR, is minimized [14]. The above results demonstrated

that the combined use of active targeting and intracellular pH-triggered quick drug release can significantly increase the intracellular drug concentration and efficiently kill tumor cells without any risk of developing drug resistance.

6. Conclusion

Taking advantages of the altered pH gradients in tumor extracellular environments and in intracellular compartments, pH-sensitive polymeric micelles have made a significant impact on targeted drug delivery, which overcome limitations of conventional nano drug delivery systems. These systems increase target drug accumulation at tumor site or at intracellular cytosolic compartments in tumor cells with less drug distribution to normal tissues and organs. More importantly, the intracellular pH-sensitive micelles offer an efficient means of overcoming the MDR. The multifunctional polymeric micelles with active targeting and pH-sensitive targeting strategies demonstrate excellent tumor targeting and MDR reversal capability. In conclusion, pH-sensitive polymeric micelles have been emerging as a fascinating class of nano drug carriers for programmable drug targeting delivery in the foreseeable future.

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