REVIEW

Bioresponsive polymeric nanotherapeutics for targeted cancer chemotherapy

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Summary In recent years, bioresponsive polymeric nanotherapeutics that facilitate tumor cell uptake and trigger drug release at the target site have emerged as a fascinating platform for safe and efficient cancer therapy. The naturally occurring environments such as tumor acidity, tumor extracellular enzymes like matrix metalloproteases (MMP), endo/lysosomal pH, elevated glutathione levels in the cytoplasm and cell nucleus, lysosomal enzymes, as well as reactive oxygen species (ROS) in the mitochondria have been exploited as potential internal stimuli to achieve active drug and protein release in the tumor tissue or cancer cells. These bioresponsive nanosystems present several unique features such as no need of an external device, precision control over site of response (from tumor tissue down to cellular organelle level) following accumulation in the tumor via either passive or active targeting, and spontaneous activation in the tumor site or inside the tumor cells. In this review, we highlight the design rationale and recent exciting development of bioresponsive polymeric nanotherapeutics for enhanced cancer treatments with low side effects.

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Introduction

Nanoparticle-based formulations have emerged as a fascinating technology platform for targeted and controlled drug release [1–4]. The preclinical and clinical studies have demonstrated that nanotherapeutics confer prolonged circulation time, enhanced accumulation in the tumor sites via the enhanced permeability and retention (EPR) effect, reduced drug side effects, improved drug tolerance, and better drug bioavailability [5–7]. It should be noted, however, that despite many advantages, current nanotherapeutics are far from meeting the clinical expectations, due to several bottlenecks such as low stability in vivo, modest tumor accumulation (typically less than 5% injected dose/gram tissue), poor tumor penetration, inefficient tumor cell uptake, and inadequate control over...
drug release [8–11]. In the past decades, thousands of nanosystems have been developed and studied for cancer chemotherapy, but few of them show a controlled drug release behavior in vivo. The first often encountered issue is premature drug release in circulation, which results in low targetability, decreased efficacy, and pronounced side effects [12]. Another typical problem is that even though nanotherapeutics have finally been transported to the tumor tissue or in the tumor cells, drug can’t be readily released, leading to low drug concentration in the tumor cells. In the past decade, stimuli-responsive polymeric nanocarriers that release drugs in response to an internal or external stimulus such as pH, redox, enzymes, temperature, reactive oxygen species (ROS), magnetic and light have been developed to achieve better-controlled drug release and thereby improved therapeutic efficacies [13–16]. In particular, bioresponsive polymeric nanotherapeutics that facilitate tumor cell uptake and trigger drug release at the target site have emerged as one of the most fascinating platforms for cancer therapy. Notably, as compared to their external stimuli (e.g. temperature, magnetic and photo)-sensitive counterparts, bioresponsive nanosystems present several unique advantages such as no need of an external device, precision control over site of response (from tumor tissue down to cellular organelle level) following accumulation in the tumor via either passive or active targeting, and spontaneous activation in the tumor site or inside the tumor cells.

The most obvious and spectacular naturally occurring environments in the tumor and inside the cancer cells are as follows: (i) tumor acidity (pH 6.5–7.2); (ii) tumor extracellular enzymes such as matrix metalloproteases (MMP); (iii) acidic pH in the endosome (pH 5.5–6.8) and lysosome (pH 4.5–5.5); (iv) elevated glutathione levels in the cytosol and cell nucleus; (v) degradative enzymes in the lysosomes, and (vi) ROS in the mitochondria (Fig. 1). These natural microenvironments have been exploited as potential internal stimuli to achieve active drug and protein release in the tumor tissue or cancer cells. We shall be aware that acidic endo/lysosomes, elevated intracellular reducing potential, lysosomal enzymes, and ROS are also present in the healthy cells. It is of critical importance, therefore, that these bioresponsive nanotherapeutics are specifically delivered to the tumor cells. The impacts of biosignals existing in the tumor and inside the tumor cells on drug delivery are summarized in Table 1. These bioresponsive polymeric nanotherapeutics have demonstrated superior in vitro and in vivo antitumor performances as compared to their non-responsive counterparts. Moreover, studies have shown that they could also effectively reverse multidrug resistance (MDR) due to fast dumping of drugs into the cancer cells. There are several excellent reviews on stimuli-sensitive nanocarriers for drug delivery [13–21], including an early review on (bio)responsive nanoparticles [22], two review papers on bioresponsive polymers for nucleic acid delivery [23,24], and one review on bioresponsive peptide-inorganic hybrid nanomaterials [25]. It is noted, however, that there is no particular review on the development of bioresponsive polymeric nanotherapeutics for targeted cancer chemotherapy. In this paper, we highlight the design rationale and recent exciting advancement of various bioresponsive polymeric nanotherapeutics. In the end, the challenges and perspectives on their future development will be discussed.

**Tumor pH-responsive polymeric nanotherapeutics**

Tumors develop unique microenvironments. The combination of a high glycolytic activity that produces acids as byproducts and impaired clearance of acids gives rise to a slightly acidic pH of 6.5–7.2 [26]. This lower tumor extracellular pH as compared to the healthy tissues and blood (pH 7.4) has been exploited to facilitate tumor cell uptake as well as drug release at the tumor site [27].

In order to achieve prolonged circulation time and enhanced tumor accumulation, nanotherapeutics are usually decorated by a non-fouling polymer like poly(ethylene glycol) (PEG) or dextran, which would, however, also
impede tumor cell uptake, thereby significantly compromising antitumor efficacy. Exposure of nanoparticle cationic surface through shedding off the stealthy polymer at tumor pH represents an interesting strategy to enhance tumor cell uptake. Bae et al. developed tumor pH-sensitive shielding/deshielding micelles from poly(L-histidine)-b-polyethyleneimine (PHis-b-PEI) and mPEG-b-poly(sulfadimethoxine) (mPEG-b-PSDM) [28]. The anionic PSDM block could complex with the cationic surface of PHis-b-PEI micelles at physiological pH to yield stealth micelles. PSDM would, however, lose its charge at tumor pH, resulting in fast shedding of mPEG-b-PSDM and efficient cellular uptake of micelles in MCF-7 breast adenocarcinoma and SKOV-3 ovarian carcinoma cells. The preliminary in vivo studies exhibited that mice bearing MCF-7 breast tumor xenografts following 29-day treatment with PTX-loaded pH-sensitive micelles had a tumor volume less than one half of that with free PTX and one third of that with pH-insensitive control. Wang et al. reported that core-shell-corona polyanion complex nanoparticles (\(^{10}\)NP/Pt@PDC-DA) constructed from positively charged Pt(IV)-conjugated nanoparticles (\(^{10}\)NP/Pt) and tumor acidity-responsive negatively charged PPC-DA copolymer (PPC: PEG-b-poly(allyl ethylene phosphate modified with cysteamine; DA: 2,3-dimethylmaleic anhydride) exhibited much slower blood clearance (0.54 mL/h) than \(^{10}\)NP/Pt (3.46 mL/h) and cisplatin (7.89 mL/h), as well as 32.1-fold higher platinum tumor accumulation than free cisplatin in nude mice bearing A549 xenografts following 24 h i.v. injection [29]. As a result, \(^{10}\)NP/Pt@PDC-DA showed significantly more efficient inhibition of A549R lung tumor growth than \(^{10}\)NP/Pt and free cisplatin.

In the past years, polymeric nanotherapeutics with tumor pH-activatable surfaces have been designed and developed to accomplish enhanced tumor cell internalization. Shen et al. decorated PEG-b-poly(ε-caprolactone) (PEG-b-PCL) micelles with a tumor-pH activatable TAT (transactivator of transcription) peptide, in which succinyl amidization of the primary amines in TAT effectively inhibited non-specific interaction in the circulation [30]. In the acidic tumor pH or further in the endo/lysosomal pH, however, the succinyl amides in the TAT would be quickly hydrolyzed, restoring TAT’s function. The in vivo experiments in Bcap-37 xenografted nude mice revealed that doxorubicin (DOX)-loaded tumor pH-activatable TAT-PEG-PCL micelles afforded 2 and 8-fold tumoral DOX concentration relative to that with DOX-loaded PEG-PCL and DOX-loaded pH-insensitive TAT-PEG-PCL micelles, respectively, resulting in elevated antitumor activity and low cardiotoxicity. Zhang et al. designed negatively charged PLLeu-PLL(DMA)-TAT(SA) micelles (PLLeu: poly(γ-L-lysine); PLL: poly(γ-L-lysine); DMA: 2,3-dimethylmaleic anhydride; SA: succinyl chloride) that switched to positively charged nanoparticles at pH 6.5 via hydrolysis of DMA amide facilitating cellular uptake while recovered TAT via hydrolysis of SA amide at pH 5.0 facilitating endosomal escape and cell nuclei delivery [31]. Flow cytometry analysis demonstrated that cellular uptake of PLLeu–PLL(DMA)–TAT(SA) micelles increased from 12.2% at pH 7.4 to 45.9% at pH 6.5. \(H_2K(R_2)_2\) peptide-modified PEG-b-poly(lactide-co-glycolide) (PEG-PLGA) copolymer micelles (H\(_2\), pH-sensitive sequence; K, linker; (R\(_2\))\(_2\), cell-penetrating peptide) while hided (R\(_2\))\(_2\) in the PEG shell at pH 7.4 were reported to expose (R\(_2\))\(_2\) to the outer surface at pH 6.8.

| Table 1 | Impacts of biosignals in the tumor and inside the cancer cells on drug delivery. |
|-----------------|---------------------------------|---------------------------------|
| Biosignals       | Responses of nanocarriers        | Impact on drug delivery         |
| Tumor tissue     | Acidic pH                        | Exposing or activating targeting ligands | Promoting tumor cell uptake |
|                  |                                 | Surface charge conversion       | Triggering drug release in the tumor |
|                  |                                 | Detaching stealthy coating      | Promoting tumor cell uptake |
|                  |                                 | Dissolution                     | Enhancing tumor tissue penetration |
|                  |                                 | Degradation and disassembly     | Triggering drug release in the tumor |
|                  |                                 | Detaching stealthy coating      |                                    |
|                  |                                 | Activating targeting ligands    |                                    |
|                  |                                 | Cleavage of enzyme-sensitive linkages |                                    |
| Extracellular enzyme |                       |                                    |                                    |
| Tumor cell       | Endo/lysosomal pH                | Swelling                        | Triggering intracellular drug release |
|                  |                                 | Dissolution                     | Facilitating endosomal escape      |
|                  |                                 | Aggregation                     |                                    |
|                  |                                 | Proton sponge effect            |                                    |
|                  |                                 | Charge conversion               |                                    |
|                  |                                 | Enhanced interaction with membrane |                                    |
|                  |                                 |                                    |                                    |
|                  | Cytoplasmic GSH                  | Shell-shedding                  | Triggering cytoplasmic drug release |
|                  |                                 | Reductive degradation           |                                    |
|                  |                                 | De-crosslinking                 |                                    |
|                  | Lysosomal enzymes                | Enzymatic degradation of specific peptide linkers | Triggering drug release in the lysosomes |
|                  | Mitochondrial ROS                | Increasing nanocarrier hydrophilicity | Triggering drug release in high ROS stress sites |
|                  |                                 | Cleavage of ROS-sensitive bonds |                                    |
because of protonation of H₂, leading to enhanced tumor cell uptake [32]. The in vivo studies in MCF-7 tumor bearing mice showed that paclitaxel (PTX)-loaded H₂K(R₂)₂:PEG-PLGA micelles caused significant antitumor and angiogenic activity as compared with Taxol® and PTX-loaded insensitive micelles. Chen et al. reported that tumor extracellular pH responsive nanoparticles with a poly(carboxybetaine) (pCB) shell and sulfo groups near a DOX loaded core exhibited a strong resistance at physiological pH but a high affinity to tumor cell membranes at tumor extracellular pH [33]. The in vivo studies in Bcap breast cancer xenografts exhibited that DOX-conjugated pCB-based nanoparticles caused a tumor growth inhibition rate of 83% with little side effects. In contrast, DOX-conjugated oligo(ethylene glycol)-based nanoparticles (pH-insensitive control) demonstrated a low tumor inhibition effect. Histological analysis showed that the DOX-conjugated pH responsive nanoparticles treated groups had compact cardiomyocytes lining up with clear structures similar to those seen in the saline controls while free DOX treated group and pH-insensitive control group both exhibited severe myocardial damage.

In addition to pH-activatable tumor cell uptake, super-pH-responsive polymeric nanotherapeutics have also been designed and developed to release payloads upon arrival at the tumor site. For instance, Lee et al. disclosed that Chlorin6 (Ce6)-loaded tumor pH-sensitive short-worm like micelles based on mPEG-poly(Lys-DEAP)₂ miktoarm block copolymer (Lys: lysine; DEAP: 3-diethylaminopropyl) caused improved phototoxicity due to triggered release of Ce6 at tumor pH and high-yield generation of singlet oxygen [34]. The in vivo experiments in KB tumor-bearing nude mice showed that the tumor volume of mice treated with Ce6-loaded micelles and illumination at 12 h post-injection was approximately 5.2-fold smaller than that with free drug under otherwise the same conditions. Interestingly, Gao et al. recently developed an ultra pH-sensitive fluorescent nanoparticles based on PEG-b-Poly(2-(dissopropyl amino)ethyl methacrylate) copolymer whose fluorescence was quenched in the circulation but strongly activated in the tumor site due to fast dissolution and dissociation of nanoparticles in response to tumor extracellular pH [35]. These tumor pH-activatable fluorescent nanoparticles were able to light up a broad range of tumors.

**Tumor extracellular enzyme-responsive nanotherapeutics**

In the tumor tissue, several specific enzymes e.g. proteases, glycosidases, and phospholipases are present with high concentrations, but usually absent or at very low concentrations in healthy tissues. Matrix metalloproteinases (MMPs), especially MMP-2 and MMP-9, are well known to be involved in many cancer invasion, progression, and metastasis [36]. These over-expressed enzymes in the tumor extracellular environment are highly appealing for achieving improved tumor cellular uptake, tumor tissue penetration and/or drug release. Unlike tumor pH-responsive nanocarriers that would most likely also degrade under physiological conditions, tumor extracellular enzyme-responsive nanocarriers are usually very stable in the absence of the specific enzyme. Torchilin et al. prepared several MMP-2-responsive nanocarriers based on MMP-2 cleavable peptide sequence GLPLG1AQ for effective tumor targeting and cellular penetration [37–39]. MMP-2 sensitive micelles based on PEG-Peptide-PEI-1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (PEG-Peptide-PEI-PE) copolymers were stable in the circulation due to PEG protection, while shed off PEG and exposed positively charged PEI due to the cleavage of peptide in the presence of MMP-2, leading to active tumor targeting and enhanced cell internalization [38]. The in vivo studies in a non-small cell lung cancer (NSCLC) xenograft mouse model showed that about 14.4% of total cells in the tumor internalized both siRNA and PTX after administration of dual drug-loaded PEG-Peptide-PEI-PE micelles, which was about 2.4-fold higher than that for MMP-2 insensitive counterpart. Similarly, MMP-2 triggered cleavage of the peptide linker in the mixed micelles based on PEG2000-peptide-PTX, TAT-PEG1000-PE and PEG1000-PE not only liberated the active drug PTX but also exposed the hidden TAT for effective cell internalization, resulting in enhanced in vitro and in vivo anticancer activity (Fig. 2) [37]. Chen et al. modified biodegradable PEG-b-PCL nanoparticles with MMP-2/9 activatable low molecular weight proteome (ALMWP, E10-PLGLAG-VSRRRRGGRRGR) for enhanced glioblastoma therapy, in which the positive charges on the LWMP necessary for transduction were masked by a polyanionic peptide (E10) via a MMP-2/9-cleavable PLGLAG peptide linker [40]. The half-maximal inhibitory concentration (IC₅₀) of PTX-loaded ALMWP nanoparticles was 3–4 times lower than that of Taxol® and PTX-loaded PEG-b-PCL nanoparticles. The in vivo studies in nude mice bearing intracranial C6 glioma showed that PTX-loaded ALMWP nanoparticles accumulated in the glioma and significantly extended the survival time of mice as compared with Taxol® and MMP-insensitive LWMP nanoparticles. Kopecek et al. developed MMP-responsive N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-CXCR4 antagonist (BKT140) conjugate (P-BKT140) by reversible addition fragmentation chain transfer (RAFT) copolymerization of HPMA and MA-GGGLG-PLGLAG-BKT140 (MA: methacryloyl) [41]. The in vitro results showed that P-BKT140 had a similar cytotoxicity to free BKT140 against PC-3 human prostate carcinoma cells and substantially higher inhibition of CXCL12-induced cell migration than free BKT140.

The high activity of hyaluronidase in metastatic human melanoma, colon carcinoma, and glioblastoma cell lines, but not in normal tissues presents a unique tumor microenvironment for developing hyaluronidase-responsive nanocarriers. Na et al. reported that PTX-loaded degradable cathionic nanogels (DpNG-PTX) based on acylated pullulan and low molecular weight PEI (1.8 kDa) following coating with hyaluronic acid (HA) restored their cathionic charge at the tumor site due to degradation of HA by the over-expressed hyaluronidase in the heterogeneous tumor, exhibiting deep tumor penetration and high antitumor effect [42]. MTT studies in heterogeneous cancer cells (CT-26, CT-26/MDR and stromal fibroblasts cells) showed that the IC₅₀ value of DpNG-PTX was 100 times lower than that of free PTX. The in vivo experiments in Balb/c mice demonstrated that HA/DpNG-PTX exhibited markedly increased antitumor efficacy compared to non-HA coating counterpart. Interestingly, the fluorescence and histological images revealed that the
invasive distance and amount of HA/DpNG-PTX localized within the deep tissue regions both increased two times than those observed for non-HA coating counterpart. Dong et al. reported that a self-assembled complex (HDB) consisting of DOX, DNA, cationic gelatin, and human serum albumin removed its shell in response to gelatinase and Dnase I in the tumor microenvironment, resulting in increased DOX accumulation in the tumor and reduced its deposition in the heart [43].

**Endo/lysosomal pH-responsive nanotherapeutics**

Nanotherapeutics are frequently internalized by tumor cells through endocytosis. Given a low pH of 4.5–6.8 in the endo/lysosomal compartments, pH-responsive polymeric nanotherapeutics have appeared as a valuable platform to accomplish triggered intracellular drug release as well as enhanced endosomal escape. It should be noted, however, that the endosome and lysosome of all cells, either tumor cells or normal cells, are acidic, which requires high tumor-targetability and selective tumor cell uptake in order to prevent the potential side effects. There are a couple of review papers on design and development of pH-responsive polymeric nanotherapeutics [44–47]. Here, we highlight only recent advancement of pH-responsive nanotherapeutics.

One of the popular strategies used in the design of endo/lysosomal pH-responsive nanotherapeutics is to employ pH-sensitive polyelectrolytes. The rapid shift in ionization under endo/lysosomal pH might alter the molecular state and hydrodynamic diameter of the polymer chains, leading to destabilization or dissociation of nanotherapeutics and fast intracellular drug release. In the past years, pH-responsive nanotherapeutics have been designed based on polyanions containing carboxylic acid or sulfonamide groups as well as polycations like PHIs containing imidazole pendant groups and poly(β-amino ester) (PAE) with tertiary amine groups [47]. Novel pH-sensitive PAE-based poly(1,4-butenediol)-diacrylate-b-5-PEI)-block-poly[(1,4-butenediol)-diacrylate-b-5-hydroxy amine) (PDP-PDHA) copolymer was reported to form pH-sensitive micelles that could load DOX in the PDHA core and compress the surviving gene shSur using the PDP shell [48]. The drug release from PDP-PDHA nanoparticles was accelerated by acidic pH (30.7% at pH 7.4 versus 68% at pH 5.2 in 72 h). CLSM confirmed enhanced endo/lysosomal escape. In the MCF-7/ADR tumor-bearing mice models, nanotherapeutics based on PDP-PDHA raised the tumor accumulation of DOX and shSur by 10.4 and 20.2 folds, respectively, resulting in a tumor inhibiting rate of 95.9%. The combination of DOX and RNA interference shSur showed synergistic effect on overcoming MDR. cRGDYK-conjugated pH-sensitive micelles based on poly(2-ethyl-2-oxazoline)-poly(D,L-lactide) (PEOz-PLA) and cRGDyK-PEOz-PLA exhibited rapid release of PTX at endo/lysosomal pH and enhanced cytotoxicity to PC-3 prostate cancer cells [49]. The in vivo antitumor efficacy studies in PC-3 xenografts revealed that PTX-loaded cRGDyK-conjugated pH-sensitive micelles exhibited the most efficient inhibition of tumor growth without significant loss of body weight among the non-targeted counterpart, Taxol® and PBS groups. DOX-loaded cisplatin crosslinked pH-sensitive nanoparticles were prepared from dextran-succinic acid conjugate (Dex-SA) [50]. The in vitro release studies showed that DOX-HCl was released from the nanoparticles in a controlled and pH-dependent manner. Hammond et al. developed pH-responsive polymerosomes based on PEG-poly(γ-propargyl L-glutamate) copolymers with pendant tertiary amine groups (PEG-PPLG-diisopropylamine/diethylamine) that were stable at pH 7.4 but destructed spontaneously at pH 5.5 due to the protonation of pendant amine groups after cellular uptake [51]. In vivo studies in triple-negative luciferase-expressing...
MDA-MB-468 breast cancer xenografts in mice revealed that DOX-loaded pH-responsive polymersomes effectively suppressed tumor growth (relative tumor volume was 0.95 and 1.26 at day 10 and day 20, respectively). Lee et al. prepared herceptin-conjugated pH-sensitive nanoparticles from poly(d, l-lactide-co-glycolide)-Phis-PEG (PLGA-Phis-PEG) triblock copolymer [52]. The in vitro release studies showed that 80% and 60% of DOX was released in 8 h at pH 5.2 and 6.4, respectively, due to protonation of Phis in the acidic condition. DOX-loaded herceptin-conjugated pH-sensitive nanoparticles showed better in vitro cytotoxicity towards MCF-7 and SK-BR-3 cells than the corresponding non-sensitive control.

The second strategy is to incorporate acid-labile linkages such as hydrazone, acetal, ketal, cis-acetonil, oxime, and orthoester in the main chains or side chains. We obtained galactose-installed photo-crosslinked pH-sensitive degradable micelles (Gal-CLMs) from PEG-b-poly(2, 4, 6-trimethoxybenzylidene-pentaerythritol carbonate-co-acryloyl carbonate) (PEG-b-PtMBPEC-co-AC) and Gal-PEG-b-PCL copolymers for active targeting chemotherapy of hepatocellular carcinoma in mice [53]. The release of PTX from PTX-loaded Gal-CLMs while inhibited at physiological pH was accelerated at pH 5.0, due to the hydrolysis of acetal in the core. The in vivo studies in human hepatoma SMMC-7721 tumor-bearing nude mice displayed that Gal20-PTX-CLMs (PTX-loaded Gal-CLMs with 20% Gal decoration) resulted in significantly enhanced drug accumulation in the tumors and much greater tumor growth inhibition than PTX-CLM (non-targeting control) and Gal20-PTX-NCLM (non-crosslinking control) (average tumor volume: ca. 35 mm³ versus 144 mm³ and 130 mm³, respectively). Histological analysis showed that Gal20-PTX-CLMs induced extensive apoptosis of tumor cells with little damage to normal liver and kidney. These results demonstrate that active targeting core-crosslinked pH-responsive degradable micelles can not only enhance therapeutic efficacy of anticancer drugs but also largely decrease their side effects. Li et al. prepared a series of pH-sensitive nanocarriers based on the acetalted α-cyclodextrin (Ac-aCD) [54]. In vitro and in vivo studies revealed that PTX-loaded Ac-aCD nanoparticles were capable of reversing the MDR of PTX-resistant MCF-7 and MDA-MB-231 cancer cells. We recently prepared pH-sensitive micelles from PEG-poly(acetal urethane)-PEG triblock copolymer with multiple acetal bonds in the main chain, in which ca. 96%, 73%, and 30% of drug was released within 48 h at pH 4.0, 5.0, and 7.4, respectively [55]. Micelles based on ketal-containing poly(ketal adipate)-PEG (PKA-PEG) block copolymer were found rapidly dissociated at pH 5.4 leading to triggered release of camptothecin (CPT) as well as effective endosomal disruption [56]. Acid degradable nanogels prepared via precipitation polymerization of poly(vinylcaprolactam) (PVCL), HHPMA, and ketal-containing 2, 2-dimethacroyloxy-1-ethoxypropane (DMAEP) as a crosslinker (PVCL-ketal-HHPMA) exhibited a rapid and nearly complete DOX release at pH 5.0 while only about 13% of DOX was released at pH 7.4 [57]. MTT assays showed that DOX-loaded PVCL-ketal-HHPMA nanogels were significantly more potent against HeLa cells than pH-insensitive controls (IC50: 0.55 versus 2.87 mg/mL). Orthoester linkages are more sensitive towards acid-catalyzed hydrolysis than acetal and ketal linkages. Du et al. reported that DOX-loaded pH-sensitive amphiphilic sugar poly(orthoesters)-b-PEG nanoparticles released 72% of DOX in 1 h and nearly 100% in 4 h at pH 5.0 while less than 20% of DOX was released at pH 7.4 in 6 h [58]. The cell viability studies exhibited significant pH-dependent cytotoxicity. Wang et al. found that DOX-loaded pH-responsive micelles self-assembled from amphiphilic PEG-b-polymethacrylate diblock copolymer bearing acid-labile ortho ester side chains released DOX at a much higher rate at pH 5.0 compared as pH 7.4 [59]. MTT assays in T98G human glioma cells showed that DOX-loaded pH-sensitive micelles had IC50 values approximately three to ten-times lower than free drug.

The third strategy involves chemically conjugating drugs to polymer backbone via an acid-labile linkage. In comparison with nanosystems with physical loading of drugs, these acid-activatable prodrugs have the advantages of high stability and minimal drug leakage. Hydrazone bond (Hyd) has often been used to construct acid-activatable prodrugs. pH-Responsive disulfiram (DSF)-loaded DOX prodrug micelles (DSM) were prepared from poly(styrene-co-maleic anhydride) (SMA)-hydrazone-DOX conjugates [60]. DSM enabled a temporal release of two drugs: encapsulated DSF was quickly released to inhibit the activity of P-gp and restore cell apoptotic signaling pathways, while the conjugated DOX was released in a sustained and pH-dependent manner and accumulated in cancer cells to exert therapeutic effect. These temporal-controlled and pH-sensitive nanotherapeutics exhibited effective combination therapy of DOX and DSF for drug-resistant MCF-7/ADR breast tumor xenografts. The mice treated with DSM had 12.8-fold smaller tumor volume and significantly higher tumor inhibition rate than those with the mixture of free DOX and DSF. Kataoka et al. developed pH-sensitive polymeric micelles (MG132/m) from PEG-b-poly(hydrazinyl-aspartamide-MG132) conjugates containing hydrazine linkage between polyaspartate backbone and MG132 for effective delivery of MG132 (proteasome inhibitor) into cancer cells (Fig. 3A) [61]. Real-time in vitro confocal microscopy experiments indicated that MG132 prodrug micelles were destructed only inside the HeLa-GFP cells target cells (Fig. 3B). In vivo toxicity studies exhibited that MG132/m had a significant increased therapeutic window with 4-fold higher maximum tolerated dose (MTD) than that of free MG132. MG132/m displayed significant antitumor effect against a subcutaneous HeLa-luc tumor model at a drug dosage of 16, 32 or 64 mg/kg, while free MG132 at a dosage of 16 mg/kg caused significant side effects as revealed by severe body weight loss or death of mice. The whole body fluorescence imaging studies in the HeLa-luc tumor-bearing mouse revealed strong fluorescence at the tumor at 24 h following i.v. injection of Alexa 647-labeled MG132/m. The biodistribution of Alexa 647-labeled MG132/m measured by the ex vivo fluorescent image showed that the fluorescence of Alexa 647 in the tumor was significantly higher than in the healthy organs such as spleen, liver, heart and kidneys. Dendronized heparine-Hyd-DOX conjugate nanoparticles were shown to release over 80% of DOX at pH 5.0 while only 20% at pH 7.4 in 56 h [62]. In vivo therapeutic studies and histological analyses demonstrated that dendronized heparine-Hyd-DOX nanoparticles had high antitumor activity in 4T1 breast tumor model and low side effect compared with the non-sensitive control. Zhou et al. reported that folic acid (FA)-functionalized
PEG-PCL-hyd-DOX micelles showed good stability at pH 7.4, while quick drug release at pH 5.0 (drug release in 10 h: 26% versus 70%) [63]. In vivo pharmacokinetics and biodistribution studies indicated that these FA-PEG-PCL-hyd-DOX micelles significantly prolonged the blood circulation time of DOX and enriched DOX into the tumors. In vivo antitumor activity in the BALB/c mice bearing 4T1 tumor demonstrated that FA-PEG-PCL-hyd-DOX micelles had the best safety and therapeutic efficacy compared with all the controls.

In addition to hydrazone linkage, other acid-labile bonds such as acetal and ı-thiopropionate have been employed for the design of anticancer prodrugs. For example, endosomal pH-activatable PTX prodrug micellar nanoparticles were prepared by conjugating PTX onto water-soluble PEG- \( b \)-poly(acrylic acid) (PEG-PAA) block copolymers via an acetal bond to the PAA block [64]. The release of PTX was found highly pH-dependent, in which ca. 86.9%, 66.4% and 29.0% of drug was released at 37°C in 48 h at pH 5.0, 6.0, and pH 7.4, respectively. MTT assays showed that these acid-activatable PTX prodrug nanoparticles had a high antitumor effect to KB and HeLa cells as well as PTX-resistant A549 cells. Wooley et al. prepared acid-activatable PTX prodrug nanoparticles with a hydrodynamic diameter of 114 ± 31 nm from poly(ethylene oxide)-block-polyphosphoester-graft-PTX (PEO-\( b \)-PPE-g-PTX), in which PTX was conjugated to PPE via the ı-thiopropionate linkage with a high drug loading content of 53 wt.% [65]. PEO-\( b \)-PPE-g-PTX nanoparticles displayed a pH-triggered drug release property and 5- to 8-fold enhancement in the in vitro antitumor activity against OVCAR-3 and RAW 264.7 cells.

**Glutathione-responsive nanotherapeutics**

The high reductive environment in the cytosol and cell nucleus is mainly due to the presence of glutathione tripeptide, \( \gamma \)-glutamyl-cysteinyl-glycine (GSH), which is the most abundant low molecular weight biological reducing agent and is kept reduced by NADPH and glutathione reductase. The reducing potential inside the cancer cells is about 100–1000 times higher than that in the extracellular spaces and blood pool. In recent years, reduction-bioresponsive nanoparticles have appeared as one of the most promising systems to achieve targeted cytosolic drug release [66-70]. As compared with pH-sensitive counterparts, reduction-sensitive nanotherapeutics have several unique advantages...
such as high stability against hydrolytic degradation, fast response to intracellular reducing environment, and triggering drug release right in the cytosol and cell nucleus where many anticancer drugs take effects.

The simplest reduction-responsive nanocarriers are shell-sheddable micelles that contain an intervening disulfide bond between hydrophilic (like PEG and dextran) and hydrophobic blocks such as polypeptides [71], polyanhydrides [72], polyesters [73], hexadecyl [74] and small hydrophobic molecules [75]. Atorvastatin calcium (Ator)-loaded reduction-responsive shell-sheddable micelles (ASM) were prepared with a high drug encapsulation efficiency of 99.09% from mPEG-SS-vitamin E succinate (mPEG-SS-VE-S) [75]. In vivo biodistribution studies showed that Ator content in lung and tumor was 13.5 and 8.2-fold higher for ASM than for free drug at 12 h post-administration. In vivo studies in a 4T1 orthotopic mammary tumor metastasis cancer model demonstrated that ASM could completely block the lung and liver metastasis of breast cancer with minimal toxicity owing to enhanced Ator accumulation in tumor and lung. Galactose-decorated shell-sheddable micelles based on PEG-SS-PCL and galactose-PEG-PCL (Gal-PEG-PCL) block copolymers were shown to release more than 75% of DOX in 12 h under a reducing condition containing 10 mM dithiothreitol (DTT) while little DOX was released under a non-reducing condition [76]. Flow cytometry revealed that cellular DOX level in HepG2 cells treated with DOX-loaded PEG-SS-PCL/Gal20 micelles (formed from PEG-SS-PCL and 20 wt.% of Gal-PEG-PCL copolymers) was much greater than that with reduction-insensitive PEG-PCL/Gal20 and non-targeting PEG-SS-PCL controls, signifying the importance of combining shell-shedding and active targeting. FA-functionalized shell-sheddable micelles based on 4-arm PEG-SS-PCL copolymer displayed a much better specificity and therapeutic efficiency in 4T1 tumor-bearing BALB/c mice [77]. Hepatoma-targeting shell-sheddable chimaeric biodegradable polymersomes were designed and developed based on Gal-PEG-PCL, PEG-PCL-poly(2-(diethylamino)ethyl methacrylate) (PEG-PCL-PDEA, asymmetric), and PEG-SS-PCL for facile loading and triggered intracellular delivery of proteins [78]. Remarkably, MTT assays revealed that the maximal activity toward HepG2 cells with a markedly low IC₅₀ of 2.7 nM. Disulfide-linked glycol nanoparticles (SS-GNs) with sheddable saccharide shells were obtained from PCL-graft-SS-lactobionic acid (PCL-g-SS-LBA) copolymer [79]. MTT assays showed that DOX-loaded SS-GNs exhibited a high antitumor activity toward HepG2 cells, which was comparable to free DOX and about 18-fold higher than their reduction-insensitive counterparts, while blank SS-GNs were nontoxic up to a treatment concentration of 1.0 mg/mL. Zhang et al. reported that DOX-loaded pullulan(SS)-cholesterol nanoparticles exhibited significantly better antitumor effect and biosafety in the nude mice bearing hepatocellular carcinoma than DOX-HCl [80].

The second approach involves position of multiple disulfide bonds in the main chain of the hydrophobic polymer or conjugation of drugs to the side chain of the polymer through multiple disulfide linker. Kataoka et al. reported that reduction-sensitive micelles (CPT/m) prepared from PEG-PLL-SS-CPT conjugates had prolonged blood circulation and high accumulation in xenografts of AY27 rat urothelial carcinoma [81]. The real-time confocal laser microscopies showed that with the induction of endosomal permeabilization using the clinically approved photosensitizer, Photofrin, CPT/m escaped from the endocytic vesicles of cancer cells into the cytosol, accelerating the drug release from the micelles only in the irradiated tissues. This spatiotemporal switch significantly enhanced the in vivo antitumor efficacy of CPT/m without eliciting any toxicity, even at a dose 10-fold higher than the maximum tolerated dose of free CPT. Chilkoti et al. reported that reduction-sensitive polymeric CPT produg obtained by living ring-opening polymerization (ROP) of drug-conjugated carbonate monomers induced greater cytotoxicity than free drug [82]. We developed DOX-loaded enzymatically and reductively degradable nanoparticles from α-amino acid-based poly(ester amides) (SS-PEAs) which had more than 10 times higher in vitro antitumor activity than free DOX in drug-resistant MCF-7/ADR cells [83]. Reduction-sensitive triblock copolymer micelles based on PEG-b-poly(disulfide urethane)-b-PEG were shown to efficiently transport and release DOX into the perinuclear and nuclear regions of MCF-7/ADR cells [84].

The third approach to construct reduction-responsive nanotherapeutics is to crosslink the polymeric nanoparticles with disulfide bonds, which renders nanotherapeutics particularly stable during circulation while prone to quick de-crosslinking in the cytosol. This reversible crosslinking strategy has shown to elegantly resolve the extracellular stability and intracellular drug release dilemma. Lee et al. reported that shell-crosslinked biodegradable micelles based on PEG-Cys₉-PDLLA copolymer stably retained DOX during circulation and delivered 7-fold higher drug to the tumor while 1.9-fold lower in the heart as compared with the non-crosslinked PEG-b-PDLLA micelles [85]. DOX-crosslinked PEG-Cys₉-PDLLA micelles almost completely inhibited M109 tumor growth in mice within 14 days after initial treatment with 2 mg DOX/kg at day 0 and 4. Park et al. prepared bioreducible shell-crosslinked HA nanoparticles based on HA-b-PCL block copolymers, in which the HA shell was cross-linked via disulfide linkages (Fig. 4) [86]. The premature drug release was greatly inhibited while quick drug release was achieved in the presence of 10 mM GSH. Cell experiments showed that HA-b-PCL nanoparticles were rapidly taken up by SCC7 cancer cells through CD44 receptor-mediated endocytosis. The ex vivo quantification of Cy5.5-labeled HA nanoparticles fluorescence exhibited comparably stronger DOX fluorescence at the tumor than in other organs (such as liver, lung, spleen, kidney and heart). In vivo studies in SCC7 tumor-bearing mice showed that DOX-loaded shell-crosslinked nanoparticles were the most effective in suppressing tumor growth among all the treatments (including free DOX and non-crosslinked controls). Reduction-sensitive reversibly crosslinked HA nanoparticles were obtained from HA—Lys—LA10 nanoparticles (Lys: L-lysine methyl ester; LA: lipoic acid) [87]. The in vivo pharmacokinetics and biodistribution studies in tumor xenografts in nude mice showed that DOX-loaded crosslinked HA—Lys—LA nanoparticles had a prolonged circulation time and a remarkably high accumulation in the tumor (12.71% ID/g). Notably, DOX-loaded crosslinked HA—Lys—LA nanoparticles caused effective inhibition of MCF-7/ADR tumor growth and increase of survival rate. Disulfide-crosslinked
nanogels were formed in situ from water-soluble PEG-b-poly(2-(hydroxyethyl) methacrylate-co-acryloyl carbonate) (PEG-b-P(HEMA-co-AC)) block copolymers in phosphate buffer in the presence of cystamine via ring-opening reaction with cyclic carbonate groups [88]. These reduction-sensitive nanogels had remarkable loading contents (up to 48.2%) and loading efficiencies (up to 98.2%) of cytochrome C (CC). The in vitro release studies showed that FITC-CC release was minimal under physiological conditions but significantly enhanced in the presence of 10 mM DTT with about 96.8% of FITC-CC released in 22 h. CC-loaded reductively degradable nanogels demonstrated apparently better apoptotic activity than free CC as well as reduction-insensitive controls. Jiang et al. reported that bioreducible nanogels prepared from copolymerization of heparin (HEP)-methacrylate derivative and cystamine bisacrylamide had a high DOX loading content of 30 wt.% and a high loading efficiency of 90% [89]. In vivo near infrared fluorescence imaging of a hepatic H22 tumor-bearing mouse and ex vivo DOX concentration measurements demonstrated that DOX accumulation at tumor site was 9.3% ID/g.

Lysosomal enzyme-responsive nanotherapeutics

Lysosomes are membrane-bound vesicles that contain abundant digestive enzymes, such as glycosidases, proteases and sulfatases [90]. Kopecék, Duncan, and Ulbrich have systematically studied PHPMA copolymer-anticancer drug conjugates with lysosomally cleavable peptide spacers like GFLG that is susceptible to cathepsin B [91]. These lysosomal enzyme-responsive nanotherapeutics are stable during circulation while release drug under the action of...
specific enzyme. For instance, Kopecek et al. reported that dual enzyme-responsive HPMA copolymer-DOX conjugates (P-DOX-PLGLAG-IRGD), in which DOX was conjugated to HPMA copolymer via FGLP peptide and IRGD was linked to HPMA copolymer via PLGLAG peptide, exhibited enhanced tumor accumulation, penetration and cytotoxicity in 2D and 3D prostate cancer cells [92]. Gu et al. reported that mPEGylated dendron-GFLG-DOX conjugate nanoparticles had much faster drug release in the presence of papain (similar to lysosomal cathepsin B) than the non-sensitive control under otherwise the same conditions (80% versus 30% drug release in 15h) [93]. These enzyme-sensitive prodrug nanoparticles could accumulate into tumor and retain for a long time as revealed by fluorescence images, and caused improved proliferation inhibition against 4T1 murine breast cancer model in vivo [94].

**ROS-responsive nanotherapeutics**

Reactive oxygen species (ROS) play important roles in a variety of physiological and pathological processes. ROS are produced from several endogenous sources, notably in the mitochondria from an incomplete reduction of oxygen and NADPH oxidase (NOX) in the plasma membrane. A moderate level of ROS is involved with normal cell functions, excessive amounts of ROS cause oxidative stress and damage critical components of cells at all levels including DNA, proteins, and lipids by oxidation. ROS production has been implicated in important pathophysiological events, such as atherosclerosis, aging, and cancer [95, 96]. Hence, ROS-responsive delivery systems are promising for selectively delivering drugs to diseased sites by targeting oxidative microenvironments at different levels. ROS-responsive nanocarriers have been designed to have the ability of triggered hydrophilic/hydrophobic switch or cleavage of the ROS-sensitive bonds in the polymer chain by virtue of imbalanced ROS activity in tumor cells, which destructs the nanocarriers and facilitates drug release in the tumor cells. Yan and Wang prepared ROS-responsive nanocarriers from amphiphilic hyperbranched polyphosphates consisting of alternative hydrophobic selenide groups and hydrophilic phosphate segments in the dendritic backbone [97]. These nanocarriers were disassembled after oxidation under the exclusive oxidative microenvironment within cancer cells due to transformation of hydrophobic selenide groups into hydrophobic selenene groups, resulting in fast intracellular DOX release. Incorporating oxidation-labile groups such as boronic ester or aryloboronic esters into polymer chains is another strategy for preparation of ROS-responsive nanotherapeutics. Zhang et al. reported ROS-responsive nanoparticles fabricated from 4-phenylboronic acid pinacol ester (PBAP) conjugated β-cyclodextrin (Ox-b-CD) [98]. At 1.0 mM H2O2, docetaxel (DTX) could be completely released from DTX-loaded Ox-b-CD nanoparticles within 4h, while only 21% of total drug was released in the absence of H2O2. Notably, ROS-responsive Ox-b-CD nanoparticles were shown to dramatically enhance the apoptotic activity of DTX against B16F10, HepG2 and MDR MDA-MB-231 cells. In vivo antitumor studies in B16F10 melanoma-bearing nude mice confirmed that the antitumor potential of DTX was greatly enhanced with Ox-b-CD nanoparticles. Guo et al. reported a H2O2-responsive nanocarriers based on PLGA nanoparticles that combined catalase and platinum anti-cancer agents together and simultaneously release platinum drug and O2 triggered by a biologically relevant concentration of H2O2 [99]. These H2O2-responsive PLGA nanoparticles could release cisplatin promptly into SGC-7901 and A549 MDR cells and overcome the MDR effect, due to that O2 generated by the catalysis of catalase under intracellular H2O2 concentration caused the shell rupture of nanoparticles followed unloading the cisplatin drug and O2 produced in the drug release process can be helpful for overcoming hypoxia-induced MDR and enhancing the efficiency of cancer chemotherapy. CA-loaded hydrogen peroxide-responsive SN38 produg micelles (HPG-2S-SN38) (CA: cinnamaldehyde; HPG: hyperbranched polyglycerol; SN 38: 7-ethyl-10-hydroxy-camptothecin) showed a H2O2 concentration-dependent drug release behavior in vitro [100]. In the presence of 1 mM H2O2, ca.70% CA and 60% SN 38 were released in 12 h, while low drug release (CA < 30%, SN38 < 20%) was observed even after 48 h in the absence of H2O2 under otherwise the same conditions. The released CA could effectively induce intracellular ROS production, which in turn accelerated the degradation of micelles and the release of SN38.

**Dual-bioresponsive nanotherapeutics**

In recent years, dual-responsive nanocarriers have received growing attention as the combination of two different stimuli might significantly enhance response rate and achieve fast and complete drug release into the cancer cells. In addition, dual-responsive nanocarriers might simultaneously solve issues of in vivo stability, tumor cell uptake, tumor penetration, and intracellular drug release, achieving enhanced tumor targetability and therapeutic efficacy [101]. Here, we emphasize recent design and development of dual-bioresponsive nanotherapeutics.pH and redox were the most used two stimuli which take place either simultaneously at the pathological site or in a sequential manner from nanoparticle preparation, nanoparticle transporting pathways, to cellular compartments. For example, Liu et al. prepared pH and reduction dual-responsive polyplex micelles from PEG-poly[(N′-dimethylmaleoyl-2-aminoethyl)aspartamide] (PEG-Pasp(EDA-DM)) and platinum(IV)-conjugated cationic poly(amidoamine) (PAMAM–Pt(IV)) dendrimer prodrugs [102]. These polyplex micelles were disassembled at tumor pH (~6.8) due to conversion of negatively charged PEG-Pasp(EDA-DM) copolymer into positively charged PEG-PaspEDA. The collagen gel diffusion and multicellular tumor spheroids (MCTS) penetration experiments verified efficient penetration into the dense tumor tissue as a result of micelle dissociation and consequently releasing PAMAM–Pt(IV) prodrugs at tumor pH. The positively charged PAMAM–Pt(IV) prodrugs exhibited high cellular uptake. The IC50 value of polyplex micelles at pH 6.8 was observed to be 88 times lower than cisplatin against drug resistant A549R cells. The growth inhibition studies in the cisplatin-resistant human lung cancer A549R MCTS revealed that dual-responsive polyplex micelles exerted a potent inhibition on the growth of MCTS at pH 6.8 with the
diameter of MCTS decreased from 300 μm to less than 100 μm at 7 days post administration. Zhou et al. constructed pH and reduction dual-responsive micelles from mPEG-PLA-SS-PEI/FA-DMA (PELE/FA-DA) terpolymer (DMA or DA: 2,3-dimethylmaleic anhydride) [103]. PELE/FA-DA micelles that are negatively charged under neutral conditions could maintain their stability and prolong their blood circulation time. Once accumulated at the tumor site, they could be efficiently internalized by tumor cells due to charge reversal in response to the tumor extracellular pH and by FA-mediated endocytosis. These micelles could escape from lysosomes via a proton sponge effect and shed off PEI shells in the cytosol, actively releasing the drug to the cell nucleus. The in vivo imaging in 4T1 tumor-bearing nude mice displayed strong DOX fluorescence in tumor following 1 h injection with PELE/FA-DA micelles and DOX fluorescence continuously enhanced even at 24 h. 

In vivo antitumor studies in 4T1 tumor-bearing Balb/c mice revealed a high tumor growth inhibition rate of 90% for PELE/FA-DA micelles at day 21 with low side effects. pH and reduction dual-sensitive poly(vinyl alcohol) nanogels (CC-SS-NGs) were prepared from PVA-(SS-alkynyl)-COOH and PVA-azide by inverse nanoprecipitation and click reaction [104]. The in vitro release results showed that 95% of DOX was released in 10 h at pH 5.5 in the presence of 10 mM GSH due to decrease of electrostatic interaction between COOH and DOX and cleavage of the intervening disulfide bonds. In contrast, only ca. 23% of DOX was released in 48 h at pH 7.4. MTT assays showed that DOX-loaded CC-SS-NGs had low IC50 values of 0.32 and 0.45 μg DOX/mL for MCF-7 and HeLa cells, respectively, which were significantly lower than those obtained with the reduction-insensitive CC-NS-NGs under otherwise the same conditions (IC50 = 2.04 and 1.42 μg DOX/mL for MCF-7 and HeLa cells, respectively). pH and reduction dual-responsive crosslinked polymersomes were formed with an average size of ~35 nm from water-soluble PEG-poly(acrylic acid)-poly(2-diethyl amino)ethyl methacrylate) triblock copolymer thiol derivative (PEG-PAAC(SH)-PDEA) by increasing its solution pH to 7.8 or above followed by oxidative crosslinking [105]. Notably, polymersome formation, protein loading and crosslinking process all do not involve any organic solvents, catalysts and byproducts. These disulfide-crosslinked polymersomes, while exhibiting excellent colloidal stability, were rapidly dissociated in response to 10 mM GSH at neutral or mildly acidic conditions. MTT assays showed that CC-loaded dual-sensitive polymersomes induced potent cancer cell apoptosis at a CC dosage of 160 μg/mL with 11.3%, 8.1% and 52.7% cell viabilities for MCF-7, HeLa and 293T cells, respectively. In contrast, free CC caused no cell death under otherwise the same conditions. pH and reduction dual-sensitive reversibly core-crosslinked polypeptide micelles were developed from LA and cis-1,2-cyclohexanedicarboxylic acid (CCA) decorated PEG-b-PLL (PEG-P(L-CCA/LA)) block copolymers [106]. The release amount of DOX was doubled under endosomal pH of 5.0 compared with that under pH 7.4, most likely triggered by cleavage of the acid-labile amide bonds of CCA. Rapid DOX release was also observed under a reductive condition containing 10 mM GSH, in which 86.0% and 96.7% of DOX were released in 24 h at pH 7.4 and 5.0, respectively. pH and reduction dual-responsive biodegradable micelles were prepared from PEG-SS-poly(2,4,6-trimethoxybenzylidene-pentaerythritol carbonate) (PEG-SS-PMBPEC) block copolymer for a lowly triggered intracellular release of DOX [107]. The in vitro release studies showed that 24.5%, 62.8% and 74.3% of DOX was released in 21 h at pH 7.4, pH 5.0, and pH 7.4 in the presence of 10 mM GSH, respectively. The fastest drug release was observed under 10 mM GSH and pH 5.0 conditions, in which 94.2% of DOX was released in 10 h. Interestingly, DOX release was obviously enhanced by 2 or 4 h incubation at pH 5.0 and then at pH 7.4 with 10 mM GSH (mimicking the intracellular pathways of endocytosed micellar drugs). MTT assays in HeLa and RAW 264.7 cells revealed that DOX-loaded PEG-SS-PMBPEC micelles had higher anti-tumor activity than reduction-insensitive PEG-PMBPEC controls.

Reversibly core-crosslinked pH-responsive biodegradable micelles with a small size of ca. 58 nm were obtained from PEG-poly(TMBPEC-co-pyridyl disulfide carbonate) (PEG-P(TMBPEC-co-PDSC)) copolymers (Fig. 5) [108]. MTT assays revealed that DOX-loaded crosslinked micelles had a much higher antitumor activity than irreversibly crosslinked controls, in which low IC50 values of 1.65 and 1.14 μg/mL were observed for HeLa and RAW264.7 cells, respectively, approaching that of free DOX. Liu et al. reported that DOX-loaded PEGylated dual-responsive poly(methacrylic acid-co-poly(ethylene glycol) methyl ether methacrylate-co-N,N-bis(acryloyl)cystamine) (PMB) nanogels released more than 85% of DOX within 30 h at pH 5.0 in the presence of 10 mM GSH, which was 1.5-fold higher than that observed in the absence of GSH under otherwise the same conditions [109]. Chen et al. reported that DOX-loaded pH and reduction dual-responsive supramolecular nanogels cross-linked by disulfide bonds and host–guest interaction between dextran-graft-benzenzimidazole (Dex-g-BM) and thiol-β-cyclodextrin exhibited enhanced DOX release under acidic conditions and intracellular reduction environment [110]. pH and reduction dual-bioreactive DOX prodrug nanogels were prepared from biocompatible hyperbranched polyglycerol [111]. These dual-reactive nanogels showed very low drug leaching at physiological condition as DOX was conjugated to the polymer via an acid-labile hydrazide linker, while efficient drug release was observed at pH 5.0 and 10 mM DTT, leading to high toxicity in HeLa cells. pH and reduction dual-bioreactive PTX prodrug nanoparticles with a drug loading content of 15.6% were obtained from polypeptide-PTX conjugate (P(L-SS-PTX)) in which PTX was linked to EG-b-PLL via 3,3’-dithiodipropionic acid [112]. P(L-SS-PTX) barely released drug (less than 10%) at physiological condition while markedly accelerated drug release was observed at pH 5.0 or in the presence of 10 mM GSH. The in vivo study on B16F1 tumor bearing C57BL/6 mice showed that P(L-SS-PTX) had superior tumor inhibition effect compared to free PTX and the non-sensitive micelles at the same drug dose.

GSH/Ros-responsive SN38 prodrug nanocapsules were obtained from OEG-ZS-SN38 [113]. These prodrug nanocapsules were shown to decompose and quickly release SN38 in the presence of GSH or H2O2, leading to high in vitro cytotoxicity in human cancer cell lines and in vivo anticancer therapeutic activity in a Bcap37 breast tumor xenograft model.
Conclusion and perspectives

In recent years, bioresponsive polymeric nanotherapeutics have emerged as a most fascinating platform for targeted cancer chemotherapy. Unlike external stimuli-sensitive nanosystems, bioresponsive nanotherapeutics do not require any external device which would not only reduce the treatment cost but also significantly improve patient compliance. Moreover, as many of the biosignals are unique to tumor or cancer cells, bioresponsive polymeric nanotherapeutics offer precision control over site, rate, and time of response. The in vitro and in vivo preclinical studies from different research groups have demonstrated that bioresponsive polymeric nanotherapeutics bring about superior therapeutic efficacy with decreased side effects as compared to the non-responsive counterparts as well as current clinical formulations.

It should be noted, however, that there remain several challenges for bioresponsive polymeric nanotherapeutics to finally enter human clinical trials. First of all, bioresponsive polymeric nanotherapeutics are typically based on novel polymers and require involved production that give rise to potential safety issues. Second, most bioresponsive polymeric nanotherapeutics show a slow response to the biosignals in the target site, leading to gradual and incomplete drug release and thereby reduced treatment efficacy. We found that nanostructures significantly impede the accessibility of biosignals including acid, enzyme, and glutathione, which is an important account for the observed lower bioresponsivity than as had expected. Third, many of the bioresponsive polymeric nanotherapeutics similar to their non-responsive counterparts exhibit a low stability and premature drug release in circulation which causes not only decreased therapeutic efficacy but also increased side effects in vivo. Last but not the least, bioresponsive polymeric nanotherapeutics like a double-edged sword if delivered to the wrong spot (e.g. healthy organs and cells) might cause more significant detrimental effect than their non-responsive counterparts. For instance, low pH is present in the endo/lysosomal compartments and high GSH concentration in the cytosols of all cells, no matter it’s cancerous cells or healthy cells. Hence, it is of critical importance that bioresponsive polymeric nanotherapeutics are selectively delivered to the target tumor cells.

In order to be applied in the clinics, bioresponsive polymeric nanotherapeutics have to be fabricated from simple and well-established biocompatible and non-immunogenic materials such as natural polysaccharides, polyesters, polycarbonates, polypeptides, PEG, albumin, and small natural compounds (e.g. amino acids, lactic acid, cholesterol, vitamin C, etc.) with as little modification as possible. The nanotherapeutics currently applied in the clinics (e.g. Doxil, Marqibo, and Abraxane) or under the clinical trials (e.g. BIND-014, Genexol-PM, NK105, and NC-6004) are all
based on materials like lipids, albumin, PEG-poly(glutamic acid), PEG-polyaspartate, PEG-PLA and PEG-PLGA. It is clear that nanotherapeutics based on novel and complex materials, which unfortunately represent a majority of research in the field as driven by the grants and publications, have difficulty in obtaining approval from the Food and Drug Administration (FDA). On the other hand, bioreponsive polymeric nanotherapeutics should be equipped with multifunction that includes high drug loading content and efficacy, excellent in vivo stability, long circulation time, decent tumor accumulation, and active targeting properties, therefore outperforming their non-responsive counterparts in the treatment of patients. It is a challenging task to design and develop bioreponsive polymeric nanotherapeutics that are multifunctional and yet simple. Encouraged by the promising in vivo preclinical results on several tumor models, we in collaboration with a local pharmaceutical company in Suzhou are endeavor to translate CD44-targeted nanotherapeutics based on reduction-sensitive reversibly crosslinked hyaluronic acid-l-lysine-lipoic acid nanoparticles to the human clinical trials. It should be noted that the clinical success of bioreponsive polymeric nanotherapeutics, as for the other nanosystems, is also intimately dependent on their surface and physicochemical properties such as size, shape, surface charge, type and density of targeting ligands as well as tumor type, characteristics and microenvironment. As a matter of fact, the clinical failure of many nanomedicines is partly due to wrong selection of malignancies. In this sense, it is of critical importance that brilliant oncologists are involved in the design and development of nanomedicines right from the beginning. In the case of active targeting chemotherapy, the therapeutic efficacy of nanotherapeutics highly depends on the amount, availability and specificity of targeting ligands on the nanocarriers, which differ with different ligands, nanosystems and pathologies. It is also possible that the best drug formulations from the preclinical studies turn out to be not optimal for the human patients, which makes translational nanomedicine research particularly challenging. We shall further notice that the reason why the authorities are “unwilling” to endorse new nanomedicines is simply that current systems have not conferred striking treatment benefits in the clinical trials. In order to gain accelerated FDA approval, we have to create “miracle” nanotherapeutics that bring about a breakthrough in life-threatening cancer therapy. We are convinced that with rational design and continuing studies, bioreponsive polymeric nanotherapeutics will be developed for the effective treatment of various cancers in the future.

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References


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