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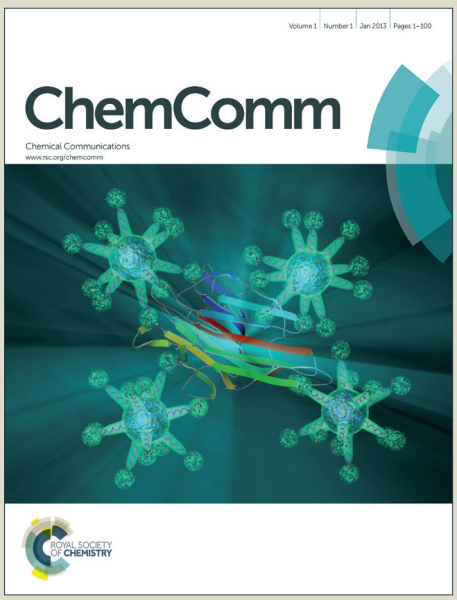

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FEATURE ARTICLE

Smart Drug Delivery Systems: from Fundamentals to the Clinic

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Forty years after the first reports on stimuli-responsive phase transitions in synthetic hydrogels, the first medicines based on responsive components are approaching to the market. Sensitiveness to internal or external signals of the body can be achieved by means of materials (mostly polymers, but also lipids and metals) that modify their properties as a function of the intensity of the signal and that enable the transduction into changes in the delivery system that affect to its ability to host/release a therapeutic substance. Integration of responsive materials into implantable depots, targetable nanocarriers and even insertable medical devices can endow them with activation-modulated and feedback-regulated control of drug release. This review offers a critical overview of therapeutically-interesting stimuli to trigger drug release and the evolution of responsive materials suitable as functional excipients, illustrated with recent examples of formulations in clinical trials or already commercially available, which can provide a perspective of the current state of the art on smart drug delivery systems.

1. Evolution of the drug dosage forms: The need of smartness

Pharmacological treatments pursue that the active substance reaches the target in such a way that a concentration sufficiently large is maintained for the time required to produce the therapeutic effect. Ideally, only the tissues where the pharmacological target is located should be exposed to the drug in order to maximize the response and minimize the collateral effects. Nevertheless, drug administration has been traditionally limited to make the drug accessible to the blood stream, relying on the irrigation and the drug affinity of the tissues for the access to the target. In fact, bioavailability is still measured from the levels of drug in the blood stream, not in the target surroundings. However, there are numerous challenges that the drug alone finds hard to overcome, such as the attack of enzymes, the poor permeability of some tissues, and the difficulty of access to the target once arriving to the destination cells, among others. As a consequence, the treatments usually involve the administration of relatively high doses of drug with the hope that a portion, although minor, goes to the right tissues or cells. The situation becomes worse in the case of new active substances obtained via biotechnological processes (peptides, enzymes, genes), which exhibit complex structure and too deficient physicochemical and stability features to be able to reach/stand the blood circulation. Overall, the efficiency of the therapy is strongly dependent on the rate the active substances success to reach the target site. Therefore, the therapeutic requirements in terms of delivery site and release rate are everyday more demanding.

Most commercially available sustained-release medicines are designed to release the drug according to a prestablished rate in the absorption site with the purpose of regulating the access of the drug to the blood circulation, from where it can distribute to the organism tissues. The so-called first generation of controlled release systems relies on the fact that a *rate-programmed drug release* may become the limiting step of the absorption process.[1,2] Its development notably prompted the search of excipients (mostly polymers) that can endow the dosage forms with capability to regulate the release via dissolution, diffusion, erosion or osmotic mechanisms. Maintenance of drug levels in a therapeutically desirable range with lower dose per day notably improves both the efficacy of treatments with short half-life drugs and the compliance of the patients, as well as allows minimizing adverse events.[3] A further step in the development of oral controlled release formulations is related to the use of components that can regulate the site at which the release process should occur; namely, the region of the gastrointestinal tract more favourable for the stability or the absorption of the drug. In the second generation of controlled release systems, *activation-modulated drug release* is accomplished by means of excipients that respond to certain physical, chemical or biochemical processes in the gastrointestinal tract, as for example, the pH gradients or the enzymatic activity.[4] The third generation pursues *feedback-regulated drug release* by means of advanced dosage forms able to deliver the drug (as a true courier) at the best conditions as possible to the target site, and that can also feedback regulate drug release fitting it to the physio/pathological conditions of the body, preferably to the progression of certain illness markers.[5-7] The medicines of this latter generation are also called drug delivery systems (DDSs) to differentiate from dosage forms that just regulate drug release before the absorption/distribution process. The mechanisms behind the three generations of controlled release systems are schematically depicted in Figure 1.[8]

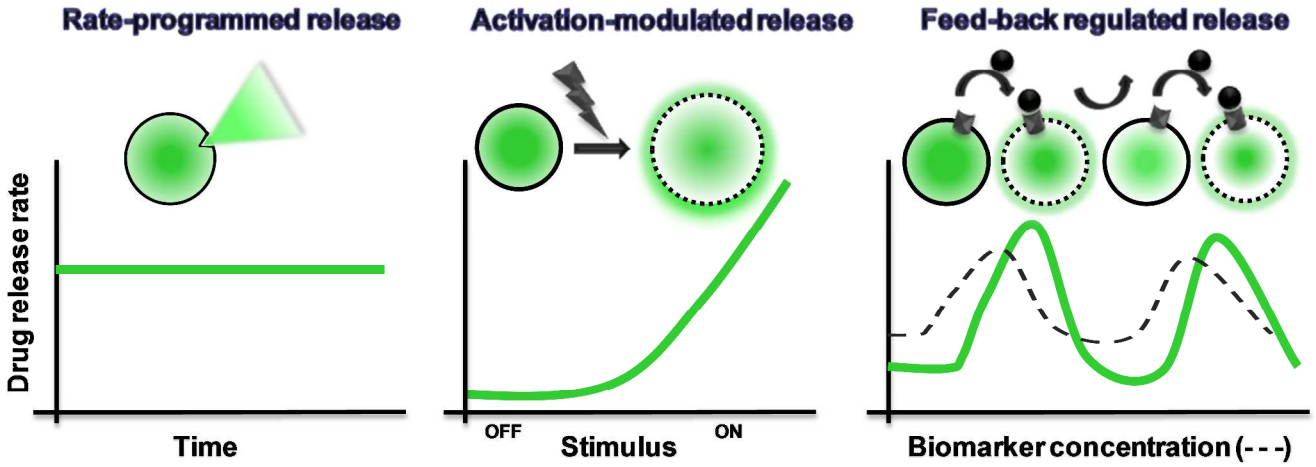

Fig. 1 Drug release patterns from a drug dosage form. In the rate-programmed systems, the release occurs according to a pre-established pattern, disregarding the conditions at the body. The release from activation-modulated systems is triggered by certain internal or external stimulus. Finally, feed-back regulated release systems adjust the release rate to the levels of a biomarker; the presence of the biomarker triggers drug release and as a consequence the level of the biomarker decreases, leading to the stop of the release until the biomarker level increases again.[8] Copyright 2013 Smithers Rapra.

Both activation-modulated and feedback-regulated systems require components that can act as "sensors" of the surrounding conditions and as "actuators" able to trigger the release of the drug in the case of the activation-modulated systems, or to finely tune the rate in the case of feedback-regulated systems. The design of such sensor/actuator (stimuli-responsive) excipients is truly challenging since the composition of the biological environment is complex and the changes caused by a pathological process are mostly of scarce magnitude.

In addition to the internal variables, development of materials able to respond to external stimuli has been also considered. Thus, two major classes of responsive DDSs can be distinguished: a) those that recognize changes in the biological medium (for example, in pH, temperature or concentration of some substances) activating or modulating the release rate are named closed-loop or self-regulated systems; and b) DDSs that switch drug release on/off as a function of specific external stimuli (e.g. light, or electric or magnetic field) are considered to work in open circuit, and they can provide pulsed drug release when externally activated.[9,10] The search for advanced excipients that can lead to responsive formulations has prompted an amazing effort for finding suitable stimuli-sensitive materials, and it is responsible of the exponential increase in publications related to "smart" delivery systems.

The adjectives smart, stimuli-responsive and environmental-responsive are commonly used indistinctly each other, although some differential aspects should be taken into account. Sensitiveness to internal or external signals of the body is typically achieved by means of semisynthetic or synthetic materials (mostly polymers) that bear functional groups which modify their properties as a function of the intensity of the signal and that enable the transduction into changes in the material features.[11-13] These changes can have different levels of complexity; for example, i) modification of the solubility, the shape, or the state of aggregation of single components (e.g. assembly/disassembly of micelle unimers or sol-gel transition), ii) reversible change in conformation of chemically cross-linked networks that lead to volume phase transitions and modifications in affinity towards other chemical groups or molecular entities, or iii) reversible stretching/shrinking of surface-immobilized chains or networks on inert substrates (Figure 2).[10,14-16] Only when these possible structural changes are reversible and proportional to the stimulus intensity, the DDS can be considered to behave as "smart".

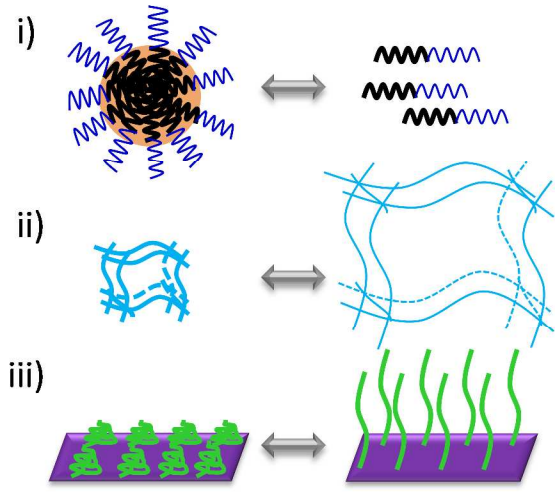

Fig. 2 Some transitions associated to the responsiveness to a stimulus: i) deaggregation of amphiphilic polymers; ii) volume phase transition; and iii) helix to random coil.

It should be noticed that there are a number of advanced DDSs named with the acronym SMART. Three categories have been recently proposed, as follows:[17]

-Type 1. Systems to Maximise Access, Retention and Therapy, which are formed by nanoparticles intended for passive or active drug targeting. Examples of these systems are nanoparticles decorated with monoclonal antibodies, magnetically guided particles, and bioconjugates designed to improve the targeting efficiency compared to that of DDSs that rely only on the Enhanced Permeability and Retention (EPR) effect observed in some pathological tissues.
-Type 2. Systems that Monitor, Analyze and Respond in Time. These DDSs are designed to feed-back modulate drug release by means of mechanically driven mechanisms or cell-based constructs. In the first case, the system is integrated by a biosensor that monitors the concentration of certain biomarker, a therapy management software, and a pump system that releases the drug at the adequate rate. In the second case, stem cells or xenotransplants of non-human cells are implanted in the body to supply a lacking substance.
-Type 3. Systems Mute until Activation by a Remote Trigger. Remote triggering release has been already tested for oral capsules and for parenteral nanocarriers using near infrared (NIR) light, magnetic field or ultrasound as trigger agents.

Most of these SMART technologies are still in development process or early clinical phases. Type 1 nanomedicines that have so far enter into market are those that have been clearly proved as Systems Modulating Adverse Reactions and Toxicity, such as Abraxane® and Doxil®.[18] In this context, it should be noticed that although "smart" (stimulus-responsive) materials are not endowed with the natural or the artificial intelligence of living cells or computer software, they have the advantage of being more simple and cheap and, in most cases, resemble better the natural mechanisms of transport and delivery of substances in the living bodies (where changes in the levels of physical or chemical factors regulate a series of biochemical processes).[19] Therefore, they may play a relevant role as components of advanced DDSs Type 2 and 3, and even Type 1, as recently revisited in the comprehensive book *Smart Materials for Drug Delivery*.[20] Moreover, there are a number of stimuli-responsive products under clinical evaluation, (e.g. Opaxio™ and ThermoDox®),[21,22] and some of them have been already approved for clinical use (NanoTherm®) or commercialized for research use only.[23-25] The information generated in the design and evaluation of these pioneering products should pave the way to the development and approval of more smart nanomedicines. In the present review, the word "smart" is thereinafter used in the context of stimuli responsiveness for drug delivery. Comprehensive reviews on particle requirements for targeting, and examples of smart changes in size, shape and surface properties for maximizing the targeting can be found elsewhere.[26] The present review aims to provide a perspective of the current state of the art on smart DDS in terms of delivery site and release rate, supported by recent examples of formulations in clinical trials or already commercially available.

2. Smart DDSs

Smart DDSs can be materialized at macro, micro and nano-scales. For example, they can be prepared in the form of macro/micro-depots that are directly placed in the target tissue/organ using more or less invasive approaches. From their static position, the macro/micro-depots are intended to trigger or to modulate the release of the drug towards the surrounding cells. Preparation of nanocarriers opens the possibility of combining systemic administration with release at specific cells, if adequately designed. The drug-loaded nanocarrier is intended to be parenterally administered and to transport the drug towards specific tissues, modifying the distribution and clearance of the drug. As far as the drug is inside the nanocarrier it cannot freely extravasate from the blood stream. The drug only gets access via passive or active targeting to the tissues where the nanocarrier accumulates.[27] Thus, an efficient nanocarrier should fulfill somehow contradictory requirements: i) it should be able to strongly retain the drug, without premature leakage, during the movement towards the target tissue/cells, where it has to release 100% drug in a short period of time; and ii) it should move in the body avoiding the adsorption of proteins or lipids that can trigger the recognition by the phagocyte mononuclear system and the retention in non-target tissues, but it has to strongly interact with the target cells. When the target cells are those of a tumor, these requirements can be summarized in the sentences "*drug Retention in blood circulation versus Release in tumor cells (2R)*" and "*Stealthy in blood versus Sticky in tumor (2S)*".[28] The difficulty of accomplish the 2R2S features together with the poor performance of some nanocarrier materials as excipients suitable for large scale production are behind the notably delay of the entrance of nanomedicines in the market. In that context, smart materials may contribute to solve, at least, the 2R challenge. Below, some useful materials to endow DDSs with smart behavior and therapeutically-interesting stimuli to trigger drug release are analyzed.

2.1. Smart components

At this point it seems clear that smart DDSs require components that do not behave as inert, but take part actively in the performance during release.[29] Thus, the evolution from classical dosage forms to advanced DDSs runs in parallel to the development of active excipients, which in turn is linked to the evolution of the biomaterials.[30] As biomaterials can be defined as any material that interfaces with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body,[31] excipients can be considered a particular type of biomaterials. The traditional functions of excipients are to facilitate the preparation of medicines (e.g. making easier some technological steps) and to ensure stability during storage. Importantly, these two key functions should not be forgotten even when more sophisticated performances are demanded (advanced nanoDDSs would not enter in the market if they could not be produced in a reproducible way). The first generation of biomaterials was the result of a change of paradigm in the understanding of the materials as entities not to be separately studied as a function of their nature (metals, ceramics, polymers), but to be evaluated regarding the processing-structure-property interrelationships.[32] As the first generation of biomaterials, traditional excipients can be considered as bioinert materials; e.g., their role in drug release from solid medicines was limited to facilitate the disintegration of the dosage form once in contact with the physiological fluids. For a variety of purposes (e.g. immediate oral release), this feature is still valid today.

In the last third of the 20st century, the integration of materials science and engineering principles with biology and biomedical criteria enabled the development of the so-called second generation of biomaterials that elicit favorable biological responses in order to achieve a better interaction with the

surrounding tissues during permanent or temporal (biodegradation) contact. Major advances in knowledge about the body components and their performances together with a new way to look at the biological systems as engineering structures led to a progressive change of the biocompatibility concept and, as a consequence, of the design and use of biomaterials.[32,33] A third generation biomaterial is expected to interact with biological components of the patient and, in turn, directly participate in the course of the medical treatment.[31] Accordingly, the third generation excipients should help to overcome constrains that prevent the drug from reaching the optimum therapeutic plasma/tissue level, tuning drug release and pharmacokinetics.[30] A lesson to learn from natural materials is that their outstanding performances come from the hierarchical structure and their ability to adapt to modifiable circumstances.[34] Thus, current design of lipids and polymers benefits of the knowledge about the conformation and functionality of natural biomacromolecules, with the advantage of that the synthetic structures are more stable and can be prepared applying versatile, less-expensive procedures.[14,35] Biomimetic (also called smart or fourth-generation) implantable biomaterials are expected to actively participate in the regeneration process of damaged tissues by stimulating specific cellular responses at the molecular level.[33] Stimuli-responsive excipients clearly form part of this avant-garde generation as components of smart DDSs and theranostic systems, which combine diagnosis and drug delivery capabilities in a single entity.[36,37]

Like the natural materials modulate their conformation and performance as a function of the conditions (stimuli) of the surrounding environment, high-performance components of DDSs should be able to tune the release as a function of the physio/pathological state of the body. Therefore, they should proactively interact with the biological functions, being able to perceive certain signals from the body, process them and consequently modify the behavior and functioning of the DDSs. In this sense, smart DDSs can be also labeled as "adaptative" medicines, which adjust their properties as they navigate through complex biological environments or in response to an external stimulus.[19,26] In addition to stimuli-responsiveness, safety and excretion/elimination assessments have to be kept in mind during the design of materials intended to reach profound tissues and cell structures.[14,38-40] The large list of requirements that smart materials have to fulfill to be suitable components of medicines is depicted in Figure 3.

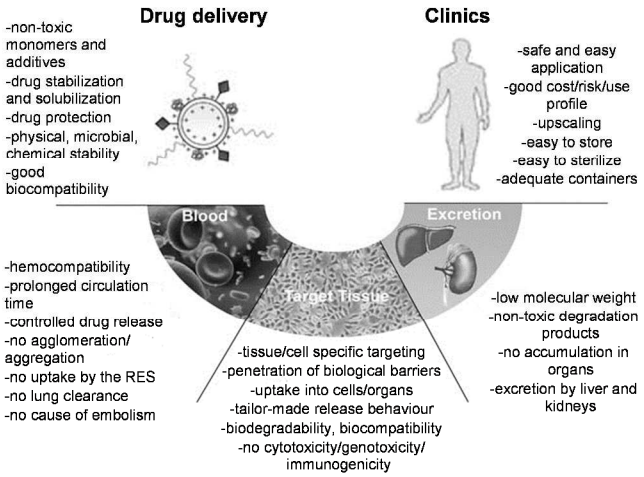

Fig. 3 Features to be taken into account when a polymer is intended to be used in a drug delivery system.[14] Copyright 2011 Wiley-VCH Verlag GmbH.

2.1.1. Phase transitions

Smart DDSs require the incorporation of sensitive components at adequate proportion and arrangement.[11,41] Polymers are the most widely used since they can be prepared with an unequaled richness of structures and functional groups that sharply modify their features, undergoing reliable and reversible phase transitions, in response to the stimulus of interest.[12,13,29] It should be emphasized that the thermodynamics governing phase transitions is the mechanistic basis of the smartness. In fact, smart DDSs have been recently referred by D. W. Grainger as examples of Some Modest Attempts to Respect Thermodynamics.[42]

Up to now ten phase transition types have been described; more than the half being exclusive of polymers.[43] Phase transitions are mainly of first or second order. In the first-order transitions, the extensive thermodynamic quantities of volume, energy, entropy or number of moles of the macromolecules show a discontinuity as a function of the intensive quantities of pressure, temperature, or chemical potential, among others. In the second-order case, no discontinuity is evident, but it appears when the derivatives of the extensive thermodynamic quantities are plotted. Mainly, to be useful as a stimuli-sensitive component for drug delivery, the polymer has to respond to the appearance/disappearance of the stimulus undergoing a first-order phase transition, which should be accompanied by a change in the specific volume of the polymer.[44,45]

A single molecule or a collection of molecules can be involved in the transition.[43] Transitions within one macromolecule may occur because the sequential connectivity of the monomers along the polymer chain makes them distinguishable from each other, differently to what happen when the monomers are free in solution, which behave similarly. Examples of this transition type are "helix to random coil" and "collapse" transitions.[29] Helix to coil transitions are typical of single-stranded polypeptides, double-stranded DNA, and triple-stranded collagen, which undergo diffuse, second-order and first-order transitions, respectively, when a change in temperature or chemical potential alters intra- and inter-strands hydrogen bonds. It should be noticed that DNA in the cells do not pack alone, but with other molecules in order to undergo first order-transitions and to be more packed, since a second-order transition of naked DNA would lead to a random coil conformation with dimensions larger than those of the cells.[46] Collapse transition refers to the competition between the attractive interactions among monomers that drive the self-collapse of the polymer, and the entropy of the chains (rubber elasticity) that tries to expand the polymer.[47] Since each monomer occupies a certain volume in the polymer and the monomers cannot penetrate each other, there is repulsion at short distances. Such repulsion prevails in a good solvent and thus the polymer coils are expanded (swollen). A change in the environmental conditions, such as temperature, pH or polarity, can modify the balance between the free energy of the internal (polymer-polymer and polymer-solvent) interactions and the elasticity component. If the attractive interactions between monomers become strong enough, a coil-to-globule transition occurs at a condition called θ point.[46] This transition may also occur when the polymer chains are cross-linked, forming a

three-dimensional hydrogel network. In a good solvent, the chains confined between two adjacent cross-linking points behave as polymer coils. If the solvent conditions change towards the θ point, each subchain undergoes a coil-globule transition and, as a result, the network as a whole shrinks; i.e., a volume phase transition occurs.[48] Hydrogel collapse is driven by any one of the four basic types of intermolecular interactions operational in water solutions and in biological systems, namely, hydrogen bonds and van der Waals, hydrophobic and Coulomb interactions.[49,50] As a consequence of the volume phase transition, the flow of fluids through the network and also the diffusion of solutes, such as drugs, is notably altered, particularly if the monomers are connected into flexible long chains. Therefore, small changes in the intensive thermodynamic variables (temperature, pressure, chemical potential) lead to drastic changes in the polymer conformation.[51]

Regarding the transitions within collections of molecules, the most exploited one for smart drug delivery is that related to block copolymers that can associate as membranes, micelles, or vesicles. Incompatible polymers are attached together as blocks that cannot move far away each other. Nevertheless, the incompatibility persists and it leads to microphase separation; namely, a pattern of microdomains, each of them containing mainly one of the blocks, separated by thin interphase regions.[46] Depending on the relative length of each block, the microdomains can adopt different shapes, forming lamellar, cylindrical or spherical phases. Free standing membranes, micelles and vesicles can exist in media with enough solvent.

It might occur that under certain physiological circumstances (e.g. hydrolysis or oxidation/reduction processes) a material suddenly changes its chemical nature and transforms in another material with different chemical groups. Thermodynamic transitions can accompany such transformation. Moreover, one transition does not exclude the occurrence of others, and in Nature the transitions of macromolecules are frequently coupled.[43] In fact, biopolymers that can undergo phase transitions are essential for all evolved-life forms, since they are the only material that can fulfill the three main requirements of the living systems: i) minimal complexity to form and function, ii) ability to produce different structures in a reproducible way, and iii) ability to transmit all information necessary to the forms and functions.

It should be noticed that most phase transitions have been studied in isolated (in vitro) systems, having all other variables strictly controlled. A stimuli-responsive material to be useful as component of a smart DDS should maintain their ability to undergo phase transitions in the complex biological environment, namely in the presence of a high concentration of salts and proteins that can themselves notably alter the polymer-solvent interactions.[42] Otherwise, promising in vitro systems may fail under in vivo conditions. Moreover, the time required for the phase transition to occur in each direction when the stimulus appears/disappears, and the possible hysteresis effects, should fit the therapeutic demands for the correct switching on and off of the drug release. Nanometer-size structures show faster transitions and less hysteresis than macroscopic networks. Nevertheless, characterization of the stimuli-responsiveness of a smart DDS under physiological conditions and relevant disease states is still quite challenging.[42]

2.2. Stimuli and suitable smart DDSs

Most smart DDSs are designed to physically entrap the drug, until the stimulus triggers conformational changes, in the entire carrier or in specific layers or channels, that lead to the release of the drug.[24,52,53] Some stimuli (e.g. pH or enzyme) can be also exploited by means of carriers made of labile bonds or having the drug molecules conjugated through cleavable bonds, which are broken under the stimulus action[19] (Figure 4).

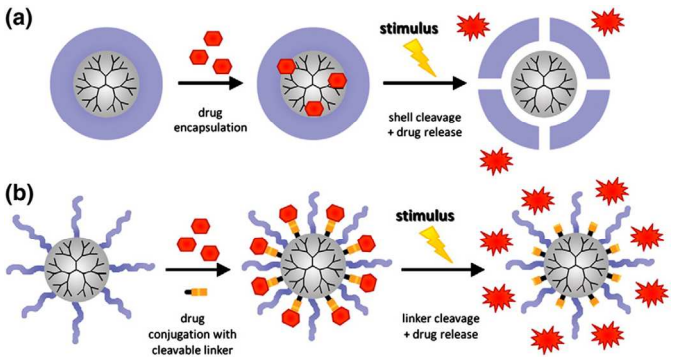

Fig. 4 Release of active agents from (a) supramolecular complexes like dendritic core-shell particles with a cleavable shell and (b) dendritic scaffolds with attached solubilizing/stealth groups using cleavable linkers for the drug conjugation.[19] Copyright 2012 Elsevier B.V.

2.2.1. pH

The variety of pH gradients that can be found in the body has notably attracted the interest of researchers, being one of the first and most evaluated stimuli. In addition to the changes in pH typical of the gastrointestinal tract that have been exploited to prepare site-specific release oral formulations, pH gradients that occur at cellular level are particularly suitable for the design of intracellular-specific delivery. The differences of pH among cytosol (7.4), Golgi apparatus (6.40), endosome (5.5-6.0) and lysosome (4.5-5.0) are considerable[54] Moreover, the extracellular pH of blood and healthy tissues (7.4) is higher than that recorded in tumor tissues (6.5-7.0), mainly because overproduction of lactic acid due to the hypoxic environment and fast metabolic rates of tumor cells.[55] Similarly, a drop in pH up to 6.5 is observed after 60 h of the onset of an inflammatory process.[56] The evolution of the pH of the wounds can be also used as an index of the progression of the healing.[57]

Development of pH-responsive systems is mostly based on polymers that bear weak acid (e.g. carboxylic acid) or base (e.g. primary and tertiary amino) groups with pKa that enables sharp changes in the ionization state at the pH of interest. An increase in the degree of ionization can dramatically alter the conformation and the affinity of the chains for the solvent as well as the interactions among them, resulting either in the disassembly of components or the swelling/shrinking of covalent networks. The pH-responsiveness can be easily tuned by varying the nature of the comonomers used to prepare the polymer.[58]

pH-responsive micelles for tumor-targeted release have been prepared using amphiphilic copolymers with amino groups in one of the blocks.[59,60] At blood pH 7.4, the non-ionized amino blocks self-aggregate forming the micellar core. Once they

enter in the tumor cells via endocytic pathways, fusion with lysosome (pH 5-6) causes the ionization of the hydrophobic blocks leading to micelle disassembly and drug release.[61] However, since tumor cells and normal cells have both the same endosomal acid pH, a more tumor-specific release can be achieved with micelles that disassemble at the weakly acidic tumor microenvironment.[60] In this sense, methyl ether poly(ethylene glycol)-b-poly(α-amino ester) (MPEG-PAE) micelles intravenously injected in MDA-MB231 human breast tumor-bearing mice demonstrated preferential accumulation in the tumor, with minimal distribution to the healthy tissues. This behavior clearly contrasted with the non-selective distribution observed for non-responsive polymeric micelles.[60] MPEG-PAE micelles loaded with Fe_3O_4 nanoparticles have been shown also useful for magnetic resonance image (MRI) and selective release in cerebral ischemic area, which exhibits an acidic environment.[62] Rapid, tumor-specific diagnosis has been recently achieved with oligopeptide-based micelles that quickly dissociate exhibiting fluorescence when encountering a subtle pH-change from 7.4 to 6.8.[63] The oligopeptides contained a fluorescent dye and its quencher and, therefore, did not emit fluorescence when self-assembled. In vivo distribution and tumor accumulation studies demonstrated that these micelles can behave as activatable probes that emit fluorescent signals from the extracellular matrix of tumor tissue as early as 10 min post injection, with steady increased fluorescent intensity up to 1–1.5 h. Dissociation of the micelles could be also exploited for antitumor drug release, being useful for theranostics. An alternative to disassembly-dependent release is the conjugation of the drug to block copolymers or dendrimers via a labile bond. Micelles, polymersomes and dendritic structures decorated with active targeting elements can be internalized in tumor cells, where the labile bonds are broken and the drug released.[64-66]

pH-sensitive polymeric micelles have also a great potential as carriers of DNA or siRNA, which interact with the amino groups of the copolymers, forming a complex inside the micelle (micelleplex) that protects nucleic acids from enzymatic degradation.[67-69] Micelles of poly(ethylene glycol)-b-poly[(3-morpholinopropyl) aspartamide]-b-poly(l-lysine) (PEG-b-PMPA-b-PLL) combine the buffering capacity of PMPA with the excellent aptitude of PLL to condense DNA, resulting in a high transfectional efficiency.[70] An approach suitable to combine long circulation, low cytotoxicity and improved transfection consists on the preparation of detachable negatively-charged coatings or PEG-based shells, which shield the positive charges of the polyplex in serum (Figure 5). Once internalized, the coating or the shell degrades at the acidic pH of the endosome, unmasking the positively charged polyplex, which facilitates endosomal escape and transfection.[71,72] Nevertheless, as shown below, pH-responsiveness is nowadays combined with components responsive to other stimuli in order to improve the specificity of the site and the control of the release of the active substances.[73]

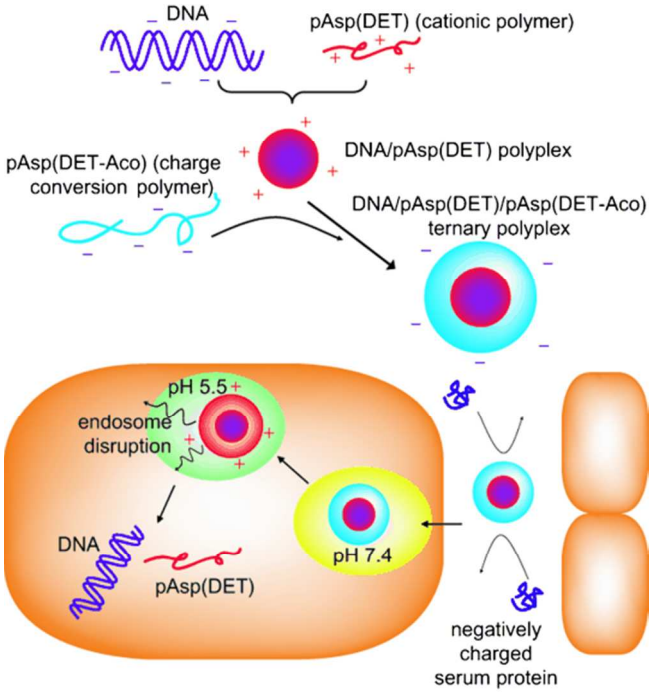

Fig. 5 Ternary polyplex prepared combining plasmid DNA with pAsp(DET) (poly{N-[N′-(2-aminoethyl)-2-aminoethyl]aspartamide}) followed by coating with pAsp(DET-Aco) (poly(N-{N′-[(N″-cis-aconityl)-2-aminoethyl]-2-aminoethyl}aspartamide)). The polyplex is negatively charged at pH 7.4, which communicates serum stability and low cytotoxicity. The cis-aconitic amide moieties are degraded at the endosomal pH, which leads to the charge-conversion components and facilitates the endosomal escape of the polyplex through membrane disruption.[71] Copyright 2008 John Wiley and Sons.

2.2.2. Redox conditions

In Nature, sulfur(II)-containing molecules such as cysteine and cysteine-derived compounds (e.g. glutathione, GSH), play relevant roles as defense compounds.[74] They usually act as transition metal binders for detoxification or modulation of the catalytic activity of metalloenzymes, or participate as redox buffers to attenuate the detrimental effects of oxidants.[75] GSH/glutathione disulfide is the major redox couple in animal cells. GSH is kept reduced by NADPH and glutathione reductase, although the intracellular levels of GSH are also dependent on other redox couples. The intracellular compartments (cytosol, mitochondria, and nucleus) contain 2 to 3 orders of magnitude higher level of GSH tripeptide (2-10 mM) than the extracellular fluids (2-20 μM). Thus, GSH is an ubiquitous intracellular substance suitable as stimulus for triggering drug release inside cells. Moreover, tumor tissues have greater concentration in GSH (4-to-7-fold higher) than the healthy ones, which reinforces the role of GSH to specifically trigger drug release in tumor cells.

Block copolymers or polymer nanogels bearing disulfide (-S-S-) bonds are suitable to undergo reduction reactions in the presence of GSH, leading to the rupture of the polymer bonds. A large variety of GSH-responsive systems, comprising shell-detachable micelles and reduction-sensitive micellar cores, polymersomes, capsules and nanogels, has been already evaluated.[76] As a consequence of the redox process, the

nanostructure swells or disassembles and the drug is released. Nevertheless, it has been recently reported that the nature of the reductant specie can exert a strong influence in the release process. In vitro studies carried out with poly(methacrylic acid) (PMAA) nanogels crosslinked with N,N-bis(acryloyl)cystamine have shown that doxorubicin release is notably faster when GSH is added to the medium, compared to the levels attained when dithiothreitol (DTT, a synthetic reductant) was used.[77] In literature, GSH and DTT are used almost indistinctly to mimic the reductant intracellular environment. In fact both have a similar capability to reduce the disulfide bonds. However, while DTT is a neutral specie, GSH (isoelectric point 5.93) becomes positively charged at the acidic environment of tumors. As a consequence, antitumor agents that are positively charged at acid pH may remain bound to the carrier via electrostatic interactions when DTT is used. Oppositely, GSH can displace the drug from the polymer, notably prompting the release (Figure 6). Thus, the natural reductant environment triggers drug release more efficiently. This calls again attention on the importance of correctly mimic the physiological environment in the in vitro release studies in order to obtain information that can be extrapolated to the in vivo conditions. Interestingly, several antibody-drug conjugates with labile bonds responsive to the intracellular redox conditions are already in clinical phase II/III for breast cancer (Trastuzumab-DM1)[78] and multiple myeloma (Maytansine).[79]

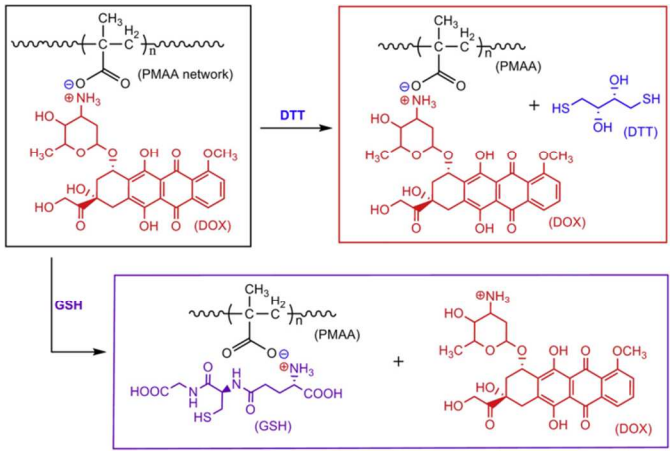

Fig. 6 Possible reaction mechanism for nanohydrogels with varying responses to DTT and GSH as reducing agent, which is caused by a drug exchange process between GSH and PMAA-doxorubicine (DOX) complex.[77] Copyright 2012 Elsevier B.V.

Tissues affected by ischemic stroke and cancer usually exhibit hypoxia; namely, tissue partial pressure of oxygen is near 0 mm Hg, which is substantially lower than that in normal tissue (30 mmHg).[80] The highly reductive environment of hypoxia has been exploited to develop imaging agents and responsive DDSs. For example, hydrophobic 2-nitroimidazol groups are converted, under hypoxic conditions, to hydrophilic 2-aminoimidazoles via a series of selective bioreductions. Hydrophilic polymers bearing hydrophobically modified 2-nitroimidazol groups self-assemble under normoxic conditions, behaving as nanocarriers of antitumoral agents, but release the drug faster into cells under hypoxic conditions. In vivo biodistribution studies proved that these polymers selectively accumulate at the hypoxic tumor tissues leading to high anti-tumor activity.[81] On the other hand, layer-by-layer nanoparticles with trilayer architecture of poly-l-lysine (PLL) modified with iminobiotin, followed by protein neutravidin, and biotin end-functionalized poly(ethylene glycol) (PEG) have been shown to gradually lose the PEG shells as the iminobiotin–neutravidin interactions decrease in the hypoxic tumor environment, allowing the exposed PLL layer to facilitate cellular uptake, thus shifting the biodistribution of the nanoparticles towards tumor retention.[82]

DDSs responsiveness to reactive oxygen species (ROS) or reactive nitrogen species (RNS) has been still barely explored. ROS such as hydrogen peroxide, superoxide or OH radical are the main contributors to the intra- and extracellular redox potential associated to stress conditions, signaling cascades, diabetes, hypertension, atherosclerosis or cancer.[83] An oxidative environment is present under inflammatory conditions, where cytokines induce the production of ROS.[84,85] Typical chemical groups responsive to physiological oxidants are metallocenes, pyridyl metal complexes, polyconjugated sequences, thiols, thioethers (sulfides) and stannanes.[83] Sulfur(II)-containing polymers, in the form of polysulfides, have been recently pointed out particularly suitable for anti-inflammatory therapy. In the presence of ROS, sulfur (II) is converted to higher oxidation states (sulfoxides or sulfones) which in turn increases the polarity of the polymer and its water solubility or swellability.[86] Oxidation-responsive swellable nanoparticles have been prepared via cross-linking of poly(propylene sulfide) (PPS) and decoration with PEO chains, either by incorporation of a pre-functionalized Pluronic (PEO-PPO-PEO copolymer) during the polymerization of PPS or by adsorption of Pluronic to the preformed particles.[87] Di- and tri-block copolymers have been also prepared combining PPS and PEO via anionic ring-opening polymerization of propylene sulfide.[88]

Since most tumors show heterogeneous levels of GSH and ROS depending on the tumor region or the development stage,[89] a nanocarrier that responds to both intracellular GSH and ROS may enable rapid release of an antitumoral agent in any tumor tissue. To prepare such a dually-responsive carrier, the camptothecin-based topoisomerase I inhibitor 7-ethyl-10-hydroxyl-camptothecin (SN38) was conjugated to ethylene oxide oligo(ethylene glycol) (OEG), by means of a thioether linker labile to either GSH levels or highly oxidant ROS species, such as H_2O_2, $O_2^{\bullet}$, and $OH^{\bullet}$, but not to NO (differentiating between tumor and inflamed tissues) (see Figure 7). Tuning the length of the OEG chain and the nature of the thioether linker, amphiphilic conjugates capable of self-assembling into nanocapsules were obtained. In vivo studies carried out in a Bcap37 breast tumor xenograft model and a dimethylhydrazine (DMH)-induced autochthonous colon cancer model evidenced that the conjugate was better tolerated than the free drug and significantly more efficient.[90]

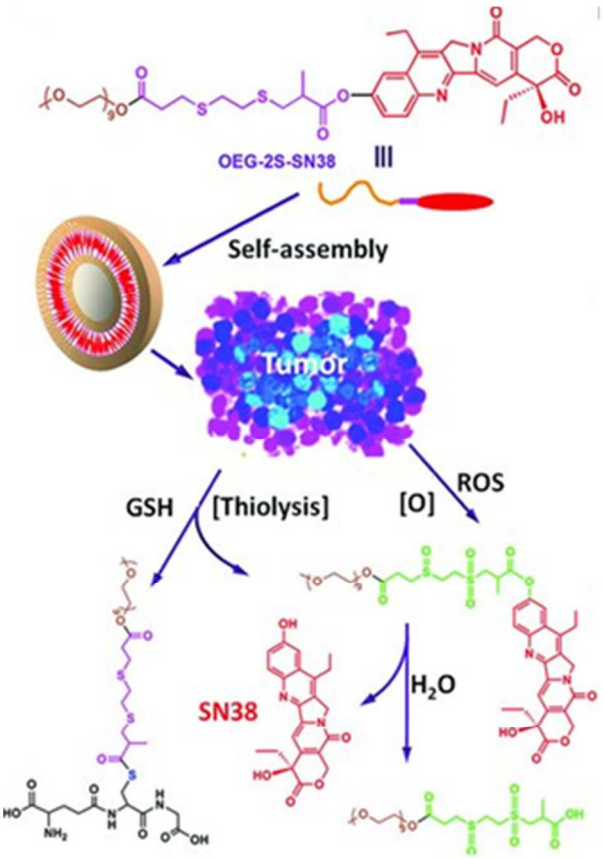

Fig. 7 Structure of GSH and ROS-responsive conjugate of SN38. The conjugate self-assembleds as nanocapsules that can release SN38 as a function of the concentration of GSH or H_2O_2.[90] Copyright 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Another approach to prepare inflammation-responsive systems, which has been already tested in vivo for the treatment of uveitis, is based on lipoidal-chitosan-poly(ε-caprolactone) nanoparticles loaded with an anti-inflammatory agent. The nanoparticles were coated with a cross-linked network of hyaluronic acid, alginate and poly(acrylic acid) loaded with an antimicrobial agent.[91] Sustained-release intraocular implants for the treatment of uveitis have the drawback of that a continuous release of corticosteroid drugs, disregarding the inflammation status, augments the likelihood of side effects, such as cataract development or intraocular pressure elevation. Thus, inflammation-responsive implants may notably improve the safety of the treatment. The network of hyaluronic acid, alginate and poly(acrylic acid) notably alters its conformation upon binding of OH radicals, which in turn decreases the consistency of the network and accelerates drug release. The changes were proved to be reversible and the implant exhibited on-off inflammation-responsive drug release. In vivo tests demonstrated higher levels of both indomethacin and ciprofloxacin in the posterior ocular fluid of inflamed eyes compared to normal ones. Histological analysis corroborated the success of the approach.[91]

2.2.3. Molecule-responsive and imprinted systems

Pathology-related markers, such as enzymes and antibodies, have been widely explored as stimuli that can enable a precise control and feed-back regulation of drug release. There is a large variety of diseases that are generated or can be detected by disregulation of enzyme activity.[92] Capthesins, plasmin, urokinase-type plasminogen activator, prostate-specific antigen, matrix metalloproteases, β-glucuronidase, and carboxylesterases, are overexpressed in tumors.[19] Design of enzymatic-triggered DDSs relies on (i) carriers susceptible to the degradation by the corresponding enzyme, (ii) drug-polymer conjugates with linkers that serve as substrate of the enzyme, or (iii) nanoparticles having caps that can be removed by the action of the enzyme.[93-96] A recent example is the development of bio-responsive delivery of tissue-type plasminogen activator (tPA) for localized thrombolysis.[97] The major challenge toward developing a clot-specific delivery system for tPA is to produce immediate thrombolytic action followed by rapid vascular reperfusion. A nanocarrier was developed with tPA camouflaged with human serum albumin (HSA) via a thrombin-cleavable peptide (GFPRGFPAGGCtPA), and the surface of the albumin was decorated with a homing peptide (CQQHHLGGAKQAGDV) that binds with GPIIb/IIIa expressed on activated platelets (Figure 8). The camouflaged construct suppressed tPA enzymatic activity in the systemic circulation, but regenerated its thrombolytic action upon contact with thrombin present on the thrombus. In a rat thrombosis model, camouflaged tPA showed similar thrombolytic activity at the thrombus, but 2-fold lower degradation of circulating fibrinogen compared to native tPA, which reduces the risk of hemorrhagic incidence.[97]

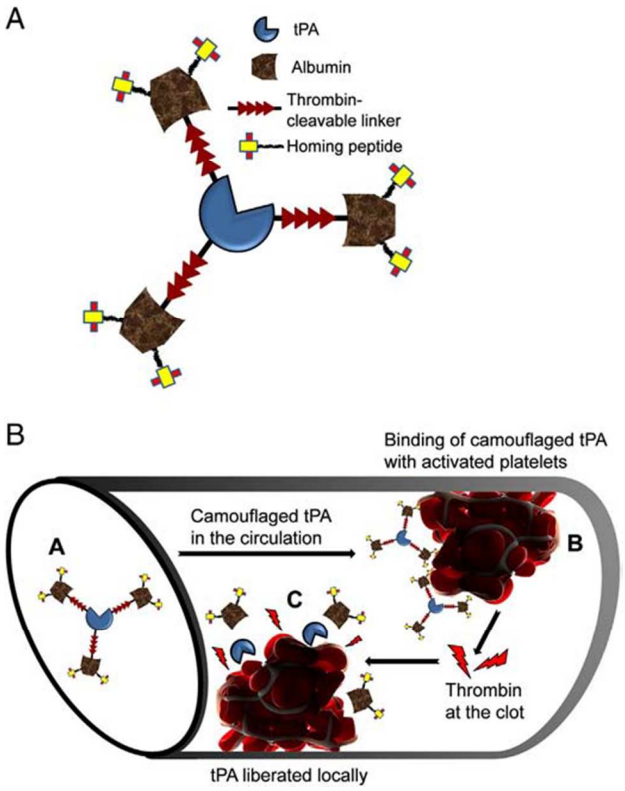

Fig. 8 (A) Construct of camouflaged tPA. The activity of tPA was masked by albumins (HSA) attached via a thrombin-cleavable peptide. (B) Targeted/triggered release action of camouflaged tPA: (i) HSA creates a steric hindrance against systemic plasminogen and tPA-binding macromolecules in the plasma; (ii) accumulation of the complex on thrombus surface via the targeting peptide; (iii) release of active tPA upon

thrombin-mediated cleavage of the peptide linker.[97] Copyright 2014 Elsevier B.V.

Alternatively, the enzyme can be incorporated into pH-responsive networks in such a way that the enzyme-substrate reaction results in the modification of the inner pH of the network. In the absence of the substrate, the network does not modify its conformation, but when the substrate concentration reaches a certain level, it reacts with the enzyme resulting in a product that modifies the local pH and, consequently, induces the network to change the degree of swelling. Once the substrate is consumed, the pH restores to the original value and the network adopts its initial degree of swelling. A proof of the interest of this type of hydrogels for drug delivery was shown for glucose-responsive insulin release from polymethacrylic acid networks containing glucose oxidase.[98] The high incidence of diabetes has notably prompted the development of glucose (enzyme-based or not) biosensors that can be implanted under the skin and continuous monitor blood glucose concentrations.[99,100] The biosensor can be coupled to a transdermal signal reading, in the form of a "smart tattoo", that sends a warning signal in case of hypo- or hyperglycemia.[101] The most widely investigated fluorescence sensors are those based on glucose oxidase (e.g., CGMS Gold® first commercialized in 1999, and advanced generations Guardian Real-Time®, Seven®, Dexcom® G4™ Platinum or Enlite™ in the market since 2005, 2006, 2012 and 2013, respectively) (Figure 9), wired enzyme technology (e.g. FreeStyle Navigator® since 2008), the plant-sugar-binding protein concanavalin A, or on boronic acid which modify its conformation and ionization degree in the presence of molecules containing cis-diol groups, such as glucose. It has been already shown that diboronic acid-based sensors immobilized in a biocompatible micro-scaled hydrogel can be safely implanted, and that fluorescence can be detected through the skin, precisely monitoring blood glucose concentration for up to 140 days.[102] The information about glucose levels can help the patient to conduct insulin-based treatment. Optionally, the biosensor could be coupled to a closed-loop insulin release systems that can autonomously maintain blood glucose levels in the non-diabetic range.[103] Development of glucose biosensors has led to a valuable gain in knowledge on protein and cellular biofouling of implantable devices, host variables that can interfere with signal sensing, and strategies to overcome these issues. Clinical experience on long-lasting glucose biosensors is the first step towards reliable closed-loop insulin release systems.[104]

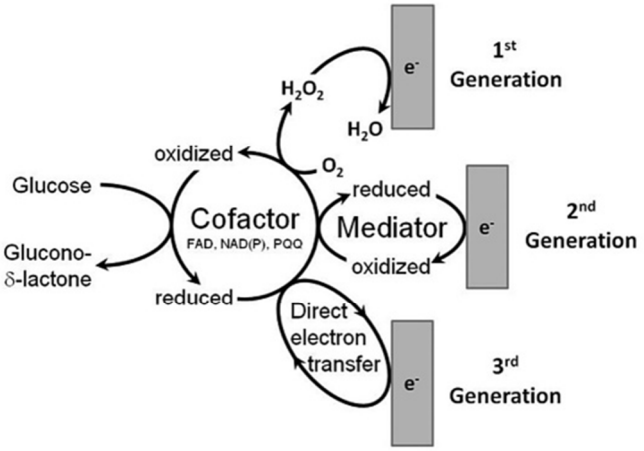

Fig. 9 Schematic representation of the principles of first-, second-, and third-generation glucose sensors. Electrons from the glucose oxidation reaction are first taken up by the enzyme's cofactor (primary electron acceptor) and transferred to either oxygen (first generation), an electron mediator (second generation), or directly to the electrode (third generation).[99] Copyright 2011 Diabetes Technology Society.

Perhaps one of the first explored recognition elements coupled to responsive hydrogels were natural antibodies. IgG antigens and antibodies can be modified with polymerizable moieties to be incorporated in hydrogels during copolymerization of common monomers. The antigen-antibody bindings act as dynamic crosslinks. If the hydrogel enters into contact with free antigens, they will compete with the copolymerized antigens for the interaction with the antibodies. Dissociation of former antigen-antibody bindings results in an increase in the degree of swelling of the hydrogel. Since nowadays it is possible to prepare antibodies for almost any biomarker, reversible antigen-sensitive hydrogels appear as highly promising biomaterials for constructing self-regulated drug release systems. In the absence of the antigen of interest (i.e., the biomarker), the hydrogel does not release the drug because of the high cross-linking density (thus, low mesh size) that the internal antigen-antibody interactions communicate to the network. Oppositely, drug release starts when the antigen biomarker appears in the surrounding medium.[105] A stepforward in this field is the use of aptamers as components of responsive gates or valves to be inserted in DDSs.[106] Aptamers are single-stranded short oligonucleotides that can be prepared to exhibit high binding selectivity and specificity for almost any kind of molecule.[107] In the absence of the molecular stimulus, aptamers form metastable structures, which become disrupted when the stimulus is recognized.[108] Molecular-gate-based DDSs mostly consist of nanocontainers (e.g. silica particles) with pores that can load the drug of interest and that, subsequently, are capped by a gating molecule. The gatekeeper (called aptavalve when formed by an aptamer) can be of very diverse nature in order to open or close the pores according to a variety of stimuli.[109]

Mesoporous silica nanoparticles have been designed to exhibit recognition to cancer cells (by means of DNA aptamer) and release of antitumor agents via responsiveness to vitamin H.[110] The levels of vitamin H (also known as biotin and coenzyme R) in cancer cells are substantially larger than in normal cells and

can be exploited not only for targeting, but also for triggering of release.[111-112] The external surface of the nanoparticles was decorated with desthiobiotin molecules and the pores capped by avidin proteins that interact with desthiobiotin (association constant, K_a, $5x10^{13}$ M^{-1}). A cancer cell-specific aptamer was then attached to avidin (Figure 10). The nanoparticles showed an enhanced capability to enter into cancer cells through receptor-mediated endocytosis. Intracellular vitamin H strongly bound to avidin ($K_a = 1x10^{15}$ M^{-1}) causing the uncapping of the pores, which accelerated drug release.

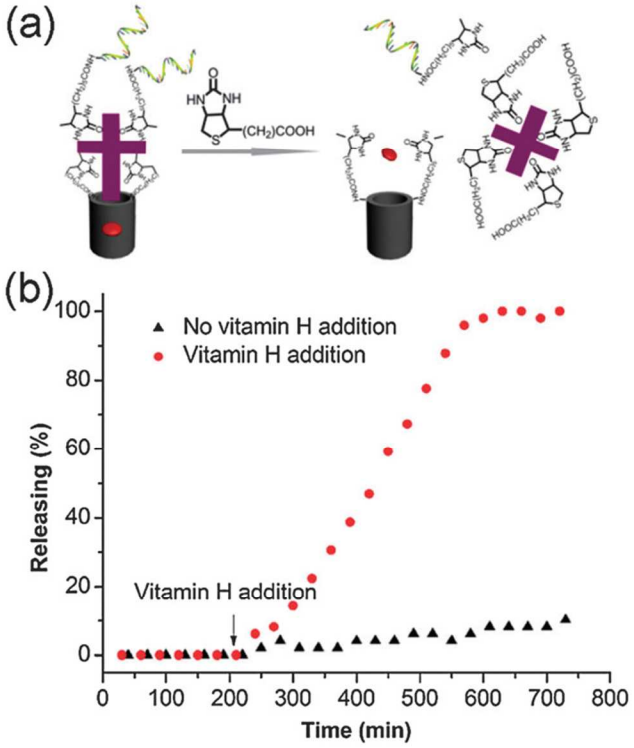

Fig. 10 (a) Schematics of vitamin-responsive drug release process for the nanovalves. (b) Release curve for MSN–Avi–Apt with and without vitamin H addition. Data have been normalized to the maximum level of dye released in the experiment.[110] Copyright 2013 Royal Society of Chemistry.

A different approach consists of designing synthetic networks with domains capable of mimicking the recognition features of the biological macromolecules. Applying the molecular imprinting technology it is possible to reproduce the small, but critical, part of the biomacromolecules responsible of the interaction with the target molecule.[113,114] This approach pursues the creation of polymer networks with cavities (artificial receptors) that are sterically and chemically complementary to the target molecule, recognizing it with high selectivity. To do that, the substance of interest is used as a template during polymerization in order to induce an adequate arrangement of the monomers, forming complexes with some of them at appropriate stoichiometry in a favorable solvent (Figure 11). The arrangement is made permanent during polymerization, and finally the artificial receptors are revealed when the template is washed out.[115-117] Molecularly imprinted polymers (MIPs) may be particularly suitable for affinity-regulated and activation-modulated release[8].

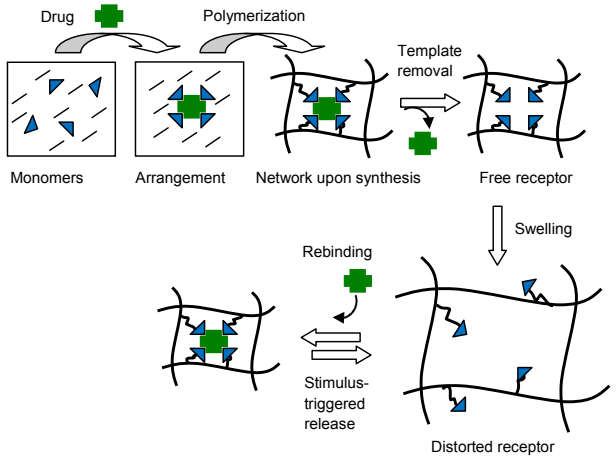

Fig. 11 Diagram of the synthesis of an imprinted hydrogel and the washing out/release and re-loading processes. The effect of stimuli on the conformation of the drug-receptors is also depicted.

When a drug is used as template, the artificial receptors created in the MIP structure selectively recognize that particular drug and rebind it strongly from a liquid medium. This enables the obtaining of high payloads. Moreover, under physiological-mimicking conditions the loaded drug is released from the MIP at much slower rate than from the corresponding non-imprinted network. This approach has been already shown very useful for the in vivo sustained release of ophthalmic drugs from imprinted soft contact lenses.[118] For example, timolol-imprinted ultrathin N,N-diethylacrylamide-based lenses (34 μg drug/lens) displayed the maximum ocular level of timolol in lachrymal fluid 5 min after wearing, followed by monoexponential decay which was prolonged for 180 min, compared to the 90 min of the non-imprinted contact lenses (20 μg drug/lens) and the 30 min of eyedrops (Figure 12). The imprinted lenses led to 3.3-fold and 8.7-fold area under the timolol concentration-time curve than the non-imprinted ones and eyedrops, respectively.[119] Imprinted contact lenses have been also shown to reduce the precorneal elimination of other drugs, such as ketotifen fumarate,[120] compared to the eyedrops and, as a consequence, much smaller amount of drug is needed to achieve and prolong in time the desired therapeutic levels.

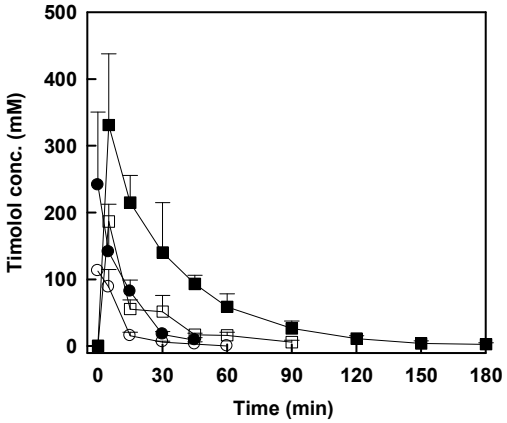

Fig. 12 Timolol tear fluid concentration-time profiles after application of drug-loaded imprinted and non-imprinted contact lenses or instillation of eye drops on rabbit eyes. The doses

were 34 μg for imprinted contact lens (solid squares), 21 μg for non-imprinted contact lens (open squares), and 34 μg (open circles) and 125 μg (solid circles) when 0.068% or 0.25% timolol eye drops were instilled. Each point represents the mean ± S.D. (n =3-5).[119] Copyright 2005 Elsevier B.V.

MIPs have been also tested as a way to endow tablets with molecular responsiveness. The tablets were prepared via compression of alternating layers of MIP microparticles and drug. In the absence of the template biomarker, no release occurs. When the biomarker appears in the medium, it is captured by the MIP particles, which in turn causes disruption of the MIP layer and exposes the drug layer to the medium. This enables the release of the drug until that the underlying MIP layer is exposed. Then, depending on the level of the biomarker, MIP layer is disrupted or not and, consequently, drug release is switched on or off.[121]

Regarding nano-sized MIP particles for systemic administration, detailed in vivo studies has so far focused on their use as traps able to recognize melittin, a cytolytic peptide that is the principal component of bee venom.[122,123] A library of tailored multifunctional copolymer nanoparticles was systematically investigate to elucidate the effect of the composition (hydrophobicity and charge) and size of the particles on their binding affinity and capacity. MIP particles (50 nm in diameter) with higher affinity for melittin were those prepared with N-isopropylacrylamide cross-linked with N,N′-methylenebisacrylamide, and using as functional monomers N-t-butylacrylamide (hydrophobic) and acrylic acid (negatively charged). The most suitable ones were then investigated regarding stability, toxicity, distribution, and capability to neutralize melittin's toxicity *in vivo* (Figure 13). Mice were intravascularly injected with melittin (0.3-4.5 mg/Kg) and 20 seconds after they were injected with MIPs. 100 percent mortality rate was observed in mice that were not treated with MIPs (8 animals). By contrast, those treated with the in vitro most efficient and less cytotoxic particles (10-30 mg/Kg) led to a significant decrease in mortality (2 out of 8 animals) and notably alleviate the peritoneal inflammation and weight loss caused by melittin. This indicates that while in the bloodstream, MIP traps specifically recognized melittin and neutralized its activity. These findings clearly evidence that a rational design of MIPs may help to preserve in vivo the affinity and selectivity of the nanoparticles when used either as drug suppliers or as detoxifying agents, avoiding deleterious effects of protein adsorption and immunogenicity.

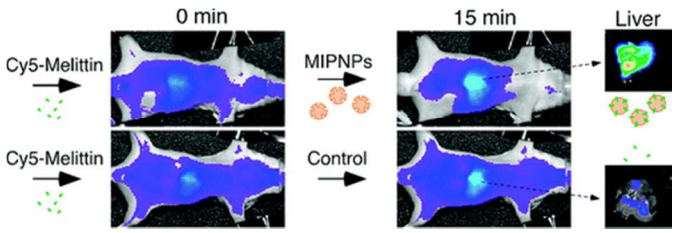

Fig. 13 Biodistribution of melittin and MIP traps according to fluorescent images of Cy5-melittin after intravenous injection (1 mg/Kg). MIP traps (27 mg/Kg) were injected 20 s after the injection of melittin. Contols did not receive the MIP nanoparticles. The fluorescent intensity of Cy5-melittin diminished immediately after administration of MIP traps. Ex vivo results showed that Cy5-melittin accumulated in the liver with a dose dependence on the amount of MIP nanoparticles administered.[122] Copyright 2010 American Chemical Society.

Preparation of stimuli-responsive imprinted networks requires memorization of the structure of the artificial receptors after several swelling/collapse cycles.[124,125] To do that, the MIP is synthesized in the presence of the template in a conformation that corresponds to the global minimum energy. The imprinted cavities develop affinity for the template molecules when the functional monomers come into proximity, but when they are separated, the affinity diminishes. The proximity is controlled by the reversible phase transition of the network that consequently controls the adsorption/release of the template (Figure 11). For example, hydrogels imprinted for doxorubicin and capable of pH-responsive release have been recently prepared maximizing the interactions between the drug and the functional monomer 4-vinyl pyridine via coordination with Cu^{2+} ions. The MIP reloaded 2.7-fold more drug than the corresponding non-imprinted polymer, and released the drug faster at pH 5.0 than at pH 7.2.[126] Temperature-responsive nanogels have been also shown as suitable platforms for switchable loading and release of proteins, as the volume phase transition notably alters the size of the imprinted cavities and, consequently, their ability to host the template protein. Interestingly, dually- and even multi-responsive MIPs have been recently investigated (Figure 14).[127] Magnetic and temperature-,[128,129] magnetic and photo-,[130] photo and temperature-,[131] temperature and salt-,[132] and photo, temperature and pH-[126] responsive MIPs have been shown to exhibit activatable and reversible molecular recognition.

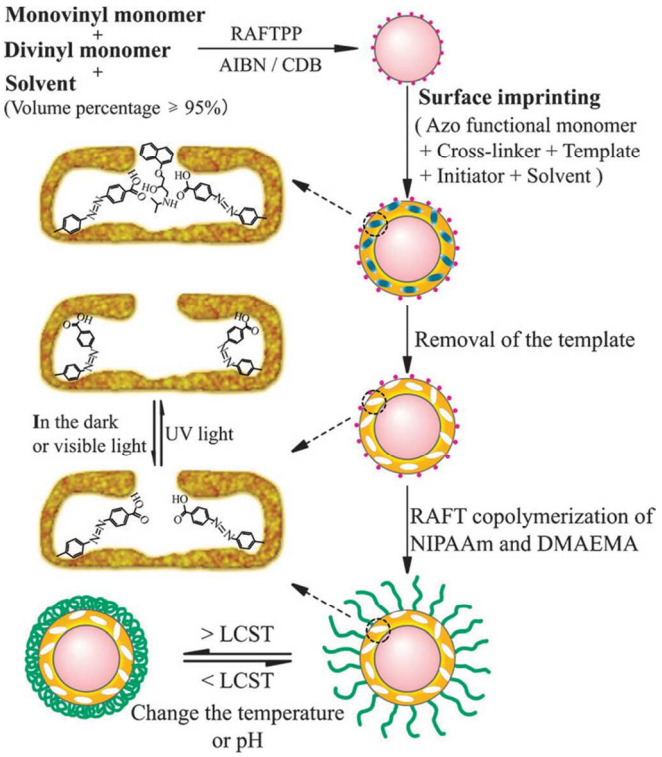

Fig. 14 Schematic protocol for the preparation of narrowly dispersed water-compatible MIP microspheres with photo-, thermo- and pH-responsive template binding properties by successive RAFT polymerization.[127] Copyright 2012 Royal Society of Chemistry.

2.2.4. Temperature

Body temperature is maintained in a short range of values under healthy conditions. Fever is associated to pyrogen substances during microorganism infections, although there are several other pathological conditions (such as inflammation, infarction, or tumor) that evolve with local increments of temperature.[133] Focalized increase in temperature can be achieved by means of external sources of heat that can be applied on the skin or can be remotely induced via irradiation of metals in the DDS that transform the energy into heat.

Temperature-responsive DDSs are based on components that modify their affinity for water as a function of temperature, which in turn causes the swelling or shrinking of the network.[45] There is a large list of polymers that exhibit critical solubility temperature (CST).[134,135] Poly-N-isopropylacrylamide (PNIPAAm), poly-N,N-diethylacrylamide, poly(methyl vinyl ether) (PMVE), poly-N-vinylcaprolactam (PVCL) and poly(ethylene oxide)-poly(propylene oxide) (PEO-PPO) block copolymers have been widely explored as components of temperature-responsive DDSs because their CST is near to the body temperature or can be easily tuned with minor changes in composition.[136,137] Natural polysaccharides and linear polypeptides may also provide temperature-responsiveness.[138] For example, homopolypeptides made of a single amino acid type have a well-defined collapse temperature: 24ºC for valine, 40ºC for proline, 45ºC for alanine, and 55ºC for glycine.[139,140] Combination of various amino acids renders "elastin-like polypeptides" with tunable CST.[141]

Micellization/demicellization cycles due to changes in temperature and pH have been reported for poly(2-diethylaminoethyl-methyl methacrylate)-poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide)-poly(2-diethylaminoethyl-methyl methacrylate) (PDEAEM25-PEO100-PPO65-PEO100-PDEAEM25),[142] and of NIPAAm, N,N-dimethylacrylamide and N-acryloylvaline.[143] Similarly, polymersomes consisting of poly(ε-caprolactone) and PNIPAAm have shown reversible disintegration in response to tiny changes of temperature.[144] In the case of cross-linked networks, the magnitude, rate and hysteresis of the responsiveness are strongly dependent on the overall size of the network and its porosity.[145] Lipid-based liquid crystals, which are prepared using amphiphilic lipids that spontaneously self-assemble into ordered structures on exposure to excess water, have been also proposed as temperature-responsive DDSs owing to their ability to undergo transitions from inverse bicontinuous cubic phase (Q_2) to inverse hexagonal phase (H_2) when temperature increases.[146]

Temperature-sensitive liposome formulation ThermoDox® is perhaps the smart DDS that has been so far more clinically evaluated. There are already in the market several liposomal formulations of anticancer drugs, which showed an improved safety profile compared to free drug solutions. However, drug bioavailability at tumor is still quite low due to a slow release from the liposomes, which in turn leads to insufficient improvement in therapeutic activity.[147] ThermoDox® is intended for passive targeting of doxorubicin towards tumor tissues and to deliver the payload in the microvasculature of the tumor when an external source of heating is applied. It should be noticed that mild hyperthermia has been safely applied in oncology for decades.[148] The formulation comprises an encapsulating solid phase membrane (dipalmitoylphosphatidylcholine, DPPC) capable of undergo solid-liquid phase transition at 41.5ºC, a permeabilizing component (monostearoylphosphatidylcholine, MSPC) embedded in the bilayer and that can induce thermally-enhanced permeability to small molecules and ions, an encapsulated drug (e.g. doxorubicin) that crystallizes in the liposome interior, and a protective PEG-layer (distearoylphosphatidylethanolamine-PEG2000) that avoids opsonization and ensures long circulation half-life.[149] ThermoDox® design followed a reverse engineering process (Figure 15), which has been kindly disclosed and may be an useful tool for further improvements and new developments.[150] Thus, starting from a clear idea of what it has to be made or achieved, the task begins with the *Performance in service* step[151] and uses an already existing product as a model (e.g. red blood cells as encapsulating entities), the iterative scheme tries to answer a sequence of questions that may be useful for the design of new prototypes or further innovations (e.g. liposomes for drug delivery).

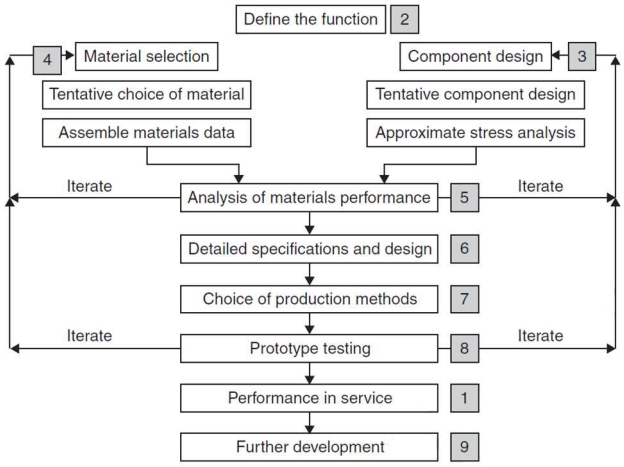

Fig. 15 The nine steps of the design methodology scheme. The numbers represent the sequence for 'reverse engineering', i.e., starting at 1, with an evaluation of the design's performance-in-service. The key questions when analyzing a design can be linked to these steps as follows: What is it for? (2); How should it work? (3); What is it made of? (4); What are the characteristics of the material? (5); How is it made? (7); Has anybody made something similar? (8); Does it really work? (1). 151 Copyright 2006 Elsevier B.V.

Although it has long been known that tumor vasculature can be hyperpermeable and that colloidal carriers may extravasate and accumulate within the tumor tissue (EPR effect),[152] the leakiness is locally heterogeneous or even absent in most spontaneous tumors.[153] Even if liposomes extravasate, the poor blood supply and high interstitial fluid pressure of the tumor together with the liposome diameter larger than the inter-fiber distance in the extracellular matrix (ECM) would make liposomes to remain trapped in the peri-vasculature space for weeks.[154] As lipids would be degraded, the drug could diffuse along the most dominant concentration gradient. Since the concentration in blood would be virtually zero, much of the drug might be reabsorbed towards the blood stream, in the opposite direction to tumor penetration. In the case of

ThermoDox®, this problem has been addressed by means of local hyperthermia (for 1h at 42ºC), which increases the size of the endothelial junctions and leads to up to 13-fold increase in liposome accumulation in the tumor.[22] Moreover, drug release can be triggered from the temperature-responsive liposomes when they are still in the blood stream of the warmed tumor in order the drug can readily diffuse throughout the neoplasm, deeply penetrate the whole cancer tissue, cross cell membranes and arrive at its target (i.e., the DNA and RNA of all cells in the tumor) (Figure 16).

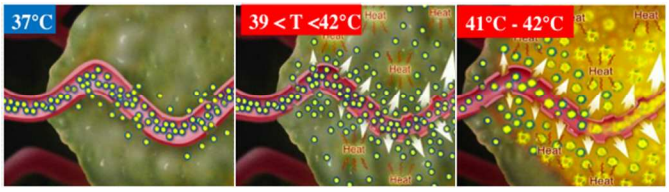

Fig. 16 Combining hyperthermia and temperature-sensitive liposome tumor-specific accumulation of drugs can be achieved. The EPR effect provides only limited access to large liposomes (A); hyperthermia can make the blood vessels more leaky and facilitates interstitial movement of liposomes and drugs (B); ThermoDox® does not need to extravasate, it can release the drug in the blood stream of the warmed tumor and the drug can readily diffuse throughout the neoplasm (C).[22] Copyright 2013 Royal Society of Chemistry.

ThermoDox® has been tested in a Phase III clinical trial in liver cancer (HEAT study), Phase II trial in chest wall recurrence of cancer (DIGNITY study), and Phase II trial of patients with colorectal liver metastases (ABLATE study).[155] This formulation has been also shown safe and efficient against widely disseminated chest wall tumors, and is currently being evaluated for a range of other drugs, imaging agents and biological modifiers using high frequency ultrasound as a heating modality. The protocol of preheating/heating of the tumor, as well as the time during which the formulation is infused appear as critical issues for efficient results.[150,156] Applying similar formulation and heating protocols, other heat-activated thermosensitive liposomes have been designed to release approximately 90% of the loaded cisplatin in less than 5 min under mild heating conditions (42 °C) for the treatment of cervical cancer.[157]

2.2.5. Light

Light-responsive DDSs have attracted a great attention as a way to take advantage of either the daily and seasonal exposition to natural solar irradiation, or artificial sources of electromagnetic radiation of very specific wavelengths in the range from 2500 to 380 nm. Ultraviolet (UV) and visible light can trigger drug release from formulations placed on skin or that circulate through blood vessels close to the body surface (e.g. eye structures). The development of these responsive DDSs can notably benefit of the knowledge about already commercialized photodynamic therapy-based treatments. For example, intravenous nonPEGylated liposome formulation of photosensitizer verteporfin (Visudyne®) is commonly used for the treatment of subfoveal choroidal neovascularization due to age-related macular degeneration, pathologic myopia, and ocular histoplasmosis. Fifteen minutes after perfusion onset, red-light laser is applied for 83 s on one or the two eyes to make the drug to be cytotoxic.[158] Similarly, photodynamic therapy using photosensitizers encapsulated in adequate nanocarriers can be used for cancer therapy.[159]

Biomimetic visible-light responsive hydrogels have been prepared by adding chlorophyll (the natural light absorbent of plants) into a PNIPAAm network.[160] Nevertheless, UV-vis light-responsiveness is most commonly achieved by means of photoactive groups, such as azobenzene, cynnamonyl, spirobenzopyran or triphenylmethane.[161] For example, the trans-cis isomerisation of the azobenzene chromophore that occurs on exposure to UV light is accompanied by an increase in the hydrophilicity, which can lead to the disassembly of polymeric micelles, liposomes or complexes with cyclodextrins.[162,163] Based on the performance of azobenzene groups, rotaxane-functionalized mesoporous silica nanoparticles have been shown suitable for in vivo remote-controlled drug release on wild-type, optically transparent zebrafish larvae. The pores of the nanoparticles were decorated with chains of triazole/ethylene glycol bearing an azobenzene unit at the end and loaded with curcumin for the treatment of heart failure (Figure 17). α-Cyclodextrin can thread along the azobenzene group. Upon irradiation with 365nm UV light, the trans-to-cis isomerization of the azobenzene group pushes α-cyclodextrin towards the entrance of the pores, hindering the exit of the drug molecules. Cis-to-trans isomerization upon exposure to visible light or heating enables the movement of α-cyclodextrin to the end of the chain, resulting in the opening of the pores and the release of curcumin.[164]

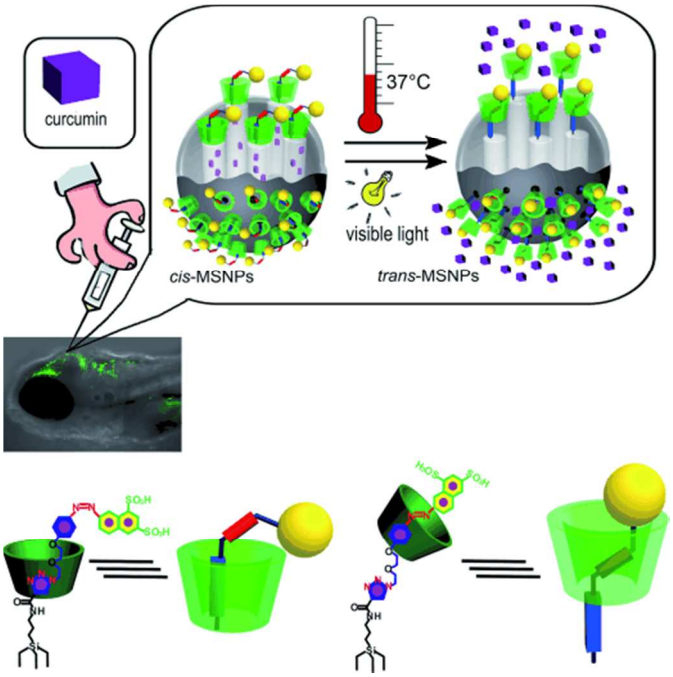

Fig. 17 A graphical representation of the injection of drug-loaded mesoporous silica nanoparticles into zebrafish larvae for in vivo drug delivery, triggered by either heating or visible light irradiation. The nanoparticles were functionalized with photothermal-responsive [2]rotaxanes on the surface. The chemical structure of the [2]rotaxane containing the α-CD ring and azobenzene unit is shown.[164] Copyright 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Near infrared (NIR) radiation can penetrate deeper into the body and switch drug release on and off at a very focalized site of a tissue.[161] In this context, near-infrared fluorescent core–shell silica-based nanoparticles (known as Cornell dots or C dots) have been recently approved by FDA for human stage I molecular imaging of cancer.[165] C dots (6-8 nm enclosing several dye molecules) coated with PEG avoid adsorption of serum proteins and are efficiently cleared from the body by the kidneys. The nanoparticles can be also decorated with ligands for selective stick to tumor cells. When exposed to NIR radiation, the dots fluoresce much brighter than unencapsulated dye enabling an overall visualization of tumor cells and blood vessels, and even metastatic spread to lymph nodes and distant organs.[166] For the human trials, dots labeled with radioactive iodine can be visible in PET scans to investigate how many dots are taken up by tumors and where else in the body they go and for how long. Such information will be very valuable for designing of efficient NIR-triggered release silica nanoparticles.

Gold-containing DDSs are particularly suitable for combination of thermal ablation and chemotherapy for the management of tumors.[167,168] Under NIR irradiation, the unique surface plasmon resonance (SPR) of gold nanoparticles causes a local increase of several degrees above body temperature. This feature is being exploited by AuroShell® formulations already in Phase I for solid tumor hyperthermia. After intravenous injection, the nanoparticles are irradiated with a fiberoptic laser to provide high temperature to the tumor area.[169] Moreover, surface of gold nanoparticles is very suitable for conjugation of drugs, oligonucleotides and peptides and, therefore, gold nanoparticles perform as adequate platforms for DDSs activatable by external or internal stimuli.[170] Light-responsive release can be attained conjugating drugs via photo-cleavable bonds.[171] It can be also achieved exploiting the changes that hyperthermia may induce in the shape of the nanoparticles, which affects to the binding of biomolecules conjugated to their surface,[172] or in the degree of swelling of temperature-responsive polymers.[173] For example, NIR-triggered release was reported for poly(lactic-co-glycolic acid) (PLGA) matrix particles containing doxorubicin and covered with a gold over-layer. Upon irradiation with NIR light, doxorubicin was abruptly released leading to high cancer cell toxicity, while the increase in temperature caused tissue ablation.[174] Similarly, PEGylated polyamidoamine (PAMAM) dendrimers have been developed to integrate gold nanoparticles for photothermal therapy, with high payloads of chemotherapy agents in a hydrophobic inner space.[175]

In a related field, the therapeutic potential of using X-ray radiation as stimulus is being also evaluated. Nanoparticles excitable by X-rays, such as NanoXray products from Nanobiotix in Phase I, aim to amplify the dose of radiation delivered to the tumor without increasing it in healthy tissue.[176] Nanoparticles with a core of hafnium crystals can be directly injected into the tumor (NBTXR3 product) or intravenously for advanced stage tumors that have invaded surroundings lymph nodes (NBTXIV product). Recently developed NBTX-TOPO product is a hafnium-containing gel designed for direct application to the tumor bed, i.e., the cavity left following the surgical removal of a tumor. Hafnium nanoparticles are radio-opaque to X-rays, which enables a precise visualization of the tumor bed, and simultaneously when excited by X-rays the nanoparticles emit very large quantities of electrons, thereby considerably amplifying the dose of energy in the tumor.[177] This product could form part of the standard procedure to prepare a site for post-operative radiotherapy for destroying residual cancer cells and might be in the future combined with drugs.

2.2.6. Electrical field

Equipment developed for transdermal delivery can be easily adapted as a source of precisely regulated electrical stimuli for responsive DDSs. Electrically-sensitive networks can be made of polyelectrolytes with a high density in ionizable groups, similar to those used for preparing pH-responsive systems.[178] These networks can be administered in the form of injectable drug-loaded microparticles or implants for subcutaneous insertion. An electro-conducting patch should be placed on the skin over the implantation site. When the battery is on, the movement of the protons to the cathode causes a change in pH near the electrodes that makes the network to shrink. Then, drug release occurs via squeezing. When the electrical field is switched off, the hydrogel swells again. Thus, tuning the intensity of the electrical field and the time the current is applied, it is possible to regulate drug release rate and duration.

Intrinsically conducting polymers (ICP) may offer advanced performances. ICPs, such as polypyrrole or polyanaline, possess the electrical, electronic, magnetic, and optical properties of a metal, and the versatile of biomedical processing of a polymer. The electrical conductivity is due to the uninterrupted and ordered π-conjugated backbone.[179] ICPs are electrochemically formed as a continuous film on the surface of a working electrode. The formed polymer is in the oxidised form with positive charges distributed along the backbone, which are balanced by anionic dopant molecules. Drugs can be loaded either during film formation or in a subsequent step. When a current is applied, the ICP undergoes reversible redox reactions that alter the electrostatic forces and the swelling degree of the film. Changes in the polymer from positive to neutral state favour the release of anionic drugs, but the uptake of cationic ones. These changes in polymer charge are balanced with the movement of solvated ions in and/or out of the polymer, which in turn leads to swelling or collapse of the film and may enable pulsate drug release.[180] ICPs can be prepared as components of microchips,[181] microneedles,[182] transdermal devices,[183] and microelectrodes.[184] For example, ICPs have been shown useful as coatings of cochlear implants used to provide auditory perception to profoundly deaf individuals, by electrically stimulating spiral ganglion neurons (SGNs) via an electrode array implanted into the scala tympani of the cochlea. Nevertheless, the loss of hair cells, which is responsible for the sensorineural hearing loss and may lead to secondary degeneration and apoptosis of SGNs, is exacerbated by the cochlear implant itself due to the continued electrical discharges. To overcome this problem, the electrode array has been coated with a layer of polypyrrole containing therapeutic neutrophins able to prevent the loss of SGNs. The ICP-coated electrode array can provide activation of central auditory pathways and, at the same time, the electrical stimulation increases the release of the neutrophins to the SGNs.[184] Incorporation of para-toluene sulphonate as ICP dopant notably enhanced both the compatibility of the electrode with primary auditory nerve tissue and its ability to release sufficient neurotrophic protein.[185] Localised release of two neurotrophins (BDNF and NT-3) has been shown to exert synergistic effect on neurite outgrowth (Figure 18).[186]

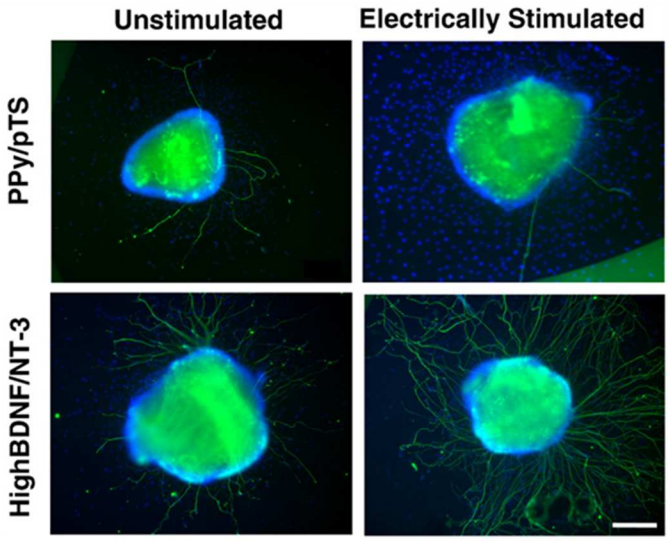

Fig. 18 Images of cochlear neural explants grown on polypyrrole (PPy)/ p-toluene sulphonic acid sodium salt (pTS) coatings with and without neurotrophins. Neurites were visualised by immunocytochemistry with a neurofilament-200 primary antibody and a fluorescent secondary antibody (green). Cell nuclei are labelled with DAPI (blue). Neurotrophins increased numbers of sprouting neuritis, particularly under electrical stimulation because the faster release (8.3 ng/cm^2/day vs. 2.80 ng/cm^2/day) from the coatings. Scale bar is 200 μm.[186] Copyright 2010 Elsevier B.V.

2.2.7. Magnetic field

A variety of magnetic nanoparticles, mostly based on magnetite and maghemite, are already in the market as contrast agents for magnetic resonance imaging (MRI).[187] Intense investigation has been carried out to endow the nanoparticles with physical and chemical stability as well as long circulation time for MRI of cancer. For such a purpose, monodisperse particles of ca. 30 nm behave as very suitable contrast agents, but they tend to aggregate. To overcome this problem, clustering of multiple nanoparticles using adequate dispersing agent leads to high MR contrast effects. The resultant colloidal suspensions are known as ferrofluids.[188] These superparamagnetic nanoparticles can further integrate optically detectable fluorophores for providing morphological and functional information of the tumor,[189] and therapeutic molecules to be delivered at the pathological tissue[190,191] (Figure 19). Differently to other responsive systems that do not allow by themselves tissue guidance, drug carriers bearing magnetic particles can be concentrated into a specific region by applying high-gradient magnetic fields. This enables a high local concentration even though the total injected dose is low. The drug can be released while an alternant magnetic field is on, leading to site-specific treatment.[192]

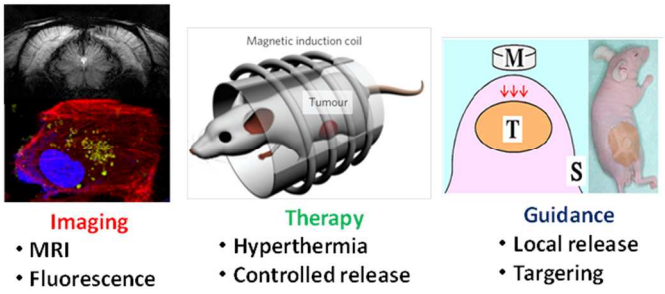

Fig. 19 Magnetic nanoparticles for biomedical applications: imaging and diagnosis (a), therapy (b), and guidance (c).[190] Copyright 2007 Elsevier B.V.

Magnetic nanoparticles rotate when exposed to a large alternating magnetic field, and the heat equivalent to the magnetic loss dissipates into the surrounding tissue.[187] Heating in the 46-56ºC range causes thermoablation by direct cell necrosis, while an increase in temperature up to 41-45 ºC activates signaling pathways that may result in apoptosis.[193] This last modality has been already approved as an adjunct to other treatment modalities, such as surgery, chemotherapy and radiotherapy. Compared to other heating resources (microwaves, radio frequency, ultrasound, and lasers), magnetic hyperthermia can deliver high heat energy into deeply situated tumors without heat loss to the healthy ones. There is already one product in the market (NanoTherm®) and several others are in pre-clinical phase.[25] As an example, the ferrofluid NanoTherm® is formed by water-dispersable nanoparticles of 15 nm in diameter that contain an iron oxide core with an aminosaline coating.[194] The nanoparticles can be dispensed with a syringe and, due to their core-shell structure, remain in the tumor tissue as a stable depot. Using an adequate alternating magnetic field applicator and a therapy planning software, it is possible to estimate/monitor the temperature distribution inside the tumor, to regulate the necessary magnetic field strength, and to repeat the heating treatment several times until complete eradication of the tumor. This product has got approval for treatment of glioblastoma, and it is in Phase I for prostate and pancreatic carcinoma.[195]

The gain in knowledge about the handling of superparamagnetic nanoparticles in patients is paving the way to the clinical evaluation of some of the numerous theranostic prototypes described in literature.[196] Drug-loaded superparamagnetic nanoparticles have already demonstrated synergic therapeutic effects. A variety of designs have been implemented to prepare drug-loaded superparamagnetic DDSs.[192] Core-shell nanoparticles consist of a core of magnetic iron oxide and a shell of a polymer (dextran, PLGA, or PVA) and/or a nonpolymer (silica or metal), with the drug covalently attached to the surface or entrapped or adsorbed within the pores of the magnetic carrier.[197,198] Nanocapsules can be prepared encapsulating the drug and the superparamagnetic particles into lipid bilayers or mesoporous silica nanoparticles, which have to be endowed with stealth features.[199-201] A small increase in temperature can be exploited to trigger reversible pulsate drug squeezing from temperature-responsive networks, while a strong increase may lead to carrier disintegration and subsequent rapid drug release.[135]

2.2.8. Ultrasound

Ultrasound can be applied to the body, using common physiotherapy equipment, to facilitate the penetration of nanostructures into specific regions and to trigger drug release, while efficiently monitor the therapy.[202,203] Compared to other external stimuli, low-frequency ultrasound can penetrate centimeters into the body with very low scattering. Cavitation (i.e., oscillations accompanied by expansion and contraction) can cause disassembly of polymeric micelles and polymersomes (Figure 20),[204,205] and disruption of networks or liposomes containing gas-filled microbubbles.[206] There is already a list of commercially available microbubble-based contrast agents clinically used in ultrasound image and that can be easily adapted to perform as ultrasound-responsive DDSs.[207] These systems enable visualization of drug-loaded microbubbles with low acoustic pressures, protection of biopharmaceuticals such as proteins and nucleic acids against premature degradation, targeted delivery to the tissue exposed to ultrasound, and enhanced uptake by cells via sonoporation. As a recent example, ultrasound-triggered release of acidic fibroblast growth factor from heparin-modified microbubbles has been proved useful for treatment of ischemic heart tissue. This formulation markedly stimulated neogenesis in myocardial vessels, resulting in significant improvement of both regional and global contractile function in the myocardium.[208]

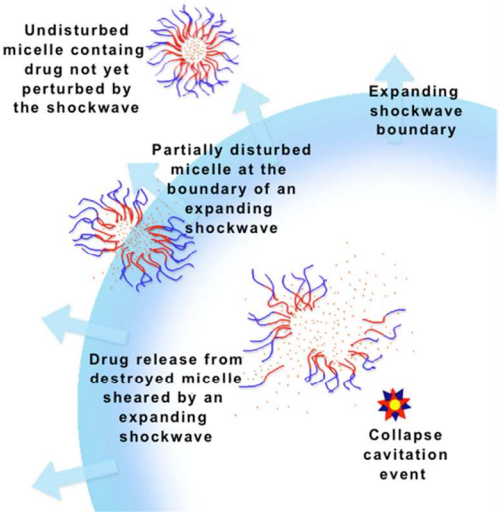

Fig. 20. Expanding shockwave from a collapse cavitation event causes mechanical disruption of micelles. The compressional shock wave is thought to transiently shear open micelles, thus releasing their contents.[205] Copyright 2013 Royal Society of Chemistry.

The large size of microbubbles (1-10 μm) restricts their application to cardiovascular targets and tumor endothelium. Ultrasound-responsive nanocarriers (mainly, polymeric micelles) and nanobubble-containing liposomes enable deeper tissue penetration.[209,210] Ultrasound-triggered release from intravenously injected polymeric micelles has been tested for a pulsatile drug delivery in tumors. The ultrasounds are applied when maximum micelle accumulation in the tumor is reached; namely between 4 and 8 hours for doxorubicin-loaded Pluronic P-105 polymeric micelles or PEO-diacylphospholipid mixed micelles. The amount of drug released can be modulated tuning the frequency, the power density, the pulse length and the inter-pulse intervals.[211,212] Drug release from the micelles is reversible; i.e., during inter-pulse intervals exceeding 0.5 s, the drug can be completely re-encapsulated into the restored micelles. Ultrasound has been shown also suitable for site-specific release of (tPA) for localized thrombolysis, as an alternative approach to that described in section 2.2.3. Encapsulation of tPA in gelatin-PEG nanoparticles suppressed the enzymatic activity to 45% that of free tPA. After intravenous injection, exposition of the nanoparticles to ultrasound led to complete recovery of tPA activity and, as a consequence, to full blood recanalization.[213]

While still the effects of ultrasound on cell apoptosis and genotoxicity should be elucidated,[214] other barriers to the clinical use of ultrasound-responsive DDSs and theranostic systems are being solved.[207,215] For example, attenuation of ultrasound by bone can be overcome combining modern imagining methods, which makes it possible to deliver drugs through intact skull towards target regions in the brain.[216]

2.2.9. Autonomous responsiveness

Advanced stimuli-responsive hydrogels can be prepared in such way that they combine in a single entity the responsive network and the stimulus. The stimulus is periodically generated inside the network enabling rhythmic reversible phase transition. The autonomously responsive networks may be useful to mimic the pulsate circadian levels of certain hormones.[217]

Chemical oscillating reactions in which large changes in pH occurs are mainly based on the Belousov-Zhabotinsky reaction that involves the oxidation and reduction of salts such as permanganates, iodates, sulfates, chlorates or bromates.[218] Poly(2-acrylamido-2-methyl-1-propane-sulfonic acid), as well as other sulfonic acid polymers, can also undergo chemical oscillating reactions.[219] Similar reactions have been shown useful for inducing the self-oscillation of temperature-responsive networks.[220] To be useful in drug delivery, the hydrogel has to be designed in such a way that the time required for the drug to diffuse out is shorter than the oscillation period.

Networks made of polyelectrolytes of different charge, or that combine a polyelectrolyte and a temperature-responsive polymer and/or a grafted enzyme can undergo autonomous oscillations due to positive and negative feedbacks that forbid the system from reaching a stationary state.[221] For example, a pH oscillator that modulates the ionization state of a model drug, benzoic acid, has been shown to regulate the permeation of the drug through a lipophilic membrane. The swelling of a poly(N-isopropylacrylamide-co-methacrylic acid) hydrogel membrane was coupled to an enzymatic reaction (involving glucose oxidase, catalase, and gluconolactonase), in such a way that the hydrogel controlled the access of the substrate to the enzyme, and the product of the enzyme reaction controlled the hydrogel swelling. This system has been shown useful for delivery of gonadotropin releasing hormone (GnRH) in rhythmic pulses, with a periodicity similar to that observed in sexually mature adult humans.

3. Smart Drug-eluting Medical Devices

An additional field of application of smart materials is that of the drug-eluting medical devices, which is a particular group of combo products known as drug-enhanced devices.[222] Integration in a single entity of the drug and the medical device

can provide synergic outcomes: i) the drug can regulate the response of the body to the device, minimizing the foreign-body reactions and biofouling, and can also prevent the adhesion of microorganisms, preventing biofilm formation; and ii) the device may serve as platform for the release of the drug to tissues hardly accessible otherwise, with improved therapeutic efficacy compared to systemic release. Consequently, device-related complications that are refractory to conventional systemic drug administration can be successfully overcome through the local release, whereas the efficacy and the safety of the treatment, as well as its cost-effectiveness, are improved. A well-known example of drug-eluting medical devices is that of already commercially available medicated stents.[223]

The approaches for preparing drug-eluting medical devices can be categorized in two large groups: i) those that enable the incorporation of the drug in the bulk of the medical device, during its fabrication (compounding) or in a latter step (presoaking); and ii) those that incorporate the drug in the outer layers of the device by means of coating procedures, covalent binding or weak chemical interactions.[16,224,225] The second approach avoids structural changes caused by the compounding with the drug, and provides greater payloads than pre-soaking. Surface functionalization of medical devices with stimuli-responsive brushes, networks or layers suitable for hosting and release drugs during the time the device is inserted/implanted in the body is gaining raising attention.[226,227]

Usefulness of grafting of responsive hydrogels to solid substrates has been already demonstrated in the field of cell culture and artificial tissues.[228,229] Directly related approach is the use of silicon nanowires with dually pH- and glucose-responsive surface for reversible capture of cancer cells, without altering their viability.[230] Interestingly, device surfaces can be decorated with switchable capability to attract, kill and release bacteria by means of nanopatterned, thermoresponsive PNIPAAm brushes and biocidal quaternary ammonium salts (Figure 21). Above the LCST (e.g. body temperature), bacteria can adhere to the surface and become exposed to the biocidal agent. Below the LCST (e.g. cleaning temperature), swelling of PNIPAAm brushes promote the release of dead bacteria.[231]

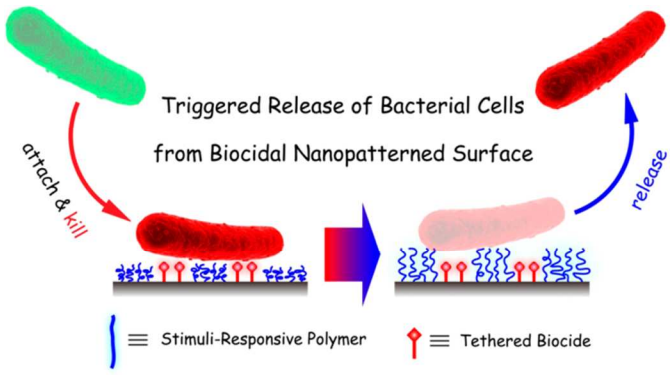

Fig. 21 Nanopatterned surfaces combining PNIPAAm chains and biocidal quaternary ammonium salt. Above the LCST, the collapsed and hydrophobic PNIPAAm chains permit attachment of *Escherichia coli K12*, while simultaneously exposing the biocidal agent. Upon a reduction in the temperature below the LCST, the hydration and swelling of PNIPAAm chains release the dead bacteria upon mild shearing.[231] Copyright 2013 American Chemical Society.

As an alternative to the chemical grafting, layer-by-layer coating of solid surfaces may enable temporal protection of the device, being the layer components degradable or eliminable.[52] For example, this technique has been shown suitable for the loading of enzyme dispersin B, which resulted useful to inhibit biofilm formation by two clinical strains of *Staphylococcus epidermidis*.[232]

Regarding drug release, several architectures have been tested.[226] Grafting of block copolymers leads to an inner layer that acts as a reservoir for guest molecules and a stimuli-responsive layer on top that can close or open the compartment where the drug is hosted. Triple-responsive temperature, pH and light wavelength release was observed for devices grafted with PNIPAAm and poly(4,5-dimethoxy-2-nitrobenzyl methacrylate) (Figure 22).[233]

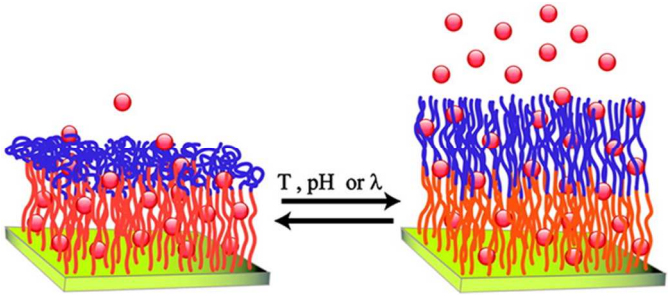

Fig. 22 Surface-grafted diblock copolymer brushes with a top layer able to switch between collapsed and extended chains in response to temperature, pH or light.[233] Copyright 2011 American Chemical Society.

Surface grafting of temperature- and pH-responsive polymers onto polypropylene (PP) and polyethylene (PE) has been shown suitable for preparing vancomycin-eluting devices.[234-236] PP and PE surface was modified with a hydrogel layer containing chemical groups with affinity for those of the drug and with switchable control of drug diffusion in (loading) and out (release) the hydrogel. Interpenetrating networks of PNIPAAm and poly(acrylic acid) were swollen at temperature below LCST and/or at neutral pH. Swelling facilitated the contact of the drug with the acrylic acid groups. At 37ºC the grafted network was capable of controlling drug release rate by the concomitance of the affinity of poly(acrylic acid) and the hindering of the diffusion through the collapsed PNIPAAm mesh. Microbiological tests demonstrated that the vancomycin-loaded devices have a small likelihood of biofilm formation by methicillin-resistant *Staphylococcus aureus*.[234] Grafting of poly(acrylic acid) networks from Prolene® sutures has been proved useful for controlled release of vancomycin.[237] On the other hand, fungi-responsive release of antifungal agents has been recently demonstrated for devices grafted with ergosterol, which can mimic the interactions of the fungi cell membrane with the antifungal agents.[238]

Conclusions and a view to the future

Pharmaceutical and bio-related industries are undergoing a notable transformation. Last decades of 20st century witnessed the success of blockbusters based on small synthetic drugs useful for treatment of pathologies affecting large groups of population, mostly in the form of sustained release systems. However, the everyday more strict requirements of quality and safety imposed by regulatory agencies, the restrictions to the health-related expenses applied by most governments and private insurance companies, the raising of the generic market, and the increasing education of people regarding medicines (internet has popularized therapeutic concepts and maintains people alert about novel discoveries) are driven the companies to focus R&D efforts on more specialized treatments for specific patients groups with unmet needs.[239] In this new context, target nanocarriers, smart DDSs and theranostic systems appear as excellent tools to pursue personalized medicines.[240] In particular, stimuli-responsiveness may help to overcome some barriers that current passive and active targeting strategies have to face up to (mostly related to heterogeneity of pathophysiological properties of target tissues).

The literature on smart DDSs is exponentially increasing and the variety of stimuli-responsive materials already described is amazing. The information about the performance in vivo, although still limited, highlights the role smart DDSs are called to play in clinics (as summarized in Table 1). However, extrapolation of the behaviour from animal models to humans is not easy. Most studies focus on local administration to small animals, which have significantly different pathologies, physiologies, anatomies, immune systems and host responses to the materials compared to humans.[42] The possibilities of curing cancer in rodents using smart DDSs have been already demonstrated in hundreds of papers. By contrast, just a few of those systems have entered in clinical phases yet.

One can point out several reasons of so slow incorporation of nanocarriers, in particular stimuli-responsive DDSs, to the therapeutics. A relevant one is the difficulty to follow one key principle of the pharmaceutical industry: quality control. Most materials are synthesized under poorly reproducible conditions and the methods to prepare the smart DDSs are not standardized. In this sense, regulatory agencies are still working on guidances for preparation and evaluation of nanosized materials. Moreover, the developed combinations of polymers, lipids or silica are considered as "new excipients" and thus cytotoxicity, genotoxicity, antigenicity and clearance are major issues to be elucidated. Differently from traditional dosage forms, bioavailability of drugs incorporated to smart DDSs has to be measured in the target tissue or cell; therefore, there is a need of developing suitable analytical techniques to do that. Another important point is the cost-effective evaluation of smart products.[33] Development of complex responsive products may be extraordinarily expensive. Thus, the increase in health *versus* the increase in costs compared with already established medical options may be not so evident.

Despite the relevant concerns mentioned above, development of the first commercially available smart DDSs and advances in knowledge about in vivo measurement of stimuli and transduction are notable steps forward towards optimized design, easily translatable production and evaluation in humans of more efficient formulations. The availability of tools and nanoprobes that can quantify in situ glucose concentration,[100] temperature in tumor and its increase when an external source of heating is applied,[241] hypoxic/acid regions in live tissues and cells,[242] or distribution of drug nanocarriers inside the target tissue (for example, using C dots[165,166] or related nanostructures[243]) is very valuable. Moreover, smart DDSs could enable the treatment of movable pathological cells, such as metastatic cancer cells or infected erythrocytes (as recently observed for *Plasmodium*-infected red blood cells)[244] without the use of expensive recognition elements, which opens novel strategies of treatment.

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Notes and references

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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Table 1. Some medicines based on stimuli-responsive components that are in clinical trials or already commercialized. Biosensors useful for biomarkers control and some formulations that have shown outstanding results in vivo are also included.

Product	Stimuli	Structure	Clinical status	Ref.
Opaxio™	Tumor enzyme	Paclitaxel poligumex	Approved orphan drug for glioblastoma multiforme	21
Trastuzumab-DM1	GSH concentration	Antibody-drug conjugate	Phase II/III breast cancer	78
Maytansine	GSH concentration	Antibody-drug conjugate	Phase II/III multiple myeloma	79
Nanocapsule prototype	Dually responsive to GSH and ROS	Camptothecin-based topoisomerase I inhibitor conjugated to nanocapsules	In vivo tests with breast tumor xenograft models and autochthonous colon cancer models	89, 90
Implant prototype for antinflammatory release	OH· radicals	Lipoidal-chitosan-poly(ε-caprolactone) nanoparticles coated with hyaluronic acid, alginate and poly(acrylic acid)	Intraocular tests in rabbit model of uveitis	91
Nanocarrier prototype for plasminogen activator (tPA)	Thrombin at the clot	tPA camouflaged with human serum albumin via a thrombin-cleavable peptide, and coated with a homing peptide that binds with GPIIb/IIIa expressed on activated platelets	Rat thrombosis model	97
Glucose biosensors Guardian Real-Time®, Seven®, Dexcom® G4™ Platinum,or Enlite™, FreeStyle Navigator®	Glucose concentration	Glucose oxidase enzyme coupled to other enzymes and transducers for continuous monitoring of glucose level and regulation of insulin release from pumps.	Approved, commercially available implantable biosensors	99,100
Nanoparticles as traps of bee venom	Melittin	Imprinted nanocarrier that selectively captures melittin in the bloodstream	Mice models	122,123
ThermoDox®	Temperature (external source)	DPPC-based liposomes for tumor-specific release of doxorubicin	Phase III in liver cancer, Phase II in chest wall recurrence of cancer, colorectal liver metastases, lung cancer and bone metastases	22, 149,150
Visudyne®	UV light	NonPEGylated liposome formulation of photosensitizer verteporfin	Approved, commercially available injectable solution	158
Rotaxane-functionalized mesoporous silica nanoparticles	UV light	Nanoparticles with pores capped with chains of triazole/ethylene glycol and an azobenzene unit that interact with α-cyclodextrin	Wild-type zebrafish larvae	164
Cornell dots (C dots)	Near infrared radiation	Fluorescent core−shell silica-based nanoparticles	Approved for human stage I molecular imaging of cancer	165,166
AuroShell®	Near infrared radiation	Gold nanoparticles for solid tumor hyperthermia	Phase I solid tumors	169
NanoXray products	X-rays	Hafnium crystals that amplify the dose of radiation delivered to the tumor.	Phase I	176,177
Cochlear implants coated with ICPs	Electrical stimulus	Coatings of intrinsically conducting polymers that switch neurotrophins release on and off	Animal models	184-186
NanoTherm®	Magnetic field	Water-dispersable iron oxide nanoparticles coated with aminosaline	Approved for thermoablation of glioblastoma; Phase I prostate and pancreatic carcinoma	194,195
Nanocarrier prototype for plasminogen activator (tPA)	Ultrasound	tPA encapsulated in gelatin-PEG nanoparticles for localized thrombolysis	Rat thrombosis model	213