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Intracellular drug release nanosystems

In order to elicit therapeutic effects, many drugs including small molecule anticancer drugs, proteins, siRNA, and DNA have to be delivered and released into the specific cellular compartments typically the cytoplasm or nucleus of target cells. Intracellular environment-responsive nanosystems that exhibit good extracellular stability while rapidly releasing drugs inside cancer cells have been actively pursued for effective cancer therapy. Here, we highlight novel designs of smart nanosystems that release drugs in response to an intracellular biological signal of cancer cells such as acidic pH in endo/lysosomal compartments, enzymes in lysosomes, and redox potential in cytoplasm and the cell nucleus.

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Nanoscale drug delivery systems such as liposomes, polymeric micelles, polymersomes, nanogels, and nanocapsules have emerged as an indispensable platform for modern cancer therapy[1-3]. For example, liposomal doxorubicin (Doxil®, Caelyx® and Myocet®) is routinely used in clinical settings for treating various forms of cancer including advanced ovarian cancer, breast cancer, multiple myeloma, and Kaposi's sarcoma[4,5]. Micellar paclitaxel (Genexol-PM®) has been approved for the treatment of breast, lung, and ovarian cancers in South Korea[6]. While several other micellar anticancer drug formulations (e.g., NK911®, NK105®, and NC6004®) have entered different phases of clinical trials[7,8]. These nanoscale drug formulations have demonstrated several merits, such as prolonged circulation time, better pharmacological profiles, decreased adverse effects and improved drug tolerance, over conventional clinical approaches. It should be noted, however, that most nanoscale drug formulations suffer slow and deficient drug release at the pathological site, which usually results in compromised treatment benefits in clinics. It is noted that in order to elicit therapeutic effects, many drugs including small molecule anticancer drugs, proteins, siRNA, and DNA have to be delivered and released into specific cellular compartments, typically the cytoplasm or nucleus of target pathological cells[9,10]. In recent years, significant effort has been dedicated to the development of intracellular environment-responsive nanosystems that are sufficiently stable under extracellular conditions (e.g., in blood circulation) while rapidly releasing drugs following uptake by the target cancer cells, leading to markedly enhanced cancer therapy.

The most obvious and spectacular intracellular environments of cancer cells are their slightly lower pH in the endo/lysosomal compartments, abundant enzymes in the lysosomes, and high redox potential in the cytosol and cell nucleus (Fig. 1). These naturally existing milieus have been actively exploited as the internal stimuli to dictate drug release from nanosystems inside cancer cells[11,12]. Notably, these intracellular

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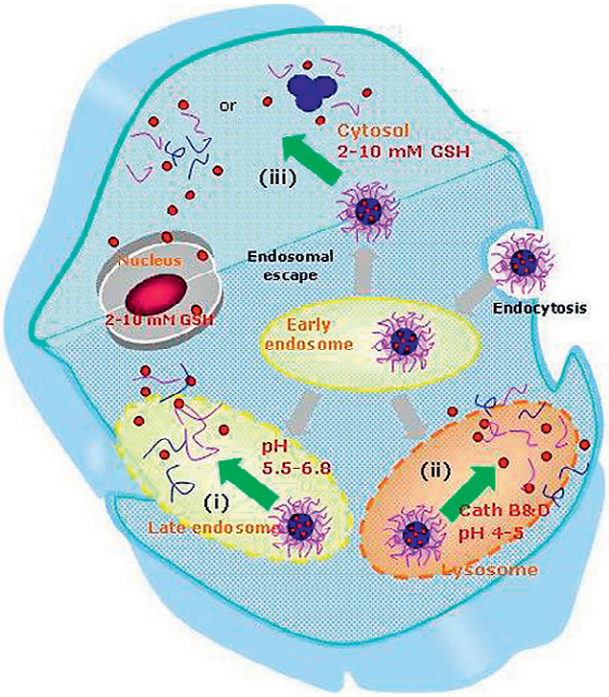

Fig. 1 Illustration of intracellular environment-responsive nanosystems for "active" drug release inside cancer cells. The release of drugs can be accomplished by following three pathways: (i) mildly acidic pH (5.5 – 6.8) induced drug release in endo/lysosomal compartments; (ii) lysosomal enzymes (cathepsins B and D) or low pH (4.0 – 5.0) caused drug release in lysosomes; and (iii) redox potential (GSH 2 – 10 mM) triggered drug release in the cytosol or cell nucleus.

environment-responsive nanosystems have in general demonstrated improved antitumor activity compared to their non-responsive counterparts, due to their faster and more complete drug release in the cancer cells. These bioresponsive nanosystems have, however, met with varying levels of success *in vitro* and *in vivo*, most likely owing to diversities in the responsive site (endosome, lysosome, cytosol, or nucleus) as well as responsive rate. Moreover, for *in vivo* applications, these bioresponsive nanosystems must also possess good stability in circulation[13].

Endo/lysosomal pH-responsive drug release systems

pH Variations are present within different tissues and cellular compartments. For instance, cancerous tissue is slightly acidic with a pH ranging from 6.5 to 7.2[14]. The pH in cytosol, like in normal tissue and blood, is ca. 7.4, in endosome is ca. 5.5 – 6.8, while in lysosome is ca. 4.0 – 5.0[15]. This intrinsic pH gradient has been used to design nanosystems to deliver therapeutics to tumor tissue or cellular compartments[16, 17]. Generally, pH-responsive nanosystems are meant to be deformed by swelling or disrupted by dissolution or polymer degradation under endo/lysosomal pH conditions, resulting in efficient intracellular drug release.

pH-sensitive nanocarriers have been developed based on polybase copolymers containing pendant primary, secondary, or tertiary amine groups (pK_a = 7 – 11). The protonation of the amine groups at endosomal pH would shift the hydrophobic polybase into hydrophilic polybase, thereby leading to rapid nanocarrier disassembly and drug release. For instance, Armes *et al.* reported pH-sensitive polymersomes based on poly(2-(methacryloyloxy) ethyl phosphorylcholine)-*b*-poly(2-(diisopropylamino) ethyl methacrylate) (PMPC-PDPA) for triggered intracellular release of doxorubicin (DOX) and DNA[18, 19]. Kim *et al.* reported that DOX-loaded micelles based on poly(2-hydroxyethyl methacrylate)-b-poly(L-histidine) (p(HEMA)-b-p(His)) released DOX in a pH dependent manner and induced higher growth inhibition of HCT 116 human colon carcinoma cells at acidic pH than basic pH[20]. Deming *et al.* prepared pH-responsive polymersomes based on polyleucine-*b*-polyarginine copolypeptides in which polyarginine segments not only direct vesicle formation but also provide functionality for efficient intracellular delivery of polymersomes[21]. We recently found that biodegradable polymersomes containing an ionizable membrane enabled highly efficient loading and rapid release of proteins at endosomal pH[22]. It should be noted that the presence of various amine groups might also facilitate endosomal escape, further increasing anti-cancer activity.

Endosomal pH-responsive nanocarriers have been constructed from copolymers containing pH-sensitive degradable hydrazone, acetal, or cis-acotinyl linkages in their side chains. The degradation of these acid-labile bonds under endosomal pH conditions would alter the hydrophilic-hydrophobic balance of nanocarriers thereby triggering drug release. For instance, Fréchet *et al.* reported that acid-sensitive microparticles prepared from acetal-derivatized dextran degraded and released FITC-dextran in a pH-dependent fashion[23]. Kataoka *et al.* reported that charge-conversional polyion complex (PIC) micelles based on cis-aconityl modified cytochrome C (CC) and PEG-poly(N-(N'-(2-aminoethyl)-2-aminoethyl)aspartamide) (PEG-pAsp(DET) could effectively reverse their charge at endosomal pH leading to dissociation of micelles and efficient intracellular protein release[24]. We reported that pH-sensitive poly(ethylene glycol)-g-doxorubicin (PEO-g-DOX) prodrugs with DOX linking to PEO main chain via a hydrazone bond while sufficiently stable at pH 7.4 were readily activatable at endosomal pH[25]. In a similar way, pH activatable DOX prodrug nanogels were obtained by conjugating DOX to PEG-*b*-poly(2-hydroxyethyl methacrylate-*co*-ethyl glycinate methacrylamide) (PEG-*b*-P(HEMA-*co*-EGMA)) copolymers via hydrazone bonds[26]. These prodrug nanogels released DOX nearly quantitatively in 48 h at endosomal pH and caused pronounced cytotoxic effects to RAW 264.7 and MCF-7 tumor cells. It should further be noted that acidic tumor pH-responsive nanosystems have also been developed to enhance tumor cell uptake and/or tumor-specific drug release. For instance, Wang *et al.* recently reported that tumor acidity-responsive shell-sheddable nanoparticles facilitated tumor cell uptake of siRNA polyplexes following accumulation at the tumor site, enhancing inhibition of tumor growth[27].

Gradual release of a drug over a period of days to weeks is usually observed for biodegradable micelles based on traditional biodegradable polymers like polylactide (PLA), poly(ε-caprolactone) (PCL), and poly(trimethylene carbonate) (PTMC) because of a slow biodegradation process in cancer cells. In order to enhance polymer degradation inside cancer cells, we designed a novel acid-labile acetal-containing cyclic carbonate monomer, 2,4,6-trimethoxybenzylidenepentaerythritol carbonate (TMBPEC), based on which pH-sensitive degradable micelles and polymersomes were developed[28, 29]. The results showed that the

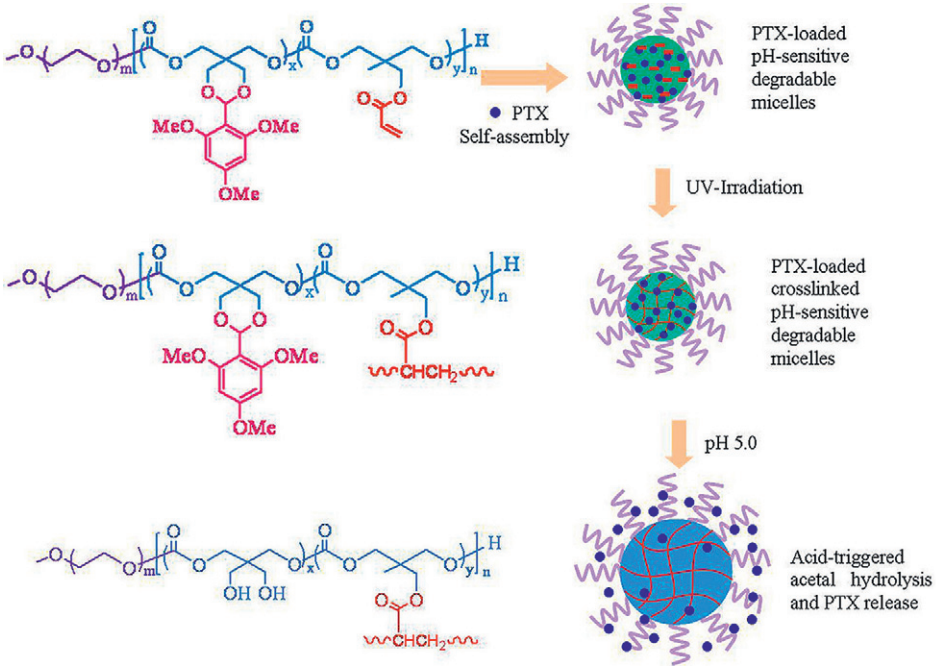

Fig. 2 Illustration of photo-crosslinked pH-sensitive degradable micelles based on PEG-b-P(TMBPEC-co-AC) block copolymer. PTX-loaded crosslinked pH-sensitive degradable micelles exhibit superior extracellular stability and minimal drug leakage on dilution while "actively" release PTX under a mildly acidic condition mimicking that of the endo/lysosomal compartments[30]. Reproduced from reference[30].

acetals in PEG-PTMBPEC block copolymer micelles or polymersomes were prone to fast hydrolysis at mildly acidic pH, which transformed hydrophobic PTMBPEC block into hydrophilic poly(pentaerythritol carbonate) (PPEC) block, resulting in markedly enhanced intracellular drug release. In a more recent study, we have prepared core-crosslinked pH-sensitive degradable micelles from PEG-*b*-P(TMBPEC-*co*-acryloyl carbonate) (PEG-*b*-P(TMBPEC-*co*-AC)) (Fig. 2)[30]. The cyclic acryloyl carbonate (AC) monomer could be easily synthesized[31]. Based on this AC monomer, we have previously obtained paclitaxel (PTX)-loaded photo-crosslinked PEG-PCL and PEG-PLA micelles that demonstrated superior stability and enhanced anti-tumor effect in hepatoma-bearing mice as compared to the non-crosslinked controls[32,33], signifying the importance of micellar stability in targeted tumor therapy. The *in vitro* release studies showed that PTX leakage from PTX-loaded core-crosslinked PEG-*b*-P(TMBPEC-*co*-AC) micelles was minimal at pH 7.4 even at low micelle concentrations while fast PTX release was observed at endosomal pH due to hydrolysis of acetal bonds[30]. MTT assays revealed that PTX-loaded crosslinked pH-sensitive degradable micelles retained high anti-tumor activity comparable to PTX-loaded non-crosslinked counterparts, supporting efficient drug release from PTX-loaded crosslinked micelles inside tumor cells. These core-crosslinked pH-responsive biodegradable micelles have elegantly addressed the extracellular stability versus intracellular drug release dilemma of micellar anticancer drugs.

Taking advantage of acid-labile acetal bonds, we have also developed pH-sensitive degradable chimaeric polymersomes from asymmetric PEG-b-poly(2,4,6-trimethoxybenzylidene-1,1,1-tris(hydroxymethyl)ethane methacrylate)-b- PAA (PEG-PTTMA-PAA) triblock copolymers, wherein longer PEG chains (M_n = 5.0 kg/mol) were preferentially located at the outer surface while shorter PAA chains (M_n = 1.5 ~ 2.7 kg/mol) were preferably oriented towards the interiors of polymersomes[34]. Notably, these chimaeric polymersomes could actively load water soluble anticancer drug DOX·HCl to give high loading contents (up to 15.9 wt.%) and loading efficiencies (up to 88.8 %). Moreover, DOX·HCl-loaded polymersomes could efficiently release DOX·HCl into the nuclei of HeLa cells resulting in high anti-tumor activity with IC_{50} (inhibitory concentration to produce 50 % cell death) of 1.48~1.67 μg/mL, close to that of free drug. These pH-sensitive degradable chimaeric polymersomes have appeared to be a "smart" alternative to liposomal doxorubicin for targeted tumor therapy. Interestingly, low molecular weight polyethylenimines (PEIs) following modification with pH-sensitive degradable acetal-containing hydrophobe, 2,4,6-trimethoxybenzylidenetris(hydroxymethyl)ethane (TMB-THME), were able to effectively condense DNA into nano-sized particles that were quickly unpacked under endosomal pH conditions due to the reversal of hydrophobic modification as a result of acetal hydrolysis[35,36]. These reversibly hydrophobilized PEIs have been shown to effectively deliver DNA into various types of cells.

pH-responsive nanosystems have also been developed based on acid degradable polymers containing orthoester, hydrazone, or acetal linkages in their backbones. For example, Fréchet *et al.* reported that poly(amidoamine)s with acetal or ketal linkages in the backbone exhibited a pH-dependent degradation behavior[37] and pH-sensitive microparticles based on ketal-containing polyurethanes and polyureas

could efficiently deliver and release FITC-BSA into macrophages[38]. Lee *et al.* demonstrated that pH-responsive micelles based on PEG-poly(beta-amino ester) responded rapidly to an acidic environment and delivered iron oxide (Fe_3O_4) nanoparticles in the brain ischemic area for MRI[39]. This pH-sensitive MRI probe may be effective for targeting the acidic environment and diagnostic imaging of pathologic tissues.

Redox-responsive drug release systems

Redox potential has recently appeared as a fascinating and ubiquitous natural stimulus for active intracellular drug and gene delivery[40, 41]. It is known that body fluids (e.g., blood) and normal extracellular matrices possess a low reducing power with glutathione tripeptide (γ-glutamyl-cysteinyl-glycine, GSH, the most abundant low molecular weight biological reducing agent) as low as 2 – 20 μM and proteins rich in stabilizing disulfides. In contrast, cytosol and the cell nucleus have a high redox potential with GSH concentrations ranging from 2 to 10 mM that is kept reduced by NADPH and glutathione reductase[42]. Moreover, the endosomal compartment has also been reported as redox active in which the redox potential is modulated by a specific reducing enzyme, gamma-interferon-inducible lysosomal thiol reductase (GILT) in the co-presence of reducing agents such as cysteine (but not GSH)[43]. It should further be noted that tumor tissues and tumor cytosol are also characterized by a reductive environment with concentration of GSH at least 4-fold higher than that in the normal tissues[44]. Therefore, redox is a unique internal signal for active drug release inside tumor cells as well as in tumor tissues. Notably, redox-sensitive degradable nanosystems offer several features over pH-sensitive degradable counterparts, such as (i) they are stable under extracellular milieu and during workup that are usually in an oxidative state; (ii) they exhibit fast response to the intracellular levels of GSH, at a time scale from minutes to hours; and (iii) they release drugs right into the cytosol and cell nucleus where most drugs take their therapeutic effects. The design rationale of reduction-sensitive nanosystems usually involves incorporation of cleavable disulfide linkage(s) in the polymer main chain, at the polymer side chain, or in the cross-linker.

Interestingly, incorporation of a single disulfide bond between the hydrophobic and hydrophilic blocks of amphiphilic block copolymers has shown able to induce fast intracellular drug release. For example, Hubbell *et al.* reported that reducible polymersomes based on PEG-SS-poly(propylene sulfide) (PEG-SS-PPS) copolymers were quickly disrupted inside cells leading to fast release of cargos[45]. We found that shell-sheddable biodegradable micelles based on PEG-SS-PCL efficiently released DOX into the cytoplasm of RAW 264.7 cells, resulting in markedly enhanced antitumor activities as compared to the "traditional" reduction-insensitive PEG-PCL controls[46]. Improved intracellular drug release and antitumor effects were also demonstrated for DOX-loaded reduction-sensitive dextran-SS-PCL micelles[47]. Wang *et al.* separately reported that shell-sheddable poly(ethyl ethylene phosphate)-SS-PCL micelles released DOX in an intracellular GSH-dependent manner[48], which was more recently shown to effectively overcome the multidrug resistance (MDR) of cancer cells[49]. Inspired by the early work, different reduction-sensitive shell-sheddable micelles have recently been developed for intracellular release of diverse anticancer drugs. For instance, Yoo and Li groups designed PEG-SS-poly(γ-benzyl L-glutamate) (PEG-SS-PBLG) and PEG-SS-poly(ε-benzyloxycarbonyl-L-lysine) (mPEG-SS-PZLL) micelles for accelerated intracellular release of camptothecin (CPT) in SCC7 cancer cells and DOX in MCF-7 cells, respectively[50, 51]. Huang and Oh groups constructed reduction-sensitive shell-sheddable micelles based on amphiphilic hyperbranched block copolymers linked with disulfide bonds[52, 53]. Huo *et al.* reported that reduction-sensitive shell-sheddable micelles based on hyaluronic acid-SS-deoxycholic acid (HA-SS-DOCA) conjugates preferentially accumulated in the tumor site in tumor-bearing mice 24 h after injection and were taken up by human breast adenocarcinoma cells (MDA-MB-231) via HA-receptor mediated endocytosis[54]. Our systematic study on shell-sheddable micelles showed that the intracellular DOX level and cytotoxicity of DOX-loaded PEG-SS-PCL micelles were intimately dependent on extent of shell-shedding[55]. These results point out that reduction-sensitive shell-shedding strategy is a straightforward, general, and effective approach to achieve highly efficient intracellular anticancer drug release from nano carriers.

This reduction-sensitive shell-shedding approach has successfully been extended for "active" intracellular protein and gene delivery. For instance, pH and reduction dual-bioresponsive polymersomes were designed based on PEG-SS-poly(2-(diethyl amino)ethyl methacrylate) (PEG-SS-PDEA) copolymers for facile encapsulation and triggered intracellular release of proteins[56]. The polymersomes were formed simply by adjusting the acidic copolymer solution to a physiological pH. These dual-bioresponsive polymersomes efficiently delivered CC into MCF-7 cells inducing markedly enhanced apoptosis as compared to free CC and reduction-insensitive PEG-PDEA controls. Novel bioreducible cationic poly(2-(dimethyl amino)ethyl methacrylate)-SS-PEG-SS-poly(2- (dimethyl amino)ethyl methacrylate) (PDMAEMA-SS-PEG-SS-PDMAEMA) triblock copolymers were shown to effectively condense DNA into partially stealthy nanoparticles that were prone to rapid de-shielding and unpacking under an intracellular reductive condition, resulting in efficient intracellular DNA release and superior transfection activity in COS-7 cells to the reduction-insensitive controls[57].

Redox-sensitive micelles have also been developed based on reductively degradable copolymers containing multiple disulfide or diselenide bonds in the main chain or side chain for intracellular anticancer drug release. Fan *et al.* reported that amphiphilic graft copolymer micelles with reductively degradable hydrophobic poly(amido amine) (SS-PAA) as the main chain and PEG as the side chain (SS-PAA-g-PEG) released DOX nearly quantitatively in 10 h in response to 1 mM DTT[58]. Thayumanavan *et al.* reported that cytotoxicity of DOX-loaded reduction-sensitive micelles based on amphiphilic copolymers containing multiple disulfide bonds in the hydrophobic segments displayed a clear correlation with the intracellular GSH level in MCF-7 cells[59]. Huang *et al.* reported that micelles formed from reduction-sensitive amphiphilic hyperbranched polyphosphates (HPHDP) efficiently transported DOX into the cell nuclei, resulting in enhanced antitumor efficacy[60]. Xu *et al.* discovered that redox-sensitive diselenide-containing block copolymer micelles were readily disassembled under a mildly reductive (0.01 mg/mL GSH) or oxidative (0.01 v.% H_2O_2) condition[61]. Interestingly, redox-sensitive amphiphilic hyperbranched polydiselenide consisting of alternative hydrophobic diselenide groups and hydrophilic phosphate segments in its backbone framework, which formed micelles with an average diameter of 50 nm,

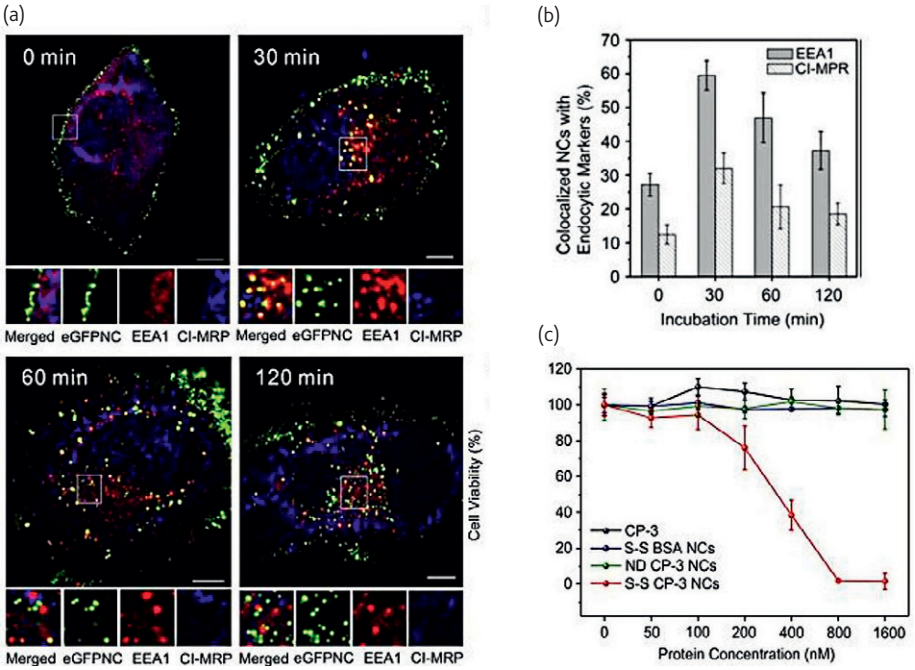

Fig. 3 Disulfide-crosslinked positively charged single-protein nanocapsules for active intracellular protein release. (a) Cellular trafficking of nanocapsules in time. Early and later endosomes were labeled by early endosome antigen 1 (EEA1, red) and cation-independent mannose-6-phosphate receptor (CI-MPR, blue), respectively; (b) Quantification of nanocapsules colocalized with early and late endosomes; and (c) cytotoxicity of active caspase 3 (CP-3) nanocapsules to HeLa cells[70]. Reproduced from reference[70].

was recently reported to effectively inhibit the proliferation of various forms of cancer cell[62]. Gan *et al.* reported that reductively degradable micelles based on poly(ethylene oxide)-*b*-poly(N-methacryloyl-N′-(t-butyloxycarbonyl)cystamine) (PEO-PMABC) copolymers exhibited enhanced intracellular DOX release and better anticancer activity than corresponding reduction-insensitive control[63].

Recently, reduction-sensitive reversibly crosslinked nanocarriers have been developed to elegantly meet the conflicting requirements of high extracellular stability and rapid intracellular drug release. Novel disulfide-crosslinked nanoparticles were obtained from biocompatible dextran-lipoic acid derivatives (Dex-LA)[64]. Lipoic acid is a natural compound produced by the human body. These crosslinked nanoparticles revealed minimal DOX release even under extensive dilution. In contrast, over 90 % DOX was released in 11 h under an intracellular mimicking reductive environment. Confocal laser scanning microscopy (CLSM) studies showed that DOX was quickly delivered into the nuclei of HeLa and RAW 264.7 cells, supporting fast reversal of disulfide crosslinking inside the tumor cells. With a similar strategy, reduction-sensitive interfacially crosslinked micelles were obtained from biodegradable PEG-PCL diblock copolymer containing two lipoyl groups at the middle (PEG-L_2-PCL)[65]. These crosslinked micelles while displaying minimal DOX release on extensive dilution quickly released DOX in response to 10 mM DTT under otherwise the same conditions. More recently, reversibly core-crosslinked micelles were constructed from PEG-*b*-poly(*N*-2-hydroxypropyl methacrylamide)-lipoic acid (PEG-*b*-PHPMA-LA) copolymer[66]. DOX-loaded core-crosslinked micelles exhibited DTT-triggered drug release and great antitumor activities in HeLa and HepG2 cells. Interestingly, modification of 1.8 kDa PEI with lipoic acid was shown to significantly enhance the intracellular DNA release leading to gene expression several hundred times greater in 293T cells[67]. Wang *et al.* reported that reduction-sensitive interfacially crosslinked PEG-PCL micelles exhibited enhanced stability and retarded DOX release against dilution[68]. The cytotoxicity of DOX-loaded crosslinked micelles increased with increasing intracellular GSH levels in A549 cells. Lam *et al.* demonstrated that disulfide-crosslinked micelles had superior stability, prolonged circulation time *in vivo* and preferential accumulation at the tumor site in nude mice bearing SKOV-3 ovarian cancer xenograft[69]. The release of PTX from crosslinked micelles could be gradually facilitated in a reductive environment. PTX-loaded crosslinked micelles displayed better tumor inhibition than the non-crosslinked counterparts and Taxol.

Tang *et al.* reported that disulfide-crosslinked positively charged single-protein nanocapsules could efficiently release proteins into cancer cells, Fig. 3[70]. They have demonstrated that using these nanocapsules, active caspase 3 (CP-3) can be delivered and released into a variety of human cancer cell lines such as HeLa, MCF-7, and U-87 MG cells, inducing significant apoptosis. Temperature and reduction dual-sensitive crosslinked polymersomes were designed based on water-soluble thermosensitive PEG-PAA-PNIPAM triblock copolymers for intracellular protein release[71,72]. Interestingly, the lower critical solution temperatures (LCST) of PEG-PAA-PNIPAM copolymers could be adjusted to ca. 38 – 39 °C in PBS (pH 7.4, 20 mM, 150 mM NaCl). The crosslinked polymersomes were prepared by simply increasing solution temperature to above their LCST

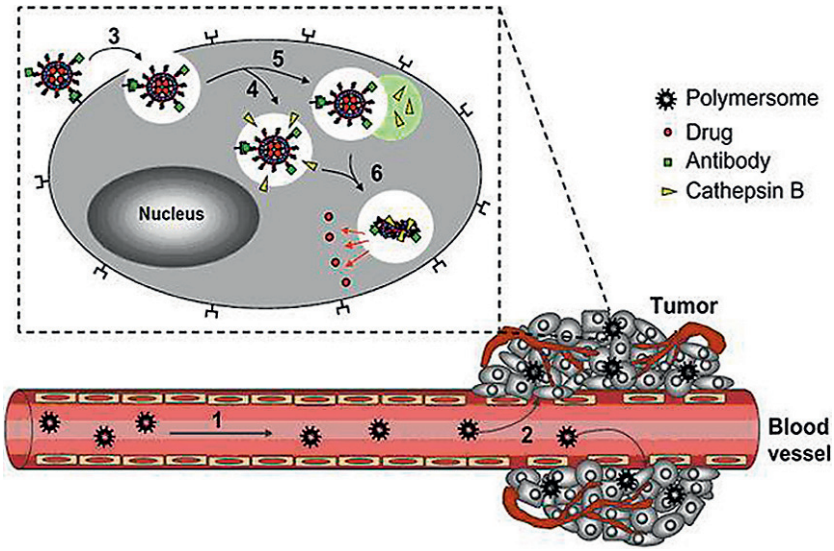

Fig. 4 Illustration of anti-EGFR functionalized enzyme-sensitive polymersomes based on PEG-GFLGF-PDLLA copolymer for active intracellular drug release to SKBR3 cells [86]. Reproduced from reference[86].

followed by crosslinking with cystamine via carbodiimide chemistry. These crosslinked polymersomes were robust against dilution and decrease of temperature but rapidly dissociated under a reductive condition under physiological conditions. They efficiently delivered and released CC into the cytosol of MCF-7 cells following 12 h incubation[72].

Lysosomal enzyme-responsive drug release systems

Enzymes have been recognized as a valuable stimulus to achieve efficient intracellular drug release[73-75], as there are abundant digestive enzymes including proteases, glycosidases and sulfatases in the lysosomes[76, 77]. For instance, it has been shown that cathepsins B and D are able to cleave specific peptide sequences such as Gly-Phe-Leu-Gly (GFLG)[78] and Gly-Phe-Ala-Leu (GFAL) inside the tumor cells[79]. It should further be noted that lysosomal enzymes like cathepsins which play a role in degradation of the extracellular matrix and enhanced invasive capability of tumors are also upregulated in a variety of solid tumors including breast cancer, ovarian cancer, skin cancer, and brain tumors[77, 80-82]. Lysosomal enzyme-responsive nanocarriers are therefore appealing for tumor-specific drug release.

For example, Kopecek *et al.* designed DOX prodrugs based on HPMA-GFLG-DOX conjugates that released DOX and induced anti-tumor effect under the action of cathepsins B in lysosomes[83]. The clinical trials on these enzyme-activable prodrugs have shown encouraging results[84]. Duncan *et al.* reported that PEG-peptidyl-DOX conjugates with a GFLG and GLFG linker released 30 % and 57 % DOX, respectively, in 5 h via lysosomal enzymes *in vitro*[85]. These PEG-peptidyl-DOX conjugates showed more than 10-fold lower cytotoxicity toward B16F10 cells than free DOX. Recently, Feijen *et al.* developed anti-EGFR decorated enzyme-sensitive polymersomes based on PEG-PDLLA block copolymer linked by Gly-Phe-Leu-Gly-Phe peptide (GFLGF)[86]. The results showed that these polymersomes could deliver and release FITC-dextran (40 kDa) into SKBR3 cells, suggesting effective cleavage of GFLGF peptides in the lysosomal compartments (Fig. 4)[86]. It should be noted that enzyme-responsive drug release systems, though highly specific, involve complex synthesis and harsh lysosomal conditions that might lead to drug degradation.

Conclusions

The recent development of intracellular environment-responsive nano drug delivery systems has opened a new door to controlled drug release technology for cancer therapy. In contrast to traditional biodegradable nanocarriers that slowly release drugs inside the cells, these "smart" nanosystems are able to quickly release or even dump drugs in response to a specific biological signal in the target cancer cells such as endo/lysosomal pH, lysosomal enzyme, and cytoplasmic redox potential. This elevated intracellular drug release has shown to markedly enhance the *in vitro* antitumor activity of nano drugs as well as to effectively reverse multidrug resistant (MDR) cancer cells. The crosslinked pH-sensitive degradable nanosystems and pH or reduction-sensitive reversibly crosslinked nanosystems, which have elegantly addressed the extracellular stability and intracellular drug release dilemma, are particularly promising for *in vivo* cancer treatments. Notably, different intracellular environment-responsive nanosystems have met with varying success, most likely because of differences in rate and site of response (i.e., endosome, lysosome, cytosol, or nucleus). We are convinced that redox-responsive nanosystems have the greatest potential for

targeted intracellular anticancer drug release because they possess good extracellular stability, show fast response to the intracellular level of glutathione, release drugs right to the site of action, and furthermore allow versatile carrier design and synthesis. It should be noted, however, that intracellular environment-responsive nano drug delivery systems are still in their infancy and warrant full preclinical assessments prior to advancement to clinical trials. With rational design and careful selection of materials, intracellular environment-responsive nano drug delivery systems might one day become an indispensable clinical practice for targeted cancer therapy. mt

Acknowledgements

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