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## Stimuli-Responsive Materials for Controlled Release of Theranostic Agents

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### Abstract

Stimuli-responsive materials are so named because they can alter their physicochemical properties and/or structural conformations in response to specific stimuli. The stimuli can be internal, such as physiological or pathological variations in the target cells/tissues, or external, such as optical and ultrasound radiations. In recent years, these materials have gained increasing interest in biomedical applications due to their potential for spatially and temporally controlled release of theranostic agents in response to the specific stimuli. This article highlights several recent advances in the development of such materials, with a focus on their molecular designs and formulations. The future of stimuli-responsive materials will also be explored, including combination with molecular imaging probes and targeting moieties, which could enable simultaneous diagnosis and treatment of a specific disease, as well as multi-functionality and responsiveness to multiple stimuli, all important in overcoming intrinsic biological barriers and increasing clinical viability.

### Keywords

stimuli-responsive materials; controlled release; smart carriers; theranostics

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## 1. Introduction

Advances in science and medicine over the past several decades have brought about the advent of numerous therapeutic agents that can be used to treat a wide variety of diseases. However, only a small fraction of these agents has ever been applied to clinical applications, due in part to their poor pharmacokinetic properties, such as accumulation in non-target tissues, unwanted side effects, and rapid clearance from the blood.<sup>[1, 2]</sup> Additionally, certain biological barriers exist *in vivo* that tend to prevent the agents from reaching their target sites.<sup>[3, 4]</sup> For example, systemically administered agents are rapidly sequestered by the reticuloendothelial system (RES) and quickly degraded in acidic and enzymatic organelles such as endosomes and lysosomes.<sup>[5, 6]</sup>

Various types of delivery carriers have been developed to overcome the aforementioned problems by altering the biological profiles of a therapeutic agent and manipulating its properties such as pharmacokinetics, pharmacodynamics, therapeutic index, and biodistribution.<sup>[7–9]</sup> In general, the therapeutic agent can be chemically conjugated to or physically complexed with, as well as encapsulated in, the delivery carrier.<sup>[10,11]</sup> The performance of such a delivery system is critically dependent on the physicochemical properties of the carrier. When prepared in the form of nanoscale objects, the carrier can cross multiple extracellular and intracellular barriers, leading to a substantially enhanced therapeutic efficacy for the loaded drug.<sup>[12]</sup> Because of its small size, such a carrier can also extravasate through the vascular endothelial junctions.<sup>[12]</sup> Significantly, the transport of a nanoscale carrier across a tumor endothelium can be more efficient than a normal endothelium owing to the leaky blood vessels and poor lymphatic drainage. This phenomenon is commonly known as the enhanced permeability and retention (EPR) effect.<sup>[13, 14]</sup> In brief, advantages of a delivery system based on nanoscale carriers are manifested by the prolonged circulation of active therapeutic agents in the blood, increased cellular uptake, and reduced toxicity.<sup>[15–17]</sup> Additionally, optimization of the surface properties of a nanoscale carrier can lead to further enhanced circulation in the blood by avoiding opsonization and nonspecific uptake by the RES.<sup>[12]</sup>

Despite the successful demonstration of many types of delivery carriers for both *in vitro* and pre-clinical animal and human studies, most of them still suffer from a number of limitations such as premature release during circulation in the blood and unmanageable release upon accumulation at the target site.<sup>[18, 19]</sup> To overcome these limitations, stimuli-responsive materials have been developed to enable on-demand release of drugs with precise temporal and spatial controls by combining advancements in materials chemistry with a continuously evolving understanding of the physiological conditions at the disease sites.<sup>[20, 21]</sup> For carriers made of stimuli-responsive materials, their physicochemical properties can change sharply in response to the specific endogenous or exogenous stimuli. Such a control can increase therapeutic efficiency through stimulus-triggered release of the payload at the disease site, while maintaining good stability until reaching the target. In many cases, such stimuli-responsive carriers also become active participants in the therapeutic landscape instead of simply serving as the passive carriers.

In the past few decades, various types of stimuli-responsive materials have been developed in an effort to overcome key challenges inherent in conventional drug delivery systems.<sup>[22, 23]</sup> In this feature article, we begin with classification and discussion of stimuli-responsive materials sorted according to the mechanisms of response to different types of stimuli, with a focus on those recently developed by our groups. We mainly concentrate on innovative approaches to molecular designs that have been used to enable the desired stimuli-responsive characteristics. Later, we offer some insights into the design and engineering of the next-generation stimuli-responsive materials for more effective therapeutic applications.

## 2. General strategies for the design of stimuli-responsive carriers

The stimuli that can be exploited for triggering the release of a theranostic agent from a carrier are diverse, but they can be broadly classified into two major types: internal and external. Internal stimuli represent physiological and pathological variations that occur naturally in target cells and tissues, whereas external stimuli refer to inducements externally applied to biological systems. Examples of stimuli that can be utilized for controlling the release of therapeutic agents are depicted in Figure 1. Making the best selection from various triggers to induce the sharpest change in chemical structure and physical property in a specific material is pivotal to the development of an effective stimuli-responsive system for drug delivery. In particular, the therapeutic benefit of a stimulus-responsive material can be tremendous when the stimulus is unique to the disease pathology and the material specifically responds to the pathological “trigger”. One of the popularly utilized pathological stimuli, which can be exploited for smart drug delivery, is the tumor-related provocation shown in Figure 1.

### 2.1. Internal stimuli

The physiological properties of diseases have been extensively investigated in the course of understanding the occurrence of diseases and developing effective therapeutic agents. Such knowledge and understanding has been used in the development of stimuli-responsive carriers that can selectively deliver their payloads to targeted disease areas. For example, the poorly vascularized tissues in a tumor facilitate the formation of hypoxic cells with low oxygen partial pressure, low pH, and high levels of bioreductive molecules.<sup>[24–26]</sup> As a result, tumor tissues exhibit a lower extracellular pH than normal tissues. The average extracellular pH of tumor tissues is between 6.0 and 7.0 whereas the extracellular pH of normal tissues and blood is around 7.4.<sup>[27, 28]</sup> Additionally, tumor tissues are typically enriched with proangiogenic and angiogenic enzymes.<sup>[29, 30]</sup> These disparate conditions between tumor and normal tissues, which are present in pathological states, can be employed to manipulate the release of therapeutic agents from a stimuli-responsive carrier.

Once a drug-loaded carrier has entered the cell, it has to reach the cytoplasm and release the drug in order to achieve the desired therapeutic effect. In the intracellular microenvironment, the cellular organelles such as endosomes and lysosomes exhibit sharp pH difference.<sup>[31, 32]</sup> A carrier with a pH-sensitive component can facilitate the release of its payload in the target compartment, either by destabilization of the carrier itself or by degradation of the pH-sensitive linker that conjugates the drug to the carrier. The redox potential in the cytoplasm

is another commonly utilized internal stimulus for facilitating the intracellular delivery of therapeutic agents. Since the redox potential in the cytoplasm is much higher than that in the extracellular environment,<sup>[33]</sup> redox-sensitive carriers can activate intracellular disassembly and release their payloads when in the reductive cytoplasm while maintaining stability in the extracellular environment. Moreover, due to altered production of reactive oxygen species (ROS), an aberrant redox balance has been observed in a number of pathological events including diabetes, atherosclerosis, cancers, and inflammatory disease.<sup>[34]</sup> Therefore, ROS-responsive delivery systems that release therapeutic agents at disease sites with high levels of ROS show great therapeutic potential. Alternatively, certain enzymes which are highly expressed in malignant cells can also be explored as an internal trigger. The unique advantages of using enzymes as a trigger include high specificity, high selectivity, and high efficiency.

## 2.2. External stimuli

There is increasing evidence that the environmental variations between pathological and normal tissues are often negligible or are not always available due to the deviation between individual patients and the limited biological knowledge of certain diseases. In some cases, especially when internal stimuli are absent, external regulations are more viable for controlled release of therapeutic agents. In contrast to drug release triggered by intrinsic biological stimuli, an externally regulated system offers spatial and temporal controls over the release profile since drug release will only be activated when the external stimulus is applied.<sup>[35, 36]</sup> Moreover, it allows for pulsatile drug release, which is defined as a rapid and constant release of the payload after a pre-determined off-release period.<sup>[37]</sup> However, externally triggered drug release systems may require some specialized equipment and devices to generate a specific stimulus, which must be taken into consideration when designing these smart systems. External stimuli may include the change in temperature, light, ultrasound, magnetic field, and electrical field.<sup>[35–39]</sup> To further improve the performance and applicability, systems responsive to multiple stimuli (*e.g.*, an array of both internal and external stimuli) have also been intensively explored.

## 2.3. Stimuli-responsive carriers

Various internal and external stimuli provide the inspiration for the design and development of stimuli-responsive materials that release their cargos in a controlled and predictable fashion. Different strategies have been investigated to facilitate targeted and controlled release of drugs. In general, these strategies often utilize hollow, porous structures and/or smart materials that exhibit controllable change in their bulk and/or surface characteristics in response to presented stimuli. As shown in Figure 2, these carriers can be divided into several classes according to their molecular interactions with the cargos. For example, in Figure 2a, bioactive cargos are directly conjugated to a carrier through a labile linker that can be cleaved in response to a specific stimulus. Stimuli-responsive release of bioactive cargos from a carrier can also be achieved through the dis-assembly of a carrier complexed with a bioactive cargo, due to degradation of the linkages between the carrier's molecular components or conformational changes within the carrier's molecular structures in response to a stimulus (Figure 2b). In addition, bioactive cargos which are physically entrapped within a carrier can be released by a stimulus-triggered phase change in the carrier (Figure

2c). Another strategy for controlled release is to selectively trap the cargo inside the hollow interior of nanocarriers, which are themselves capped with smart polymers or smaller nanoparticles (*e.g.*, Au nanoparticles, quantum dots, and iron oxide nanoparticles) *via* stimuli-responsive linkers. These small nanoparticles act as stoppers and can be removed by either internal or external triggers (Figure 2d). A novel system for the controlled release has recently been developed where the encapsulated bioactive cargos can be released *via* the formation of pores inside the shell of a carrier. Bubble-generating molecules encapsulated in the carrier can generate pores in the walls under a particular stimulus, which triggers the prompt release of the encapsulated cargo (Figure 2e).

### 3. Examples of stimuli-responsive materials

This section highlights the various types of stimuli-responsive materials that were recently developed by our and other groups, sorted according to the mechanism of response to a given stimulus. Their molecular designs and characteristics are discussed in some details, and a complete list of these materials can be found in Table 1.

#### 3.1. pH-responsive materials

The concept of using pH-responsive materials as drug carriers relies on the variation in pH between pathological or physiological states, which can exist on both cellular and tissue levels. For instance, upon cell uptake *via* endocytosis, the carrier faces endosomal and then lysosomal compartments with significantly different pH values. The early endosome has a pH value of about 6.0–6.8 while the late lysosome, which is the most acidic compartment, has a pH value around 5.0.<sup>[40, 41]</sup> Such intracellular pH gradients have been used in the design of pH-responsive carriers for the controlled release of a theranostic agent. A number of acid-labile linkers have been incorporated into the carriers for this purpose, including hydrazone,<sup>[42]</sup> acetal,<sup>[43]</sup> ketal,<sup>[44]</sup> orthoester,<sup>[45, 46]</sup> vinyl ester,<sup>[47]</sup> thiopropionate,<sup>[48]</sup> anhydride,<sup>[49]</sup> and silyl ether.<sup>[50]</sup> Additionally, some polymeric carriers whose hydrophilicity/hydrophobicity or structural conformation is sensitive to pH changes have been developed for pH-triggered release.<sup>[51, 52]</sup>

We have recently developed hollow microspheres (HMs) of poly(D,L-lactic-*co*-glycolic acid) (PLGA) containing sodium bicarbonate (NaHCO<sub>3</sub>) for the release of doxorubicin (DOX) in response to the acidic pH in endocytic organelles.<sup>[53]</sup> As shown in Figure 3a, once a PLGA HM has been transported into an endocytic organelle of a live cell, the protons seeping in from the endosomal or lysosomal compartment will react with the encapsulated NaHCO<sub>3</sub> to generate CO<sub>2</sub> gas. The evolving CO<sub>2</sub> bubbles will cause a quick increase in internal pressure, leading to the fragmentation of the PLGA shell, which in turn allows the prompt release of the encapsulated DOX. While the PLGA HMs containing NaHCO<sub>3</sub> had a smooth surface and a dense structure at pH 7.4, those incubated under acidic solutions (*e.g.*, pH 6.0 or 5.0) were dramatically fissured (Figure 3b). We loaded a green fluorescent lipophilic dye in the PLGA shells of the HMs and incubated them with cells for different durations of time to monitor the intracellular release of the encapsulated DOX (red fluorescence in Figure 3c). Confocal laser scanning microscopy (CLSM) showed the accumulation of DOX inside the cell nuclei for the PLGA HMs containing NaHCO<sub>3</sub> after incubation for 12 h, demonstrating the efficient intracellular release of DOX during the

endosomal/lysosomal trafficking (Figure 3c). The released DOX accumulated in the cell nuclei and the amount of DOX released from the PLGA HMs increased in proportion to the increase in the amount of  $\text{NaHCO}_3$ . In contrast, the PLGA HMs containing no  $\text{NaHCO}_3$  (HMs-w/o- $\text{NaHCO}_3$ ) displayed a limited release of DOX from the carrier (Figure 3c).

Recent therapeutic approaches have started to target the acidic extracellular environments of tumors. A typical example involves a pH-responsive nanogel consisting of poly(2-aminoethyl methacrylate hydrochloride) (PAMA), which is cross-linked with diacrylate poly(ethylene glycol) (PEG) and then modified with 2,4-dimethylmaleic anhydride (DMMA) on the surface.<sup>[54]</sup> This PAMA-DMMA nanogel had a negative surface charge under physiological conditions, resulting in enhanced biocompatibility. The nanogel has to be activated in order to be positively charged under slightly acidic conditions for efficient cellular uptake (Figure 4a). Figures 4b and 4c show CLSM images of MDA-MB-435 cells incubated with a PAMA-DMMA nanogel labeled with fluorescein isothiocyanate (FITC) at pH 6.8 and 7.4, respectively. At pH 6.8, the green fluorescent nanogel was internalized to a remarkable extent and distributed throughout the cytoplasm. At pH 7.4, however, the nanogel adhered mainly to the cell membrane. This difference can be largely attributed to the positive charges on the PAMA-DMMA nanogel upon acid-hydrolysis at pH 6.8, which could enhance the cellular internalization through attractive interaction with the negatively charged cell membranes. The positively charged PAMA-DMMA nanogel also exhibited enhancement in DOX release due to an increased repulsive force between the positively charged DOX and nanogel. As a result, the DOX-loaded PAMA-DMMA nanogel substantially inhibited the proliferation of cancer cells at pH 6.8, relative to the proliferation observed at pH 7.4. The FITC-labeled nanogel was intratumorally injected into tumor-bearing mice, and the tumoral distribution of the nanogel was examined by CLSM, in comparison with the FITC-labeled PAMA-succinic anhydride (PAMA-SA) nanogel, a control nanogel lacking the pH-responsiveness. As indicated by the white arrows in Figures 4d and 4e, most of the PAMA-DMMA nanogel was located in the cytoplasm of tumor cells due to efficient cellular internalization of the gel stemming from its positive surface charges, whereas the PAMA-SA nanogel remained exclusively in the extracellular space or adhered to the cell membranes (Figure 4e).

### 3.2. Redox-responsive materials

It has been well-established that the extracellular space is an oxidative environment while the intracellular space is a reductive one, which can be ascribed to the variations in concentration for the reducing agents. For example, in body fluids (*e.g.*, blood) and extracellular matrices (ECMs), the concentration of glutathione (GSH, a thiol-containing tripeptide) is approximately 2  $\mu\text{M}$ . In contrast, the intracellular concentration of GSH is 0.5–10 mM, and it is kept at a constant level by glutathione reductase to maintain a highly reductive environment inside the cell. Meanwhile, in some tumor cells, the concentration of GSH can be several times higher than normal.<sup>[55]</sup>

The high redox potential gradient between the oxidizing extracellular space and the reducing intracellular space has been exploited for controlling the intracellular drug delivery, mainly by incorporating a reducible disulfide bond into the drug-loaded carriers. Several groups



have developed disulfide bond-bearing reducible carriers for efficient and controlled drug release in the cells.<sup>[56, 57]</sup> For example, shell-shedding micelles based on a disulfide-linked poly(ethylene glycol)-*b*-poly( $\epsilon$ -caprolactone) (PEG-SS-PCL) block copolymer were developed for the rapid intracellular release of DOX.<sup>[56]</sup> These micelles efficiently released DOX within 12 h in a reductive environment analogous to that of intracellular compartments such as the cytosol. In contrast, minimal drug release (<20%) was observed within 24 h for reduction-insensitive PEG-PCL micelles under the same conditions, as well as for PEG-SS-PCL micelles under non-reductive conditions. As a result, these shell-shedding micelles achieved more efficient intracellular release of DOX and higher anticancer efficacy in comparison with the non-reducible control.

The principle of the detachable shell was also utilized to efficiently deliver DOX to multi-drug resistant (MDR) cells expressing P-glycoprotein (P-gp).<sup>[57]</sup> P-gp is a membrane-bound efflux pump capable of effluxing a broad range of structurally and functionally distinct anticancer drugs.<sup>[58, 59]</sup> It was hypothesized that a sufficiently high level of intracellular accumulation of cytotoxic chemicals could overcome the MDR of cancer cells because P-gp may have limited capacity to efflux cytotoxic chemicals.<sup>[57, 60]</sup> A redox-responsive micellar drug carrier assembled from a disulfide bond-bridged block copolymer of poly( $\epsilon$ -caprolactone) and poly(ethyl ethylene phosphate) (PCL-SS-PEEP) was developed to achieve high levels of drug accumulation and retention in MDR cancer cells.<sup>[57]</sup> The PCL-SS-PEEP system exhibited an increase in DOX influx with decreased DOX efflux by the MDR cancer cells in comparison with direct incubation of DOX with the cells. Additionally, the PCL-SS-PEEP system achieved rapid intracellular release of DOX in response to the intracellular reductive environment and significantly enhanced the cytotoxicity of DOX to MDR cancer cells (Figure 5).

We recently developed redox-sensitive, branched poly(disulfide amine) (B-PDA) as a safe and efficient small interfering RNA (siRNA) carrier. RNA interference (RNAi) is a rapidly evolving topic of drug development for the treatment of genetic disorders.<sup>[61]</sup> However, rapid enzymatic degradation in serum and poor cellular uptake of naked siRNA severely limit its utility as a therapeutic drug.<sup>[62]</sup> Therefore, development of a safe and effective carrier that can protect and deliver siRNA to its intracellular target site (*i.e.*, cytosol) is indispensable to ensure efficient RNAi.<sup>[63]</sup> We synthesized a bioreducible, cationic polymer containing disulfide bonds in the backbone to achieve safe and efficient siRNA delivery *via* stimuli-responsive biodegradation and cytoplasmic release of siRNA (Figure 6).<sup>[64]</sup> We have focused on the Fas in human mesenchymal stem cells (hMSCs) as a target protein because Fas expression critically regulates the apoptosis and the angiogenic efficacy of hMSCs when they are formulated as spheroids with enlarged sizes (> 800  $\mu$ m in diameter). We have demonstrated that Fas-silencing siRNA delivery into hMSCs by bioreducible B-PDA efficiently inhibited Fas expression and thus led to effective inhibition of hypoxia-induced apoptosis in the core of enlarged hMSC spheroids. As a result, the enlargement in spheroid size not only increased the number of viable hMSCs but also dramatically increased the secretion of angiogenic growth factors per hMSC relative to small spheroids that were not treated with Fas siRNA.

### 3.3. ROS-sensitive materials

Elevated levels of ROS, including superoxide, hydrogen peroxide, and hydroxide radicals, are often observed in cellular inflammation as part of the non-specific immune reaction, which occurs as a response to harmful events such as pathogens, foreign bodies, and damaged cells.<sup>[65, 66]</sup> The contribution of ROS to the progression of a variety of diseases has stimulated a research interest to exploit the ROS as a unique cellular stimulus to achieve selective release of therapeutic agents in a site-specific manner.

Recently, various ROS-sensitive polymeric carriers have been developed to utilize oxidative conditions for selective release of their cargos. For example, a boronic ester-bearing polymeric carrier that degrades upon exposure to H<sub>2</sub>O<sub>2</sub> at low concentrations was developed.<sup>[67]</sup> The polymeric carrier could release its cargo upon exposure to 50 μM H<sub>2</sub>O<sub>2</sub>, due to the transformation of a boronic ester to a phenol and the subsequent degradation of the polyester backbone. This system represents a good example of a biologically relevant and biocompatible approach to ROS-triggered drug delivery.

A poly(propylene sulfide)-based micellar drug carrier was developed for the enhanced release of encapsulated drugs in antigen-presenting cells with phagosomal oxidative activity.<sup>[68]</sup> ROS-triggered drug release was achieved by the conversion of hydrophobic poly(propylene sulfide) to more hydrophilic poly(propylene sulfone) under ROS conditions and the subsequent disruption of the micellar structure. As a result, the poly(propylene sulfide)-based micelle showed a significantly increased release of drugs in inflamed macrophages with high ROS production.

Recently, several studies reported that cancer cells, compared to normal cells, are under an increased oxidative stress, associated with oncogenic transformation and alterations in metabolic activity.<sup>[69–71]</sup> Inspired by this finding, we have focused on the intrinsic oxidative stress of cancer cells as a unique stimulus that can be exploited therapeutically. To this end, we developed an ROS-responsive cationic polymer bearing ROS-cleavable thioketal linkages to achieve efficient intracellular gene delivery in prostate cancer cells with high levels of intracellular ROS.<sup>[72]</sup> As illustrated in Figure 7, the ROS-responsive, cationic, water-soluble poly(amino thioketal) (PATK) was specifically designed to efficiently complex with negatively charged plasmid DNA and then readily release it when exposed to the heightened oxidative conditions present in prostate cancer cells. The DNA/PATK polyplexes were efficiently disassembled upon exposure to the high levels of ROS in prostate cancer cells, leading to significantly higher gene transfection relative to its non-ROS-responsive counterpart. We also demonstrated enhancement in cancer-specific gene transfection by further functionalization of the PATK with a cancer-specific, GRP78 protein-targeting peptide. These results suggest that ROS-responsive polymers also hold great potential for site-specific drug/gene delivery to treat other ROS-related diseases such as neurodegeneration and inflammation.

### 3.4. Enzyme-responsive materials

Enzymes play an essential role in many cancer-related processes such as progression, metastasis, and angiogenesis of tumor tissues, as well as being involved in the degradation



of the extracellular matrix in arthritis.<sup>[73, 74]</sup> Exploiting enzymes in the design of stimuli-responsive carriers has a number of advantages which include, but are not limited to, the abundance of enzymes at the disease sites, high selectivity of the enzyme-catalyzed reactions, and the mild conditions involved (typically aqueous conditions, mild pH, and 37 °C).<sup>[75]</sup> Enzyme-responsive carriers mainly rely on the cleavage of esters or short peptide sequences by esterases or proteases. In a recent study, we developed a matrix metalloproteinase (MMP)-sensitive, multimodal probe that can detect and diagnose cancer lesions by photoacoustic (PA) imaging and fluorescence imaging.<sup>[76]</sup> MMP was chosen as a cancer-specific enzyme for our proof-of-concept experiment because it has been recognized as a biomarker associated with cancer cell invasion and metastasis.<sup>[73, 74]</sup> The surface of Au nanocages (AuNCs) was modified with an MMP-cleavable peptide and then a fluorescent dye. This hybrid system relies on efficient non-radiative energy transfer from the excited state of the dye to the AuNCs. As a result, the emission from the dye conjugated to the peptide was quenched in the absence of MMP-2 protease. However, in the presence of MMP-2, the fluorescence emission of the dye was recovered due to the cleavage of the peptide and the consequent release of the dye from the surface of the AuNCs. Fluorescence spectroscopy studies showed a high quenching efficiency for the dye and a high sensitivity in response to a relatively low concentration of MMP-2. This multimodal probe could enable cancer detection by fluorescence imaging, monitoring its distribution in the cancer lesions by PA imaging, as well as cancer treatment by photothermal therapy.

### 3.5. Thermo-responsive materials

Abnormal temperatures at disease sites represent a unique pathological stimulus. For example, a tumor microenvironment is often 1–2 °C warmer than normal tissues.<sup>[77]</sup> In addition to endogenous temperature change, externally induced local hyperthermia in a target tumor has been widely explored as a therapeutic strategy to trigger efficient and controlled drug release in the tumor. Thermo-sensitive release has been developed mainly with typical thermo-responsive polymers exhibiting a low critical solution temperature (LCST) around 38–39 °C.<sup>[78, 79]</sup> These polymers are in their hydrophilic, extended state in normal tissues but become hydrophobic and collapsed in the hyperthermic tumor environments. This thermal switching can be employed to regulate the release profiles of drugs from the polymers. A number of thermo-responsive polymers have been demonstrated for this purpose, with typical examples including poly(*N*-isopropylacrylamide) (pNIPAAm) and its derivatives,<sup>[80–82]</sup> poly(organophosphazenes),<sup>[83, 84]</sup> polyphosphoesters,<sup>[85–87]</sup> Pluronic<sup>®</sup>,<sup>[88, 89]</sup> and block copolymers consisting of PLGA and PEG.<sup>[90, 91]</sup>

We recently introduced a new class of thermo-responsive materials, fatty alcohols and fatty acids, which are also known as the phase-change materials (PCMs).<sup>[92]</sup> These materials undergo reversible solid–liquid transition in response to temperature changes within a very narrow range. Specifically, we have fabricated uniform 1-tetradecanol (with a melting point at 38–39 °C) beads containing the gelatin particles loaded with FITC-dextran (Figure 8a).<sup>[92]</sup> Upon an increase in temperature, the PCM beads melted, and the gelatin particles containing FITC-dextran were freed from the beads, subsequently releasing FITC-dextran into solution. The insets in Figure 8a schematically depict the release of FITC-dextran from the melted PCM beads. The release profiles of FITC-dextran from gelatin, chitosan, and PLGA

colloidal particles encapsulated in blocks made of 1-tetradecanol were determined at 37 and 39 °C. As shown in Figure 8b, no FITC-dextran was released at all for up to 24 h at 37 °C, a temperature below the melting point of 1-tetradecanol. Above the melting point of 1-tetradecanol (*i.e.*, 39 °C), the FITC-dextran encapsulated in the colloidal particles was quickly released.

More recently, we developed a thermo-responsive liposomal system that contained no anticancer drugs but was still capable of killing cancer cells.<sup>[93]</sup> This work is based on the notion that ammonium bicarbonate ( $\text{NH}_4\text{HCO}_3$ , ABC) can quickly decompose to generate  $\text{CO}_2$  bubbles (Figure 9a) upon heating at 40 °C. This compound was incorporated into the aqueous compartment of a liposome, creating an ABC-containing liposome (ABC liposome). After the liposomes were internalized, they underwent endocytosis and subsequent intracellular trafficking to lysosomes, where they were thermally triggered at 42 °C to generate  $\text{CO}_2$  bubbles. As a result of the transient formation, growth, and collapse of  $\text{CO}_2$  bubbles, a disruptive force was produced similar to the cavitation effect induced by ultrasound (Figure 9b). To demonstrate this concept, we investigated the ability of cavitation to destabilize the lipid-bilayer membranes of ABC liposomes by incorporating calcein into the aqueous core. As shown in Figure 9c, a significant release of the encapsulated calcein was observed only for ABC liposomes heated to 42 °C, whereas the controls of phosphate buffered saline-containing liposomes (PBS liposomes) and ABC liposomes heated at 25 °C and 37 °C exhibited no such behaviors. At 42 °C, the decomposition of  $\text{NH}_4\text{HCO}_3$  generated a large number of  $\text{CO}_2$  bubbles, which grew rapidly and collapsed violently, producing a cavitation force that perforated the lipid-bilayer membranes and triggered release of the encapsulated calcein. The cavitation force acting on lysosomes can mechanically disrupt lysosomal membranes to release proteolytic enzymes into the cytosol, resulting in cell necrosis without the use of any toxic agents. It was observed that cells treated with ABC liposomes at 42 °C suffered from considerable cell necrosis, as compared to untreated control cells (Figure 9d).

### 3.6. Light-responsive materials

Utilization of light as an external stimulus to trigger drug release is very attractive due to its spatiotemporal control of therapeutic molecules, high biocompatibility, and ease of application.<sup>[94–97]</sup> The major drawback of light is the tissue penetration depth, which should be addressed for efficient and targeted drug release in the bulk of a tissue. Near-infrared (NIR) light with wavelengths in the range of 650 to 900 nm has recently emerged as an attractive optical stimulus because of its minimal attenuation (due to absorption and scattering) by blood and soft tissues, thus allowing for noninvasive and deep tissue penetration.<sup>[98]</sup> Light-triggered drug release from a carrier is usually activated *via* the cleavage of a photolabile linker in the material upon irradiation with light of a certain wavelength, or *via* destabilization of the material triggered by changes in their physicochemical properties in response to light.<sup>[99, 100]</sup>

We have previously demonstrated that drug molecules could be loaded into the interiors of AuNCs and selectively released by NIR light through the photothermal effect.<sup>[101]</sup> As shown in Figure 10a, thermo-responsive pNIPAAm or its derivatives can abruptly and reversibly

collapse when heated beyond their LCSTs. These compounds were grafted to the surface of AuNCs through thiolate linkages. Upon exposure to a laser beam whose wavelength matches the localized surface plasmon resonance (LSPR) peak of the AuNCs, the light is absorbed and converted into heat due to the photothermal effect.<sup>[102]</sup> It was hypothesized that heating by NIR irradiation would cause the polymer chains to collapse and expose the pores on the AuNCs, resulting in release of the pre-loaded drugs (Figure 10a). When the laser is turned off, the drop in temperature reverts the polymer to its original extended conformation, halting the release process. To demonstrate this concept, we trapped DOX in pNIPAAm-coated AuNCs and monitored its release *in vitro*. As shown in Figure 10b, DOX was rapidly released from the polymer-coated AuNCs by irradiation with a pulsed NIR laser, regulated by irradiation time. We then tested the controlled release of DOX and the therapeutic efficacy of the DOX-loaded AuNCs *in vitro*. A significant amount of breast cancer cells incubated with the DOX-loaded AuNCs were killed after NIR irradiation, and the percentage of the live cells was reduced with increased laser exposure time (Figure 10c). In contrast, the cells irradiated with the same NIR laser for 2 min in the absence of AuNCs showed almost no change in their viability, and cells treated with AuNCs alone showed only a slight reduction in cell viability, possibly due to the photothermal effect of the AuNCs. These results demonstrated the feasibility to fabricate NIR-triggered drug release systems with high spatiotemporal resolutions using AuNCs coated with smart polymer chains.

### 3.7. Ultrasound-responsive materials

Ultrasound is a popularly used non-invasive stimulus, as it allows for spatial and temporal control with millimeter precision.<sup>[103–106]</sup> A focused ultrasound beam can trigger drug release *via* localized heating induced by the deposition of acoustic energy at a focused area.<sup>[106]</sup> We recently demonstrated a noninvasive, on-demand drug release system based on high-intensity focused ultrasound (HIFU) and AuNCs loaded with a PCM.<sup>[107]</sup> In this system, the hollow interiors of AuNCs were filled with a PCM such as 1-tetradecanol that has a melting point of 38–39 °C. Below its melting point, the PCM existed in a solid state, acting as a barrier to prevent the premixed dyes from being released. When exposed to direct heating or HIFU, the PCM quickly melted and escaped from the interior of the AuNCs through small pores on the surface, simultaneously releasing encapsulated molecules into the surrounding medium (Figure 11a). Figures 11b and 11c show the release profiles of a fluorescent dye, rhodamine 6G, from the AuNCs by direct heating and HIFU, respectively. These results show that the release dosage of the dye from the AuNCs can be regulated by the temperature or the power of HIFU.

In another study, we functionalized the surface of AuNCs with thermo-responsive pNIPAAm or one of its derivatives *via* Au-thiolate bonding.<sup>[108]</sup> The LCSTs of the pNIPAAm derivatives can be tuned from 32 to 50 °C through the incorporation of different amounts of acrylamide. When the AuNCs were exposed to HIFU, the polymer chains collapsed as the temperature increased beyond the LCST, opening the pores and releasing the pre-loaded drugs. When the HIFU was turned off, the polymer chains relaxed back to the extended state, terminating the release. The dye-loaded AuNCs covered with pNIPAAm derivatives demonstrate controlled and highly localized release of payloads upon HIFU exposure. In addition, using this system for controlled drug release at depths up to 30 mm is

possible thanks to the deep tissue penetration of HIFU. This drug delivery system based on thermo-responsive polymer-covered AuNCs and HIFU exhibited great potential for remote and controlled drug release in clinical applications.

### 3.8. CO<sub>2</sub>-responsive materials

Recently, a CO<sub>2</sub>-responsive, “breathing” polymersome system was developed in which the permeability of polymersome membranes was reversibly controlled by external CO<sub>2</sub> levels.<sup>[109]</sup> The polymersomes were self-assembled from diblock copolymers of hydrophilic PEG and hydrophobic, CO<sub>2</sub>-sensitive poly(*N*-amidino)dodecyl acrylamide (PAD). The polymersomes undergo reversible swelling/collapsing when exposed to CO<sub>2</sub>/N<sub>2</sub> in an alternating fashion, and thus altering the membrane structure and permeability. The membrane permeability of the polymersomes could be tuned by regulating CO<sub>2</sub> exposure time to induce their growth. Utilizing this controlled semi-permeability, hierarchical cargo release and selective separation of guest molecules according to their size differences were achieved. Furthermore, the vesicle shows the potential to serve as a nanoreactor which can insulate different enzymatic catalytic reactions by simply modulating the CO<sub>2</sub> exposure.

## 4. Conclusions and perspectives

A variety of stimuli-responsive materials have been developed for efficient and localized delivery of drugs to reduce the side effects. Some of these materials respond to internal stimuli, self-regulating and selectively releasing their payloads, when triggered by a mechanism specific to a pathological event. Other materials are activated externally, giving them greater spatial and temporal controls, with minimal drug released in the ‘off’ state. It has been indicated that these materials responsive to external stimuli are more clinically relevant than passively or internally stimulated ones.

The change in behavior of stimuli-responsive materials in complicated biological systems is often a result of responses to a combination of environmental stimuli. It can be reasonably postulated that the greatest advance in the therapeutic selectivity of stimuli-responsive materials will be achieved by using synergistic responses to a combination of environmental stimuli.<sup>[110–112]</sup> These materials could also be applied to diverse delivery systems as they would allow tuning of their properties in multiple ways. Increasing the sensitivity of materials in response to a particular stimulus could also lead to more accurate and programmable drug delivery. These developments are still in their initial stages, but continuing studies in engineering these materials will facilitate their translation into clinical applications.

Although the main focus of this feature article is on cancer therapy, the great potential of stimuli-responsive systems in the treatment of other diseases should not be ignored. In principle, the aforementioned concepts and strategies can also be applied to treat any other disease by modification with the appropriate targeting ligands and encapsulation with specific bioactive compounds. For example, an interesting strategy was recently reported for differential delivery of antimicrobials to bacterial infection sites with a polymeric triple-layered nanogel (TLN) that can be degraded by bacterial lipases.<sup>[113]</sup> The lipases are abundant in microbial flora since these enzymes are involved in bacterial lipid metabolism.

Once the TLN senses the lipase secreting bacteria, the TLN degrades to release the antibiotics.<sup>[113]</sup> This is but one example of the broad scope of applications for these functional materials.

Although stimuli-responsive carriers hold great promise in drug delivery for applications in nanomedicine, they still face a number of challenges *in vivo* due to multiple extracellular and intracellular barriers stymieing delivery. Ideally, after inoculation the carriers should be able to access target cells and tissues of interest, while bypassing the RES and protecting their cargo from biological degradation.<sup>[114]</sup> By combining stimuli-responsiveness with multifunctional components in materials, these obstacles could be circumvented.<sup>[115]</sup> For instance, PEGylation of carriers can overcome certain extracellular barriers by extending the blood circulation of the carrier and evading RES clearance.<sup>[116]</sup> Further enhanced accumulation of carriers in the target cells or tissues and concomitantly reduced side effects could also be achieved by tethering the carriers to active targeting ligands, including antibodies, peptides, and aptamers.<sup>[117]</sup> Nanoscale carriers with active targeting ligands would be especially advantageous for cancer therapy, due to their enhanced cellular uptake and retention of drugs *via* receptor-mediated endocytosis. Greater efforts to identify disease biomarkers and associated targeting ligands are needed to enable further development of these targeted delivery systems. Additionally, combining these materials with various imaging modalities enables their use in theranostic applications. Such integration allows controlled drug delivery and diagnosis simultaneously, enabling treatment and monitoring of a disease in parallel, which could effectively realize personalized medicine.

Moreover, it should be pointed out that much of the work to date is purely experimental and does not meet clinical standards and requirements of “reproducible, safe, clinically effective, largely scalable, and economically acceptable”.<sup>[118]</sup> In order to translate these laboratory-based processes to industry-scale techniques, combination with the approaches of translation medicine will be useful to address fundamental questions surrounding the clinical feasibility of these materials.

One fundamental concern regarding nanoscale carriers is dose-dependent toxicity. Utilizing biocompatible materials (*e.g.*, lipids, polypeptides, and natural polymers) and developing bioresorbable or bioadsorbable polymers responding to biological stimuli is necessary to lower the systemic toxicity of the carriers. As such, safety evaluations such as long-term toxicity and systemic clearance must be conducted prior to any clinical use of these materials.

Taken all together, recent advances highlighted in this feature article clearly indicate that stimuli-responsive materials hold great potential as a new platform for theranostics. Further optimization of the stimuli-responsive materials, including improvement in biocompatibility and integration of multiple stimuli-responsiveness, will be indispensable for these materials to have successful clinical translation. The sheer breadth of possible targets makes stimuli-responsive materials on a truly promising advance in both science and medicine.

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## Biographies

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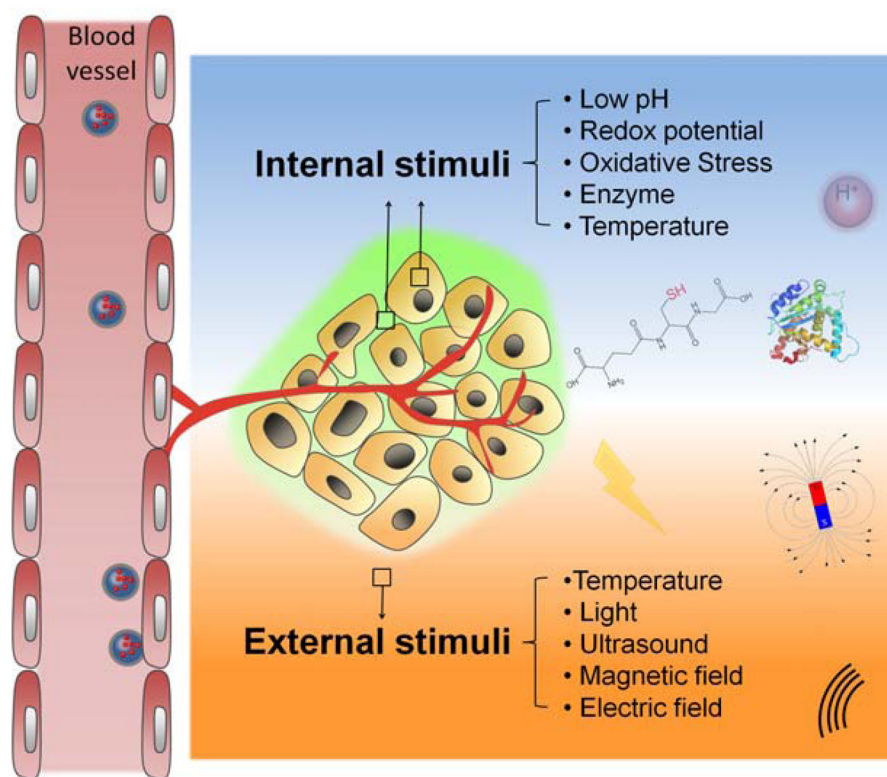
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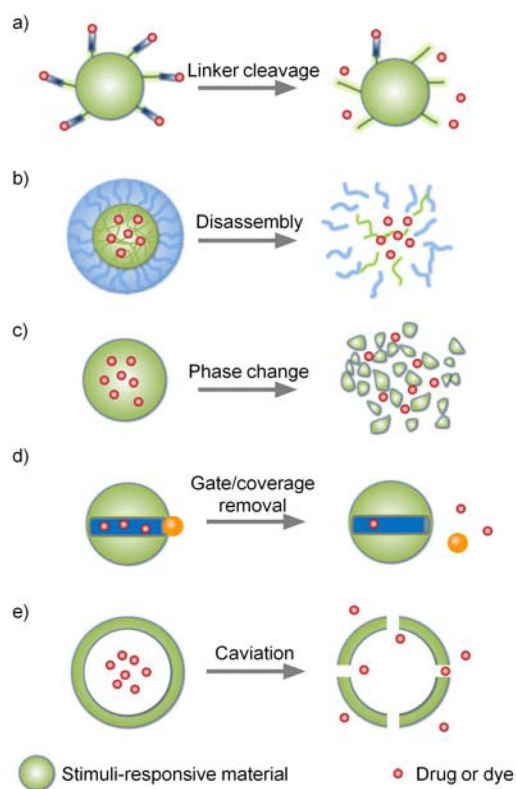
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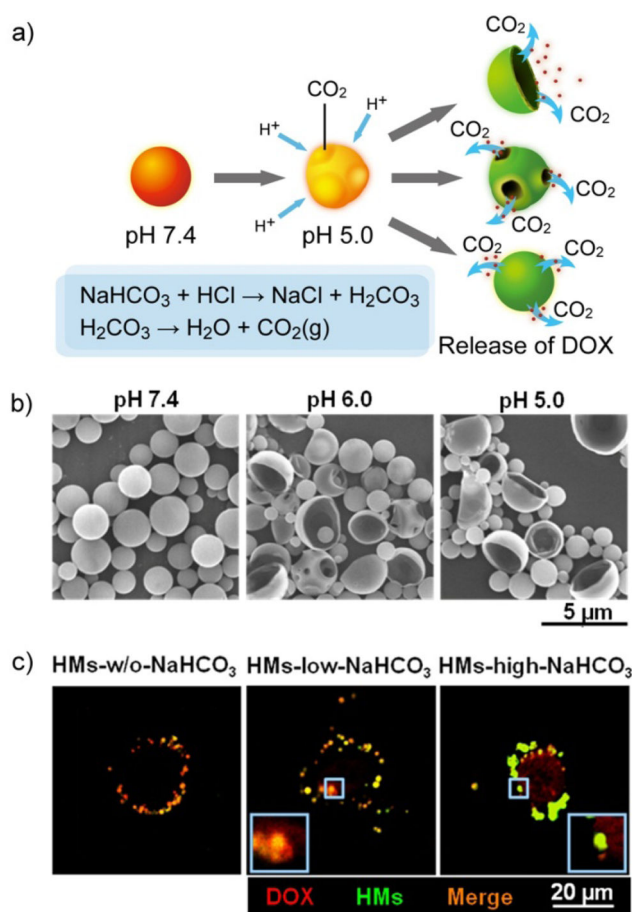
**Figure 1.**

Schematic illustrations summarizing various stimuli employed for controlled release of therapeutic cargos following systemic administration. The therapeutic cargos can be selectively released in response to internal stimuli, including low pH, redox potential, oxidative stress, enzyme, and temperature, or external stimuli, including temperature (remote heating), light, ultrasound, magnetic field, and electronic field.



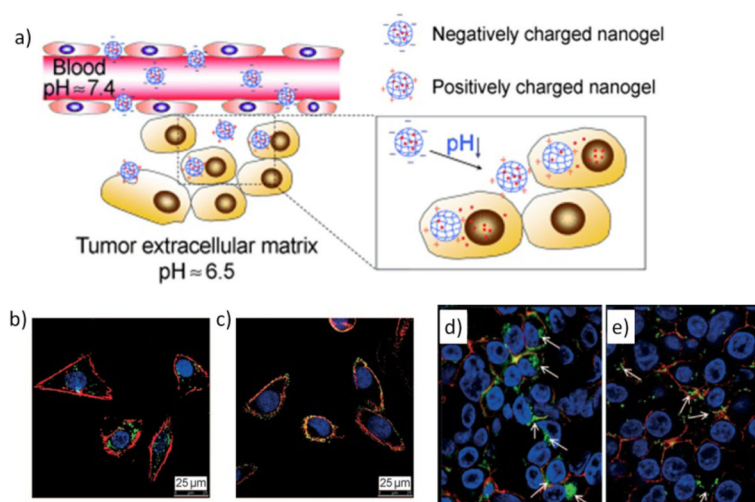
**Figure 2.** Schematic illustrations summarizing the major strategies that have been developed for constructing stimuli-responsive drug release systems.





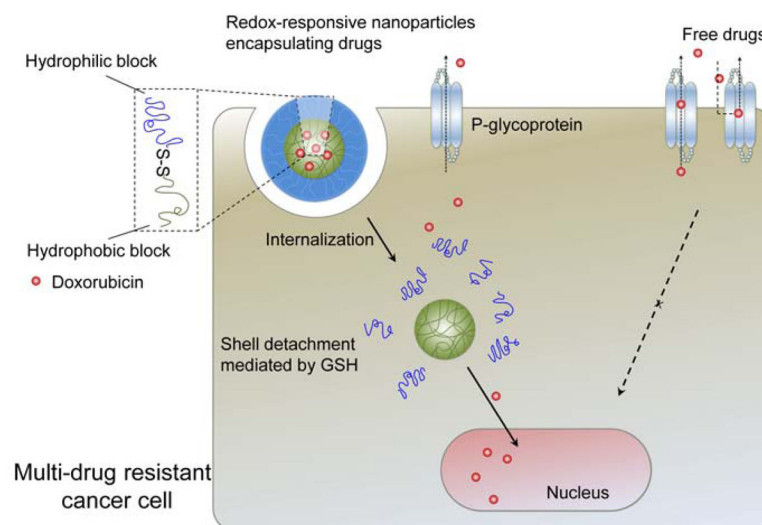
**Figure 3.**

a) Schematic illustration of the mechanism that uses PLGA HMs containing DOX and NaHCO<sub>3</sub> for pH-responsive drug release. b) SEM micrographs of PLGA HMs (prepared at a NaHCO<sub>3</sub> concentration of 2.5 mg mL<sup>-1</sup>) after incubation in media with different pH values to mimic the extracellular environment (pH 7.4), early endosomes (pH 6.0), and lysosomes (pH 5.0), respectively. c) CLSM images showing the intracellular release of DOX from PLGA HMs after incubation with cells for 12 h. HMs-w/o-NaHCO<sub>3</sub>: HMs containing no NaHCO<sub>3</sub>; HMs-low-NaHCO<sub>3</sub>: HMs prepared with NaHCO<sub>3</sub> at 1.25 mg mL<sup>-1</sup>; HMs-high-NaHCO<sub>3</sub>: HMs prepared with NaHCO<sub>3</sub> at 2.5 mg mL<sup>-1</sup>. Reproduced with permission.<sup>[53]</sup> Copyright 2011, Wiley-VCH.

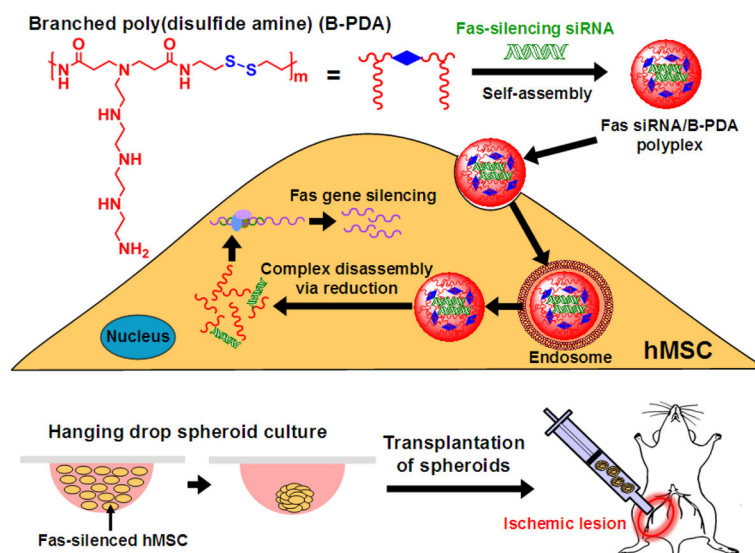


**Figure 4.**

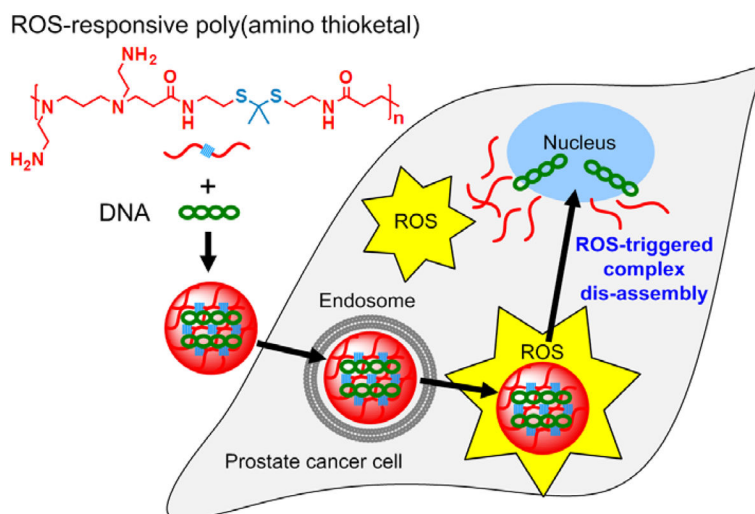
a) Schematic illustration showing the performance of a drug-loaded, pH-responsive, charge-switchable PAMA-DMMA nanogel. In the acidic tumor extracellular environment, the nanogel is activated to become positively charged and is thus readily internalized by tumor cells with subsequent intracellular drug release. b, c) CLSM images of MDA-MB-435 cells incubated with the PAMA-DMMA nanogel at (b) pH 6.8 and (c) 7.4 for 2 h, respectively. d, e) CLSM images showing the distributions of (d) PAMA-DMMA and (e) non-pH-responsive PAMA-SA nanogels in the tumor tissue following intratumoral injection. The white arrows indicate the locations of the nanogels. Both nanogels were labeled with fluorescein isothiocyanate (FITC; green). In all CLSM images, the F-actin and nuclei of the cells were stained, respectively, with rhodamine phalloidin (red) and 4',6-diamidino-2-phenylindole (DAPI; blue) Reproduced with permission.<sup>[54]</sup> Copyright 2010, Wiley-VCH.



**Figure 5.** Schematic illustration showing the intracellular trafficking of redox-responsive nanoparticles for overcoming multidrug resistance of cancer cells.

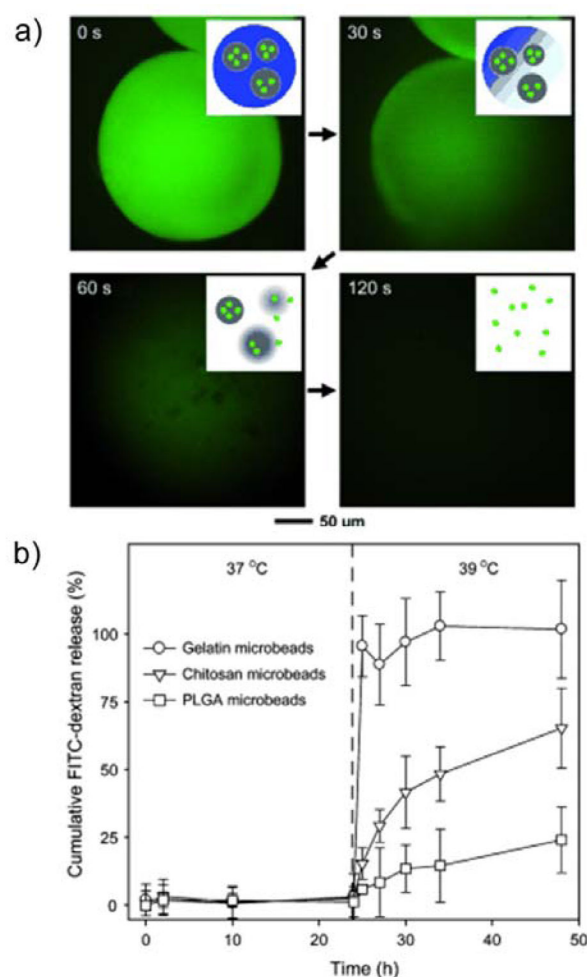


**Figure 6.** Efficient Fas inhibition of hMSCs using B-PDA *via* redox-responsive dis-assembly of Fas siRNA/B-PDA polyplexes. The Fas-inhibited hMSCs can be formulated as enlarged spheroids ( 800 μm in diameter) for enhanced therapeutic angiogenesis. Reproduced with permission.<sup>[64]</sup> Copyright 2012, Wiley-VCH.



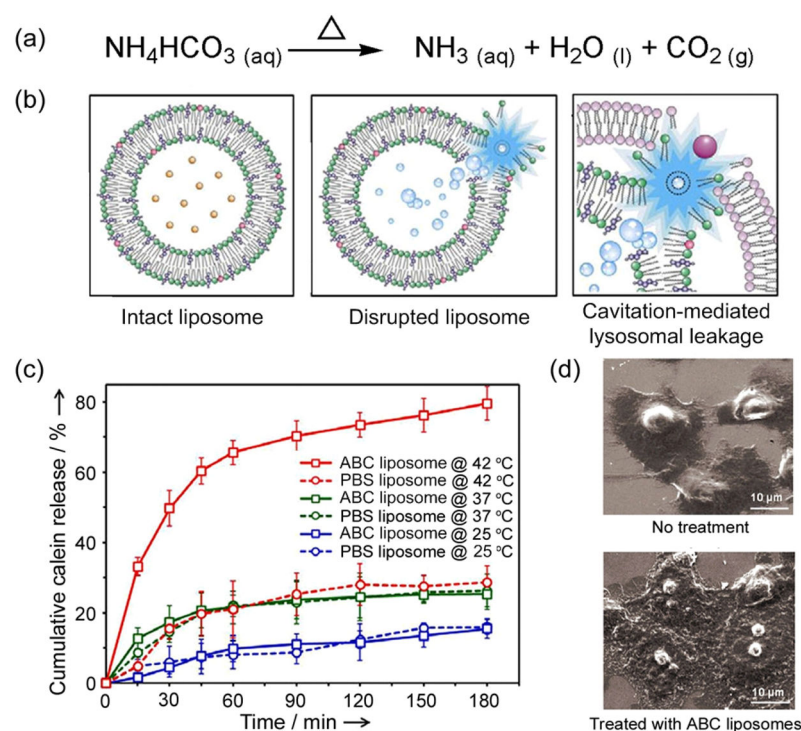
**Figure 7.**

Schematic illustration of ROS-responsive PATK for facilitated intracellular delivery of plasmid DNA in prostate cancer cells. After DNA/PATK polyplexes are internalized by a prostate cancer cell, DNA is efficiently released from the polyplexes due to ROS-triggered dis-assembly of the polyplexes, leading to efficient gene transfection. Reproduced with permission.<sup>[72]</sup> Copyright 2013, Wiley-VCH.

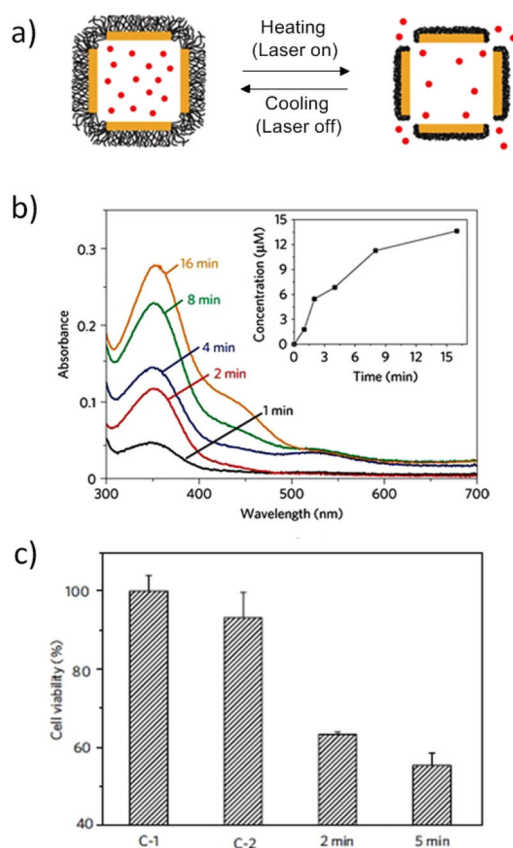
**Figure 8.**

a) Time-lapse fluorescence micrographs showing the melting of a 1-tetradecanol bead and thereby release of FITC-dextran from the gelatin particles as the temperature was gradually increased by adding warm water (60 °C) under gentle stirring. The insets are schematic diagrams showing the three major steps involved in the release of FITC-dextran: melting of 1-tetradecanol beads (30 s), release of gelatin particles (60 s), and release of FITC-dextran as gelatin is being dissolved (120 s). b) Release profiles at 37 and 39 °C for FITC-dextran from gelatin, chitosan, and PLGA microbeads encapsulated in 1-tetradecanol blocks (n=3).<sup>[92]</sup> Copyright 2010, Wiley-VCH.



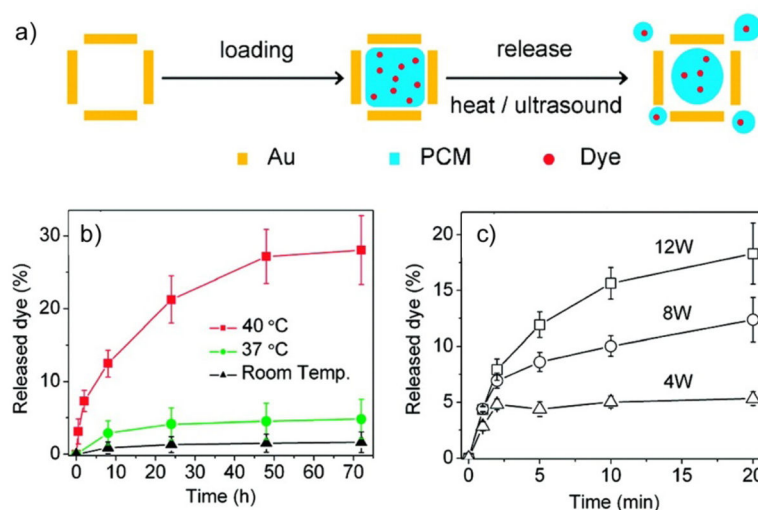
**Figure 9.**

a) Decomposition reaction of ammonium bicarbonate (ABC) upon heating to 40 °C or above, which can quickly generate CO<sub>2</sub> bubbles. b) A schematic illustration showing the composition/structure of the thermo-sensitive, bubble-generating ABC liposomes and how they can be used to eradicate cancer cells by using the mechanical force from transient cavitation. c) Release profiles of calcein from ABC and phosphate buffered saline-containing liposome lacking ABC (PBS liposome) incubated at three different temperatures (n = 6). d) SEM micrographs of HT1080 cells before and after being treated with the ABC liposomes at 42 °C. Reproduced with permission.<sup>[93]</sup> Copyright 2012, Wiley-VCH.



**Figure 10.**

a) Schematic illustration of the release mechanism for AuNCs coated with a thermo-responsive copolymer of pNIPAAm-co-pAAm. b) Absorption spectra of alizarin-PEG released from the copolymer-covered AuNCs upon exposure to a pulsed NIR laser (power density =  $10 \text{ mW/cm}^2$ ) for 1, 2, 4, 8, and 16 min. The inset shows the accumulated concentrations of alizarin-PEG released from the copolymer-covered AuNCs. c) Cell viability plots for samples after different treatments: (C-1) cells irradiated with a NIR laser for 2 min in the absence of AuNCs; (C-2) cells irradiated with the NIR laser in the presence of DOX-free AuNCs; and (2 min and 5 min) cells irradiated with the NIR laser for 2 and 5 min in the presence of DOX-loaded AuNCs. Reproduced with permission.<sup>[101]</sup> Copyright 2009, Nature Publishing Group.



**Figure 11.**

a) Schematic illustration showing how to load the hollow interior of a AuNC with a dye-doped PCM and then have it released from the AuNC through direct or ultrasonic heating. b) Release profiles of rhodamine 6G under direct heating to various temperatures for different periods of time. c) Release profiles of rhodamine 6G *via* high-intensity focused ultrasound (HIFU) at different powers. Reproduced with permission.<sup>[107]</sup> Copyright 2011, American Chemical Society.

Table 1

Examples of stimuli-responsive materials for controlled release and drug delivery.

Origin	Stimuli	Materials	Achieved properties	Outcomes	Ref.
Internal	Acidic pH in the endosome/lysosome	PLGA hollow microspheres encapsulating sodium bicarbonate	Rupture of microspheres by CO <sub>2</sub> generated from the decomposition of sodium bicarbonate at the acidic pH	Enhanced DOX release in the endosome and lysosome	53
	Acidic tumor pH	PAMA-DMMA nanogel	Surface charge conversional at the acidic extracellular tumor pH	Enhanced cellular uptake and DOX release	54
	Redox potential	PEG-SS-PCL micelle	Detachment of PEG shell <i>via</i> degradation of disulfide bonds and the subsequent micelle destabilization	Enhanced cytoplasmic release of DOX	56
		PCL-SS-PEEP micelle	Detachment of PEEP shell <i>via</i> degradation of disulfide bonds and the subsequent micelle destabilization	Enhanced intracellular accumulation and retention of DOX in multidrug resistant cancer cells	57
		Branched poly(disulfide amine)/siRNA complex	Disassembly of complex dis-assembly by cleavage of disulfide bonds in the cytoplasm	Enhanced cytoplasmic release of siRNA and subsequent gene silencing	64
External	ROS	Boronic ester-bearing polyester nanoparticle	Degradation of polymer backbone upon exposure to low concentrations of hydrogen peroxide	ROS-responsive release of cargos	67
		Poly(PS- <i>b</i> -DMA micelle)	Conversion of poly(propylene sulfide) to hydrophilic poly(propylene sulfone) and the subsequent micelle destabilization upon exposure to hydrogen peroxide	Facilitated release of cargos in inflamed macrophages with high ROS production	68
		Poly(amino thioketal)/DNA complex	Efficient complex dis-assembly <i>via</i> cleavage of thioketal linkages upon ROS exposure	Enhanced intracellular DNA delivery in cells with high levels of intracellular ROS	72
	Enzyme	AuNC-fluorescent dye conjugates linked with a MMP-cleavable peptide	Release of conjugated dyes <i>via</i> cleavage of a MMP-cleavable peptide upon exposure to MMP	Fluorescence recovery under MMP-abundant conditions for cancer detection and imaging	76
	Temperature (external heating)	PCM microsphere	Melting of PCM particles upon heating above the melting point of a PCM	Temperature-sensitive, on-demand drug release with precisely controlled dosage	92
		Liposome containing ammonium bicarbonate	Cavitation of a liposome by CO <sub>2</sub> bubbles generated from ammonium bicarbonate upon heating	Rupture of lysosome and the proteolytic enzymes, resulting in cell necrosis	93
	NIR	pNIPAAm-co-pAAAm copolymer-coated AuNC	Collapse of polymer chains by photothermal effect and subsequent exposure of AuNCs pores	On-demand release upon NIR irradiation	101
	NIR	pNIPAAm-co-pAAAm copolymer-coated AuNC	Collapse of polymer chains by photothermal effect and subsequent exposure of AuNCs pores	On-demand release upon NIR irradiation	101

Origin	Stimuli	Materials	Achieved properties	Outcomes	Ref.
	HIFU	PCM-coated AuNC	Melting of PCM by HIFU-triggered heating	Controlled release from AuNCs upon HIFU exposure	107
		pNIPAAm-co-pAAm copolymer-coated AuNC	Collapse of polymer chains by HIFU- triggered heating and subsequent exposure of pores in AuNCs	Controlled release from AuNCs upon HIFU exposure	108
	CO <sub>2</sub>	PEG-PAD polymersome	Increase in membrane permeability after self-expansion of polymersomes at high CO <sub>2</sub> levels	Controlled release from polymersomes at high CO <sub>2</sub> levels	109

AuNC: Au nanocage; DOX: doxorubicin; HIFU: high-intensity focused ultrasound; MMP: matrix metalloproteinase; NIR: near-infrared; PAMA-DMMA: poly(2-aminoethyl methacrylate hydrochloride) modified with 2,4-dimethylmaleic anhydride; PCL-SS-PEEP: disulfide-linked poly( $\epsilon$ -caprolactone)-poly(ethyl ethylene phosphate) block copolymer; PCM: phase-change material; PEG-SS-PCL: disulfide-linked poly(ethylene glycol) -poly( $\epsilon$ -caprolactone) block copolymer; PEG-PAD: block copolymer of poly(ethylene glycol) and poly(*N*-amidino)dodecyl acrylamide; PLGA: poly(D,L-lactic-co-glycolic acid); pNIPAAm-co-pAAm: random copolymer of poly(*N*-isopropylacrylamide) and poly(acrylamide); poly(PS-*b*-DMA): block copolymer of propylene sulfide and *N,N*-dimethylacrylamide.