nature materials

INSIGHT | REVIEW ARTICLES

PUBLISHED ONLINE: 23 OCTOBER 2013 | DOI: 10.1038/NMAT3776

Stimuli-responsive nanocarriers for drug delivery

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Spurred by recent progress in materials chemistry and drug delivery, stimuli-responsive devices that deliver a drug in spatial-, temporal- and dosage-controlled fashions have become possible. Implementation of such devices requires the use of biocompatible materials that are susceptible to a specific physical incitement or that, in response to a specific stimulus, undergo a protonation, a hydrolytic cleavage or a (supra)molecular conformational change. In this Review, we discuss recent advances in the design of nanoscale stimuli-responsive systems that are able to control drug biodistribution in response to specific stimuli, either exogenous (variations in temperature, magnetic field, ultrasound intensity, light or electric pulses) or endogenous (changes in pH, enzyme concentration or redox gradients).

Advanced nanoscale systems for drug delivery have recently received tremendous attention, in particular from the field of nanomedicine. The need for drug nanocarriers that efficiently target diseased areas in the body arises because drug efficacy is often altered by nonspecific cell and tissue biodistribution, and because some drugs are rapidly metabolized or excreted from the body. Owing to impressive progress in materials science and pharmaceutics, a broad range of nanocarriers with diverse sizes, architectures and surface properties have been designed. These include liposomes, polymer nanoparticles, micelles, dendrimers, and inorganic nanoparticles made of iron oxide, quantum dots, gold or metal oxide frameworks. The size of these carriers is typically small (from a few tenths to a few hundreds of nanometres) to allow systemic (intravenous) or local (mucosal) administration, and to promote their diffusion within the cell. Moreover, current surface-functionalization methodologies can impart nanocarriers with the ability to control, at least in part, their pharmacokinetics and biodistribution. For example, the PEGylation (the process of attaching polyethylene glycol (PEG) chains)-induced steric repulsion of blood opsonins — molecules, such as antibodies, that enhance phagocytosis — endows the nanocarriers with *in vivo* longevity and specific capability of extravasation through the endothelium of inflammatory tissues (the so-called enhanced permeability and retention effect), whereas their functionalization with biologically active ligands facilitates the targeting of specific cells.

Nanotechnology-based targeted delivery has shown promising results in preclinical animal models. However, the translation of both the enhanced permeability and retention effect and ligand recognition into the clinic still remains questionable. This may be, to a certain extent, a consequence of the stochastic nature of ligand–receptor interactions and of difficulties in the control of the release of the drug from targeting nanocarriers. In fact, Fickian diffusion — which governs the leakage of the drug — is not specific to cells, tissues or organs; therefore, more efficient delivery strategies are needed. One alternative involves on-demand processes (also termed 'switch on/off'), which in principle allow for tailored release profiles with excellent spatial, temporal and dosage control. On-demand drug delivery is becoming feasible through the design of stimuli-responsive systems that recognize their microenvironment and react in a dynamic way, mimicking the responsiveness of living organisms. However, this approach is rather complex. It requires the use of biocompatible materials that are able to undergo a specific protonation, a hydrolytic cleavage, or a molecular or supramolecular conformational change in response to a desired stimulus, or that are susceptible to specific physical stimulation. The concept of stimuli-responsive drug delivery was first suggested in the late 1970s with the use of thermosensitive liposomes for the local release of drugs through hyperthermia[1]. Since then — and particularly in the past decade — a great deal of research has been carried out on stimuli-responsive materials for drug delivery, especially concerning their design and application as nanocarriers.

Nanoscale stimuli-responsive devices may be sensitive to specific endogenous stimuli, such as a lowered interstitial pH, a higher glutathione concentration or an increased level of certain enzymes such as matrix metalloproteinases. At the cellular level, pH sensitivity can either trigger the release of the transported drug into late endosomes or lysosomes, or promote the escape of the nanocarriers from the lysosomes to the cell cytoplasm. At the tissue level, one can take advantage of specific microenvironmental changes associated with neoplastic diseases (the treatment of which is the focus of most of the research effort on stimuli-responsive nanocarriers) as well as pathological situations such as ischemia, inflammatory diseases or infections. Extracorporeal physical stimuli can be also applied. For example, the targeted delivery of pharmacologically active molecules to a diseased area in the body can be magnetically guided by using ultrasmall iron oxide-based nanoparticles. Sustained drug release can also be achieved by thermo-, light- or ultrasound-sensitive nanoparticulate systems. Furthermore, the possibility of choosing between different routes of administration (intravenous, oral, ocular or mucosal) is attractive.

In this Review, we discuss the most significant progress made in the past five years in the field of stimuli-responsive drug-delivery nanocarriers. In the interest of brevity, we do not include stimuli-sensitive transported drugs, or single prodrugs or nanocarriers for which drug-release evidence has yet to be reported.

Exogenous stimuli-responsive drug delivery

In this section we discuss drug-delivery systems that take advantage of externally applied stimuli, including temperature changes, magnetic fields, ultrasounds, light and electric fields.

Thermoresponsive systems. Thermoresponsive drug delivery is among the most investigated stimuli-responsive strategies, and has been widely explored in oncology. Thermoresponsiveness is usually governed by a nonlinear sharp change in the properties of at least one component of the nanocarrier material with temperature. Such a sharp response triggers the release of the drug following a variation in the surrounding temperature. Ideally, thermosensitive nanocarriers should retain their load at body temperature (~37 °C), and rapidly deliver the drug within a locally heated tumour (~40–42 °C) to counteract rapid blood-passage time and washout from the tumour.

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NATURE MATERIALS DOI: 10.1038/NMAT3776

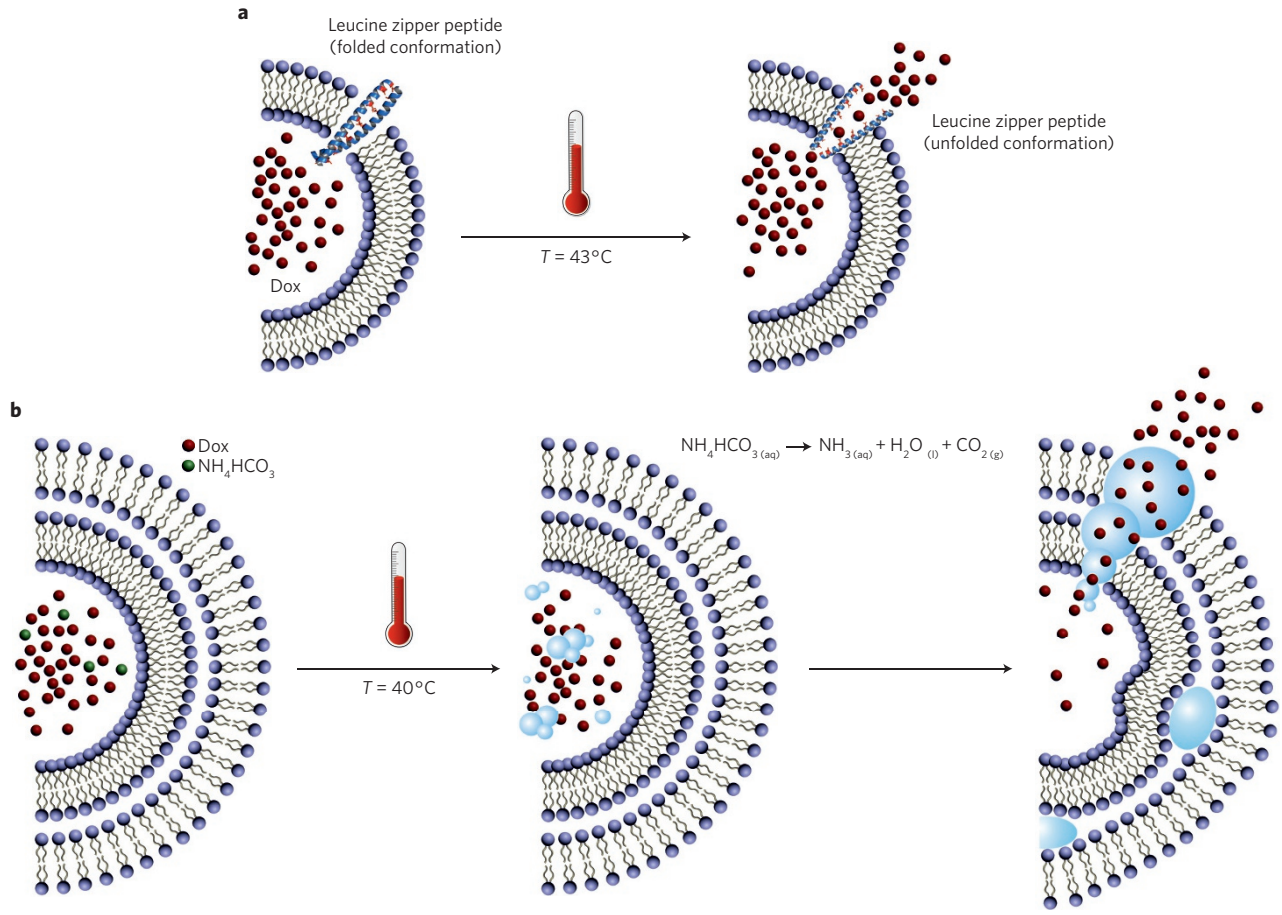

Figure 1 | Temperature-based actuation mechanisms for liposomal drug delivery. a, The temperature-triggered unfolding of a leucine zipper peptide inserted in the membrane of a doxorubicin (Dox)-carrying liposome opens a channel through which the drug is released. **b**, Drug-permeable pores can also be created by the temperature-triggered generation of bubbles from the decomposition of encapsulated ammonium bicarbonate. Figure adapted with permission from: **a**, ref. 3, © 2012 ACS; **b**, ref. 4, © 2013 ACS.

Thermoresponsive systems are generally liposomes, or polymer micelles or nanoparticles (usually poly(*N*-isopropyl acrylamide), PNIPAM) that exhibit a lower critical solution temperature. For liposomes, thermoresponsiveness usually arises from a phase transition of the constituent lipids and the associated conformational variations in the lipid bilayers. *In vivo*, heat is generally applied by using temperature-controlled water sacks, radiofrequency oscillators or miniature annular-phased array microwave applicators. In the past few years, the focus has been on rapid and quantitative drug-release performance. Thermosensitive liposomes (TSLs) are perhaps the most advanced thermoresponsive nanosystems, as shown by their use in several clinical trials. Doxorubicin-loaded TSLs (ThermoDox, Celsion Corporation), in association with hyperthermia or radiofrequency ablation, are at present being investigated in phase II trials for the treatment of breast cancer and colorectal liver metastasis, and reached phase III trials for the treatment of hepatocellular carcinoma. More recently, improved liposomal formulations have been shown to release their loads shortly after the onset of hyperthermia (~40–45 °C)[2]. An alternative approach used leucine zipper peptide–liposome hybrids, which combine the advantages of traditional TSLs with the dissociative, unfolding properties of a temperature-sensitive peptide (Fig. 1a)[3]. Also promising are thermoresponsive bubble-generating liposomal systems. These rely on the creation of permeable defects in the lipid bilayer by means of the generation of carbon dioxide bubbles through decomposition of ammonium bicarbonate at mild hyperthermia (~42 °C). Interestingly, because of the hyperechogenic features of carbon dioxide bubbles, bubble-generating liposomal vesicles that rapidly release doxorubicin (Fig. 1b)[4] also improved ultrasound imaging of tissues. Thermosensitive liposomes can also be functionalized with ligands for specific targeting, such as the human epidermal growth factor receptor 2 affibody for breast-cancer treatment[5].

Although PNIPAM is the preferred polymer building block for thermosensitive polymeric drug nanocarriers[6], other polymeric materials, such as poly(γ-2-(2-(2-methoxyethoxy)-ethoxy)ethoxy-ε-caprolactone)-*b*-poly(γ-octyloxy-ε-caprolactone)[7], have demonstrated marked transition temperatures, allowing improved drug release at low hyperthermia (40 °C). Importantly, tuning the nature and the composition of the copolymers so that transition temperatures are close to body temperature may be useful for local administration (either subcutaneous, or intra- or peritumoural).

Local hyperthermia has also been used as a stimulus for the on–off control of the activity of cell-penetrating peptides (CPP). In this case, the temperature-triggered assembly of diblock-copolymer elastin-like polypeptides allowed arginine residues to be displayed at the periphery of the resulting micelles. This resulted in a greater than 8-fold increase in HeLa-cell uptake[8].

Thermoresponsiveness can also occur on a brief temperature decrease (also called cold shock or cryotherapy). In this case, a thermally reversible swelling or de-swelling of the nanocarrier leads to free diffusion of the encapsulated drugs as a consequence of increased porosity. For example, Pluronic F127–polyethyleneimine

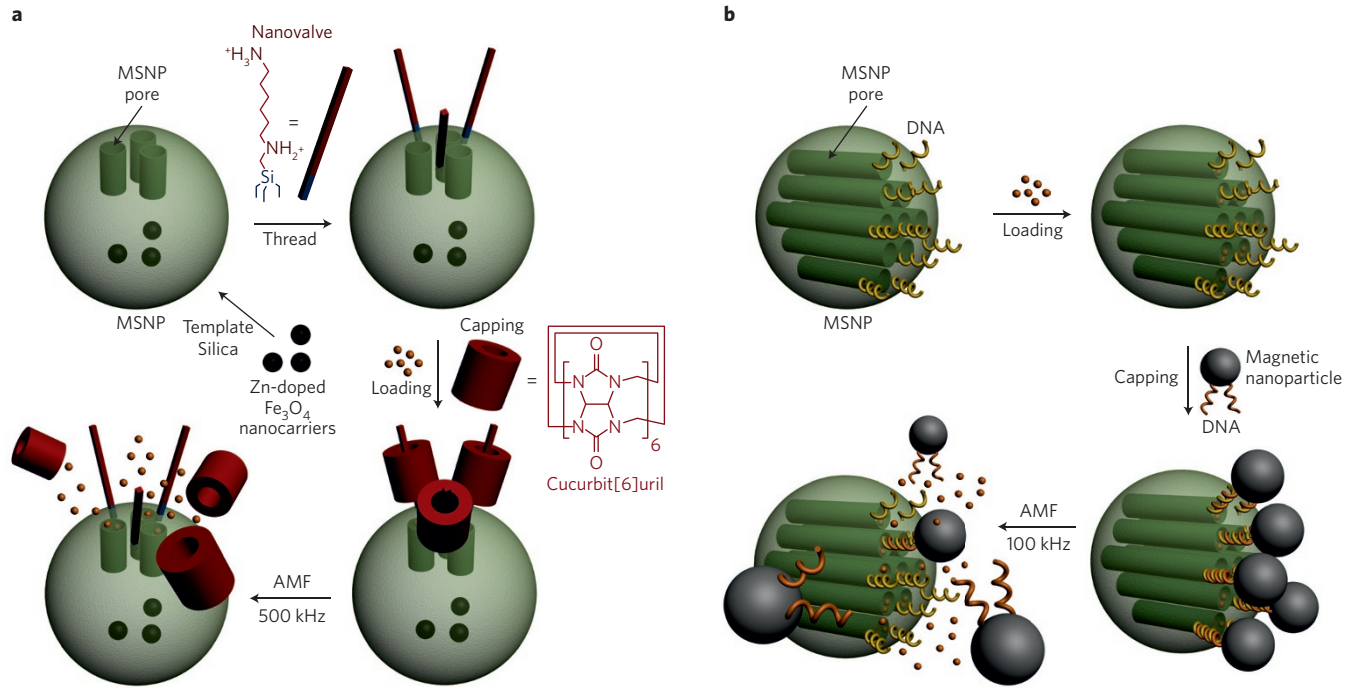

Figure 2 | Actuation mechanisms based on the heat generated by an alternating magnetic field (AMF) leading to on-demand pulsatile drug release from mesoporous silica nanoparticles (MSNPs). a, Pseudorotaxane-based nanovalves made of cucurbit[6]uril. **b**, Capping system based on complementary DNA sequences. Figure adapted with permission from: **a**, ref. 34, © 2010 ACS; **b**, ref. 36, © 2011 ACS.

(PEI) nanocapsules were used for efficient small interfering RNA (siRNA) delivery into the cytosol and subsequent silencing of a target messenger RNA[9].

In general, the challenge in the design of thermoresponsive nanodevices lies in the use of materials that are both safe and sensitive enough to respond to slight temperature changes around the physiological temperature of 37 °C. Liposomal systems are at present the more advanced and thus have the highest potential for clinical applications.

Magnetically responsive systems. The advantage of using a magnetic field relies on the different nature that the magnetic response can take, which can be a magnetic guidance under a permanent magnetic field, a temperature increase when an alternating magnetic field is applied, or both when alternately used. Therefore, magnetically responsive systems allow for diversity in the drug-delivery pathway. Furthermore, there is the possibility of performing magnetic resonance imaging, and hence to associate diagnostics and therapy within a single system (the so-called theranostic approach)[10].

Magnetic guidance is typically obtained by focusing an extracorporeal magnetic field on the biological target during the injection of a magnetically responsive nanocarrier. This concept has demonstrated great potential in experimental cancer therapy because of improved drug accumulation inside solid-tumour models. Candidate nanosystems for such a therapeutic approach are core–shell nanoparticles (a magnetic core made of magnetite (Fe_3O_4) coated with silica or polymer)[11,12], magnetoliposomes (Fe_3O_4 or maghemite (Fe_2O_3) nanocrystals encapsulated in liposomes)[13] and porous metallic nanocapsules[14]. Most core–shell nanoparticles have shown promising results *in vitro*, yet only some of them have demonstrated improved tumour accumulation and anticancer pharmacological efficacy in various *in vivo* models. However, without normalized benchmark experiments, the comparison between all these systems remains rather difficult.

To avoid limitations related to physical drug entrapment (for instance, uncontrolled burst release or poor drug loading), the drugs and the nanocarriers can be covalently linked[12,15]. For example, Fe_3O_4 nanocrystals loaded into squalene–gemcitabine conjugate nanoassemblies exhibiting high drug payloads have demonstrated absence of burst release, enhancement of the magnetic resonance imaging contrast in the targeted L1210 solid-tumour nodule and significant therapeutic efficacy[15].

Interestingly, a permanent magnetic field can also trigger drug delivery. For instance, when applied to a ferrogel composed of Pluronic-F127 micelles encapsulating superparamagnetic iron oxide nanoparticles and a hydrophobic drug, the drug is released as iron oxide nanoparticles approach each other and squeeze the micelles[16]. Similarly, a syringe-like system using magnetic nanoparticles can push the drug out of a biodegradable reservoir based on poly(L-lactic acid)[17].

Magnetically guided nanocarriers have also found application in the delivery of nucleic acids, including siRNA and genes. Such magnetofection experiments are generally performed using nanoassemblies with cationic coatings to condense nucleic acids, which results in higher transfection efficiencies under a permanent magnetic field. For instance, this technique led to improved effectiveness in the transfection of siRNA *in vitro* and/or *in vivo* when directed against prostate[18] and breast[19] cancers, as well as in the gene transfer to oligodendrocyte precursors for neural repair[20]. For DNA vaccine delivery, two different strategies have been successful: the complexation of plasmid DNA (pDNA) at the surface of PEI-coated Fe_3O_4 nanoparticles[21], and the use of bacterial magnetic particles as carriers of a recombinant DNA[22]. Magnetic vaccines have also been demonstrated in the form of magnetic-nanoparticle formulations associated to a replication-defective adenovirus[23]. Cell-based gene therapies employing human monocytes — cells that naturally migrate from the bloodstream into tumours — that were transfected with therapeutic genes, loaded with magnetic nanoparticles and placed under the influence of a magnetic field led to a

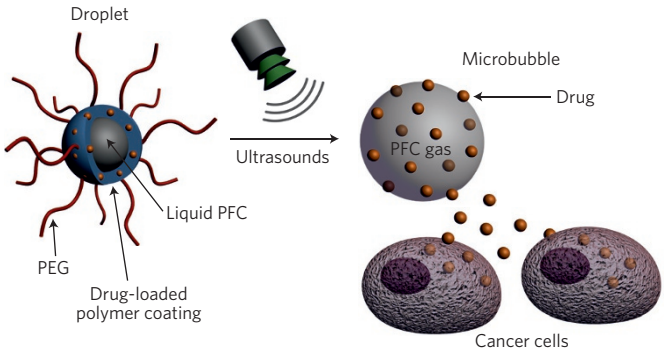

Figure 3 | Drug delivery from echogenic perfluorocarbon (PFC)-containing nanoemulsions. It is believed that the mechanism of delivery involves a droplet-to-bubble transition under the action of ultrasound, leading to drug transfer from the bubbles to neighbouring cells. Figure adapted with permission from ref. 41, © 2009 Elsevier.

marked increase in the monocytes' extravasation into the tumour[24]. Magnetic nanoparticles have also been used for the delivery of antioxidant enzymes (catalase and superoxide dismutase, for example) with the aim of increasing resistance to oxidative stress *in vitro*[25].

When magnetic nanoparticles are placed in an oscillating or alternating magnetic field (AMF), because of hysteresis loss and/or Néel relaxation, they serve as a transducer by generating heat in the surrounding medium; therefore, they have been extensively used for the selective heating of tumours (hyperthermia). However, by combining this phenomenon with the particular physical and/or chemical properties of organic and inorganic nanocarriers, a broad range of actuation mechanisms for on-demand drug release has been developed. Typical examples are the use of thermosensitive polymers[26] and lipids[27], which can serve as coating materials for magnetic nanoparticles and trigger the release of a drug in an on–off fashion in response to a magnetically induced increase of temperature. This can also be achieved with crosslinked PNIPAM hydrogels loaded with Fe_3O_4 nanoparticles[28]. The release of the encapsulated drug can be modulated by the duration of the AMF on–off states, which affects the shrinkage of the mesh size and the recovery of the gel. The heat generated by an AMF can also trigger nanocarrier structural alteration, such as shell or bilayer porosity increase[29,30], disintegration of the Fe_3O_4 core[31], or single-crystal nanoshell lattice deformation[32]. Furthermore, active targeting by means of nanocarrier functionalization can be combined with hyperthermia to achieve a synergistic cytotoxic effect[33].

The heat generated by an AMF can also be used to achieve on-demand pulsatile drug release. Examples include: pseudorotaxane-based nanovalves at the surface of mesoporous silica nanoparticles (MSNPs) that act as thermally sensitive gatekeepers (Fig. 2a)[34]; composite membranes containing thermoresponsive PNIPAM-based nanogels and magnetic nanoparticles that enable on–off drug delivery on de-swelling or swelling of the polymer[35]; a capping system for drug-loaded MSNPs based on complementary DNA sequences (Fig. 2b)[36]; and a nucleic-acid duplex as a heat-labile linker that releases a drug from dextran-coated Fe_3O_4 magnetic nanoparticles embedded into a matrigel plug[37]. An AMF can also remotely regulate protein production by using a modified temperature-sensitive channel (TRPV1) decorated with iron oxide nanoparticles[38]. When the local temperature rises, TRPV1 gates calcium to stimulate the production and release of bioengineered insulin driven by a Ca^{2+}-sensitive promoter.

The use of magnetically responsive nanoparticles, either for magnetic guidance or local hyperthermia, is generally limited to accessible tumour nodules, but not metastasis or disseminated tumours. Even if most of these tumours are indications for direct surgery, some are not surgically removable because they are too haemorrhagic or localized in tissues with high risk of healthy-tissue injury (as is the case for some brain cancers). In these situations, magnetically responsive nanoparticles represent a promising therapeutic option. However, magnetic guidance is hampered by the complexity involved in the set-up of external magnetic fields, which need adequate focusing and deep penetration into the tissues to reach the diseased area with sufficient strength. In this respect, efforts to identify the best magnetic and irradiation technologies are needed.

Ultrasound-triggered drug delivery. Ultrasounds represent an effective method for attaining spatiotemporal control of drug release at the desired site, thus preventing harmful side effects to healthy tissues. The use of ultrasounds is also appealing because of their non-invasiveness, the absence of ionizing radiations, and the facile regulation of tissue penetration depth by tuning frequency, duty cycles and time of exposure.

Ultrasound waves can trigger the release of the drug from a variety of nanocarriers through the thermal and/or mechanical effects generated by cavitation phenomena or radiation forces. Indeed, it has been shown that physical forces associated with cavitation can induce nanocarrier destabilization, drug release[39] and transient increase in vessel permeability, leading to the cellular uptake of therapeutic molecules[40].

The cavitation threshold is easily achieved when low ultrasound frequencies (in the kHz range) are used. However, ultrasound-mediated enhancement of vessel permeability can also be the cause of possible drawbacks such as metastatic dissemination. Therefore, microbubbles or other ultrasound contrast agents, which are able to efficiently interact with ultrasonic waves, have been used at diagnostic frequencies to reduce the threshold required for cavitation. However, short lifespan and absence of extravasation may still limit the use of microbubbles for tissue targeting. This difficulty has been overcome by the development of perfluorocarbon (PFC) nanoemulsions that convert into microbubbles under the action of therapeutic ultrasounds. The bubbles are formed through acoustic droplet vaporization and are subjected to cavitation, thus promoting cellular uptake and/or release of the entrapped drugs in the tumour site (Fig. 3). This has resulted in significant therapeutic efficacy and suppression of metastatic dissemination[41]. Moreover, functionalization with aptamers has increased the targeting specificity of PFC nanodroplets[42]. Low-frequency ultrasounds have also been used to promote the delivery of drugs through the skin. For example, high penetration of siRNA-loaded liposomes has led to significant inhibition of the progression of melanocytic lesions[43].

Furthermore, echogenic liposomes — also termed bubble liposomes — contain air pockets or nanoemulsions of liquid PFC[44] and can integrate ultrasound responsiveness into a drug nanocarrier. In fact, a combination of ultrasounds and intravenous administration of xenon-loaded bubble liposomes achieved a significant neuroprotective effect in a model of cerebral ischemia[45]. Bubble liposomes increased the transfection efficiency of pDNA-loaded liposomes, probably also by enhancing ultrasound-mediated endosomal escape[46]. However, co-localization of pDNA and bubble liposomes is needed *in vivo* to attain high transfection efficacy. Indeed, intravenous administration of bubble liposomes loaded with the basic fibroblast growth factor gene resulted in significant gene delivery at the ultrasound-focused site[47]. Systemic administration of pDNA-loaded bubble liposomes and ultrasound exposure also achieved prolonged cancer vaccination[48]. Compared with other approaches, ultrasound-triggered drug delivery allows the drug to be released into the cytosol as a result of pore formation in the cell membrane, thus bypassing the degradative endocytotic pathway. This is especially useful in DNA transfection.

Combination of thermosensitive drug carriers and high-intensity focused ultrasound (HIFU) enables triggered drug release with only a mild temperature increase. For instance, inducing the release of doxorubicin from TSLs by using a clinically available HIFU material resulted in higher drug accumulation in an experimental tumour animal model compared with non-irradiated controls[49]. The combination of HIFU and ThermoDox is under investigation at Celsion Corporation for the treatment of liver metastatic cancer, painful bone metastasis and pancreatic cancer.

Light-triggered drug delivery. Owing to their non-invasiveness and the possibility of remote spatiotemporal control, a large variety of photoresponsive systems has been engineered in the past few years to achieve on-demand drug release in response to illumination of a specific wavelength (in the ultraviolet, visible or near-infrared (NIR) regions). The different strategies available rely on either a one-time or repeatable on–off drug-release event triggered by photosensitiveness-induced structural modifications of the nanocarriers.

For instance, the ultraviolet–visible reversible photoisomerization of the azobenzene group (and its derivatives) — from *trans* to *cis* on irradiation at 300–380 nm, and from *cis* to *trans* by shining light in the visible region — enables photoregulated control of drug release. This has been achieved through azobenzene functionalization of the pore interior of MSNPs[50], by means of azo-modified DNA valves at the pore mouth[51], and by the light-controlled host–guest recognition between a cyclodextrin cavity and azobenzene derivatives[52]. The hydrophobic–hydrophilic transition that accompanies the *trans–cis* photoisomerization can also be used. For example, the disassembly at ultraviolet light of 350 nm that occurs for cationic micelles of azo-modified surfactants can initiate rapid intracellular DNA release[53]. Another strategy for obtaining nanoparticles with photoswitchable drug release in illuminated cells is to take advantage of the ultraviolet light-triggered spiropyran–merocyanine isomerization. Ultraviolet light can also activate reversible shrinkage in spiropyran–PEGylated lipid nanoparticles, thus enabling deeper tissue penetration[54]. Furthermore, the photodimerization–cleavage cycle of thymine can serve as a mechanism for MSNP opening and closing[55], although other photocleavable groups, such as *o*-nitro benzyl and its derivatives, have also been tested. Controlled drug release has been achieved with *o*-nitro benzyl attached to gold nanoparticles as a linker for the direct conjugation of drugs[56], as part of MSNP gatekeeper mechanisms[57], and as a crosslinker incorporated in the backbone of polymers[58]. Photolabile caging groups have been exploited for the light-activated production of proteins. Lipid vesicles loaded with caged DNA and with the reacting and enzymatic components needed for transcription and translation enabled, upon local ultraviolet irradiation-mediated DNA uncaging, the activation of green fluorescent protein and the synthesis of enzymatically active luciferase (Fig. 4a)[59].

The major drawback of light-triggered drug delivery is the low penetration depth (~10 mm) that results from the strong scattering properties of soft tissues in the ultraviolet–visible region of the spectrum (less than 700 nm). Conventional light-induced drug delivery is thus only applicable to regions of the body that can be directly illuminated (such as the eye or the skin). However, by using photosensitive groups that respond to higher wavelengths or exploiting two-photon technology[60], it is possible to replace the ultraviolet–visible light source by a NIR laser (700–1,000 nm range) with deeper tissue penetration, lower scattering properties and minimal harm to tissues. This makes NIR-responsive systems extremely promising for clinical applications. The capacity of NIR-absorbing plasmonic materials to convert the photon energy adsorbed during irradiation into heat has been used to trigger the release of chemotherapeutic molecules from NIR-responsive nanodevices. For instance, doxorubicin-loaded hollow gold nanospheres showed accelerated drug release when irradiated at 808 nm, allowing enhanced anticancer activity and reduced systemic toxicity compared with the free-drug treatment[61]. Light-to-heat transduction mediated by NIR irradiation of gold nanorods caused a rapid rise in the local temperature, which was exploited to induce dehybridization of DNA helices conjugated at the gold surface, allowing the release of doxorubicin molecules bound to consecutive cytosine–guanine base pairs (Fig. 4b)[62]. Alternatively, DNA duplexes can be used to cap the pores of a mesoporous silica shell, with dehybridization leading to the release of the host molecules[63]. The increase in temperature resulting from the surface plasmon resonance of gold on NIR irradiation may also cause a phase transition in polymers or lipids, allowing the leakage of a preloaded drug. Accordingly, poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles (glass transition temperature of 45 °C) entirely or half coated with a gold layer[60,64], gold-shell-coated lipidic nanomicelles[65], polymer-coated gold nanocages[66] or gold nanorods combined with thermosensitive liposomes[67] have been designed, and some have shown significant antitumour activity in experimental models of breast cancer[64] and glioma[67]. Also, overheating gold nanoparticles

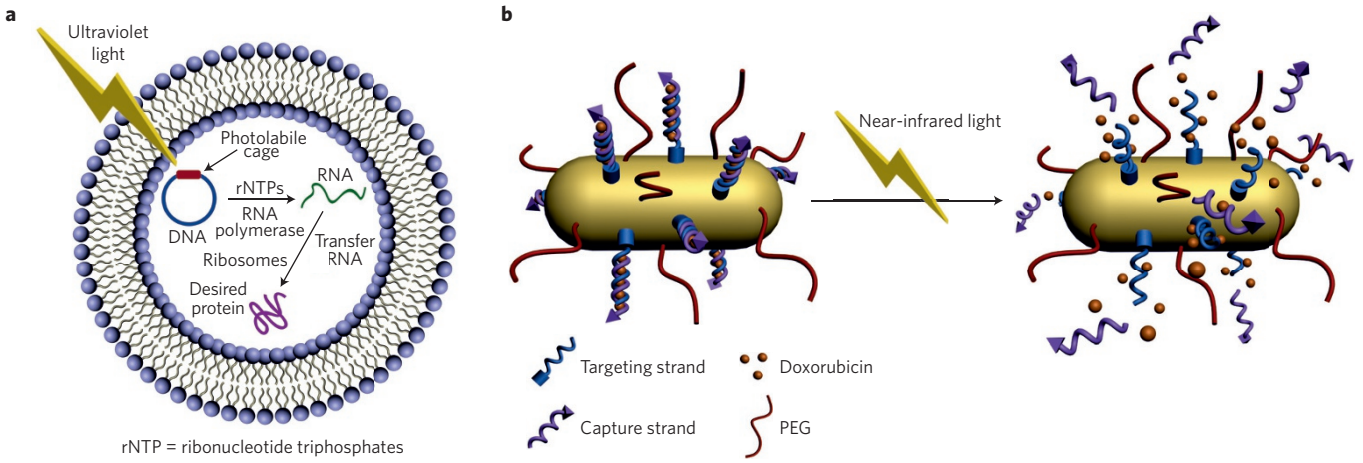

Figure 4 | Examples of light-triggered drug delivery. a, Schematic representation of an encapsulated *in vitro* transcription–translation liposomal system triggered by irradiating caged DNA with light. **b**, Delivery of doxorubicin through the near-infrared-triggered induction of dehybridization of the DNA conjugated at the surface of gold nanorods. Figure adapted with permission from: **a**, ref. 59, © 2012 ACS; **b**, ref. 62, © 2012 Wiley.

with a short laser pulse in the NIR range can result in plasmonic nanobubbles, which could be used in applications similar to those of ultrasound-triggered drug delivery[68].

Although promising from a conceptual point of view, the safety and/or biodegradability of the typical materials used in light-responsive nanoparticles for drug-delivery applications (Au–Ag, gold nanorods, azobenzene and *o*-nitro benzyl derivatives) is questionable. Finding biocompatible photosensitive materials will therefore be a critical part in the potential clinical translation of these systems.

Electroresponsive systems. Weak electric fields (typically about 1 V) can be used to achieve pulsed or sustained drug release through a variety of actuation mechanisms. For instance, nanoparticles based on polypyrrole — a conductive polymer — exhibited tailored drug-release profiles as a result of a synergistic process of electrochemical reduction–oxidation and electric-field-driven movement of charged molecules[69]. Multiwalled carbon nanotubes could be used as a conductive additive to increase the electrical sensitivity of drug-delivery systems[70]. Montmorillonite, when formulated in a chitosan nanohydrogel, finely tuned the drug release on electrostimulation, and preserved responsiveness and reversibility after consecutive on–off switching operations[71]. An electric field also activated the reversible scission of supramolecular polymersomes that formed through host–guest complexation between the end groups (β-cyclodextrin and ferrocene) of a pair of homopolymers (one hydrophilic and one hydrophobic) (Fig. 5)[72]. Similarly, an oxidizing voltage activated the splitting of a vesicle membrane (composed of redox-responsive self-assembled amphiphilic rod–coil tetraaniline-PEG) into smaller pucklike micelles, which could reassemble on the application of a reductive voltage[73].

Electroporation — the application of a (typically high) transmembrane voltage to cause the formation of pores in cell membranes and thus increase their permeability to drugs — has been shown to be an efficient pathway for electroresponsive drug delivery. In the past few years, it has been applied to nucleic acid delivery against cancer, either by using PEG-coated silica nanoparticles with opposite polarities to enhance gene transfection[74] or by using transferrin-decorated liposomes loaded with exogenous oligonucleotides[75]. Similarly, iontophoresis — which uses an electric field to enhance the transdermal delivery of charged compounds — is a particularly versatile approach. It has been recently applied to various types of nanoscaled systems, including organic nanocarriers such as PLGA nanoparticles loaded with estradiol[76] and liposome-containing insulin[77], as well as inorganic gold nanoparticles against traumatic tendinitis[78]. Iontophoresis has also been applied to ocular delivery, for instance in the transport of dexamethasone across the human sclera with egg lecithin–taurocholate micelles as nanocarriers[79].

Overall, electroresponsive systems offer significant freedom in the design of the device that controls the electrical signal. However, similar to other external stimuli, the low tissue penetration depth and the need to avoid undesired tissue damage may restrict their application in therapy.

Endogenous stimuli-responsive drug delivery

In this section we discuss systems that take advantage of variations in pH, redox potential, or the concentrations of enzymes or specific analytes.

pH-sensitive systems. pH variations have been exploited to control the delivery of drugs in specific organs (such as the gastrointestinal tract or the vagina) or intracellular compartments (such as endosomes or lysosomes), as well as to trigger the release of the drug when subtle environmental changes are associated with pathological situations, such as cancer or inflammation. Two main strategies exist: the use of polymers (polyacids or polybases) with ionizable groups that undergo conformational and/or solubility changes in response to environmental pH variation; and the design of polymeric systems with acid-sensitive bonds whose cleavage enables the release of molecules anchored at the polymer backbone, the modification of the charge of the polymer or the exposure of targeting ligands.

There exists a plethora of anticancer drug-delivery systems that have taken advantage of the slight difference of pH existing between healthy tissues (~7.4) and the extracellular environment of solid tumours (6.5–7.2). This is mainly a consequence of irregular angiogenesis in fast-growing tumours, which causes a rapid deficit of both nutrients and oxygen and thus a shift towards a glycolytic metabolism, therefore leading to the production of acidic metabolites in the tumour interstitium. Hence, efficient pH-sensitive systems must give a sharp response to a subtle change of pH in the tumour extracellular microenvironment. For example, chitosan swelling induced on amino-group protonation (pK_a ~6.3) leads to the release of encapsulated tumour necrosis factor alpha (TNFα) in the local acidic environment of tumour tissues[80]. Sudden disassembly at pH 6.4–6.8 of PEG–poly(β-amino ester) micelles triggered the leakage of entrapped campthotecin[81]. pH-mediated triggered delivery of proteins into ischemic areas was achieved

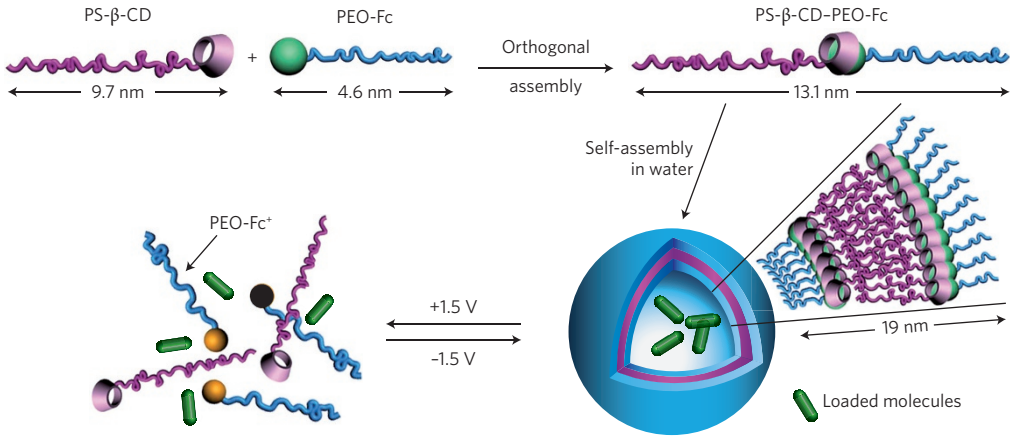

Figure 5 | Voltage-responsive vesicles. Structure of polystyrene-β-cyclodextrin (PS-β-CD) and poly(ethylene oxide)-ferrocene (PEO-Fc), and representation of the voltage-responsive controlled assembly and disassembly of PS-β-CD–PEO-Fc supramolecular vesicles. Figure reproduced with permission from ref. 72, © 2010 ACS.

with piperidine- and imidazole-modified PEG–poly(β-amino ester) micelles[82].

A change of pH has also been exploited to tune CPP display at the surface of nanocarriers to promote cell internalization. Polyhistidine-based micelles could respond to acidic tumour microenvironments by efficient exposure of the transactivating regulatory protein (TAT) sequence (Fig. 6a)[83], and TAT-peptide-decorated liposomes comprising an acidic hydrolyzable PEG shell allowed improved exposure of the TAT sequence at low pH (Fig. 6b)[84]. Other targeting strategies involved the uncaging of an exposed ligand by hydrolysis of acid-labile groups[85], and the protonation of titratable ligand-functionalized lipids with consequent lipid-bilayer reorganization and ligand exposure[86]. Cell internalization can also be promoted by means of pH-triggered surface-charge reversal from negative or neutral to positive[87].

Bacterial infections are generally characterized by very low pH values because of anaerobic fermentation and subsequent inflammation. In this regard, systemic antibiotic therapy was achieved by incorporating an ionizable polyhistidine segment in a block copolymer to make PLGA-*b*-polyhistidine-*b*-PEG triblock copolymer nanoparticles. A charge switch at the sites of localized acidity promoted interactions with the negatively charged bacterial wall, and led to increased nanoparticle uptake in both Gram-positive and Gram-negative bacteria[88].

Because of the broad range of pH found throughout the gastrointestinal tract, pH-responsive systems for oral drug delivery have been designed to protect drugs from the harsh conditions found in the gastric cavity and to improve their absorption in the intestine[89]. For instance, poly(methacrylic acid)-based copolymers have been used as pH-sensitive coatings at the surface of porous silica nanoparticles[90], as well as to prepare copolymer micelles able to disassemble at the intestinal pH[91]. This charge-reversal approach was also applied to MSNPs to achieve drug release at neutral pH by taking advantage of electrostatic interactions[92], and to chitosan nanoparticles for gastric or intestinal delivery[93].

At the cellular level, the acidification of endosomes (pH ~5–6) and their fusion with lysosomes (pH ~4–5) is another pH gradient that can be used for effective intracellular drug accumulation. Nanoparticles that expand in response to a mildly acidic pH to afford rapid release of their contents have been conceived either by masking the hydroxyl groups in the polymer backbone with acid-labile protecting groups[94] or by using protonable dimethylaminoethyl methacrylate monomer units. This led to tunable swelling and DNA release kinetics within the endosomal pH range[95]. Alternatively, the presence of acid-sensitive bonds in the polymer backbone (such as hydrazone[96], oxime[97] or acetals[98]) or the use of acid-degradable crosslinkers[99] can lead to nanocarrier disassembly. pH-sensitive bonds also enabled the release of drugs covalently conjugated to polymer backbones[87,96], protein scaffolds[100], MSNP pores[101] and nanoparticles derived from the particle replication in nonwetting templates (PRINT) process[102]. MSNPs with β-cyclodextrin nanovalves are also responsive to endosomal acidification[103]. As bacteria are located in acidic intracellular compartments, pH responsiveness was also used in the treatment of resistant intracellular infections by means of intralysosomal release of penicillin[104]. Drug release can also be mediated by physical destabilization due to a hydrophobic–hydrophilic transition combined with hydrogen bond breaking[105] or by acidic etching of MSNP pores[106].

The low pH values and high enzymatic content of lysosomes can be harmful to many therapeutic molecules. Therefore, substantial effort has been directed towards the design of systems able to escape the endosomal compartment by exploiting the so-called proton sponge effect (by which an increase in osmotic pressure leads to lysosomal swelling and rupture). To this end, copolymers made of amine-containing polymers (such as poly-L-lysine[107], poly(β-amino

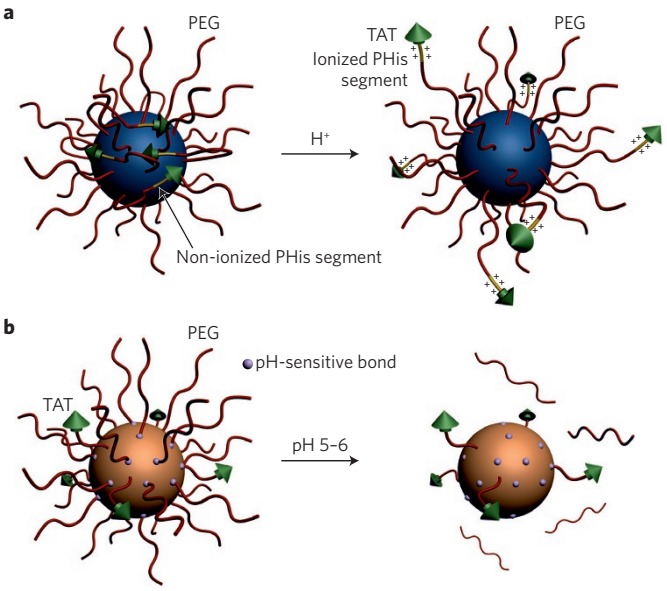

Figure 6 | pH-sensitive nanocarriers for efficient TAT-peptide exposure. **a**, Polyhistidine (PHis)-based micelles responding to acidic tumour microenvironments by an efficient TAT-sequence exposure following ionization of the polyhistidine segments. **b**, TAT-peptide-decorated liposomes comprising a hydrolyzable PEG shell allowing improved exposure of the TAT sequence at low pH. Figure adapted with permission from: **a**, ref. 83, © 2008 Elsevier; **b**, ref. 84, © 2012 Elsevier.

esters)[108], polyhistidine[109] or poly(γ-benzyl-L-glutamate)[110]) have been widely used to buffer the endosomal–lysosomal pH. For example, the introduction of a protonable polyhistidine segment endowed a virus-mimetic nanogel with endosomal disruption and multiple cell-infection abilities[111]. Lipid-coated poly(β-amino ester) nanoparticles combined endosomal escape and mRNA delivery together with efficient *in vivo* transfection after intranasal administration[112]. The charge-reversal behaviour of chitosan has also been exploited for pH-triggered drug release[113]. However, because of the low buffering effect of chitosan and its derivatives, complexation with membrane-destabilizing polyelectrolytes was needed to enhance the endosomolytic potential and siRNA release[114]. We should note that pH-sensitive nanodevices inducing disruption of the lysosomal membranes may cause leakage of lysosomal enzymes into the cell cytoplasm, potentially leading to autophagy and cell death.

pH-sensitive liposomes are generally formulated with 1,2-dioleoyl-*sn*-glycero-3-phoshoethanolamine (DOPE) or 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine, which undergo a transition from a lamellar phase to a fusogenic hexagonal phase at acidic pH[115,116]. The conjugation of DOPE to low-molecular-weight PEI significantly improved gene and siRNA delivery through a combination of fusogenicity and buffering properties[117]. Positively charged and PEG-protected liposomes were used to facilitate the interaction with the endosomal membrane[118]. Alternatively, pH sensitivity can be conferred by using anchored[119] or caging[120] polymer chains that undergo a phase transition in lysosomal acidic environments, thus causing lipid-membrane destabilization and cargo release.

Redox-sensitive systems. Disulphide bonds, prone to rapid cleavage by glutathione (GSH), can be used to attain redox sensitivity. The cytosolic release of drugs can then be triggered by the different concentrations of GSH found in extracellular (~2–10 μM) and intracellular (~2–10 mM) compartments, and in tumour tissues

NATURE MATERIALS DOI: 10.1038/NMAT3776

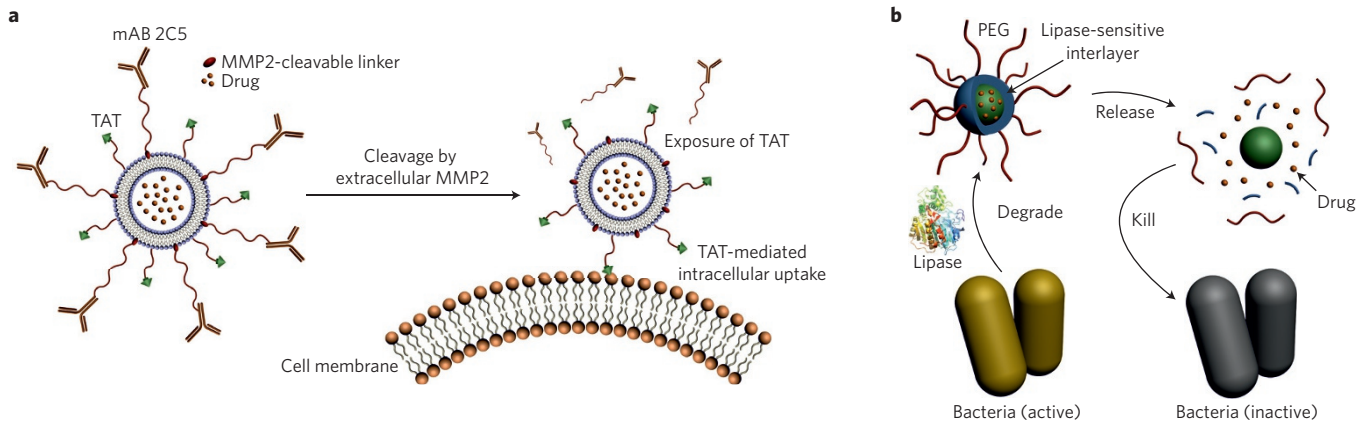

Figure 7 | Enzyme-sensitive drug delivery. a, Multifunctional liposomal nanocarrier responsive to matrix metalloproteinases (MMP2) for drug delivery via TAT-mediated internalization. mAB 2C5; nucleosome-specific monoclonal antibody 2C5. **b**, On-demand drug delivery triggered by bacterial lipase. Figure adapted with permission from: **a**, ref. 137, © 2012 ACS; **b**, ref. 146, © 2012 ACS.

compared with healthy ones. This has been achieved by reductively degradable micelles from self-assembled amphiphilic copolymers containing disulphide links within the hydrophobic backbone[121] or bearing a single disulphide bond at the connection of the two polymer blocks[122,123]. Other routes used GSH-sensitive crosslinking agents incorporated either in the shell[124] or in the core[125] of the micelles, leading to rapid micelle disassembly followed by specific intracellular release of hydrophobic drugs. Redox-sensitive systems can also use capped mesoporous materials[126], dendrimer–drug conjugates containing thiol-cleavable bonds[127], liposomes built from a quinone-lipid conjugate[128] or disulphide crosslinked nanogels[129].

Complexation of nucleic acids with a reducible cationic polymer (such as poly(disulphide amine)[130], disulphide-containing poly(amido amine)[131] or histidine-containing polycations[132]) can improve transfection or gene silencing compared with non-redox sensitive analogues as a result of the quick disassembly of the complexes under reductive intracellular conditions. For example, PEG-based polyplex micelles that can shed the surrounding PEG chains in response to a reducing environment allowed to fully recover the cytosolic-release properties of the polycation thus leading to significant *in vivo* gene silencing[133]. Other drug-delivery systems synthesized through disulphide linkages are siRNA-grafted polymers[134] and multimerized siRNA[135].

Oxidation responsiveness was also explored for triggered drug release in inflammatory tissues, which are characterized by an accumulation of reactive oxygen species. For example, thioketal-based nanoparticles formulated with a reactive oxygen species-sensitive polymer exhibited the capability of specific TNFα–siRNA delivery to the sites of intestinal inflammation, thus providing therapeutic levels of gene silencing after oral administration[136].

It is noteworthy that Mylotrag, a redox-responsive anti-CD33 antibody-linked drug developed by Celltech and approved by the Food and Drug Administration of the United States for acute myelogenous leukemia, failed to confirm benefits to patients and was withdrawn from the market. This illustrates the difficulties of drug-release control by a specific redox molecular mechanism in a complex biological environment.

Enzyme-sensitive systems. The altered expression profile of specific enzymes (such as proteases, phospholipases or glycosidases) observed in pathological conditions, such as cancer or inflammation, can be exploited to achieve enzyme-mediated drug release with accumulation of drugs at the desired biological target. Most of the systems devoted to enzyme-mediated drug delivery exploited the presence of enzymes in the extracellular environment. Recent studies reported the use of short peptide sequences, cleavable by matrix metalloproteinases, as linkers between surface PEG chains and either TAT-functionalized liposomes (Fig. 7a)[137] or CPP-decorated, dextran-coated iron oxide nanoparticles[138]. After cleavage of the PEG shell in the tumour microenvironment, surface bioactive ligands became exposed, and this enhanced intracellular penetration compared with nanocarriers without cleavable linkers. Using this approach, systemic administration of siRNA-loaded nanoparticles resulted in an almost 70% gene-silencing activity in tumour-bearing mice[139]. Similarly, protease-sensitive polymer coatings or lipopeptides were designed to achieve triggered release from porous silica nanoparticles[140] or liposomes[141].

It is also possible to deliver drugs to intracellular compartments by using enzymes. For instance, mesoporous silica scaffolds grafted with polysaccharide derivatives enabled the specific delivery of doxorubicin after lysosomal enzyme-mediated cleavage of the glycoside bonds and reduction of the polysaccharide chain lengths[142]. Similarly, lysosomal enzyme cathepsine B, overexpressed in several malignant tumours, enabled cargo release by means of fast enzymatic degradation of polymersomes[143]. Transgene expression with high cell specificity has been achieved through polymer-based delivery systems bearing a cationic peptide as substrate of intracellular proteases (or kinases) that are exclusively expressed in cells infected with human immunodeficiency virus[144] or inflamed cells[145]. The enzyme-mediated disintegration of the polymer–DNA electrostatic interaction promoted gene release and transcription.

Enzyme responsiveness can be extended to bacterial-infection treatments. For example, on-demand release of antibiotics, achieved with vancomycin-releasing lipase-sensitive nanogels (Fig. 7b)[146], significantly inhibited the growth of *Staphylococcus aureus* and was also effective in killing intracellular bacteria.

These representative examples highlight the potential of enzyme-triggered drug delivery. However, work is still needed to obtain precise information of the target enzyme levels at the desired site to fine-control cell uptake and to demonstrate that *in vivo* drug release is correlated to enzymatic activity.

Self-regulated systems. Systems capable of responding to changes in the concentration of specific analytes are able to achieve self-regulated drug delivery. This is particularly important in the non-invasive management of diabetes, which requires a system that triggers the release of insulin according to blood-glucose levels. A commonly used strategy to design glucose-responsive systems takes advantage of the ability of phenylboronic acid (PBA) and its derivatives to combine reversibly with *cis*-diol units. The equilibrium in aqueous

solution between neutral (hydrophobic) and charged (hydrophilic) PBA is shifted towards the latter when charged PBA complexes with glucose, resulting in the swelling of PBA-containing polymers. This was exploited to activate the release of insulin from poly(ethylene glycol)-*b*-poly(acrylic acid-*co*-acrylamidophenylboronic acid) micelles[147] and poly(ethylene glycol)-*b*-poly(styrene boroxole) polymersomes[148]. However, responsiveness required elevated glucose concentrations (up to 50 mg ml^{-1}), far from physiological conditions (1–3 mg ml^{-1}). Greater glucose sensitivity was obtained either by rearrangement of the polymer structure through the introduction of non-responsive solubilizing groups[149], the replacement of PBA by a boronate ester with lower pK_a (ref. 150) or the introduction of carbohydrate molecules as pendant groups on the copolymer backbone[151]. When carbohydrate molecules are introduced, the interaction of PBA with the glycopolymer is weakened in the presence of glucose, which leads to matrix swelling and insulin release. Responsiveness under physiological glucose conditions can also be achieved with polypeptide-based micelles[152] and PBA-functionalized MSNPs[153].

The enzymatic reaction between glucose and glucose oxidase, which results in the production of gluconic acid and hydrogen peroxide, can also confer glucose responsiveness to pH-sensitive systems containing glucose oxidase. For example, sustained release of insulin was mediated by the pH-dependent loosening of a crosslinked glucose oxidase multilayer deposited onto MSNPs[154].

Despite the promising proofs of concept, strong clinical evidence of the feasibility of self-regulated insulin delivery has not yet been achieved.

Multistimuli-responsive drug delivery

Sensitivity to more than one stimulus can even further improve drug delivery. Here, we only discuss systems that respond to simultaneously applied stimuli.

Because of the coexistence of a pH gradient and an oxidative environment in certain pathological conditions, in certain cases pH and redox responsiveness can be used in combination. For example, conjugation of antisense-bcl2 oligonucleotides and doxorubicin to a four-arm PEG with acid-cleavable and redox-reducible linkers resulted in efficient cancer cell apoptosis[155]. A redox-sensitive crosslinked interlayer embedded in pH-sensitive polypeptidic micelles triggered nanocarrier disassembly and a burst of doxorubicin release in a reductant-rich environment, resulting in an *in vivo* improvement of the therapeutic efficacy of the loaded drug[156]. Improved drug-release activation by dual responsiveness to pH and temperature has been shown for ionically self-assembled nanoparticles[157] and liposomes[158]. Light-sensitivity can also be associated with pH responsiveness by exploiting the resonance surface properties of palladium and silver[159]. Other systems have shown response to temperature and magnetic field for methotrexate delivery to skeletal muscle[160], to light and reducing environment to control the release kinetic from block copolymer micelles[161], and to ultrasounds and enzymes to enhance drug release from bubble liposomes[162].

Despite the advantageous versatility of these systems, they often appear as too complicated and many still remain as proofs of concept. To ascertain the viability of these strategies, evidence of the regulation of the response to each stimulus would be needed both *in vitro* and *in vivo*.

Clinical status of stimuli-responsive nanodevices

The translation of stimuli-responsive drug-delivery systems from the bench to the bedside is not straightforward. This can be explained by their usually sophisticated designs, which makes the potential pharmaceutical development more complex, especially in terms of the manufacturing process, reproducibility and quality control. Also, nontrivial optimizations and improvements are often required for the translation of each stimulus from preclinical experimental models to daily clinical practice. In particular, endogenous triggers are indeed difficult to control because they may vary from one patient to another (such as the pH of a tumour or the presence of reducing agents in the blood circulation). Although systems responsive to external stimuli are, from this point of view, more promising, major improvements would be needed to improve both tissue-penetration depth and focusing (to avoid damage to healthy tissues).

This may explain why the two stimuli-responsive nanosystems that have reached the clinical stage (Table 1) are only responsive to exogenous stimuli. They are the thermosensitive liposomes

Table 1 | Stimuli-responsive drug-delivery systems in clinical trials.

Stimulus	Nanocarrier	Drug (trade name)	Targets	Clinical status	Reference*
Temperature	Liposomes	Doxorubicin (ThermoDox)	Unresectable hepatocellular carcinoma	Phase III, the HEAT study	NCT00617981
			Recurrent chest-wall breast cancer	Phase II, the DIGNITY study	NCT00826085
			Colorectal liver metastases	Phase II, the ABLATE study	NCT01464593
			Painful bone metastases, breast carcinoma, non-small-cell lung cancer, small-cell lung cancer, adenocarcinoma	Phase II	NCT01640847
			Bone metastases, pancreatic cancer, metastatic liver cancer	Phase I	http://celsion.com/docs/pipeline_overview
Magnetic	Magnetic fluid MFL AS1	NanoTherm AS1, MagForce Nanotechnologies	Glioblastoma	European Union regulatory approval	http://www.magforce.de/en/studien.html
	Iron-oxide magnetite		Prostate and pancreatic carcinoma	Phase I	http://www.magforce.de/en/studien.html
	Iron and carbon particles	Doxorubicin/MTC–DOX	Unresectable hepatocellular carcinoma	Phase II and Phase III	NCT00034333
			Hepatocellular carcinoma	Phase I and Phase II	NCT00054951
			Cancer metastatic to the liver	Phase I and Phase II	NCT00041808

*ClinicalTrials.gov identifiers are given if available; ClinicalTrials.gov

ThermoDox, which are at present in clinical trials for the treatment of breast cancer (phase II) and hepatocellular carcinoma (phase III), and the iron oxide NanoTherm, which has been approved for the treatment of glioblastoma. Although clinical trials for ThermoDox have been recently suspended because the life span increase has not reached the threshold of 33%, these trials have demonstrated the safety profile of this formulation, which was well tolerated by patients. For the iron oxide-based MTC–DOX (magnetic target carrier–doxorubicin; developed by FeRX), which entered phase II and III clinical trials for the treatment of liver cancer and unresectable hepatocellular carcinoma, respectively, no more data have been published since 2005.

Outlook

Systems for drug delivery are often decorated with a specific ligand for disease recognition. However, for most targeting systems, less than ~5% of the injected dose is able to reach the tumour site or the infected or inflamed tissues. The consensus is that both the structural heterogeneity of the biological targets and the limited accessibility of the target cells are detrimental for drug targeting (often because of a combination of an exaggerate desmoplastic reaction, an excessive interstitial pressure and a poor status of endothelial blood vessels). Also, the enhanced permeability and retention effect, widely observed in preclinical investigations, is unlikely to be translated to the clinic.

In this context, the design of nanocarriers sensitive to exogenous or endogenous stimuli may represent an attractive alternative to targeted drug delivery. The wide range of stimuli able to trigger the drug release at the right place and time, and the diversity of responsive materials that can be assembled in different architectures, allow great flexibility in the design of stimuli-responsive systems. However, although *in vitro* proofs of concept have been reported for a number of stimuli-responsive systems, only a few have been tested in *in vivo* preclinical models, and very few (thermosensitive liposomes and iron oxide nanoparticles) have reached the clinical stage. For most of these systems, the complexity of their architectural design and difficulties in the scaling-up of their synthesis are likely to hamper their translation from the bench to the bedside. Moreover, their toxicity is multifactorial, depending on composition, physicochemical properties, route of administration and dose. The benefit-to-risk ratio has therefore to be balanced according to the intended medical application. Unfortunately, many available stimuli-responsive systems have limited chances of reaching the clinic because of absence of degradability or insufficient biocompatibility. The ability of these systems to be sensitive to discrete variations of pH, temperature or redox potential is not straightforward to achieve, and issues related to the penetration depth of the externally applied stimulus would eventually need to be solved.

It is difficult to pinpoint which stimuli-responsive nanosystems have the best chances of reaching the clinic. The medical applications of most of the systems that we have discussed in this Review correspond to either therapeutic niches, or to orphan diseases that are resistant to already available treatments or for which no therapeutic alternative exists. As a general rule, the simpler and easier the development of a system is, the better its chances of reaching the clinic. The thermosensitive liposomes ThermoDox, already in the clinical phase, are a good example.

As we have shown in this Review, immense progress in materials chemistry and drug delivery has led to the design of smart stimuli-responsive concepts using well-engineered nanosystems. Perhaps the focus should now shift towards clinically acceptable systems that are more sensitive to discrete variations in specific stimuli.

Received 31 January 2013; accepted 10 September 2013; published online 23 October 2013

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Acknowledgements

Some results described in this Review were obtained thanks to the European Research Council Advanced Grant TERNANOMED (European Research Council under the European Community's Seventh Framework Programme FP7/2007-2013; grant agreement no. 249835). CNRS and Université Paris-Sud are also acknowledged for financial support.

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Competing financial interests

The authors declare no competing financial interests.