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Stimuli-Responsive Polymersomes for Cancer Therapy

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Abstract

Cancer is the leading cause of mortality and remains a major challenge for modern chemotherapy. Recent advances in cancer therapy have resulted in modest impact on patient survival. Nanomedicine represents an innovative field with significant potential to improve cancer treatment. Nanomedicine utilizes numerous nanoconstructs, including polymersomes, micelles, and drug conjugates, to deliver therapeutic agents at the target site of interest. In particular, polymeric vesicles, also known as polymersomes, are self-assembled amphiphilic polymers in which an aqueous compartment is enclosed by a thick bilayer membrane. Unlike liposomes, polymersomes consist of high-molecular-weight amphiphilic polymer analogues. Since polymersomes are prepared using synthetic amphiphilic polymers, the bilayer membrane thickness can be readily altered by tuning the molecular weight of hydrophobic blocks. As a consequence, the polymersomes prepared from high-molecular-weight amphiphiles strengthen their membranes, making them inherently more stable than liposomes. The intriguing aggregation of polymersomes offers numerous advantages, including stability, tunable membrane properties, and the capability to encapsulate hydrophilic and hydrophobic agents. Owing to these properties, polymersomes are attractive candidates for various applications such as drug delivery, gene therapy, and tissue engineering. Although these properties have placed polymersomes at the forefront of drug delivery applications, to attain enhanced therapeutic effect polymersomes are supposed to rapidly release the drug at the target site. To fulfill this requirement, stimuli-responsive polymersomes that respond to various internal or external stimuli have been developed. This review focuses on recently developed stimuli-responsive polymersomes and their potential application in cancer therapy.

Key words: Cancer, drug delivery, copolymer, nanoparticles, polymersomes.

1.   Introduction

The development of smart nanocarriers is an active area of recent studies.[1] The self-assembly of amphiphilic copolymers in aqueous solutions can be utilized to form a range of nanocarriers of varying morphologies, including spherical micelles, worm-like micelles, and polymersomes.[2-4] Among the variety of nanocarriers, polymersomes are artificial vesicles that have received considerable attention due to their entangled architecture.[5] Polymersomes, composed of amphiphilic polymers, are spherical nanostructures in which an aqueous core is enclosed by a hydrophobic membrane. The membrane thickness of polymersomes can be elegantly tailored by altering the hydrophobic ratio of the copolymers.[6] Polymersomes are generally prepared using high-molecular-weight amphiphilic block copolymers, their stability and toughness are superior to those of lipid counterpart, liposomes. Owing to the unique properties of polymersomes, their critical aggregation concentrations are low, which allows these particles to be retained longer in the bloodstream. The unique structural feature of polymersomes allows the encapsulation of hydrophobic and hydrophilic agents, such as anticancer drugs, proteins, and genes.[7] It is known that polymersomes with an optimal size can alter the tissue distribution of anticancer drugs.[8] In addition, the surface modification of polymersomes with target-specific ligands can facilitate the cellular uptake of anticancer drugs by binding to receptors that are overexpressed on cancer cells.[9] Additionally, poly(ethylene glycol) (PEG) conjugation, known as the PEGylation of polymersomes, or the preparation of PEG-containing polymersomes is widely used strategy to prevent the adsorption of blood protein components.[10, 11] The hydrophilic and neutral nature of PEG in polymersomes reduces protein opsonization, which makes polymersomes invisible to phagocytic and dendritic cells. As a consequence, systemically administered polymersomes can passively accumulate at the tumor site via the

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enhanced permeation and retention (EPR) effect.[10, 12] Given this unique structure, polymersomes could efficiently protect encapsulated agents from hydrolytic or enzymatic degradation without the loss of biological activity. It is important that systemically administered polymersomes should trigger the drug after it arrives at the target site.[13, 14] Thus, nanocarriers should be stable in the bloodstream and rapidly release their payload after arriving at the target site of action. To fulfill this contradictory requirement, smart polymersomes, also known as stimuli-responsive polymersomes, have been developed. Stimuli-responsive polymersomes are able to circulate in the bloodstream for long periods and maintain their nanostructures; however, they are disassembled at the target site. Fig. 1 graphically represents the state of the art of polymersomes and their tumor-targeting characteristics.

2.   Stimuli-responsive polymersomes

Traditional nanocarriers, which lack stimuli-responsive linkers, release anticancer drugs in a sustained manner via diffusion-controlled mechanism. The utilization of such nanocarriers may not endow tumor tissues with cytotoxic local concentrations of anticancer drugs. All drugs are characterized by a plasma level above which the drug is toxic and below which it is ineffective. Thus, nanocarriers primarily serve to lower the systemic level of the drug while maintaining the desired therapeutic range after a single dose.[15] Therefore, numerous stimuli-responsive polymersomes that respond to physical stimuli (e.g., temperature, ultrasound, light, electrochemical, magnetic), chemical stimuli (e.g., pH, redox, and ionic) or biological stimuli (e.g., enzymes, inflammation and glucose) have been developed to improve the therapeutic efficacy of anticancer drugs (Fig. 2).[16-20] Among the various types of stimuli-responsive carriers, pH-, temperature-, and redox-responsive nanocarriers have been widely used for various

biomedical applications, including cancer therapy. In this review, we focused on recently developed chemical and biological stimuli-responsive polymersomes for cancer therapy as well as other representative physical stimuli-responsive polymersomes that might be applicable in cancer therapy in the near future.

3. Bioreducible polymersomes

Recently, bioreducible polymersomes have been extensively studied for the triggered release of anticancer drugs in an intracellular reductive environment. These polymersomes usually possess a bioreducible disulfide bond in the middle or side chain of an amphiphilic polymer. Although the dissociation energy of a disulfide bond (-S-S-) is lower than that of a carbon-carbon bond (-C-C-), it is stable in an oxidizing extracellular environment.[21, 22] Disulfide bonds are longer (l_{s-s} ~ 2.03 Å) than carbon-carbon bonds (l_{s-s} ~ 1.54 Å), making them susceptible to various physical and chemical stimuli. Disulfide bonds are stable under physiological conditions; however, they are readily reduced in an intracellular environment due to the thiol-disulfide exchange reaction with glutathione (GSH), a tripeptide.[23] Owing to the fact that several intracellular compartments, including the cytosol, mitochondria and cell nucleus, contain a higher concentration of GSH than the blood, polymersomes containing disulfide bridges are expected to be readily disintegrated and consequently release drugs more readily inside the cell than in circulation. Specifically, the concentration of GSH in the blood plasma is in the micromolar range (2-20 μM), whereas this concentration is approximately 1-10 mM in the intracellular compartments.[24, 25] Most GSH (85%-90%) is present in the cytosol, whereas much lower concentrations of GSH (15%-10%) are found in other organelles, including the mitochondria, nuclear matrix, and peroxisomes.[26] The high GSH concentration provides an essential reducing environment inside the cell. This change in the concentration gradient has

encouraged the development of disulfide-containing polymersomes for the site-specific triggering of drug release.[27-29] Such polymersomes with therapeutic payloads may significantly improve the therapeutic efficacy of the drug, because various therapeutic targets are located intracellularly.

Hubbell et al. were the first to develop disulfide-containing polymersomes, which consisted of PEG-*b*-poly(propylene sulfide) bearing an intervening disulfide bond.[30] Polymersomes prepared from this block copolymer were demonstrated to destabilize in the presence of GSH or cysteine. The destabilization of polymersomes was examined via the encapsulation of calcein, a hydrophilic fluorescent probe, whose fluorescence in the polymersomes is autoquenched at a specific concentration. However, after the exposure of the polymersome to reducing agents, the released calcein generated a fluorescence signal and the intensity of this signal was measured to estimate the amount of released cancein. This release test indicated that the polymersome ruptured within 10 min of the addition of reducing agents. The cell uptake of polymersomes by J774A-1 cells was monitored; the fluorescence signal of calcein increased over time in the intraendosome and subsequently in the cytoplasm. These polymersomes may deliver therapeutic agents at the endosome and improve the efficacy of the drug by avoiding exposure to the harsh conditions encountered after lysosome fusion.

In another study, Jia et al. developed a novel type of disulfide-containing robust liquid crystal polymersome in which the hydrophobic block is a cholesterol-containing liquid crystal polymer.[31] Since the polymersome was prepared using cholesterol pendant groups, they were robust owing to the physical cross-linking of the cholesterol in the membrane and remained stable for a longer period of time. The destruction of polymersomes was examined based on the encapsulation of calcein, whose release was triggered by the addition of GSH. Although the term

polymersome was coined in early 2000, the anticancer properties of drug-loaded polymersomes remain poorly understood. The combination of multiple drugs with different molecular targets can maximize therapeutic efficacy and is more likely to overcome drug resistance.[32] Combination drug therapy has been adopted in clinics as a primary regimen for cancer treatment. To observe the fate of drug-loaded polymersomes, we prepared bioreducible polymersomes for the controlled delivery of dual-drugs (Fig. 3).[33] In this study, a triblock copolymer bearing disulfide bonds, PEG-*b*-p(lysine)-SS-p(caprolactone), that is cleavable in an intracellular environment was synthesized. In an aqueous solution, the copolymer self-assembled into polymersomes and could simultaneously encapsulate the hydrophobic drug camptothecin (CPT) and the hydrophilic drug doxorubicin·hydrochloride (DOX·HCl). The encapsulated drugs were rapidly released in response to 10 mM GSH, which is similar to the concentration encountered in an intracellular environment. An *in vitro* cytotoxicity test showed that dual drug-loaded polymersomes enhanced the cytotoxicity of drugs to SCC7 cells. Confocal microscopic images showed enhanced fluorescence intensity for the DOX-loaded bioreducible polymersomes in the cytoplasm of SCC7 cells, whereas weaker fluorescence was detected for the disulfide-insensitive control polymersomes. The significantly higher fluorescence of the bioreducible polymersome was due to the high GSH concentration in the cytoplasm, which resulted in the cleavage of the disulfide bond in the polymersomes and enhanced drug release.

Although the majority of polymersomes reach their target by the EPR effect, the preparation of functionalized polymersomes with targeting ligands is a topic of interest.[34] Such polymersomes can specifically target cacer cells using an active targeting mechanism, in which polymersomes can bind to the cancer cells via overexpesed receptors. Polymersomes prepared using ligands behave like a missile and specifically bind to certain receptors that are

overexpressed on cancer cells but not expressed by normal cells. Koul et. al. prepared dual-targeting ligand-containing, redox-responsive polymersomes for the targeted delivery of DOX to breast cancer cells.[35] Redox-responsive polymersomes, composed of PEG-poly(lactic acid) (PEG-PLA)-based tetra block copolymers with multiple disulfide bonds were initially prepared, and their surface was functionalized using folate and trastuzumab ligands. The presence of higher hydrophobic balance enhanced the DOX loading, whereas the drug release was triggered by exposure to GSH. The folic acid and trastuzumab ligand in the polymersomes enhanced the cellular uptake of polymersomes in breast cancer cells. Owing to these properties, the systemic administration of DOX-loaded polymersomes resulted in complete tumor regression in Ehrlich ascites tumor-bearing Swiss albino mice.

Polymersomes prepared using amphiphilic polymers tend to dissociate after systemic administration, which might be due to their significant dilution in the body condition upon administration.[36, 37] As a consequence, the polymer concentration falls below the critical aggregation level, resulting in rapid drug release at unwanted sites.[38, 39] To surmount this issue, polymersomes have been cross-linked to preserve their nanostructure. In particular, cross-linking of polymersomes with a bioreducible cross-linker elegantly resolve extracellular instability and enhances intrcellular drug release. We prepared a novel biocompatible and biodegradable polymersome nanotemplate using a PEG-*b*-PLys-*b*-PCL triblock copolymer for chemical cross-linking (Fig. 4).[40] The primary amine in the PLys block was reacted with a disulfide-containing cross-linker to obtain reversibly cross-linked biostable polymersomes, and the cross-linking took place on the surface of a PCL membrane. The cross-linked polymersomes were remarkably resistant to dissociation by sodium dodecyl sulfate, a nanoparticle disturbing agent. Doxorubicin (DOX), which was selected as a model anti-cancer drug, was effectively encapsulated in the

cross-linked polymersomes with high loading efficiency. The *in vitro* release test indicated that DOX-loaded cross-linked polymersomes greatly retarded the release of DOX at physiological conditions (pH 7.4), whereas the DOX release rate was markedly increased in the presence of 10 mM GSH, which mimics an intracellular environment. These results suggest that cross-linking the polymersomes reduces the initial burst release and enhances the drug release at target sites.

Controlled release of proteins at the target site are of great interest. Zhong et al. prepared dual-responsive reversibly cross-linked polymersomes for the site-specific delivery of proteins.[41, 42] In this study, a water soluble triblock copolymer, PEG-*b*-poly(acrylic acid)-*b*-poly(*N*-isopropylacrylamide) (PEG-*b*-PAA-*b*-PNIPAM), was inititally prepared using a one-pot sequential reversible addition-fragmentation chain-transfer (RAFT) polymerization reaction. The block copolymers were freely soluble in water at room temperature, whereas they quickly transformed to nano-sized polymersomes when the temperature was increased to 37 ºC. This effect may be due to the lower critical solution temperature of the PNIPAM. The carboxylic acid group in the PAA was cross-linked with cystamine, a bioreducible cross-linker, and the cross-linking took place at the interface of the hydrophobic PNIPAM membrane. These cross-linked polymersomes exhibited remarkable stability against high dilution, salt concentration, and temperature. Various proteins, including bovine serum albumin (BSA), lysozyme, cytochrome C, ovalbumin, and FITC-dextran, were effectively loaded into the cross-linked polymersomes with high loading efficiency. The release rate of protein was minimal in a physiological buffer, whereas protein release was enhanced in a reductive environment. The confocal laser scanning microscopy (CLSM) observations demonstrated that the cross-linked polymersomes efficiently delivered proteins into the cytosol of MCF-7 cells. As a result, therapeutic protein-loaded polymersomes enhanced the apoptosis of MCF-7 cells compared with the free proteins.

Although PEG-*b*-PAA-*b*-PNIPAM-based polymersomes exhibited appreciable *in vitro* efficacy, the neutral nature of PNIPAM in polymersomes restricts their application *in vivo*. To make PEG-*b*-PAA-*b*-PNIPAM-based polymersomes suitable for practical applications, Zhong et al. elegantly replaced the PNIPAM with a poly(2-(diethylamino)ethyl methacrylate) (PDEA) block and obtained PEG-*b*-PAA-*b*-PDEA (Fig. 5).[43] The pH-sensitivity, cell permeability and proton sponge effect of the PDEA copolymer are known, and these materials have been applied for various biomedical uses. To obtain self-cross-linked polymersomes, cystamine was conjugated to PAA and reduced to obtain thiolated PEG-*b*-PAA-*b*-PDEA. The copolymer was freely soluble in acidic environments but formed self-assembled polymersomes by raising the pH to 7.8. These polymersomes could efficiently encapsulate BSA and cytochrome C without loss of its biological activity. The intracellular localization of protein was measured by exposing the protein-loaded polymersomes to MCF-7 cells. Microscopy image indicated that protein-loaded polymersomes were taken up by the MCF-7 cells via endocytosis and subsequently escaped from endosomes due to the proton sponge effect. The released polymersomes in the cytosol were de-cross-linked, which enhaced protein release. As a result, cytochrome C-loaded polymersomes were more cytotoxic to MCF-7 and HeLa cancer cells than 293T cells.

Polymersomes prepared using amphiphilic copolymers are typically inert. These inert carriers are the major component of drug carriers, whereas drugs are the minor component. The major limitation of these nanomedicines is their low drug content. Therefore, a large amount of a nanocarrier must be systemically administered to maximize the therapeutic effect of the drug. For practical applications, repeated administrations of high doses of these excipients may cause systemic toxicity and impose an additional burden on the patient to excrete the carriers. To overcome these limitation, CPT was conjugated to a very short oligomer of ethylene glycol that

could form polymersomes and be used for drug encapsulation.[32] SN38 is an active metabolite of irinotecan, a potent anticancer drug, which has been widely used to treat colon cancer. Recently, Gu et al. prepared prodrug (SN38)-anchored polymersomes (OEG-2S-SN38).[44] The drug was released from polymersomes in the presence of reactive oxygen species (ROS) and GSH. As a consequence, prodrug polymersomes were highly effective for treatment in a tumor model. These results suggested that drugs can be directly used as part of the carrier to fabricate other highly efficient nanocarriers and substantially reduce the use of other inert carrier materials while increasing the content of the loaded drug and avoiding premature burst release.

4. pH-responsive polymersomes

The extracellular pH of tumor tissues (~6.5-7.2) is slightly lower than that of normal tissues and other biological fluids (~7.4); it is further decreased in intracellular endosomes (pH 5.5-5.0) and lysosomes (pH 4.0-4.5).[45-48] Owing to the wide range of pH gradients in physiological systems, the application of pH-responsive polymersomes in cancer therapy is of great interest. In fact, the fabrication of pH-tunable polymersomes has been proposed as the main strategy to control the encapsulation and release pattern of nanoconstructs *in vivo*. Numerous acid-responsive linkers have been investigated to prepare polymeric nanoconstructs, including hydrazone, imine, orthoester, and acetal.[17, 49-54] Furthermore, anionic polymers, including poly(acrylic acid), poly(methacrylic acid), and cationic polymers, such as poly(β-amino ester), poly(lysine), and poly(histidine), are sensitive to the pH.[47, 53, 55-58] Such pH-responsive polymersomes can be designed to carry, deliver, and control the release of therapeutic agents to the tumor tissue by relying the low pH in the vicinity of tumor tissues.

pH-responsive polymersomes, composed of biocompatible and biodegradable polymers, were initially developed by Feijen and Zhong's group.[13, 59] They prepared PEG- and polyester-based biodegradable polymersomes as a basis for artificial cells.[5] Although these polymers can encapsulate and release drugs well, the fine tuning of the drug release by these polymersomes has been limited by their insensitivity to stimuli. Thus, pH-sensitive polymersomes based on PEG and poly(2,4,6-trimethoxybenylidinepentaerythritolcarbonate) (PTMBPEC) have been prepared.[60] Varying the hydrophobic ratio of the block copolymer resulted in both micelles and polymersomes. Specifically, the PEG(1.9k)-PTMBPEC(6k) and PEG(5k)-PTMBPEC(5.8k) could form polymersomes and micelles, respectively. The acetal linker present in the PTMBPEC is highly sensitive to acidic environments, and its degradation byproduct exhibits low/no cytotoxicity to normal cells. The PTMBPEC-based polymer degrades at mild acidic pH values with a half-life of 0.5 day, and the degraded polymersomes resulted in a significant increase in size (over 1000 nm). Polymersomes could encapsulate hydrophobic (Paclitaxel (PTX)) and hydrophilic (DOX·HCl) drugs in their hydrophobic membrane and aqueous core, respectively. Conversely, micelles could encapsulate only PTX. Regardless of the morphology, the drug-loaded nanostructure exhibited pH-dependent drug release, i.e., at mildly acidic pH values, complete drug release was observed.

Liu et al. developed biomimetic pH-responsive biodegradable polymersomes for the simultaneous encapsulation and release of two drugs.[61] The copolymer PLA-*b*-poly(2-methacryloyloxyethyl phosphorylcholine) (PLA-*b*-PMPC) was prepared by atom-transfer radical-polymerization. The natural PMPC present in the copolymer effectively reduces the protein opsonization due to its hydrophilic nature and may allow the prolonged circulation of polymersomes in the bloodstream. Polymersomes encapsulated hydrophilic DOX·HCl and

hydrophobic DOX in their aqueous lumen and hydrophobic membrane, respectively. The drug-loaded polymersomes rapidly released the drug under acidic conditions but not at physiological pH values. The drug-loaded polymersomes rapidly enter HepG2 cells, escaped from endosomes and released the drug in the cytosol.

The presence of a large aqueous core in polymersomes may serve as an ideal candidate for the encapsulation and release of water-soluble proteins and peptides. In particular, polymersomes with an ionic membrane may efficiently encapsulate proteins via electrostatic interaction. Unlike other formulations that require the use of organic solvents for protein loading, which results in the denaturation of the protein, polymersomes with ionizable membranes may allow for the efficient encapsulation of proteins due to their tunable pH. For instance, biodegradable polymersomes with pH-responsive ionizable membranes have been developed for the encapsulation and intracellular release of therapeutic proteins.[62] In this study, PEG-poly(carbonate)-based block copolymers containing acrylate, carboxylic acid, and amine were prepared. The simple dispersion of block copolymers in PBS (pH 7.4) yielded nano-sized polymersomes that could encapsulate therapeutic proteins by simple mixing. Rapid protein release was observed when the polymersomes were exposed to endosomal pH, whereas protein release was minimized at physiological pH values. To accelerate the release of protein in the cytosol, the same group introduced disulfide bonds to ionizable polymersomes in another study, and these polymersomes exhibited dual-stimuli sensitivity. The pH and reduction sensitivity of polymersomes allows the endosomal escape of polymersomes via proton sponge effect, and disulfide in polymersomes are reduced in the cytosol to enhance cytochrome C release for cancer cell apoptosis.

Chimeric polymersomes, a novel type of polymersome prepared from asymmetric ABC triblock copolymers, have recently been developed for the simultaneous encapsulation of drugs and proteins.[63, 64] Zhong et al. developed biodegradable chimeric polymersomes based on asymmetric PEG-PCL-PDEA triblock copolymers for the site-specific delivery of therapeutic agents.[64] The film hydration of triblock copolymers allows the formation of polymersomes with a size of 130-175 nm. These polymersomes could load various proteins, including BSA, cytochrome C, lysozyme, ovalbumin and IgG, and the encapsulation of proteins did not alter their morphology. In addition, chimeric polymersomes simultaneously loaded proteins and DOX, which were then transported to the cytoplasm and nucleus, respectively. Although these chimeric polymersomes exhibited remarkable properties, their practical application has been limited because of the lack of suitable targetability. To improve the efficiency of chimeric polymersomes, anisamide-decorated chimeric polymersomes have been developed for the targeted delivery of an apoptotic protein, granzyme B (GrB).[65] Anisamide-decorated chimeric polymersomes, composed of PEG-poly(2,4,6- trimethoxybenzylidene-1,1,1-tris(hydroxymethyl)ethane methacrylate)-PAA (PEG-PTTMA-PAA), were readily synthesized by RAFT polymerization. The GrB-loaded chimeric polymersomes exhibited rapid protein release in mild acidic conditions, perhaps due to the degradation acetal bonds in PTTMA. An *in vitro* cytotoxicity test suggested that anisamide-decorated chimeric polymersomes effectively targeted the overexpressed sigma receptor on the surfaces of H460 and PC-3 cells. As a result, GrB-loaded anisamide-decorated chimeric polymersomes were cytotoxic to cancer cells.

Polypeptide-based chimeric polymersomes, also known as pepsomes, have been developed for the efficient encapsulation and transportation of DOX·HCl into the nuclei of drug-resistant MCF-7 cells.[66] Pepsomes, composed of PEG-poly(L-leucine)-poly(L-glutamic acid)

(PEG-PLeu-PGA) were synthesized via the sequential one-pot ring-opening polymerization of Leu-NCA and benzyl glutamate NCA in the presence of a PEG macroinitiator. The PEG-PLeu-PGA triblock copolymer formed pepsomes in aqueous solutions and encapsulated DOX·HCl in their aqueous core. Interestingly, pepsomes could disassemble at pH 5.0 due to the ionization of PGA groups. As a result, DOX·HCl-loaded pepsomes exhibited a higher antitumor activity than free DOX·HCl in both RAW 264.7 cells and drug-resistant MCF-7 cells (Fig. 6).

5.   Enzyme-responsive polymersomes

The development of enzyme-responsive polymersomes is a relatively new area of research.[20] In general, enzymes play critical roles in various biological and metabolic processes, serving as the prime protagonists in the chemistry of living organisms.[20, 67] The upregulation of enzymes were often occurs in various diseases including cancer, thrombosis, inflammation, and infectious pathogens.[68] Such enzyme upregulation has been used as a pathological indicator in diagnosis and therapy. In this approach, enzyme-responsive substrates are covalently linked to the copolymer to form polymersomes. These polymersomes undergo physiocochemical changes when exposed to certain enzymes found in inflammatory tissues.

Cathespin B (Cath B), a known lysosomal enzyme, is more abundant in tumor tissues than in normal tissues. This enzyme can be used to degrade certain tetrapeptides, such as Gly-Phe-Leu-Gly (GFLG). Owing to this unique property, polymersomes prepared using GFLG peptides may be a good candidate for intracellular delivery. Lee et al. prepared lysosomally cleavable polymersomes, in which a GFLG degradable linker was introduced to middle of a hydrophilic PEG and hydrophobic PLA block copolymer.[69] The copolymer self-assembled into

polymersomes in aqueous solutions and disassembled in the presence of Cath B. Anti-epidermal growth factor receptor-antibody immobilized polymersomes enhanced the cell uptake of polymersomes. Fluorescein-labeled dextran-containing polymersomes demonstrated that peptide linkers were cleaved in the lysosomal compartments, which led to membrane disruption. IgG-immobilized enzyme-responsive polymersomes could specifically bind to the primary antibody-treated SKBR3 cells. These findings identify enzyme-responsive polymersomes as promising tumor-targeting delivery vehicles. The ability of enzyme-responsive polymersomes containing chemotherapeutic drugs or proteins to treat tumors is promising and remains to be tested.

To overcome tumor drug-resistance, the delivery of multiple drugs by a single nanocarrier is promising. In particular, delivering multiple drugs with different chemical properties may improve therapeutic efficacy. For instance, combination of inhibitors of topoisomerases I and II exhibit synergistic activity when administered together.[70] Pavillard et al. demonstrated that the combination of CPT and DOX exhibit synergistic effect to C6 glioma cell line.[71] CPT is a hydrophobic anti-cancer drug exhibit anticancer activity by inhibiting DNA topoisomerase 1, an enzyme required for replication and transcription of DNA.[72] On the other hand, DOX·HCl is a hydrophilic compound that exhibits anticancer activity by intercalating DNA strands and subsequently inhibiting macromolecular biosynthesis.[17] Since both drugs exhibit anticancer activity at the intracellular level, delivery of these two drugs into the cell could synergistically enhance the anticancer activity. Pramod et al. prepared a new enzyme-responsive polymersome based on a hydrophobic renewable source and hydrophilic dextran.[73, 74] The renewable source used in this study was pentadecyl phenol, which is isolated from cashew nut shells. The dextran polymersomes effectively encapsulated hydrophilic DOX·HCl and hydrophobic CPT. Drug-loaded polymersomes released the drugs upon exposure to esterase,

whereas they were stable in PBS buffer. The polymersomes were internalized by the cancer cells via caveolae-mediated endocytosis. As a consequence, enzyme-responsive polysaccharide polymersomes act synergistically to kill cancer cells. The same group also prepared pH- and enzyme-responsive polymersomes utilizing the same approach to deliver DOX into breast cancer cells.[75]

6.   Glucose-responsive polymersomes

In recent years, glucose-responsive polymeric materials have paid enormous attention due to their potential application in the treatment of glucose-related human diseases including diabetes.[76] In this regard, phenylboronic acid (PBA)-containing polymers have been developed because of their rapid physicochemical changes in response to the glucose.[77] Generally, at high pKa (8.2-8.86) PBA exists in an equilibrium between uncharged form and charged form. The uncharged form is hydrophobic, whereas the charged form is hydrophilic.[77, 78] The PBA could react reversibly with 1,2-*cis*-diols of glucose, resulting in conversion of hydrophobic PBA into hydrophilic form which might induce the release of entrapped agents. Kim et al. demonstrated glucose-responsive disassembly of amphiphilic copolymer for the triggered delivery of insulin.[79] Glucose-responsive amphiphilic copolymer, composed of PEG and poly(styreneboroxole-*alt*-*N*-functionalized maleimide), was synthesized using RAFT polymerization. The amphiphilic polymer could form polymersomes and loaded insulin effectively; the insulin release was triggered by the addition of glucose in the release medium. In another study, Shi et al. prepared glucose-responsive polymersomes based on the complexation between glucosamine (GA) conjugated block copolymer PEG_{45}-*b*-P(Asp-co-AspGA) and PBA conjugated block copolymer PEG_{113}-*b*-P(Asp-co-AspPBA).[80] Vancomycin, chosen as a model drug, was encapsulated into the

polymersomes effectively. From the release experiment, it was found that vancomycin was barely released in physiological buffer (pH 7.4). Interestingly, when glucose was added into the release medium, the vancomycin release was triggered.

7.   Miscellaneous polymersomes

7.1.   Photo-responsive polymersomes

Light, an external source, has been used for the design of intelligent delivery systems because the release of entrapped therapeutic agents can be rapidly induced at specific time points and locations by exposure to light.[16] Although various stimuli-responsive systems respond quickly, they require the chemical modification of the environement via the addition of acids/bases or other reagents. However, light is a remote stimulus that does not require the addition of external substances. To prepare light-responsive polymersomes, the photo-labile chromophores azobenzene, triphenylmethane, 2-nitrophenylalanine, and stilbene have been incorporated into the amphiphilic polymer systems to make the systems susceptible to light.[81-84]

For instance, Burdick et al. presented a modular approach for synthesizing photo-labile polymersomes, which allows for the incorporation of various functional groups at the junction of amphiphilic blocks (Fig. 7).[85] With the utilization of this strategy, photo-cleavable 2-nitrobenzyl groups or 2-nitrobenzyl groups-bearing fluorescein was introduced in middle of PEG and PCL copolymers. Owing to the amphiphilic nature, the resultant block copolymer formed polymersomes. Exposing these nanoparticles to UV light resulted in the disassembly of polymersomes. The fluorescein-containing polymersomes allowed the labeling of cells. Similarly, Meier et al. synthesized a photocleavable amphiphilic block copolymer, composed of

poly(methyl caprolactone)-*b*-PAA-bearing ortho-nitrobezyl, which could self-assemble into polymersomes in aqueous solutions (Fig. 8).[86] The prepared polymersomes encapsulated a range of payloads, including small molecules (fluorescein) and proteins (eGFP). The polymersomes were completly disintegrated upon UV irradiation and rapidly released payloads. The photocleaving property of polymersomes were controlled by varying the UV intensity and irradiation time. Zhou et al. prepared block copolymer free hyperbranched supramolecular amphiphile through the noncovalent coupling between functionalized adamantane or azobenzene (Azo) with hyperbranched polyglycerol (HPG) grafted cyclodextrins (CDs).[87, 88] Such host-guest complex self-assembled into vesicles with narrow size distribution. Interestingly, the vesicles can be disassembled readily by the introduction of competitive hosts. The Janus hyperbranched polyglycerol prepared by Azo/CD complexation between CD-*g*-HPG and Azo-HBPO could form vesicles in an aqueous solution.[88] The Azo group could undergo light-triggered reversible isomerization between *trans-to-cis* form under UV light irradiation. As a result, the Janus hyperbranched vesicles could be disassembled under the irradiation of UV light (365 nm). This kind of system could be useful in pulsatile drug delivery applications.

Although various UV-responsive polymersomes have shown to possess excellent properties *in vitro*, this radiation is only applicable for the treatment of certain regions of the human body, such as the eye or the skin. Therefore, the use of UV radiation has been limited by its toxicity and low penetration depth. To circumvent this limitation, photo-sensitive groups that respond to near-infrared (NIR) laser light (~700-1000 nm) have been utilized. NIR light deeply penetrates tissue and is expected to cause minimal photodamage to the body. Nie et al. fabricated nano-sized gold vesicles from gold nanoparticles containing a PEG-poly(styrene) (PEG-PS) anchoring polymer (Fig. 9).[89] The gold-vesicles encapsulated hydrophobic Ce6 with high loading

efficiency (~18.4 wt%). The vesicles showed strong NIR absorption, as a result of the plasmonic coupling effect of gold nanoparticles. Thus, NIR excitation produced heat and a singlet oxygen from gold nanoparticles and Ce6, respectively, to synergistically kill cancer cells. The heating of gold-vesicles resulted in the disassembly of the nanoconstructs and enhanced the release of Ce6, which allowed NIR/thermal/photo-acoustic trimodal imaging. Also, the combination of PTT/PDT treatment using single continuous wave laser irradiation improved the anti-tumor efficacy.

7.2.   *Voltage-responsive polymersomes*

Numerous studies have attempted to develop stimuli-responsive polymersomes based on block copolymers. Recently, the preparation of block copolymer-free reversible nanoconstructs have paid enormous attention because of the easy fabrication and tunable structural properties of these materials. Block copolymer-free polymersomes can be obtained via host-guest interactions between the end group of hydrophilic and hydrophobic homopolymers.[90] CDs, composed of cyclic oligosaccharides with high biocompatibility, are known for their ability to form inclusion or host-guest complexes with various hydrophilic and lipophilic molecules via hydrogen bonding or hydrophobic interactions.[91-93] Yin et al. prepared voltage-responsive vesicles based on chain end-decorated poly(styrene)-β-CD (PS-β-CD) and PEG-ferrocene (PEG-Fc) hompolymers (Fig. 10).[94] These materials could form reversible diblock copolymers (PS-β-CD/PEG-Fc) via the host-guest interaction between CDs and Fc. In aqueous conditions, the copolymers self-assembled into vesicular structures that were approximately ~102 nm in size and stable for 3 months. Interestingly, the application of +1.5 V electrochemical stimuli initiated the disassembly of the vesicles, which had completely disrupted within 2 h. The voltage-responsiveness of the

polymersomes was further verified by the polymersomes with rhodamine B (RhB). The release rate of RhB was precisely tuned by varying the applied potential. In particular, an abrupt release was observed in response to a high voltage (+4.0 V). The unique features of voltage-responsive polymersomes offer a new approach for stimuli-responsive drug delivery; such systems are well suited for applications that require structural changes without the addition of chemical reagents.

Although PS-*β*-CD/PEG-Fc-based polymersomes showed promising voltage-responsive properties, their disassembly in aqueous solutions resulted in the formation of hydrophobic aggregates due to hydrophobic PS homopolymer. To surmount this limitation, Park et al. developed electrically switchable polymersomes, in which the polymersome membrane was split by an oxidizing voltage.[95] They prepared tetra-aniline-conjugated PEG (TAPEG). In an aqueous solution, TAPEG self-assembled into polymersomes and effectively encapsulated a fluorescein derivative. The membrane was split by the action of applied voltage and could form puck-like micelles. The encapsulation and release pattern of FITC demonstrated the potential of electrically switchable polymersomes.

7.3.   *Ultrasound and magnetic field-reponsive polymersomes*

Ultrasound, an external stimulus, is used as an adjuvant of chemotherapy in tumor treatmet.[96] In chemotherapy, ultrasound can be used as a sensitizer to enhance chemotherapy and overcome drug resistance. Compared with light, which does not have time and target selectivity, ultrasound is effective for deep tissue penetration by tuning frequency, duty cycles and time of exposure. Recently, Du et. al. prepared a novel polymersomes that is responsive to both ultrasound and pH (Fig. 11).[97] Polymersomes were prepared from a triblock copolymer based on PEG-*b*-poly[2-(diethylamino)ethyl   methacrylate-*stat*-tetrahydrofuranyloxy)ethylmethacrylate]

(PEG-*b*-p(DEA-*stat*-TMA)). This polymersome respond to both ultrasound and pH, and its potential applications were examined based on the entrapment and release of DOX under different conditions. In response to 180 W ultrasound radiation, the vesicles shrank to accelerate DOX release. Upon ultrasound exposure, the PTMA chains hydrolytically reduced and the hydrophobic balance was altered, which resulted the shrinking of the polymersomes.

Magnetic field-responsive materials are an important class of stimuli-responsive polymeric systems in drug delivery.[98] The drug release from these types of polymersomes could be triggered by applying magnetic field. In general, magnetic field-responsive polymersomes are prepared by encapsulating magnetic nanoparticles into the polymersomes. For instance, Lecommandoux et al. simultaneous encapsulated magnetic nanoparticles and DOX into the poly(trimethylene carbonate)-*b*-poly(GA)-based polymersomes.[99] The controlled release of DOX was studied under magnetic field. The applied magnetic field could generate local heating by hypothermia effect, and the produced heat energy altered crystallinity of polymersomes membrane, which resulted in an abrupt drug release.

8.   Conclusion and future perspectives

Harnessing the tumor microenvironment in nanocarriers-based drug delivery has proven to be a powerful strategy to improve chemotherapy. In this review, we highlighted several representative examples of stimuli-responsive polymersomes, which are the most promising nanoconstructs for biomedical applications. Polymersomes are self-assembled amphiphilic polymers in which an aqueous compartment is enclosed by a thick bilayer membrane. Unlike conventional polymersomes, stimuli-responsive polymersomes rapidly release drugs at the target

site in response to a specific stimulus. Numerous biological and chemical stimuli, including pH, temperature, enzyme, and reduction, have been employed to trigger the hydrophobic-hydrophilic balance of polymersomes and destabilize their nanostructures. It is notheworthy that physical stimuli, including ultrasound, electrochemical potential, and light, can be applied via external sources and have seldom been used to prepare polymersomes. In fact, physical stimuli-responsive polymersomes constitute a non-invasive cancer therapy, which may open a new paradigm in cancer therapy. Although numerous stimuli-responsive polymersomes have been developed for biomedical applications, their potential application often been proven *in vitro*, which does not guarantee their efficacy *in vivo*. In addition, the safety of polymersomes needs to be considered primarily. In particular, for practical applications, it will be important to develop polymersomes based on well established biocompatible materials like PEG and poly(carbonates)-based materials. Furthermore, novel stimuli-responsive polymersomes with suitable targetability and stability with multi stimuli-linkers that might cleavable in extracellular and intracellular space in a stepwise manner might be promising.

Acknowledgments

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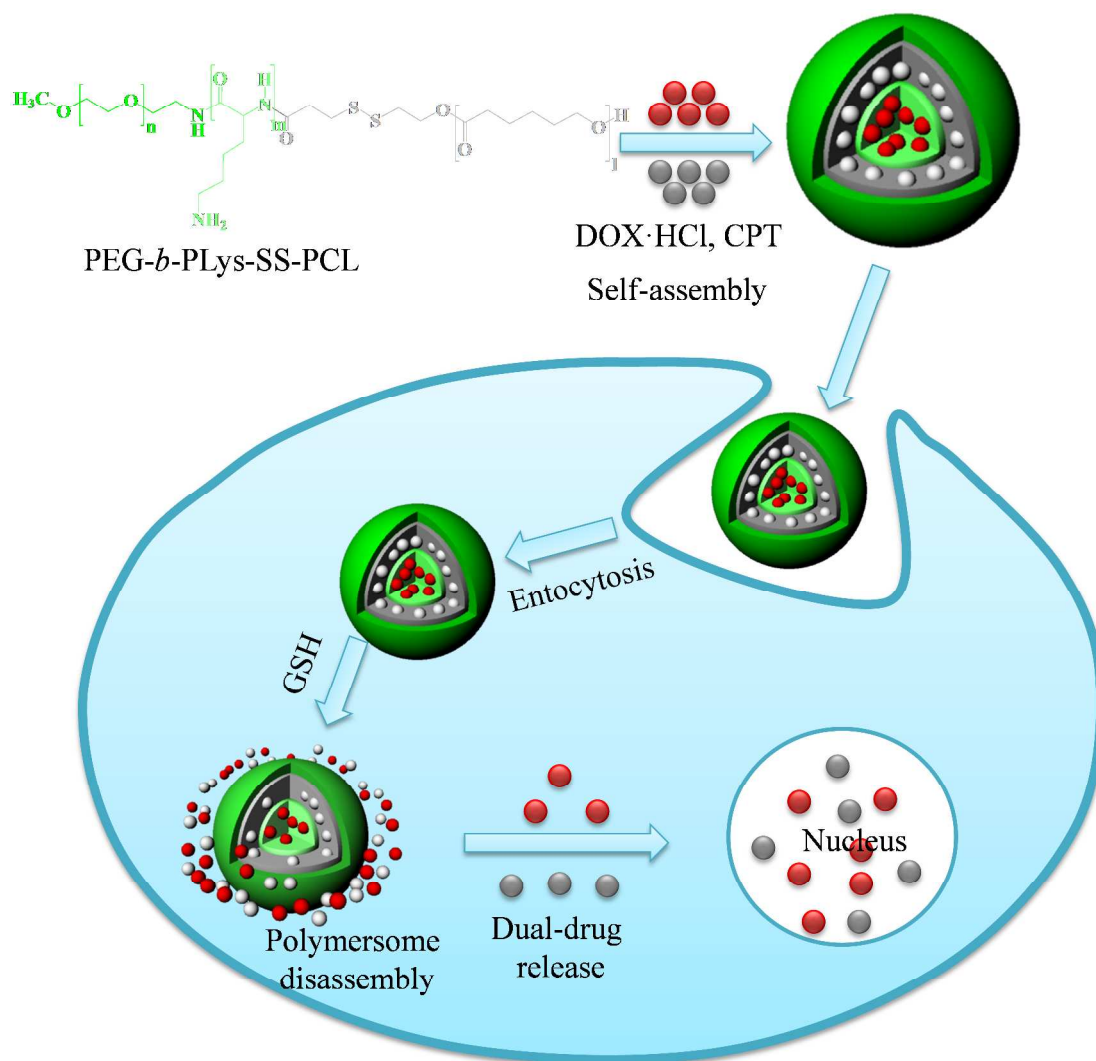
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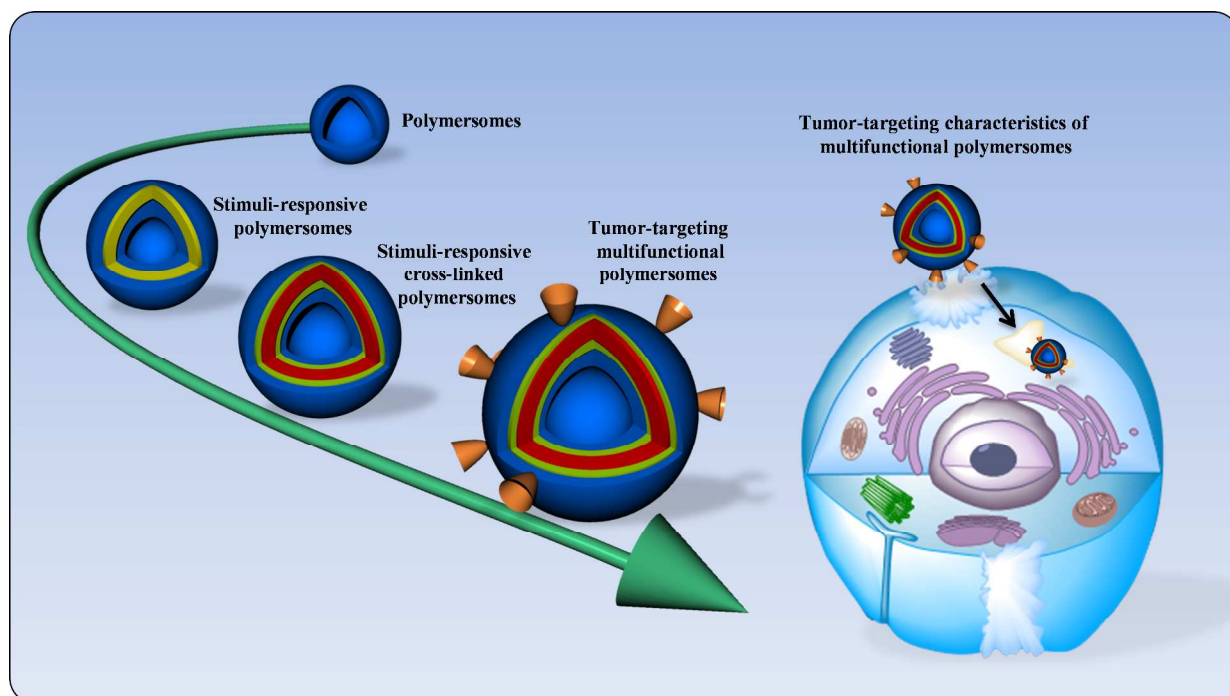
Fig. 1. Schematic illustration of state of the art of polymersomes and their targeting characteristics in cancer therapy.
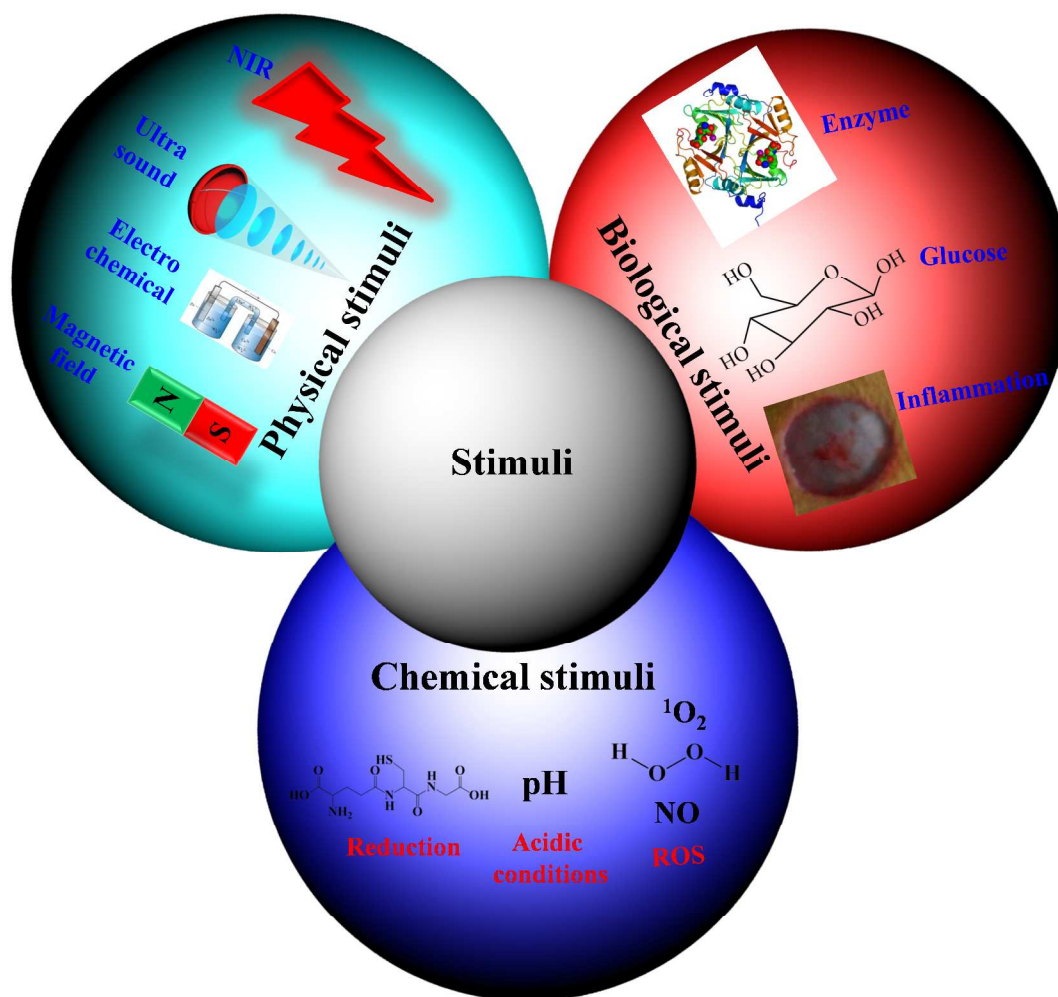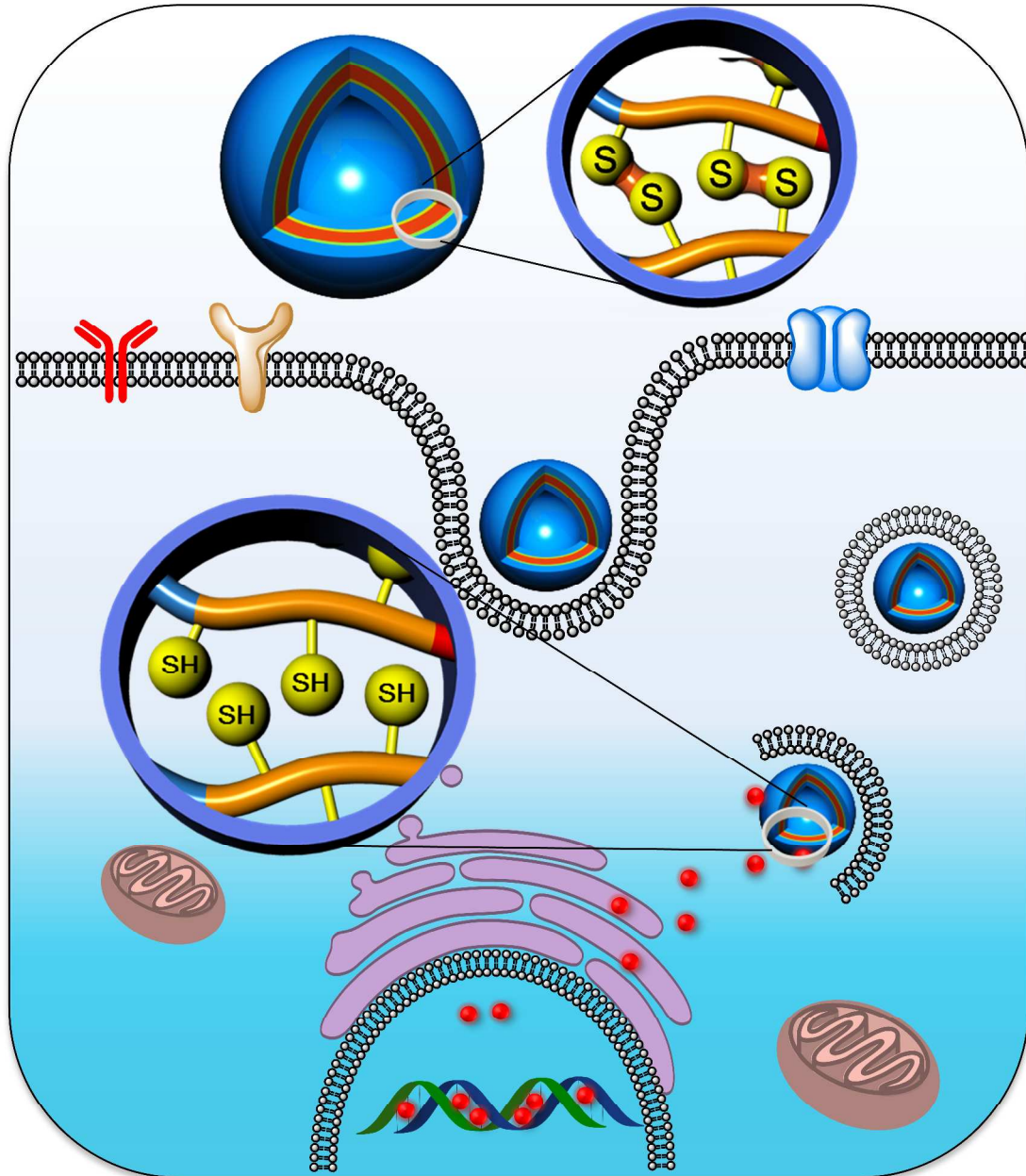
**Fig. 2.** Schematic illustration of various types of stimuli and their representative examples.

PEG-*b*-PLys-SS-PCL

DOX·HCl, CPT

Self-assembly

Entocytosis

GSH

Polymersome
disassembly

Dual-drug
release

Nucleus

**Fig. 3.** Schematic illustration of bioreducible dual-drug polymersomes for intracellular drug delivery.[33] Reprinted with permission from ref. 33.

Fig. 4. Controlled delivery of drugs into the nucleus of cancer cells from cross-linked polymersomes.[40] Reprinted with permission from ref. 40.
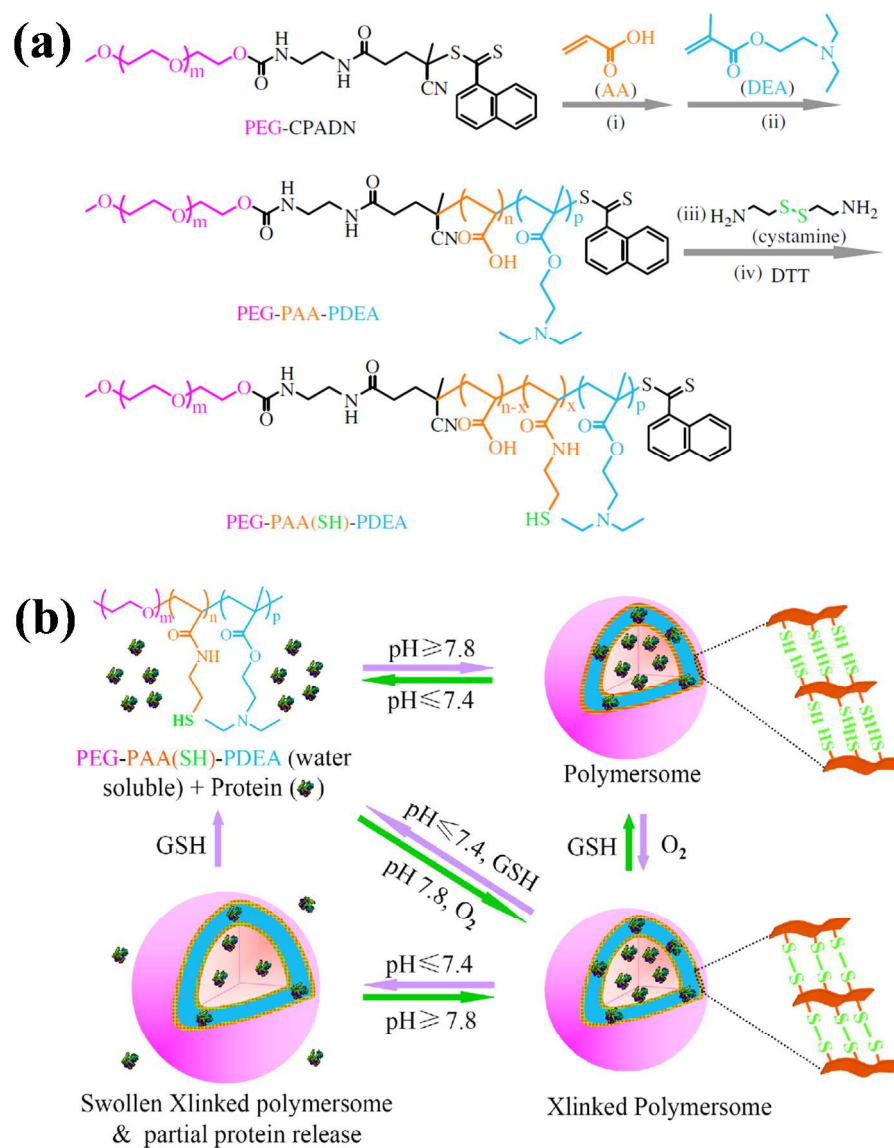
Fig. 5. (a) Synthesis scheme for preparation of thiolated PEG-PAA-PDEA triblock copolymer. (b) Schematic illustration of pH and reduction dual-reponsive cross-linked polymersomes for triggered release of encapsulated protein.[43] Reprinted with permission from ref. 43.
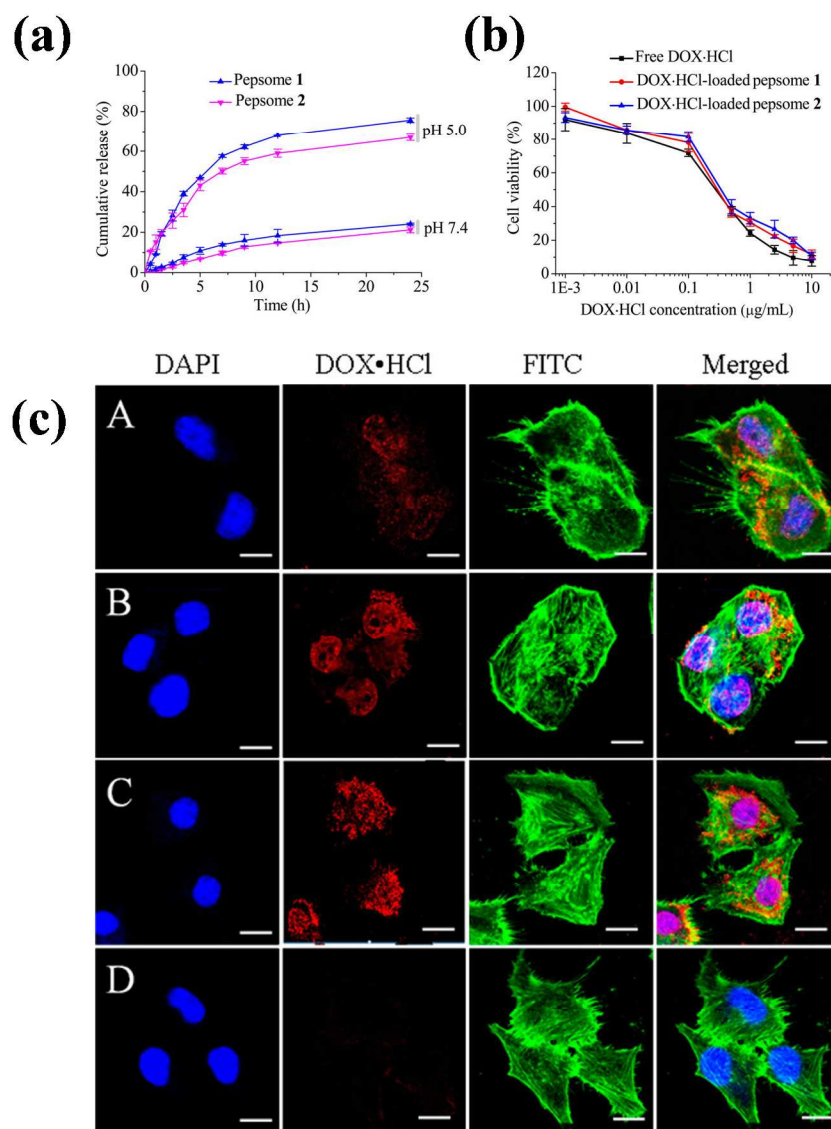
Fig. 6. (a) pH-dependant drug release behavior of pepsomes at 37 ºC. (b) Cytotoxicity of DOX·HCl-loaded pepsomes on RAW 264.7 cells. (c) CLSM image of multidrug-resistance MCF-7 cells incubated with DOX·HCl-loaded pepsomes and free DOX·HCl. DOX·HCl-loaded pepsome (A) 1 h; (B) 2 h; (C) 4 h; and (D) free DOX·HCl, 4 h incubation.[66] Reprinted with permission from ref. 66.
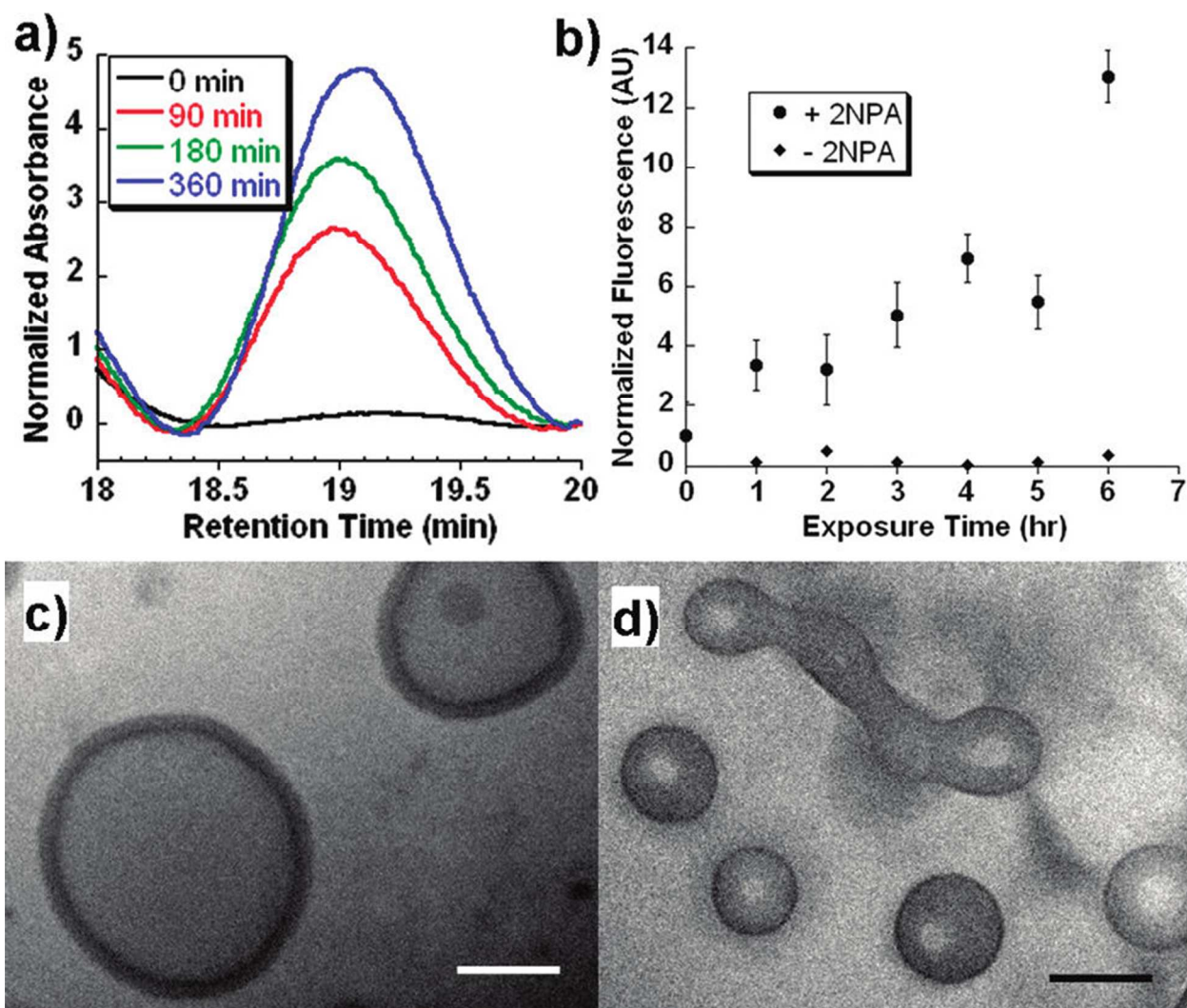
Fig. 7. (a) GPC trace of the block copolymers as a function UV exposure time. (b) *In vitro* release of biocytin from polymersomes under UV exposure. Cryo-TEM image of polymersomes at (c) 0 h and (d) 6 h after UV exposure.[77] Reprinted with permission from ref. 77.
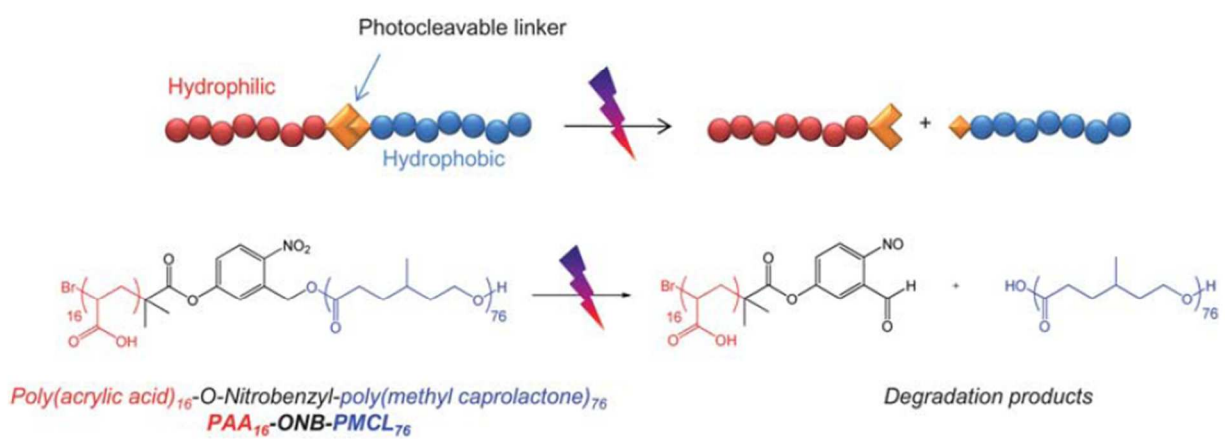
Fig. 8. Schematic illustration of photocleavable polymersomes and chemical structure of *O*-nitrobenzyl-bearing poly(methyl caprolactone)-*b*-poly(acrylic acid) copolymer and its degradation reaction after UV irradiation.[78] Reprinted with permission from ref. 78.
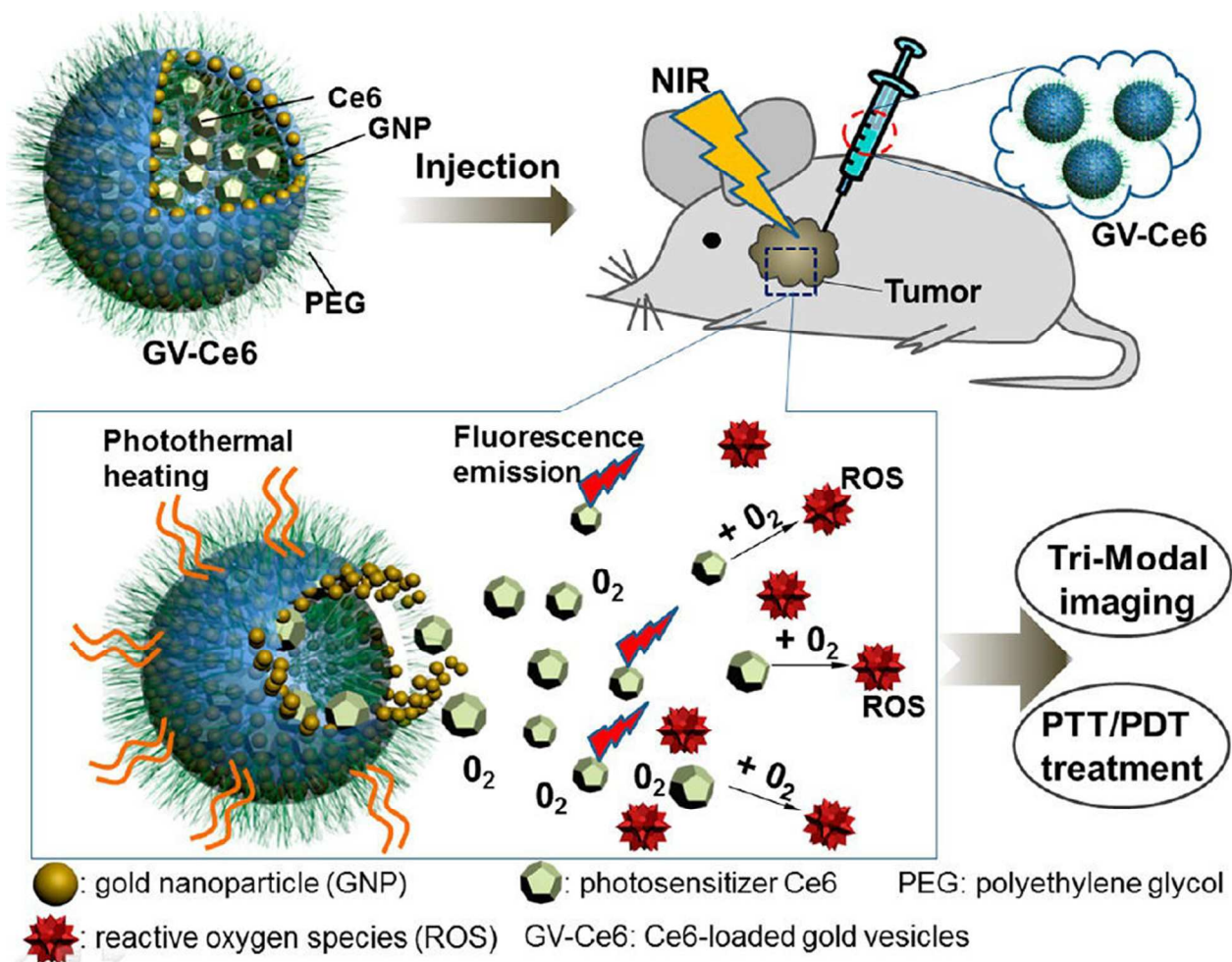
Fig. 9. Systemic administration of photosensensitizer-loaded gold vesicles for multimodal (fluorescence/thermal/photoacoustic) image-guided photothermal/photodynamic cancer therapy.[79] Reprinted with permission from ref. 79.
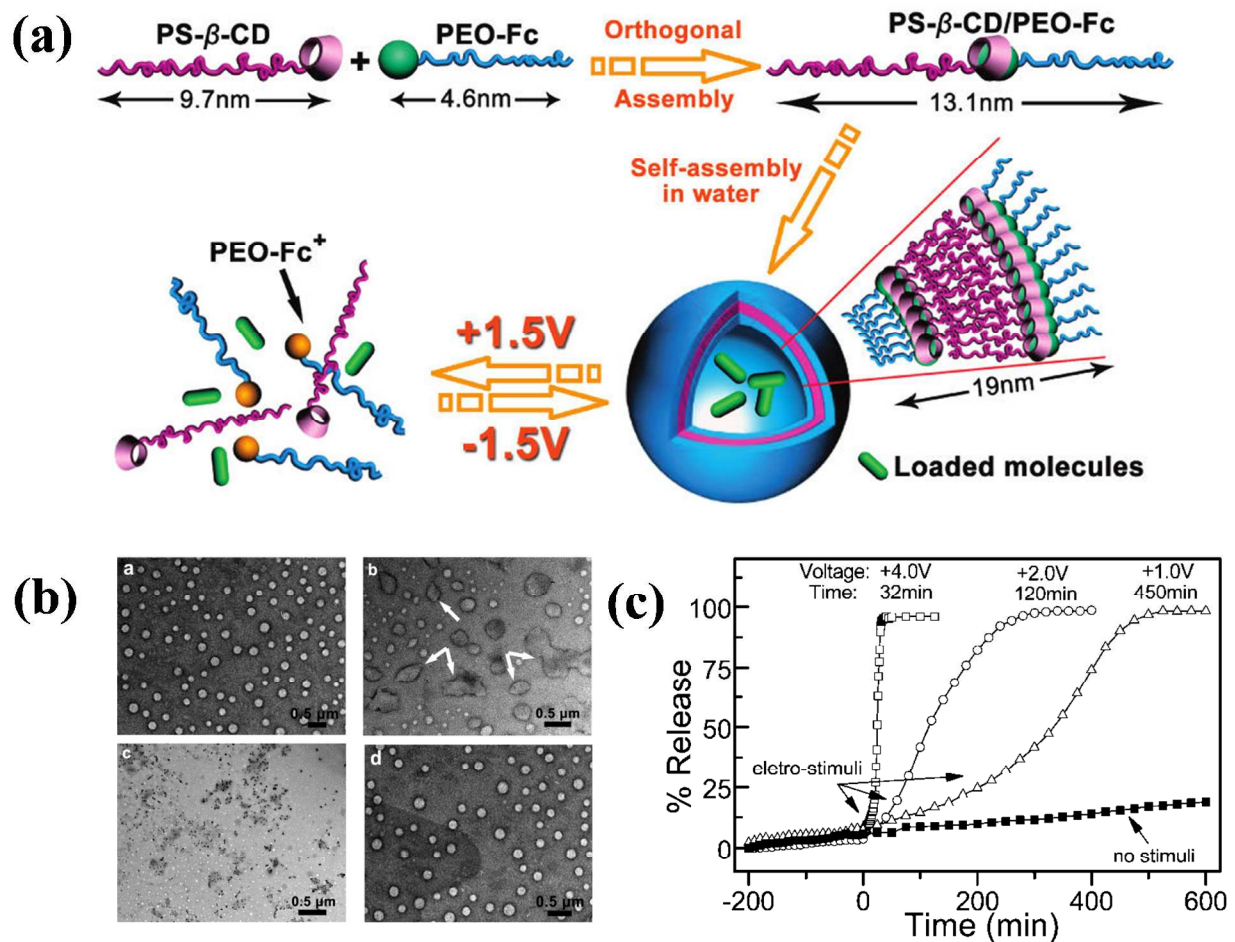
Fig. 10. (a) Schematic illustration of formation of block copolymer free polymersomes from PS-*β*-CD and PEG-Fc homopolymers and their voltage-responsive assembly and disassembly. (b) TEM image voltage-reponsive polymersomes. Top left figure shows the polymersomes with no external voltage and the top left figure shows the polymersomes 2 h after +1.5 V applied voltage. Bottom left figure shows 2 h after +1.5 V applied voltage and bottom right figure shows 5 h after -1.5 V applied voltage. (c) *In vitro* release of RB from polymersomes upon applying variying voltage.[84] Reprinted with permission from ref. 84.
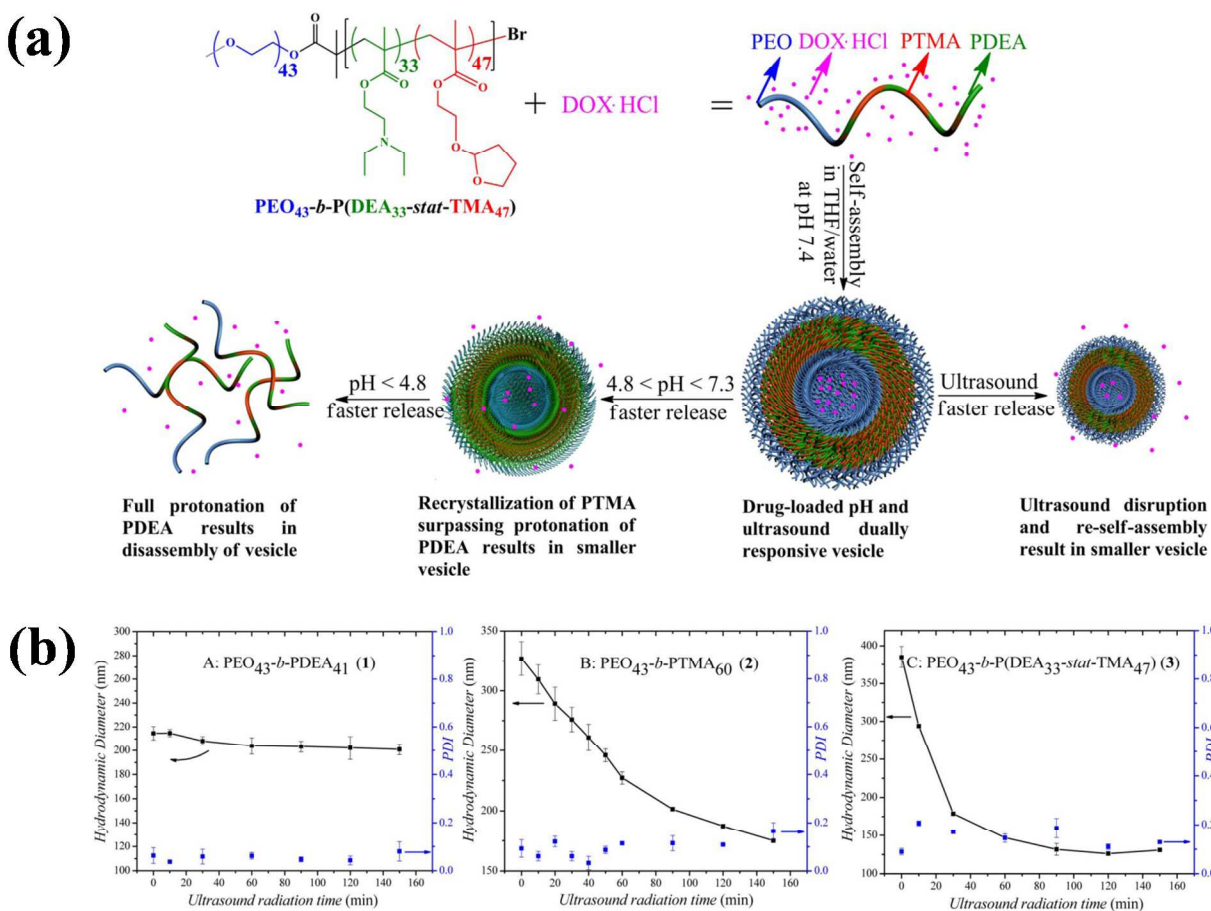
Fig 11. (a) Preparation of ultrasound and pH dual-responsive polymersomes from PEG-*b*-P(DEA-*stat*-TMA) copolymers and their drug release triggered by lowering the pH or ultrasound radiation. (b) Effect of particle size change of copolymers by ultrasound irradiation as a function of time.[87] Reprinted with permission from ref. 87.

Graphical Abstract

This review focuses on stimuli-responsive polymersomes for cancer therapy, which can be disintegrated by recognizing the specific environments of cancer (e.g., low pH, biroreductive environment, over-expressed enzymes, *etc.*).