Review

Endogenous pH-responsive nanoparticles with programmable size changes for targeted tumor therapy and imaging applications

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Abstract

Nanotechnology-based antitumor drug delivery systems, known as nanocarriers, have demonstrated their efficacy in recent years. Typically, the size of the nanocarriers is around 100 nm. It is imperative to achieve an optimum size of these nanocarriers which must be designed uniquely for each type of delivery process. For pH-responsive nanocarriers with programmable size, changes in pH (~6.5 for tumor tissue, ~5.5 for endosomes, and ~5.0 for lysosomes) may serve as an endogenous stimulus improving the safety and therapeutic efficacy of antitumor drugs. This review focuses on current advanced pH-responsive nanocarriers with programmable size changes for anticancer drug delivery. In particular, pH-responsive mechanisms for nanocarrier retention at tumor sites, size reduction for penetrating into tumor parenchyma, escaping from endo/lysosomes, and swelling or disassembly for drug release will be highlighted. Additional trends and challenges of employing these nanocarriers in future clinical applications are also addressed.

Key words: nanocarriers, endogenous pH-responsive, size change, targeted drug delivery, tumor therapy

1. Introduction

Over the last decade, tremendous progress has been made in the development of nanocarriers as effective antitumor drug delivery agents. These carriers are highly attractive due to their nanometer size and versatile surface properties, which enhance their pharmacokinetics and bio-distribution while reducing systemic toxicity [1-5]. To date, a broad range of nanocarriers has been designed and tested, including polymer nanoparticles, micelles, liposomes, dendrimers, star polymers, and inorganic nanoparticles made of iron oxide, quantum dots, silica, gold, and metal oxide frameworks [1, 6-18]. Intravenously injected nanomedicine undergoes a multistep process before reaching solid tumors [19, 20]. Therefore, for efficient drug delivery in the complex in vivo environment, it is important to develop multiple stimuli-responsive nanocarriers that can minimize nonspecific interactions with and uptake by non-targeted cells or the immune system [6, 21-24]. Specifically designed intelligent stimuli-sensitive nanocarriers can increase the concentration of effective anticancer drugs by targeting their delivery to tumor locations, thus allowing for greater therapeutic efficacy and reducing undesired side effects.

Although much work has been carried out on the development of drug-delivery systems affected by external stimuli, various limitations have greatly
hindered their biomedical application. For example, several temperature- and light-sensitive pharmaceuticals can damage normal cells and even tissues and organs. The magnetic-associated preparations (used as contrast agents in MRI) are limited by the stringent requirements of carrier materials. The ultrasound-associated formulations are usually used for synergistic therapy. Considering the limitations of external stimulant sources (e.g. light, ultrasound, magnetic fields), endogenous stimulations, such as enzymes [25-29], glucose concentrations [30, 31], redox reactions [32-38], temperatures [39-41] and pH differences [34, 42-51], are more useful when designing safe, efficient, and intelligent carriers. Among these endogenous stimuli, pH differences have been the most widely used control parameter for tumor-targeted drug delivery and controlled intracellular drug release. This is possible due to the pH differences between healthy and diseased tissues as well as between various cellular compartments [6, 52-56]. Given the vulnerability of acid-base homeostasis, acidic microenvironment changes are associated with pathological tissues, such as in ischemia, inflammatory diseases, infections, rheumatoid arthritis, or solid tumors [6, 52, 57-60]. Especially in solid tumor tissues, the substantial energy requirement for their intensive growth results in increased lactate and hydrogen ions (H+) produced by catabolism of glucose. Consequently, the tumor microenvironment becomes acidic with a pH ~6.5 in tumor tissues versus pH ~7.4 in normal tissues [57, 61-63]. This difference can then be exploited for targeting the tumor tissue or triggering drug release in the tumor’s extracellular matrix [60, 64-68]. At the cellular level, the intracellular acidic components (pH ~5.5 for endosome, pH ~5.0 for lysosome) can also be used to trigger drug release and promote the escape of the carrier into the cytoplasm [6, 69-72]. Therefore, these endogenous pH differences provide a strong option for targeted antitumor drug delivery and controllable release kinetics.

In general, the physicochemical characteristics of nanocarriers, including size, architecture, and surface properties determine their fate by affecting one or more of the above steps in the antitumor drug delivery process [1, 47, 49, 52, 60, 64, 73-75]. Size-dependent enhanced antitumor findings have received much attention because of the variable and strict size requirements of the entire cascade processes, including their biodistribution, immune activation, blood circulation, tumor target accumulation/retention, tumor tissue penetration, tumor cell uptake, and final cargo release (Figure 1) [52, 76-81]. For example, small nanocarriers (typically <10-20 nm) can spread into various organs, but they are also rapidly eliminated through the kidneys’ glomeruli [1, 82]. Large nanocarriers (typically >200 nm) tend to be rapidly taken up by the mononuclear phagocytic system (MPS) and subsequently accumulate in the liver, spleen, and to a lesser extent in the bone marrow [1, 83-88]. Compared to the tight endothelial junctions of normal vessels (5-10 nm), the pores of tumor vascular walls have typical junction sizes ranging from 200 nm to 1200 nm allowing extravasation of appropriately sized nanocarriers into solid tumors [1, 19]. Also, the lack of functional lymphatic drainage in tumor tissues prevented the reentry of accumulated nanocarriers into blood circulation. This phenomenon, termed the enhanced permeability and retention (EPR) effect, is the rational basis for passive targeting in antitumor drug delivery [1, 7, 89]. However, proliferating tumor cells compress the intratumor blood and lymphatic vessels, leading to a uniformly elevated interstitial fluid pressure. Additionally, increased fibrillar collagens (such as type I and type III) in some tumor interstitial matrix can greatly hinder larger (>60 nm) nanocarriers from penetrating deeply into the tumor parenchyma [19, 90, 91]. Therefore, pH-responsive nanocarriers with programmable size changes can be automatically altered during each stage of the anti-tumor drug delivery.

Recently, remarkable advances in size-dependent smart nanocarriers have attracted great interest for their advanced and efficient antitumor drug delivery as well as their reduced systematic side effects. Although pH-responsive [6, 92-102] and size-related [1, 20, 83, 103, 104] antitumor studies have been amply discussed in previous review articles, there is a need to review several aspects of endogenous pH-responsive nanocarriers for antitumor therapy. Considering the unique advantages of nanocarriers’ size characteristics for antitumor drug delivery, this review focuses on pH-responsive nanocarriers with programmable size changes in antitumor applications, especially highlighting recently advanced design strategies.

2. Endogenous pH-responsive carriers with programmable size changes for antitumor drug delivery

To achieve efficient targeted payload delivery, nanocarriers must meet the following challenges: (a) long-term stability in blood circulation as well as retention within tumor lesions, (b) ability to accumulate at tumor tissue site and penetrate deeply into tumor parenchyma, and (c) avoiding the premature release of payload before reaching the target and then accelerating drug release once at the
To achieve safe and efficient antitumor drug delivery, pH-responsive nanocarriers with programmable size changes based on the tumoral/intracellular phase-transition (protonation/deprotonation and cleavable bond) (Table 1) offer a feasible approach for overcoming many contradictory requirements during various phases.

2.1. pH-responsive aggregation for tumor retention

2.1.1. Design principle

The long-term stability in the circulatory system necessitates nanocarriers with a different size than that required for uptake by tumor cells [8, 76, 90, 177-179]. From previous anti-tumor studies, the well-accepted size for long-term blood circulation is around 100 nm due to the size-dependent balance between organ filtration and tumor vessel extravasation [8, 76, 83]. After intravenous injection, carriers circulating for a longer time in the blood have a better chance of accumulating in the tumor tissue via EPR effects.

Therefore, for prolonging the blood circulation time, it is extremely important to avoid nonspecific interactions with blood components and other off-targeted cells. Also, nanocarriers undergo a series of screening events by the reticuloendothelial system (RES) and organs such as the liver, spleen, lung, and kidney. Improvement of their “stealth” capabilities is a key aspect for prolonging blood circulation time. Furthermore, once accumulation begins in tumor tissue, it is ideal for nanocarriers to “stick” to the tumor cells.

**Figure 1.** Illustration of size-dependent antitumor drug delivery. stage 1: extravasation based on the tumor vascular pore size selection (Three arrows indicate that less phagocytosis by MPS, longer circulation time in bloodstream, and higher extravasation at tumor vasculature are beneficial for enhancing passive targeting of tumor by EPR effects); stage 2: antitumor drug delivery in the highly permeable tumor tissue; stage 3: antitumor drug delivery in the poorly permeable tumor tissue; stage 4: tumor cell uptake and subsequent intracellular drug release. pH ~7.4 for normal tumor tissue and blood stream, ~6.5 for tumor tissue, ~5.5 for endosome, ~5.0 for lysosome.
| Phase-transition | Structure (Name) | pH    | Examples          |
|------------------|------------------|-------|-------------------|
| **Tumoral acid responsive structures** (pH 6.5-7.2) | Protonation      | ~ 7.2 | [99, 106-108]     |
|  | (PC6A)           | ~ 6.9  |                   |
|  | (PC7A)           | ~ 7.0  | [109-112]         |
|  | (PAE)            | ~ 7.0  | [113-115]         |
|  | (PSD)            | ~ 7.0  | [116-119]         |
|  | (PHis)           | ~ 6.5  |                   |
|  | (PVBA)           | ~ 7.1  | [120-123]         |
| **Deprotonation** |                  |       |                   |
|  | (DMMA)           | ~ 6.8  | [53, 60, 124, 125]|
|  | (Benzoic-imine)  | ~ 6.5  | [126-129]         |
| **Endo/lysosome acid responsive structures** (pH 4.5-6.5) | Protonation      | ~ 6.3  | [49, 52, 64, 77, 130]|
|  | (PDPA)           | ~ 5.6  | [131-134]         |
|  | (P4VP)           | ~ 5.6  |                   |
Deprotonation

(P2VP)  $\sim 5.0$ [135-137]

(PMAA)  $\sim 6.3$ [138-141]

(PEAA)  $\sim 5.6$ [142, 143]

(PAsp)  $\sim 5.0$ [144-147]

Acid-labile bond

(Citraconic amide)  $\sim 5.5$ [55, 148-150]

(Hydrazone)  $\sim 6.0$ [117, 151-153]

(Imine)  $\sim 6.5$ [154-157]

($\beta$-Thiopropionate)  $\sim 5.5$ [158-161]

(Ketal)  $\sim 5.0$ [162-165]

(Acetal)  $\sim 5.0$ [166-169]

(Cyclic acetal)  $\sim 5.0$ [170-172]

(Ortho ester)  $\sim 5.0$ [72, 173-176]
As a result of the time- and concentration-dependent internalization process caused by enhanced interactions between nanocarriers and cells, carriers can have increased chances of tumor cells uptake and retention in tumor tissue [1, 89, 180]. Cytotoxicity of aggregated nanocarriers compared to non-aggregated ones has also been reported [179]. All in all, nanocarriers must be “stealthy” in blood circulation to evade undesirable RES recognition or protein/cell adhesion but must be “sticky” to interact with tumor cells. To this end, nanoparticles with sizes of 20-200 nm are appropriate to avoid undesirable clearance through the nonspecific adsorption and urinary excretion. However, the design strategy employed may need to take into account the opposing requirements depending on the intercellular space. For the highly permeable tumor parenchyma with large intercellular space, the aggregated nanoparticles can be retained in the tumor tissue to enhance tumor cell endocytosis. This is one of the areas where the tunable properties of nanocarriers are advantageous as their surfaces can be functionalized appropriately to fulfill size requirement.

2.1.2. pH-responsive aggregation based on protonation/deprotonation

pH-responsive aggregation triggered by slightly acidic stimuli of the extracellular tumor matrix is a suitable mechanism for designing nanocarriers with low uptake by normal cells but high uptake by tumor cells. Based on the protonation/deprotonation transition triggered by mild acidic stimuli, Ji and coworkers prepared a series of mixed-charge zwitterionic monolayer-modified gold nanoparticles (Figure 2) [89, 181]. Owing to zwitterionic monolayer protonation/deprotonation balance, the optimized nanoparticles were stable at the physiological pH of the bloodstream and normal tissues but rapidly aggregated within a few seconds in response to a slight pH change from 7.4 to 6.5. This work demonstrated that controllable aggregation at tumor sites could enhance retention efficiency and cellular uptake of nanoparticles in tumors for photo-thermal tumor-targeted diagnosis and therapy. Inspired by this study on the surface charge conversion resulting from ultra acid-labile amide bond breakage, we fabricated a pH-responsive polymeric nanocarrier with tumor-targeted aggregation properties for enhanced retention and endocytosis (Figure 3) [52]. The doxorubicin-loaded smart nanocarrier was constructed from succinic anhydride-modified poly (2-diisopropylaminoethyl methacrylate)-block-poly (2-aminoethyl methacrylate hydrochloride) (PDPA-b-PAMA/SA@DOX) and was responsive to a slightly acidic stimulus. It exhibited size aggregation from ~154 nm to ~1517 nm due to the pH-responsive protonation of PDPA block-induced electrostatic attraction with its negatively charged corona. Importantly, cell uptake studies showed that the aggregated nanomedicine was endocytosed by tumor cells at pH 6.5 rather than pH 7.4. Using the same
mechanism of pH-sensitive surface change transition, Chiu and coworkers constructed a nanocarrier using N-acetyl histidine-modified D-α-tocopheryl polyethylene glycol succinate on the surface of poly (lactic-co-glycolic acid) [182]. The increased protonation of imidazole groups from histidine residues resulted in the lack of sufficient interparticle electrostatic repulsion for maintaining stable individual particles at pH 6.5, and the DOX-loaded nanoparticles with an almost neutral surface charge aggregated into large clusters. Both in vitro and in vivo results confirmed that tumor acidity-triggered surface charge neutralization and aggregation could significantly enhance tumor cell uptake. Ji’s group also reported a pH-responsive supra-molecular prodrug micelle based on the host-guest inclusion [183]. The size of this supra-assembly could be tuned by pH changes showing significant aggregation triggered by slight acidity of the tumor extracellular matrix. Benefitting from aggregation-induced accumulation in tumor tissues, this supra-assembly exhibited desirable antitumor effect by efficiently inhibiting tumor proliferation. In these design strategies, the pH-responsive size aggregation based on the protonation/deprotonation transition, pH-responsive size change induced by the acid labile cleavable bond in response to the slight acidity of the tumor tissue exposing the surface positive charge and inflated size to promote tumor cell endocytosis. However, compared to the protonation/deprotonation transition, pH-responsive size change induced by the acid labile cleavable bond is generally irreversible. Therefore, the acid labile nanoparticles have a potential risk of instability in their preparation, storage, and application.

2.2. pH-responsive size shrinkage for deep tumor penetration

2.2.1. Design principle

The size of nanocarriers is a significant factor in promoting their accumulation and penetration in tumor tissues [1, 76, 185]. To sufficiently illustrate the important role of size in drug delivery, Tang et al. have fabricated micelles of the same chemical structures and physical properties from a single copolymer but ranging in size from 20 nm to 300 nm. Their results indicated that the optimal size of nanocarriers through the cascade processes was different at different stages, i.e. the optimal size range for suitable blood circulation time and tumor accumulation was between 100 and 160 nm. However, greater accumulation of the large micelles (100 nm) at tumor sites did not result in significantly improved therapeutic efficacy because of their poor tumor penetration compared to that of the smaller micelles (30 nm). A relatively large size (100 nm) is needed for accumulation at tumor sites and a smaller size (preferably <20 nm) is required for tumor penetration. Therefore, the two most commonly-used strategies are to construct slightly acidic pH-responsive nanocarriers with large aggregation for accumulation at the tumor site [52, 89, 186] or with a small size for deep-seated tumor penetration [25, 90, 187]. In contrast to the pH-responsive aggregation in the highly permeable tumor parenchyma, the poorly permeable tumor parenchyma exhibits narrow intercellular spaces resulting from the increased fibrillar collagen crosslinking. For this reason, developing the ultra-pH-responsive nanoparticles with size shrinkage in response to mild acidic stimulus is beneficial for deep penetration into the tumor parenchyma to inhibit tumor proliferation.
Figure 3. (A) Scheme and (B) Digital image of the tumor-targeted aggregation of pH-responsive polymeric nanomicelles for enhanced retention at tumor tissue and rapid intracellular drug release in tumor cells. Reproduced with permission from [52], copyright 2014 Royal Society of Chemistry.

Figure 4. (A) Schematic of the ultrasensitive pH-triggered charge/size dual-rebound gene delivery system for efficient antitumor applications. (B) Fast hydrodynamic charge/size dual-rebound property and (C) TEM of the gene delivery system. (GP)D and P[(GP)D] represent (PLG/PEI)/DNA and PEG[(PLG/PEI)/DNA], respectively. Reproduced with permission from [184], copyright 2016 American Chemical Society.
2.2.2. pH-responsive size shrinkage based on cleavable bond at tumor extracellular acidity for tumor parenchyma penetration

The pH-responsive size shrinkage due to the acidic environment of the extracellular tumor matrix provides a feasible strategy to facilitate deep penetration of nanocarriers into the tumor’s dense collagen parenchyma. Thayumanavan and coworkers constructed a series of ultra pH-sensitive size-changeable nanogel clusters [42]. Large nanoclusters were obtained by using a pH-sensitive dynamic covalent imine bond to cross-link individual nanogels. Under physiological pH conditions, nanoclusters showed a relatively large size and less positive surface charge to prolong the blood circulation time. Because of the breakage of cross-linked dynamic imine bonds triggered by the tumor-acidity at pH 6.5, nanoclusters transformed into the original nanogel, exhibiting small size and a more positive surface charge to promote deeper penetration into the tumor matrix. Wang’s group reported pH-responsive clustered nanoparticles, comprising the platinum-poly(amidoamine) dendrimer prodrug-conjugated at the terminal of the amphiphilic poly(ethylene glycol)-b-poly(ε-caprolactone) block polymer using the acid-labile bond [187]. These smart clustered nanoparticles showed an initial diameter of ~100 nm, and discharged the small size (~5 nm) prodrug by cleaving its conjugation in the acidic environment within the tumor for deep penetration and enhanced endocytosis, followed by further intracellular reduction to kill tumor cells. Ge’s group reported a supramolecular polymeric nanogel based on the host-guest interaction between adamantane and β-cyclodextrin moieties [188]. Due to the cleavage of the benzoic imine linkage in tumor-acidic conditions, this nanogel could reorganize into smaller nanoparticles from ~220 nm to ~25 nm. For targeted drug delivery into the tumor cell nucleus, Huang and colleagues synthesized a multistage nanovehicle with pH-responsive stepwise size reduction and charge reversal using an ultra pH-sensitive charge-reversal 2,3-dimethylmaleic amine modification and hydrazone linkage (Figure 5) [189]. The neutrally charged nanovehicle with relatively large size (~55 nm) showed good blood persistence and excellent accumulation in tumor sites at pH 7.4. Responding to the acidic stimulus of tumor tissue, the nucleus-homing cell-penetrating peptide-modified drug conjugate was separated from the disassembled large nanovehicle, and had a size of ~10 nm in diameter, which allowed deeper tumor matrix penetration and better cellular internalization. Subsequently, the antitumor drug was cleaved from the conjugate in the acidic microenvironment of the endosome, and efficiently entered the nucleus via nuclear location signaling. Benefitting from the
stepwise size reduction and on-demand target moiety exposure, this multistage nanovehicle greatly improved the antitumor drug efficiency inhibiting HeLa tumor growth in nude mice by 75%.

2.2.3. pH-responsive size shrinkage based on protonation at tumor extracellular acidity for tumor parenchyma penetration

Another study by Ge and co-workers used ultra pH-sensitive charge reversal micelles with a large size (114 nm at pH 7.4) to introduce pH-responsive hydrophobicity to hydrophilicity transition of tertiary amine. Under slightly acidic condition (pH 6.8), this specifically accelerated the release of an encapsulated smaller poly(amidoamine) dendrimer-conjugated prodrug (several nanometers) for deep tumor penetration, and subsequently released the active drug by cleaving the conjugate in the intracellular reduction environment [190]. Recently, Wang and colleagues have developed a class of pH-responsive nanoparticles superstructures with ultrasensitive size shrinkage for improving deep tumor penetration and efficient antitumor drug delivery (Figure 6) [90]. Due to the rapid pH-responsive protonation of the ionizable tertiary amine group, this smart superstructure had an initial size of ~80 nm at the physiological pH of 7.4 during its circulation in the blood, but undergoes a dramatic and sharp size reduction to less than 10 nm in the slightly acidic microenvironment of the tumor. The ultra pH-sensitive size-changeable superstructures did not only facilitate enhanced targeted tumor accumulation by EPR by utilizing the long-circulation benefit of large nanoparticles but also promoted deeper tumor penetration by taking advantage of the increased penetration capability of smaller nanoparticles, which ultimately improved efficiency of antitumor therapy in the poorly permeable BxPC-3 pancreatic tumor model.

Figure 6. (A) Structure of polymer-drug conjugate. (B) Schematic illustration of the pH-sensitive cluster nanobomb at neutral pH and the disintegration into small particles at tumor due to its acidic pH. (C) Schematic illustration of cluster nanobomb for in vivo drug delivery in the poorly permeable pancreatic tumor model. (I) large superstructure for prolonged blood circulation, (II) targeted tumor accumulation via EPR, (III) pH-responsive disintegration into small particles for deep tumor parenchyma penetration. Hydrodynamic diameter and TEM of polymer-drug conjugate at (D) pH 7.4 and (E) pH 6.7. (F) pH-dependent size change detected by DLS. Reproduced with permission from [90]. Copyright 2016, American Chemical Society.
2.2.4. pH-responsive size shrinkage based on cleavable bond at tumor intracellular acidity for endo/lysosome escape

pH-responsive size shrinkage triggered by acidic stimuli of endo/lysosomes is helpful for facilitating the escape of nanocarriers from these subcellular compartments. Sha and colleagues prepared a size-shifting micelle nanocluster based on a cross-linked framework interspersed with small micelles using the emulsion-evaporation method (Figure 7) [91]. The cross-linked nanocluster was stable with a size of around 104 nm and prolonged half-life for increased tumor accumulation by EPR. Due to the proton sponge effects of polyetherimide in an acidic environment, the cross-linked framework of nanoclusters was able to swell and disintegrate, thus accelerating the release of individual micelles (14 nm) and penetration into the cytoplasm. To ensure delivery of antitumor drug into the nucleus, Chen and coworkers designed a large compound nanoparticle with pH-activated size reduction for \textit{in vivo} nucleus-targeted gene delivery (Figure 8) [191]. This large compound nanoparticle with detachable PEG shell and folate decoration had a size of \textasciitilde150 nm under neutral conditions. Once internalized by tumor cells, breakage of the benzoic imine bond of PEG conjugate in response to lower pH led to dissociation of the PEG shell resulting in the formation of smaller entities (~40 nm) exposing a previously shielded nuclear localization signal oligo-L-lysine. The resultant smaller nanoparticles could enter into the nucleus \textit{via} the nucleopores, causing a 20-fold higher cytotoxicity \textit{in vitro} and greater tumor suppression in animal models compared with the native drug. For endo/lysosome escape and nuclear entry, the size shrinkage in intracellular acidic conditions could greatly improve the therapeutic efficacy due to the reduced drug loss.
2.3. pH-responsive swell or disassembly for burst drug release

2.3.1. Design principle

pH-responsive size change nanocarriers can also be constructed for encapsulated drug release intracellularly. After being endocytosed by tumor cells, the instantaneous pH-responsive swell accelerates release of encapsulated drugs from the loose nanocarriers and the pH-responsive disassembly results in the burst drug release [30, 49, 64, 77, 192]. The controllable pH-responsive drug release determines the therapeutic efficacy and side effects of the drug [193-195]. The ideal nanocarrier must tightly retain the encapsulated drug without premature release during transport but must show burst release once inside tumor cells to overcome multidrug resistance and subsequently efficiently induce tumor cells apoptosis [70, 105, 196-200]. However, nanocarriers do not conform to the ideal drug release profile; there is burst release of up to 30% during the first several hours and sustained slow drug release over the following several days [196]. The lost drug not only leads to reduced therapeutic efficiency but also increases toxicity to healthy cells and organs. Therefore, improvements in release kinetics are still a tremendous challenge. To match drug release as close to the ideal profile as possible, the tight encapsulation of the drug in the nanoparticles is required and their rapid disassembly to accelerate the release of the payload drug in response to the intracellular acidic environment. Many mechanisms including appropriate cross-linking, reversible conjugation, or specific physicochemical property transformations based on the pH are adapted for a viable strategy to modulate release kinetics and reaching an appropriate therapeutic window. The payload drug would be ineffective below a certain concentration [119, 201-204].

2.3.2. pH-responsive swell or disassembly based on protonation

pH-responsive protonation resulting in polymeric hydrophobic-hydrophilic conversion in the acidic microenvironment of endo/lysosomes is one of the most utilized strategies for controlling the size of nanocarriers. For example, we previously prepared a series of amphiphilic block copolymers based on the pH-sensitive block poly[2-(diisopropylamino)ethyl methacrylate] (pKa ~6.2) [64, 77, 124]. The pH-sensitive block copolymers could self-assemble into nano-micelles with a hydrophobic PDPA core for tightly encapsulating DOX during circulation and then rapidly released DOX intracellularly once transformed from amphiphilicity to double hydrophilicity due to protonation of PDPA block in the acidic environment of tumor cells (Figure 9) [64]. Dong and colleagues prepared integrin-targeted zwitterionic polymeric nanoparticles based on the ultra pH-sensitive PDPA [205]. In their study, 24% of the loaded drugs was released at pH 7.4 within 36 h, but the released drugs increased to 60% at pH 6.0 within 24 h and eventually 100% at pH 5.0 within 5 h. Shuai’s group developed a highly packed

Figure 9. Illustration of DOX-loaded micelles for rapid intracellular drug release in response to the acidic stimulus of endo/lysosome in anti-cancer therapy. Reproduced with permission from [64], copyright 2014 Royal Society of Chemistry.
interlayer-crosslinked nano-micelles for reduction-and pH-responsive burst drug release (Figure 10) [196]. Benefiting from the hydrophobicity of the 2-(diisopropylamino) ethyl amine groups in copolymer at pH 7.4 and disulfide functional cross-linkage in a normal reduction environment, these micelles were capable of tightly encapsulating DOX to avoid drug leakage during storage and blood circulation. The pH-responsive hydrophilicity conversion and reduction-responsive cross-linker breakage in the lysosomes then led to the disassembly of nano-micelles into separate copolymers; Subsequently, there was a burst drug release (more than 95% of loaded DOX released in a lysosome-simulated aqueous solution of 10 mM dithiothreitol at pH 5.0) to induce cell apoptosis.

2.3.3. pH-responsive swell or disassembly based on acid-labile cleavable bond

Another pH-responsive intracellular release strategy is to use acid-labile bond cleavage. pH-responsive disassembled nanocarriers based on charge-reversal or degradation mechanisms are feasible for obtaining a burst release profile. Zhong’s group improved a number of pH-responsive degradable polymeric nanocarriers using acid-labile polycarbonate modified for intracellular drug delivery (Figure 11) [3, 206-212]. The polycarbonate modification provided the amphiphilic feature for a remarkably high drug loading content. Notably, these nanocarriers are stable at pH 7.4 but swell and eventually disassemble for fast hydrolysis of the polycarbonate in the acidic conditions of endo/lysosomes.

Given the high drug-carrying capacity and tunable drug-release kinetics, the pH-responsive prodrug conjugates provide a platform for further increasing drug delivery efficiency [69, 70, 213, 214]. Zhong and coworkers developed a pH-sensitive nano-micelle based on the acid-labile acetal bridging polymer and drug conjugates for accelerated intracellular antitumor drug release [215]. In release studies performed in vitro, the acetal-link breakage resulted in paclitaxel (PTX) monomer recovery and swelling of prodrug nanoparticles, showing that PTX release was highly pH-dependent, i.e. 86.9%, 66.4% and 29.0% in 48 h at pH 5.0, 6.0, and pH 7.4, respectively. Wang and colleagues developed a dual tailor-made pH-responsive polymer-drug conjugate to inhibit drug-resistant cancer stem cells (Figure 12) [53]. This advanced polymeric prodrug could respond to both extracellular and intracellular acidic stimuli.

Figure 10. Highly packed interlayer-crosslinked nanomicelles for reduction- and pH-responsive collectively triggered burst drug release. Reproduced with permission from [196], copyright 2011 Wiley-VCH.

Figure 11. Acid-labile polycarbonate-modified pH-responsive degradable polymeric nanocarriers showing swelling and eventual disassembly for controlling intracellular drug release.
through chemically defined mechanisms. It was expected to have a prolonged blood circulation, accumulation in the tumor via EPR, and endocytosis through pH-responsive surface charge conversion from negative to positive in response to the mild acid conditions in tumor tissue. Interestingly, nanosized prodrug rapidly disassembled into the drug monomer due to the acid-labile hydrazone bond cleavage in endo/lysosomes. This multifunctional nanosized prodrug greatly enhanced antitumor efficacy providing an advanced guide for antitumor drug delivery research and clinical applications.

Gu et al. improved a potential theranostic reagent using a transformable liquid-metal nanomedicine with a polymeric shell for encapsulating DOX and a liquid inorganic core for fusion and subsequent degradation in a mildly acidic environment (Figure 13) [216]. Within 4 h, the cumulative amount of drug released in the acidic buffer of pH 5.0 was significantly higher than that in the neutral condition of pH 7.4 (> 50% for DOX and 44% for paclitaxel). The increased drug release at pH 5.0 resulted from the liquid-metal core fusion mediating the disruption of the polymeric shell and subsequent dissociation of the drug-loaded surface ligand. Besides, this liquid-metal nanoformulation exhibited a contrast-enhancing capability for imaging applications, and eventually degraded under mildly acidic condition showing excellent biocompatibility.

3. Advanced endogenous pH-responsive carriers with programmable size changes for tumor imaging

Besides being useful for antitumor therapy, the pH-responsive size-changeable design strategy has also been exploited for tumor diagnosis [217-219]. For example, to overcome the low optical absorbance in biological tissues’ near-infrared optical window, Chang et al. developed novel melanin-like nanoparticles by modifying the hydrolysis-susceptible surface citraconic amide for photoacoustic imaging (Figure 14) [55]. These improved nanoparticles were able to aggregate within a mildly acidic environment, and, due to their overlapping thermal fields, resulted in the photoacoustic signal strength amplification (8.1 times at pH 6.0 than that at
pH 7.4) in the near-infrared window of biological tissues without absorption tuning. The unique characteristic of photoacoustic amplification in melanin-like nanoparticle clusters might allow their use as a contrast agent for highly sensitive in vivo tumor target imaging. This innovative targeted tumor imaging based on the pH-responsiveness unveiled great opportunities for many basic research studies and clinical explorations. Recently, Gao and colleagues have fabricated a large number of the ultra-pH-responsive nanosensors for amplifying signals based on the positive cooperativity of the noncovalent selfassembly of the fluorescence-labeled functional block copolymers [66, 67, 71, 106-108, 114, 220-226]. Typically, a hybrid nanotransistor probe, composed of a molecular mixture of three different ultra-pH-responsive block copolymers, served as a binary pH threshold sensor exhibiting dramatic signal amplification (>30-fold) and sharp (<0.25 pH) acidic response capabilities [220]. The hybrid nanotransistor stayed “OFF” at pH 7.4, resulting from the dramatic fluorescence quenching through the hetero-/homo-molecular fluorescence energy transfer in micelle state. After endocytosis, the ultra-pH-responsive block copolymer components disassembled and fluoresced sequentially as the pH value decreased. After each pH transition encoding with a unique fluorescent reporter, the acidification kinetics at single-organelle resolution could be quantified using digitization paradigm by a series of barcodes with binary (0 and 1) output in each channel reporting the luminal pH of the individual endocytic organelle (Figure 15). This novel nanoprobe provided a feasible platform for imaging and new biological investigations in tumor pathology. The preliminary exploration of the pH-responsive size changeable nanoprobes showed exciting perspectives in tumor imaging diagnosis. For the same design mechanism, the pH-responsive nanoparticles with programmable size changes provided a feasible platform to integrate therapy and imaging for tumor theranostic improvement.

Figure 13. Illustration of the transformable liquid-metal nanomedicine for the antitumor application. (A) Preparation and (B) Main components of liquid-metal nanomedicine. (C) pH-responsive delivery of the DOX-loaded liquid-metal nanomedicine for targeted antitumor therapy. (D) Acid-triggered fusion and degradation process of liquid-metal nanomedicine. (E) Chemical structures of the main components. Reproduced with permission from [216], copyright 2015 Nature Publishing Group.
4. Conclusions and perspectives

pH-responsive nanocarriers with programmable size changes have proven to be a powerful and flexible platform for designing more effective anticancer drug delivery systems with higher therapeutic efficacy and fewer clinical side effects. In this review, we focused on various advanced pH-responsive size-changeable design strategies: (a) Nanocarriers with appropriate size are necessary for avoiding undesirable clearance during circulation by nonspecific MPS and for enhancing accumulation and retention at tumor sites via the EPR effect. (b) For highly permeable tumors (such as C26 colon tumor), the slightly acidic environment in tumor tissues can promote uptake of nanocarriers with pH-responsive aggregation through concentration- and time-dependent interaction effects. (c) For poorly permeable tumors (such as the BXPC-3 pancreatic tumor), nanocarriers with pH-responsive size shrinkage are capable of accelerating nanocarrier penetration into the tumor parenchyma, escaping from endo/lysosomes, and delivering the drug into the nucleus. (d) After internalization by tumor cells, nanocarriers with pH-responsive swell and even disassembly can accelerate drug release to provide the
required therapeutic dose for inducing tumor cell apoptosis. (e) pH-responsive nanoprobes with programmable size changes are expected to be useful for tumor imaging diagnosis.

Although considerable progress has been made with pH-responsive nanocarriers with programmable size changes, some challenges still exist. For example, rapid mutations of malignant tumors may result in unpredictable and unstable pathological environment including extracellular pH gradients, varying tumor vascular leakage by changing pore diameter, and tumor parenchyma collagen density. The synchronous development of real-time technology in tumor imaging and diagnosis is needed to detect the precise pathological changes for designing an accurate and targeted clinical approach. Furthermore, the strategy in the selection of sizes for nanocarrier aggregation or deep tumor penetration should be designed based on the permeability of various tumor types and location. For example, different size nanocarriers (with diameters of 30, 50, 70 and 100 nm) exhibit efficient penetration into the highly permeable tumor, but only small-sized nanocarriers with a diameter of 30 nm can penetrate the poorly permeable pancreatic tumors [227]. For tumors with low blood supply and mesenchyme-enriched tumors, the size reduction strategy for deep tumor penetration is a better choice for enhancing antitumor therapeutic efficacy. On the other hand, the excessive pursuit of stimuli-responsiveness may come at the cost of loss of stability which may cause serious physiological toxicity. Therefore, it is necessary to achieve a rational balance between ultra sensitivity and stability. Although pH-responsive size variation is a major aspect of designing nanocarriers, it is not the only criterion that is worthy of consideration. Sophisticated novel antitumor nanocarriers must navigate a hostile and complex environment in vivo and, therefore, a variety of mechanisms are needed to integrate multiple stimuli (temperature, redox, enzyme, glucose, ion, etc.) as well as different factors (surface charged potential, hydrophilic-hydrophobic balance, shape etc.). Also, improving biocompatibility and degradability as well as functionality is important for the successful implementation of clinical nanomedicine. In summary, pH-responsive nanocarriers with programmable size changes provide an excellent platform for precise and personalized treatment for current antitumor therapy. However, a great number of challenges remain for the future clinical nanomedicine.

Abbreviations

EPR: enhanced permeability and retention; DLS: dynamic light scattering; DMMA: 2,3-dimethylmaleic anhydride; DOX: doxorubicin; H+: hydrogen ion; MPS: mononuclear phagocytic system; P2VP: poly(2-vinylpyridine); P4VP: poly(4-vinylpyridine); PAE: poly(β-aminostyrene); PAMA: poly(2-aminoethyl methacrylate hydrochloride); PAsp: poly(aspartic acid); PC6A: poly[2-(pentamethylenemino) ethyl methacrylate]; PC7A: poly[2-(hexamethylenimino) ethyl methacrylate]; PDPA: poly[2-(diisopropylamino) ethyl methacrylate]; PEAA: poly(ethylacrylic acid); PEG: polyethylene glycol; PEI: polyethylenimine; PHis: poly(L-histidine); PMAA: poly(methacrylic acid); PLG: poly-L-glutamate; PSD: poly(methacryloyl sulfadimethoxine); PTX: paclitaxel; PVBA: poly(4-vinylbenzoic acid); RES: reticuloendothelial system; SA: succinic anhydride; TEM: transmission electron microscope.

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Competing Interests

The authors have declared that no competing interest exists.

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