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IN FOCUS: NANOMEDICINE - REVIEW

Stimuli-Responsive Polymers and Their Applications in Nanomedicine

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Abstract This review focuses on smart nano-materials built of stimuli-responsive (SR) polymers and will discuss their numerous applications in the biomedical field. The authors will first provide an overview of different stimuli and their corresponding, responsive polymers. By introducing myriad functionalities, SR polymers present a wide range of possibilities in the design of stimuli-responsive devices, making use of virtually all types of polymer constructs, from self-assembled structures (micelles, vesicles) to surfaces (polymer brushes, films) as described in the second section of the review. In the last section of this review the authors report on some of the most promising applications of stimuli-responsive polymers in nanomedicine. In particular, we will discuss applications pertaining to diagnosis, where SR polymers are used to construct sensors capable of selective recognition and quantification of analytes and physical variables, as well as imaging devices. We will also highlight some examples of responsive systems used for therapeutic applications, including smart drug delivery systems (micelles, vesicles, dendrimers …) and surfaces for regenerative medicine.

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1 Introduction

Challenges confronted by medicine today include the increasing demand for sensitive, efficient systems and approaches that will improve responses to pathology. In this respect, for detection purposes, there is a need for new agents that will simultaneously increase sensitivity while their concentrations in the body decrease to avoid accumulation and side-effects. Such agents are intended to efficiently detect pathological conditions in their early stages or distinguish slight changes in areas where surgery has been done, serving to enhance prognoses, especially in complex diseases such as cancer, HIV, and degenerative diseases. The necessity of decreasing doses while increasing efficacy is essential for therapeutic approaches, while decreased side effects will improve a patient's condition, especially in chronic disease or diseases requiring the administration of toxic compounds, for example cancer or HIV. The design of new systems and approaches must meet challenges associated with administration in the body: (i) a simple route of administration, (ii) effective delivery to the desired biological compartment, (iii) response adapted to the pathological event, either rapid or slow, depending on the bio-specificity, and (iv) the use of non-toxic, biocompatible and biodegradable systems. Current know-how in nanotechnology is making possible new ways to fight a number of diseases. As the development of the fast growing field known as nanomedicine employs nanostructures and nanodevices to diagnose, treat, and prevent diseases [1]. In this respect, nanoscience offers novel systems and methods for medical use by providing carriers such as particles, micelles, dendrimers, and vesicles to transport active compounds (drugs, contrast agents, proteins, DNA), and "active" surfaces adapted to biosensing, regeneration and wound healing, An efficient way to improve these

systems is to make them stimuli-responsive. A smart response to external or internal stimuli allows: (i) better localization of the system in the desired biological compartment, (ii) controlled release of payload at the location of the pathological event, and (iii) rapidly addressing/imaging the pathological event. In particular, polymers have proven themselves clever options in developing stimuli-responsive systems because their chemistry permits modulating the properties by including responsiveness via sensitive chemical moieties. A large variety of polymers/copolymers has been synthesized to response to physical stimuli (temperature, pH, light), chemical stimuli (various "signaling" molecules), or biological stimuli (enzymes). Stimuli-responsive polymers undergo dramatic and abrupt physical and chemical changes in response to external stimuli [2]. They are also termed 'smart-' [3, 4], 'intelligent-' [5], or 'environmentally sensitive' polymers [6]. One important feature of this type of material is reversibility, i.e. the ability of the polymer to return to its initial state upon application of a counter-trigger. In nature, biopolymers such as proteins and nucleic acids are all basic stimuli-responsive components of living organic systems and often remain stable over wide ranges of external variables but undergo drastic conformational changes abruptly at given critical points [3, 7]. These 'natural' stimuli-responsive polymers have led to the development of numerous synthetic polymers that have been designed to mimic their adaptive behaviours.

By incorporating functional groups that are amenable to a change in character (e.g. charge, polarity and solvency) along a polymer backbone, the resulting relative changes in chemical structure will be amplified synergistically, leading to dramatic transformations in macroscopic material properties. Typically, the 'response' of a polymer in solution alters its individual chain dimensions/size, secondary structure, solubility, or the degree of intermolecular association [8]. In most cases, the present or destruction of secondary forces (hydrogen bonding, hydrophobic effects, electrostatic interactions, etc.), simple reactions (e.g., acid–base reactions) of moieties linked to the polymer backbone, and/or osmotic pressure differences are responsible for this response. Another type of 'response' is due to dramatic alterations in the polymeric structure, such as degradation of polymers upon the application of a specific stimulus by bond breakage in the polymer backbone or at pendant cross-linking groups [8].

Stimuli-responsive systems containing polymers can be designed either with a responsive polymer, or by combining a polymer with a responsive compound, the polymer serving only as a template/carrier for that compound. Here we will focus only on the stimuli-responsive systems involving polymers as smart components, i.e. their properties and structures are changing in response to a specific stimulus. In addition, we are interested to mainly present supramolecular polymers assemblies in solution because they are extensively used both in therapeutic and in detection approaches. Note that the huge chemical diversity of polymers proposed for their stimuli-responsiveness (we will describe in the first part of our review) is dramatically reduced when medical applications are intended due to the biological constraints, we mentioned above. In this particular field it is extremely important to understand the parameters and mechanisms related to the distribution and transport of the nanosystems in the body. Controlling these parameters is necessary to answer the various concerns that will arise regarding environmental risk and side effects associated with the use of nanostructures in the body [9].

In this respect in the last part of the review we will focus on systems that are already used in medical applications, or have possible medical applications.

2 Stimuli-Responsive Polymers

The strategy underlying polymer-containing responsive systems is a dramatic physicochemical change caused by stimuli. At the macromolecular level, polymer chains can be altered in different ways, including changes in hydrophilic-to-hydrophobic balance, conformation, solubility, degradation, and bond cleavage, and these, in turn, will cause detectable behavioral changes to self-assembled structures [10]. Many designs that vary the location of responsive moieties or functional groups are possible. Locations include, but are not limited to: side chains on one of the blocks, chain end-groups, or junctions between blocks. The response may be reversible or not, depending on the strategy employed.

Stimuli are commonly classified in three categories: physical, chemical, or biological (Fig. 1) [11, 12]. Physical stimuli (light, temperature, ultrasound, magnetic, mechanical, electrical) usually modify chain dynamics, i.e. the energy level of the polymer/solvent system, while chemical stimuli (solvent, ionic strength, electrochemical, pH) modulate molecular interactions, whether between polymer and solvent molecules, or between polymer chains [13]. Biological stimuli (enzymes, receptors) relate to the actual functioning of molecules: enzymatic reactions, receptor recognition of molecules [14]. In addition, there are dual stimuli-responsive polymers that simultaneously respond to more than one stimulus.

2.1 Physically Dependent Stimuli

Physically dependent stimuli mainly include: temperature, electric field, light, ultrasound, magnetic fields and

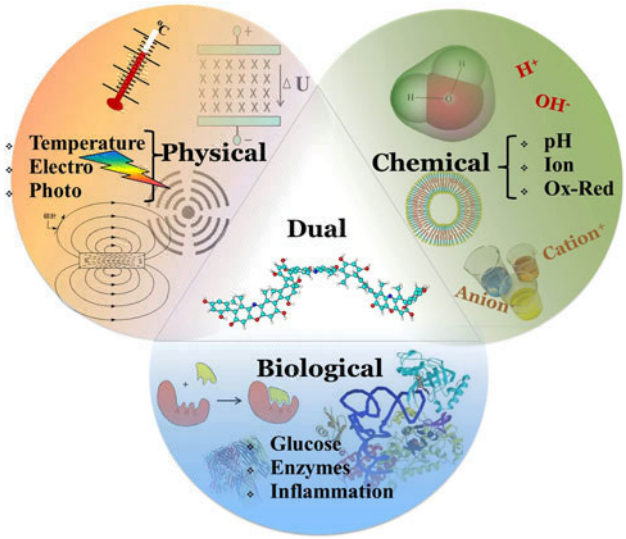

Fig. 1 Classification of stimuli of stimuli-responsive polymers

mechanical deformation. However, in this review we focus only on the stimuli-responsiveness of polymer/copolymer systems, hence, the physical stimuli reported as actively changing their properties/supramolecular structures are temperature, light, and electric field. We mention that magnetic fields and ultrasound have been used only for compounds that have been entrapped/encapsulated in polymer assemblies, and therefore we will not include them here.

2.1.1 Temperature Responsive Polymers

Temperature-responsive polymers have attracted great attention in bioengineering and biotechnology applications, because certain diseases manifest temperature changes [15]. Normally, these copolymers are characterized by a critical solution temperature around which the hydrophobic and hydrophilic interactions between the polymeric chains and the aqueous media abruptly change within a small temperature range. This induces the disruption of intra- and intermolecular electrostatic and hydrophobic interactions and results in chain collapse or expansion (a volume phase transition). Typically, these polymer solutions possess an upper critical solution temperature (UCST) above which one polymer phase exists, and below which a phase separation appears. Alternatively, polymer solutions that appear as monophasic below a specific temperature and biphasic above it generally possess a so-called lower critical solution temperature (LCST). Depending on the mechanism and chemistry of the groups, various temperature-responsive polymers have been reported: poly(*N*-alkyl substituted acrylamides), e.g. poly(*N*-isopropylacrylamide) (PNiPAAm) [16, 17], poly (*N*-vinylalkylamides), e.g. poly(*N*-vinylcaprolactam) (PNVC) [18], and copolymers such as poly (L-lactic acid)-poly(ethylene glycol)-poly(L-lactic acid) (PLLA-PEG-PLLA) triblock copolymers [19], and poly (ethylene oxide)-poly(propylene oxide)-poly (ethylene oxide) (PEO–PPO–PEO) copolymers [20].

2.1.2 Electro-Responsive Polymers

Electrical and electrochemical stimuli are widely used in research and applications, due to their advantages of precise control via the magnitude of the current, the duration of an electrical pulse or the interval between pulses [21, 22]. Typical electrically responsive polymers are conducting polymers, as for example polythiophene (PT) or sulphonated-polystyrene (PSS), which can show swelling, shrinking or bending in response to an external field [23, 24]. There are different effects upon electrochemical stimulation: (a) an influx of counter ions and solvent molecules causes an increase in osmotic pressure in the polymer, resulting in a volumetric expansion, (b) control of the loading/adsorption of polyelectrolyte on to oppositely charged porous materials, (c) formation and swelling of redox-active polyelectrolyte multilayers. For example, when an electrochemical stimulus is applied to multilayer polyacrylamide films, the combined effects of H^+ ions migrating to the region of the cathode and the electrostatic attraction between the anode surface and the negatively charged acrylic acid groups lead to shrinking of the film on the anode side [25, 26].

2.1.3 Photo-Responsive Polymers

Because light can be applied instantaneously and under specific conditions with high accuracy, it renders light-responsive polymers highly advantageous for applications [6]. The light can be directly used at the polymer surface or can be delivered to distant locations using optical fibers. Ideally, the wavelength of the laser is tuned to the so-called biologically 'friendly' window [27], the near-infrared part of the spectrum, which is less harmful and has deeper penetration in tissues than visible light. In this case, the light is both minimally absorbed by cells/tissue and maximally so by the polymers. Most photo-responsive polymers contain light-sensitive chromophores such as azobenzene groups [28, 29], spiropyran groups [30, 31], or nitrobenzyl groups [32, 33]. A variety of azobenzene or spiropyran-containing photo-responsive polymers, as for example PAA [34, 35], PHPMAm [36, 37], and PNIPAM [38, 39], have been reported.

2.2 Chemically-Dependent Stimuli

Chemically-dependent stimuli comprise pH, ionic strength, redox and solvent.

2.2.1 pH-Responsive Polymers

pH is an important environmental parameter for biomedical applications, because pH changes occur in many specific or pathological compartments. For example, there is an obvious change in pH along the gastrointestinal tract from the stomach (pH = 1–3) to the intestine (pH = 5–8), chronic wounds have pH values between 7.4 and 5.4 [40], and tumour tissue is acidic extracellularly [41, 42]. Therefore, unlike temperature changes, this property can be exploited for a direct response at a certain tissue or in a cellular compartment. The key element for pH responsive polymers is the presence of ionisable, weak acidic or basic moieties that attach to a hydrophobic backbone, such as polyelectrolytes [6, 10, 43]. Upon ionization, the electrostatic repulsions of the generated charges (anions or cations) cause a dramatic extension of coiled chains. The ionization of the pendant acidic or basic groups on polyelectrolytes can be partial, due to the electrostatic effect from other adjacent ionized groups [44].

Another typical pH responsive polymer exhibits protonation/deprotonation events by distributing the charge over the ionisable groups of the molecule, such as carboxyl or amino groups [45]. pH induces a phase transition in pH responsive polymers very abruptly. Usually, the phase switches within 0.2–0.3 U of pH [46]. pH responsive polymers typically include chitosan [47], albumin [48], gelatin [49], poly(acrylic acid) (PAAc)/chitosan IPN [50], poly(methacrylic acid-*g*-ethylene glycol) [P(MAA-*g*-EG)] [51, 52], poly(ethylene imine) (PEI) [53], poly(*N*,*N*-diakylamino ethylmethacrylates) (PDAAEMA), and poly(lysine) (PL) [54, 55].

2.2.2 Ion-Responsive Polymers

The responsiveness to ionic strength is a typical property of polymers containing ionisable groups. These polymer systems exhibit unusual rheological behaviour as a result of the attractive Coulombic interactions between oppositely charged species, which may render the polymer insoluble in deionized water but soluble in the presence of a critical concentration of added electrolytes where the attractive charge/charge interactions are shielded [56–58]. Therefore, changes in ionic strength cause changes in the length of the polymer chains, the polymer solubility and the fluorescence quenching kinetics of chromophores bound to electrolytes [57, 59, 60].

2.2.3 Redox-Responsive Polymers

Polymers containing labile groups present an beneficial opportunity to develop redox-responsive biodegradable or bioerodible systems. Acid labile moieties inside polyanhydrides [61, 62], poly(lactic/glycolic acid) (PLGA) [63], and poly(β-amino esters) (PbAEs) [64] induce redox responsiveness. Disulfide groups have also been used to induce redox responsiveness, because they are unstable in a reducing environment, being cleaved in favour of corresponding thiol groups [65, 66]. Polymers with disulfide cross-links degrade when exposed to cysteine or glutathione, which are reductive amino-acid based molecules [67]. Another typical redox responsive polymer is poly(NiPAAm-co-Ru(bpy)$_3$), which can generate a chemical wave by the periodic redox change of Ru(bpy)$_3$ into an oxidized state of lighter colour [68]. This redox reaction alters the hydrophobic and the hydrophilic properties of the polymer chains and results in swelling and deswelling of the polymer.

2.3 Biologically Dependent Stimuli

Biologically dependent stimuli typically involve analytes and biomacromolecules such as glucose, glutathione, enzymes, receptors, and over-produced metabolites in inflammation.

2.3.1 Glucose Responsive Polymers

Precisely engineered glucose sensitive polymers have huge potential in the quest to generate, for example, self-regulated modes of insulin delivery [11, 69]. For glucose responsive polymers, glucose oxidase (GOx) is conjugated to a smart, pH-sensitive polymer. GOx oxidizes glucose to gluconic acid, which causes a pH change in the environment [6]. The pH sensitive polymer then exhibits a volume transition in response to the decreased pH [69]. In this way, drastic changes in the polymer conformation are regulated by the body's glucose level, which, in turn, significantly affects enzyme activity and substrate access.

2.3.2 Enzyme-Responsive Polymers

In nature, bacteria located mainly in the colon produce special enzymes, including reductive enzymes (e.g. azoreductase) or hydrolytic enzymes (e.g. glycosidases) which are capable of degrading various types of polysaccharides, such as pectin, chitosan, amylase/amylopectin, cyclodextrin and dextrin [70–72]. In most enzyme-responsive polymer systems, enzymes are used to destroy the polymer or its assemblies. The biggest advantage of enzyme-responsive polymers is that they do not require an external trigger for their decomposition, exhibit high selectivity, and work under mild conditions. For example, polymer systems based on alginate/chitosan or DEXS/chitosan

microcapsules are responsive to chitosanase [73]. And azoaromatic bonds are sensitive to azoreductase [74]. In this respect, they have great potential for in vivo biological applications. However, the main disadvantage is the difficulty of establishing a precise initial response time.

2.3.3 *Inflammation-Responsive Polymers*

The inflammatory process is initiated by T- and B-lymphocytes, but amplified and perpetuated by polymorphonuclear (PMN) leukocytes and macrophages. Various chemical mediators in the process, including arachidonic acid metabolites, proteolytic enzymes and oxygen metabolites, can cause tissue damage. For inflammation-responsive systems, the reactive oxygen metabolites (oxygen free radicals) released by PMNs and macrophages during the initial phase of inflammation are the stimuli [75]. Such chemical mediators have been successfully used as stimuli for responsive drug delivery. For example, in vivo implantation experiments revealed that hyaluronic acid (HA) cross-linked with glycidylether can degrade in response to inflammation [76].

2.4 Dual-Stimuli

For biomedical applications, a step forward is realized if the smart materials respond simultaneously to more than one stimulus. Therefore, increasing the efficacy of drug therapies may require polymeric materials, which are responsive to several kinds of stimuli. These will support the diagnosis of patients by monitoring several physiological changes at once. The dual-stimuli responsive approach is ideally suited for theragnostic (a combination of diagnostics and therapy) because some functionalities can provide on-site feedback and diagnostics, while others could initiate curing and therapy. Availability of various physical, chemical and biological stimuli is indispensable for multiple response functions. Therefore, multi-stimuli-responsive polymers, especially dual temperature- and pH-responsive systems, are attracting increasing attention recently for their advantages in biotechnological and biomedical applications. For example, a dual-stimuli-responsive delivery system, using both pH and glutathione-responsive polymeric modules, was developed to therapeutically deliver medicinal molecules [77]. It was possible to tune the release kinetics by systematically varying the composition of the pH-sensitive hydrophobic moiety (butyl acrylate), by modifying the glutathione-responsive moiety (pyridyl disulfide acrylate), or by modifying both of them.

Table 1 summarizes stimuli responsive polymers grouped by stimulus–response, and contain information about the synthesis method and application.

3 Stimuli Responsive Polymers with Different Physical Forms

3.1 Dendrimers

Dendrimers are macromolecules characterized by highly branched structures. Their properties attract attention for their applicability as delivery vessels, carriers of imaging agents, and therapeutically active compounds [78–80].

3.1.1 *Temperature Responsive Dendrimers*

Various examples of temperature responsive dendrimer systems (with differing architecture and chemical composition) used to encapsulate and release drugs are described in literature: star-shaped poly(ε-caprolactone)-*b*-poly(2-(dimethylamino)ethyl methacrylate) (HPs-Star-PCL-*b*-PDMAEMA) [81], core–shell dendritic poly(ether-amide) (DPEA) modified with carboxyl end-capped linear poly (*N*-isopropylacrylamide) (PNIPAAm–COOH) and carboxyl end-capped methoxy polyethylene glycol (PEG–COOH) [82]. It was shown that the temperature sensitivity of dendrimers can depend on their generation and molecular mass [83]. Dendrimers based on poly(aminoamide) (PAMAM) or poly(propyleneimine) (PPI) were obtained by introducing isobutyramide (IBAM) groups onto the chain ends and, in the case of PAMAM dendrimers, the thermoresponse was further modulated by introducing various peripheral alkylamide groups [84].

3.1.2 *Photo-Responsive Dendrimers*

Photo-responsive carbosilane dendrimers containing 4-phenylazobenzonitrile units at each terminal end were synthesised for potential applications in conversion of photo-energy into dynamic energy or in drug delivery systems [85]. The molecular size of a dendrimer with azobenzene derivatives depends on the photo- and heat-isomerization abilities of the azobenzene unit. The photo-response can also be obtained by introducing *O*-nitrobenzyl groups to the surface of hyperbranched polyglycerols (HPGs) for drug release [86]. The presence of a hexa(ethylene glycol) outer-shell instead of the hexene increased the stability of the formed host–guest complexes but resulted in lower guest release. The stability of the host–guest complexes depended on the counterion of the guest molecules. This system offers the opportunity to tune the nanocapsules to control guest binding and release.

3.1.3 *pH- and Ion-Responsive Dendrimers*

PAMAM (polyamidoamine) and PPI (polypropylene imine) dendrimers are known to be ion- or pH- responsive

Table 1 Summarize on stimuli responsive polymers grouped by stimulus–response, and contain information about the synthesis method and application

Type of stimulus–response	Stimulus-responsive polymers	Synthesis method	Application
Physically dependent stimuli			
Temperature-responsive polymers	PNiPAAm [15, 16]	Living radical polymerization	Water soluble polymer sensor, Tissue adhesion prevention material
	PNVC [17]	Living radical polymerization	Thermosensitive hydrogel at any temperature
	PLLA/PEG/PLLA [18]	Ring open polymerization	Potential anti-cancer drug carrier
	PEO–PPO–PEO [19]	Crosslinking the ethoxysilane-cap	Drug carrier
Electro-responsive polymers	PT [23]	Electrochemical Synthesis	Drug release and cancer chemotherapy
	PSS [22]	Emulsion polymerization	Drug carrier
Photo-responsive polymers	Azobenzene or spiropyran-containing		
	PAA [33, 34]	Copolymerization	Photocchromic polymer
	PHPMAm [35, 36]		Sensor
	PNIPAM [37, 38]		Photodegradation material
Chemically dependent stimuli			
pH-responsive polymers	chitosan [46]	Biosynthesis	Drug release
	Albumin [47]		Enzyme immobilization
	Gelatin [48]		Immunoassay
	PAAc/chitosan IPN [49]	UV irradiation	Wound dressing material and drug release
	P(MAA-*g*-EG) [50, 51]	Free-radical, solution photopolymerization	Controlled insulin delivery
	PEI [52]	Solution polymerization	pH-sensitive controlled release systems
	PDAAEMA		
	PL [53, 54]	Biosynthesis	Vectors for gene delivery
Ion-responsive polymers			
Redox-responsive polymers	Polyanhydrides [60, 61]	Melt condensation polymerization	Potential oral drug delivery systems
	PLGA [62]	Double emulsion solvent evaporation	Controlled delivery systems
	PbAEs [63]	Addition solution polymerization	Efficient carrier for cytotoxic agents
	Poly(NiPAAm-co-Ru(bpy)3) [67]	Living radical copolymerization	Artificial muscles, artificial reptile
Biologically dependent stimuli			
Glucose-responsive polymers	GOx conjugated chitosan [6, 68]	Carbodiimide chemistry	Self-regulated insulin delivery
Enzyme-responsive polymers	DEXS/chitosan [72]	Layer-by-layer assembly	Local and sustained drug release
	Azoaromatic crosslinked hydrogel [73]	Copolymerization	Specific delivery of peptides and proteins
Inflammation-responsive polymers	Glycidylether crosslinked HA [75]	Suspension solution reaction	Implantable drug delivery
Dual-stimuli	PLL block PEG–PLL [76]	Side chain reaction and crosslinking	Enhance gene expression

in an aqueous environment, due to the charge repulsion of the multiple amine groups [53, 87, 88]. Biocompatible acetylated poly(amidoamine) (PAMAM) dendrimers were used for drug delivery, with dexamethasone 21-phosphate (Dp21) as the model drug [89]. Cationic (non-acetylated) and acetylated (acetylation is a convenient strategy to neutralize the peripheral amine group) dendrimers exhibited different pH-dependent micellization, complexation,

and encapsulation behaviour. The acetylated dendrimer encapsulated the Dp21 under acidic conditions (pH = 3.0), while the cationic dendrimer encapsulated the drug under both acidic (pH = 3 and pH = 5.0) and neutral conditions (pH = 7.4). In addition, pH-responsive release was different for an acetylated- and a non-acetylated dendritic matrix. Non-acetylated dendrimers showed a much slower release rate than acetylated dendrimers under conditions of lower pH and a much faster release rate from non-acetylated dendrimer as pH values decreased. Degradable 1,3,5-triazaadamantane (TAA) dendrimers were able to be triggered by the addition of HCl [90]. TAAs units are stable under basic conditions but hydrolyze rapidly under acidic conditions to yield basic by-products [tris(amino-methyl)-ethane]. In the polyphosphazene-functionalized diaminobutane poly(propyleneimine) (DAB-PN) dendrimeric system used for hydrophobic drug delivery, release was triggered by sodium chloride ions [91]. Cations such as Na^+, K^+ complexate ethyleneoxy moieties on polyphosphazene chains, which results in the swelling of the polyphosphazene external groups.

3.1.4 Redox-Responsive Dendrimers

Degradable polylysine dendrimers with multiple spermine groups on the surface and non-covalently bound DNA were synthesized via attachment of the spermine by a disulfide linker [92], which was cleaved by mild reducing agents such as glutathione (GSH), therefore causing the release of DNA. Chemically and electrochemically triggered release of dendrimer end groups was obtained, based on different generations of poly(propyleneimine) dendrimers with redox-labile, trimethyl-locked quinone (TLQ) end groups [93]. The TLQ units were released by chemical ($Na_2S_2O_4$) or electrochemical (electrolytic current) redox reaction. Redox-triggered release of dendrimer end groups can be caused by the physiological redox cofactors (e.g., redox proteins, ascorbic acid, thiols).

3.1.5 Enzyme/Protein-Responsive Dendrimers

An interesting example of an enzyme-responsive dendrimer was obtained by the synthesis of dendrimers with a hexyl ester functionality as the hydrophobic part and polyethylene glycol (PEG) as the hydrophilic part [94]. These dendrimers disassembled in response to an enzymatic trigger (enzyme-porcine liver esterase) due to the incorporation of enzyme-cleavable ester moieties at the hydrophobic part of the dendrimers. Enzymatic cleavage of the ester groups caused disintegration of the dendritic structure and release of the guest molecule (Fig. 2). The rate of guest release systematically decreased with an increase in the dendron generation (higher generation dendrimers are more tightly packed, which sterically protects them—the ester functionalities are less accessible for enzymatic degradation). A similar strategy was used for the preparation of dendritic micellar containers [95], based on receptor-ligand binding interactions. PEG was chosen as the hydrophilic part and a decyl chain as the hydrophobic part. In order to disintegrate the dendritic structure, biotyn was incorporated (via click chemistry) as a ligand that bonded to a specific protein-extravidin. The disintegration of the system was caused by the biotin–extravidin interaction, which dramatically changed the hydrophilic–lipophilic balance (HLB) of the dendrimer molecule. The selectivity of this binding and release is based on molecular recognition.

3.2 Micelles

Block copolymer micelles are generally formed by the spontaneous self-assembly of amphiphilic copolymer molecules in an aqueous environment. Usually they are spherically shaped core–shell structures with sizes varying in the range of 10–100 nm. The hydrophobic blocks form the micelle cores, while the hydrophilic blocks form the micelle corona (shells). Lipophilic drugs can be solubilized in the hydrophobic micelle cores, significantly increasing the drug concentration in an aqueous environment.

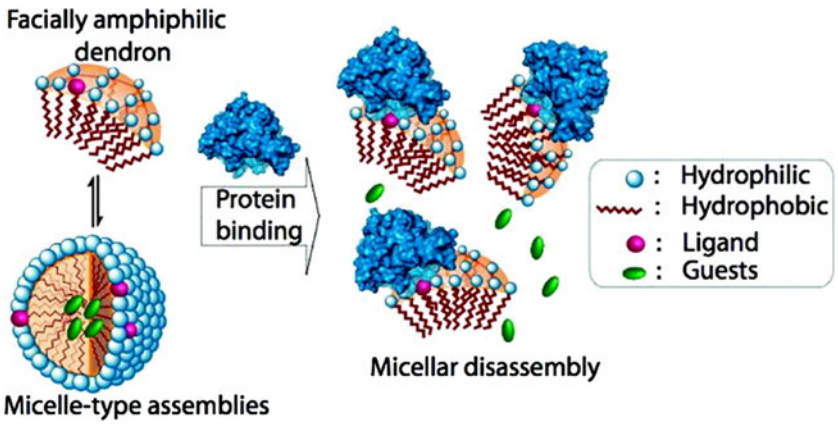

Fig. 2 Disintegration of dendrimer-ligand assemblies upon protein–ligand binding [95]

3.2.1 Photo-, Thermo- and pH-Responsive Micelles

Copolymerization of a spiropyran-containing methacrylate (SPMA) with di(ethylene glycol) methyl ether methacrylate (DEGMMA) resulted in dual-response (photo- and thermo-responsive) PSPMA–PDEGMMA material, which formed micelles and reverse micelles in aqueous solution (Fig. 3) [96]. Upon exposure to UV light, ring-opening isomerization of spiropyran (non-polar, hydrophobic, and colourless under visible light irradiation) occurred, resulting in the coloured, polar, hydrophilic form. The photoswitchable PSPMA block and the thermo-responsive PDEGMMA block, both PSPMA-core and PDEGMMA-core micelles, were obtained by changing the temperature (from 15 to 30°C) of the solution and by photo irradiation. These micelles were used for encapsulation and controlled release and re-encapsulation of the model drug coumarin 102.

Spiropyran-decorated amphiphilic polypeptide-based block copolymers PLGASP-*b*-PEO (poly(L-glutamic acid)-*b*-polyethylene oxide) that form micelles and micellar aggregates also showed conformational changes (from alpha-helix to random coil and vice versa) under UV and visible light, respectively [97]. Because the light used was a medically non-invasive, highly penetrating UV source, these photoresponsive rod-coil block polypeptides could be applied as viable model systems to study photo-induced drug release or light-controlled biomedical applications. Acid labile micelles of a model amphiphilic block copolymer, poly(hydroxyethyl acrylate)-*b*-poly (*n*-butyl acrylate) (PHEA-*b*-PBA) with encapsulated doxorubicin (DOX) demonstrated that hydrolysis of less than half of the cross-links in the core was sufficient to release DOX at acidic pH (5.0) faster than at neutral pH (7.4) [98].

3.2.2 Enzyme-Responsive Micelles

Examples of polymer peptide conjugates, particles of which disintegrated in response to the proteinase K signal [99], are the graft-type polymers (NIPAM–PEP and NIPAM–PEPEP, NIPAM is *N*-isopropylacrylamide, PEP and PEPEP are peptide units) containing a substrate peptide of protein kinase A (PKA) (PKA forms one of the most important intracellular signals in cellular signal transduction). The lower critical solution temperature (LCST) of NIPAM–PEP was raised from 36.7 to 40°C in response to phosphorylation by activated PKA. The NIPAM–PEPEP containing a different poly(ethylene glycol) unit formed a polymer micelle-type particle above the LCST. These particles disassembled and released drug in response to phosphorylation catalysed by PKA. The micellization of the complex of the polymer poly(potassium acrylate) (PPA) and the surfactant cetyltrimethylammonium bromide (CTAB), using the fluorescent pyrene as a guest molecule, resulted in an enzyme responsive system [100, 101].

3.3 Vesicles

Polymer vesicles, also called polymersomes, are spherical shell structures in which an aqueous compartment is enclosed by a bilayer membrane made of amphiphilic block copolymers. Their advantages compared to liposomes are: greater toughness, greater stability, tunable membrane properties, capacity to transport both hydrophilic and hydrophobic compounds (genes, proteins, imaging agents, anticancer and anti-inflammatory drugs and others), making them good candidates for applications including drug delivery, nanoreactors and templates for micro- or nanostructured materials. They can be used as stimuli-responsive controlled drug release systems [102–104].

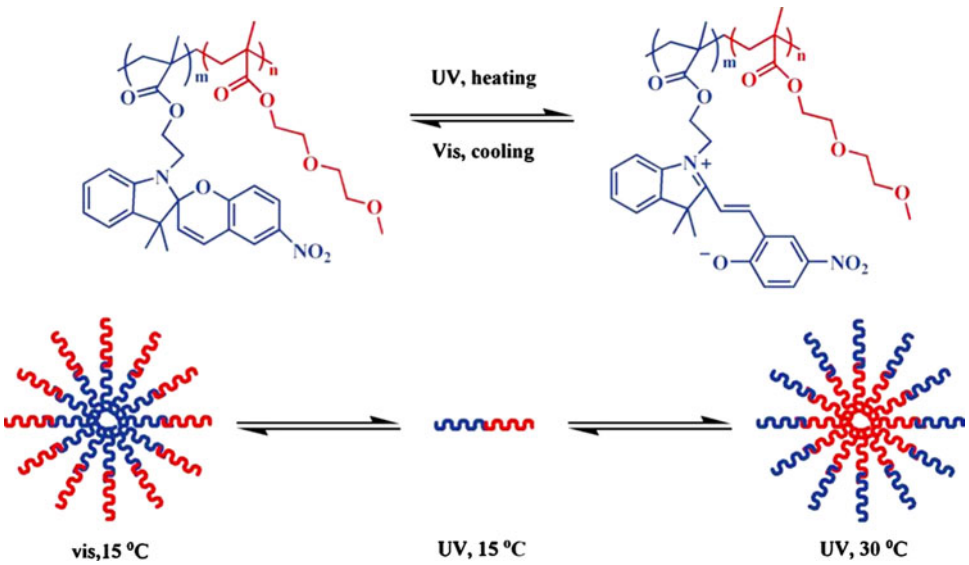

Fig. 3 Temperature- and UV-responsive micellar transition of PSPMA-*b*-PDEGMMA copolymer in aqueous solution [96]

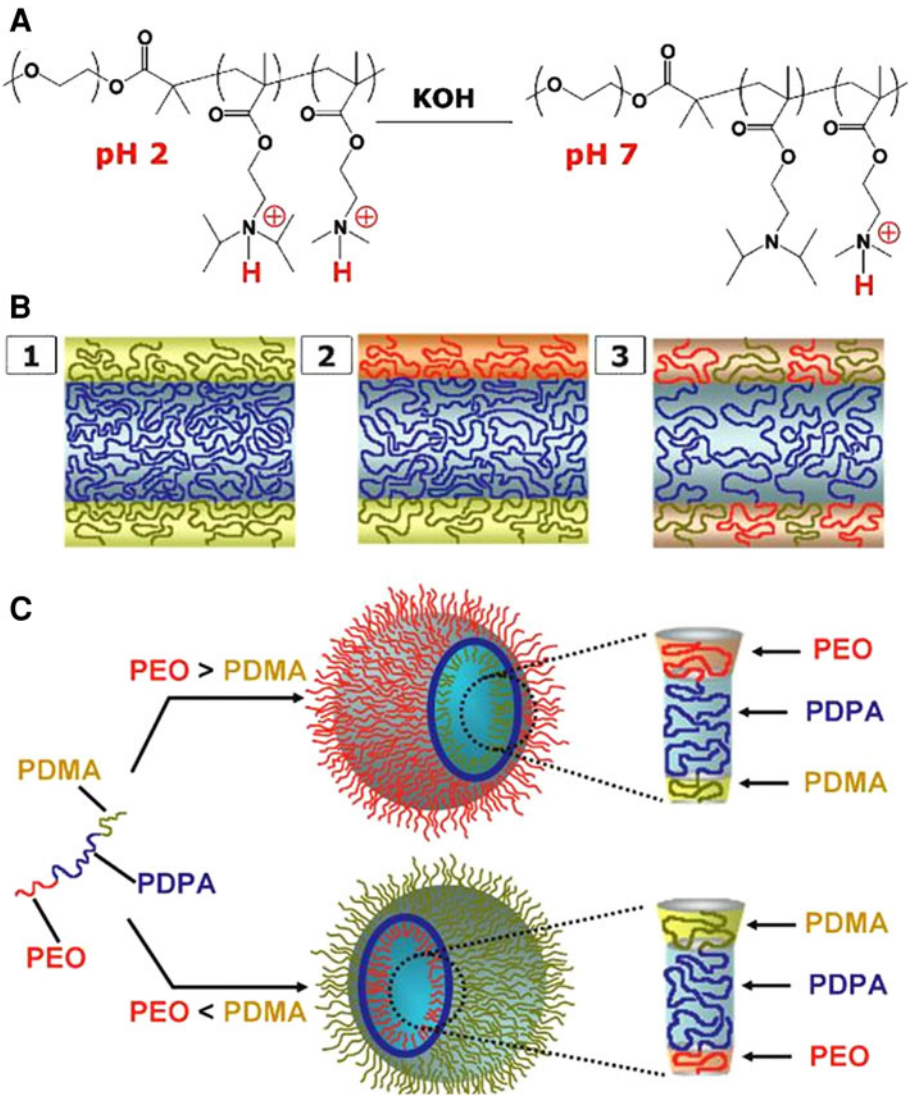

Fig. 4 **A** Effect of solution pH on the degree of protonation of the P and M chains. **B** Three possible membrane structures depending on the block copolymer morphology: **1** AB diblock copolymers form an interdigitated membrane with chemically identical faces; **2** ABC triblock copolymers where the central hydrophobic 'B' block bridges the membrane with segregated 'A' and 'C' interfaces; **3** central 'B' block of ABC triblock copolymer forms a 'loop' within the membrane, with the 'A' and 'C' chains forming a non-segregated membrane. **C** Effect of varying the relative volume fractions of the hydrophilic 'A' and 'C' blocks on the polymersome structure [110]

3.3.1 pH-, Ion-Responsive Vesicles

The response of polypeptides to pH and ionic strength was used to produce pH-and ion-responsive nanoparticles with controlled sizes and shapes. Amphiphilic poly(butadiene)-*b*-poly(γ-L-glutamic acid) (PB-*b*-PGA) diblock copolymer vesicles underwent reversible coil-helix transition in response to pH and, as a result, the sizes of the particles changed from 100 to 150 nm [105]. Also, peptide based biocompatible polybutadiene-*b*-poly(L-glutamic acid) (PB-*b*-PGA), polyisoprene-*b*-poly(L-lysine) (PI-*b*-PLys) and poly(L-glutamic acid)-*b*-poly(L-lysine) (PGA-*b*-PLys) vesicles demonstrated multi-responsive behaviour [106]. pH-responsive polymer vesicles obtained by the aqueous self-assembly of carboxy-terminated hyperbranched polyesters have the advantage of simple synthesis (a one-step esterification of the commercially available hydroxy-terminated hyperbranched polyester) and the possibility of controlling vesicle size (from 200 nm to 10 mm) by pH changes [107].

The potential of a drug to be released as triggered by pH changes was demonstrated with poly(ethylene oxide)-*b*-poly-(glycerolmonomethacrylate) (PEO-*b*-PG2MA) drug conjugates [108]. At a pH close to neutral, ester-bond linkages were stable and vesicular structures were formed. When pH was lowered to 2.0–3.5, hydrolysis of the ester bond took place and the drug was released. pH-sensitive vesicles made of the copolypeptide polyarginine-*b*-poly-leucine (PARG-PLE) were obtained based on the presence of a polyarginine block [109], the properties of which allowed vesicular self-assembly and intracellular delivery.

ABC triblock copolymers (PEO–PDPA–PDMA) [poly (ethylene oxide)-poly(2-(diisopropylamino)ethyl methacrylate)-poly(2-(dimethylamino)ethylmethacrylate] of varying block compositions with asymmetric membranes were used to demonstrate that the surface chemistry of polymersomes plays a crucial role (Fig. 4). PEO and PDMA blocks were hydrophilic and the pH-sensitive PDPA block changed from hydrophilic in acidic solution to hydrophobic

at pH 7.0. In vitro cell delivery studies suggest that the vesicles can be either biocompatible or cytotoxic, depending on whether the PEO or PDMA block is at the exterior surface [110].

3.3.2 Temperature-Responsive Systems

Thermo-responsive cross-linked polymer vesicles were formed by self-assembly of the block copolymer poly(2-cinnamoylethyl methacrylate)-*b*-poly(*N*-isopropylacrylamide) (PCEMA-*b*-PNIPAM) and following photo-cross-linking of PCEMA shells, and were used for temperature-(higher than 32°C) triggered release of 4-aminopyridine [111].

Self-assembly of amphiphilic hyperbranched star copolymers with a hydrophobic hyperbranched poly[3-ethyl-3-(hydroxymethyl)oxetane] (HBPO) core and many hydrophilic polyethylene oxide (PEO) arms also showed thermo-sensitive behaviour [112]. The thermo-sensitivity of the vesicles results from the partial dehydration of the PEO vesicle corona.

Diblock copolymer poly(*N*-(3-aminopropyl)methacrylamidehydrochloride)-*b*-(*N*-isopropylacrylamide) (PAMPA-*b*-PNIPAM) vesicles showed not only temperature responsiveness in a narrow range (25–45°C), depending on the length of the building blocks structures of the polymer, but were also "locked" by ionic cross linking of the PAMPA block [113]. Vesicles were stable between pH 0 and 11. However, the particle size was shown to vary with the pH of the solution. At lower pH values, the vesicles were bigger (310 nm at pH 3.0), and increasing the pH value of the solution decreased the size of the vesicles (e.g. 220 nm at pH 10.8).

Thermo-responsiveness can also be obtained by using the synthetic poly(trimethylene carbonate)-*b*-poly(L-glutamic acid) (PTMC-*b*-PGA), diblock copolymer [114]. Temperature induced reversible crystallization/melting of the PTMC-*b*-PGA vesicles in water depended on the vesicle size (membrane thickness). The disruption of the vesicular structure occurred when the temperature was increased above the melting point of the PTMC block (34–35°C).

Dual-response poly[(*N*,*N*-diethylaminoethyl methacrylate)-*b*-(*N*-isopropyl acrylamide)] [P(DEAEMA-*b*-NIPAM)s] systems capable of "schizophrenic" (two or more responsive blocks that can form two different structures triggered by stimuli) aggregation in aqueous solution were controlled by varying the pH and temperature [115].

3.3.3 Glucose-Responsive Systems

Oxidation-responsive vesicles from amphiphilic block copolymers based on ethylene glycol and propylene sulphide (PPS) exposed to oxidative conditions were destabilized [116]. Thioethers in the hydrophobic PPS blocks were changed into hydrophilic sulfoxides, influencing the hydrophilic–lipophilic balance of the amphiphile and inducing its solubilization. A poly(ethylene glycol)-*b*-poly(styrene boronic acid) (PEG-*b*-PSBA) system with boronic acid moieties showed both pH and sugar-responsive behaviour [117]. Disruption of the assemblies occurred after adding 0.5 M NaOH to the vesicle solution (Fig. 5). In addition, in the presence of 200 mM D-glucose, vesicles were also disrupted. The binding of the sugar molecules to the ionized boronic acid increased solubility of the PSBA blocks in water. The polymersomes disassembled completely in the presence of D-fructose (100 mM) in medium of pH 10.

3.3.4 Glutathione-Responsive Systems

Drug release systems based on reversibly crosslinked temperature-responsive nano-sized polymersomes of poly(ethylene oxide)-*b*-poly(acrylic acid)-*b*-poly(*N*-isopropylacrylamide) (PEO–PAA–PNIPAM), were formed in water (no organic solvents), which is important in the delivery of biopharmaceutics [118]. The polymersomes showed high stability in organic solvent, high salt concentrations, and at different temperatures, but in the presence of 10 mM dithiothreitol (DTT) the fast release of encapsulated species was observed. Polymersomes based on hydrophilic poly(ethylene glycol) (PEG) and hydrophobic poly(propylene sulfide) (PPS) connected by a disulfide bridge, PEG_{17}–SS–PPS_{30} were disrupted in the presence of cysteine, at a concentration corresponding to the intracellular level [65]. A similar system, also based on PEG–PPS block copolymers, was reported earlier [119]. This was the first example of the use of oxidation (in the presence of H_2O_2) in order to destabilize PEG–PPS–PEG vesicles and oxidize

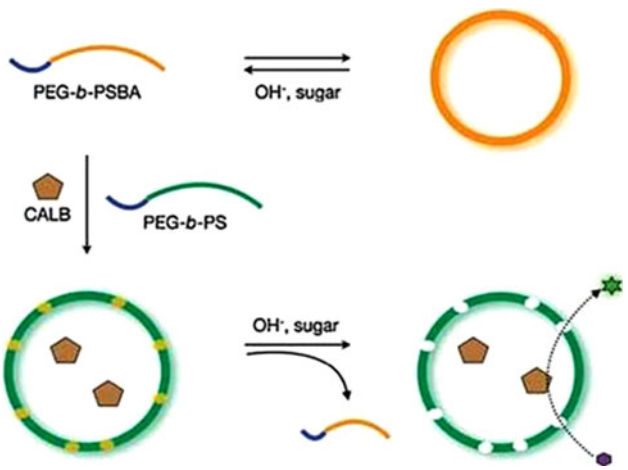

Fig. 5 Schematic structure of PEG-*b*-PSBA block copolymers and their equilibrium with D-glucose in a basic aqueous environment, and formation of polymersomes with a permeable membrane induced by the sugar responsiveness of the block copolymers [117]

the central-block sulphide moieties to sulphoxides and finally to sulphones, this oxidation causing an increase in the hydrophilicity of the initially hydrophobic central block.

3.3.5 Light-Responsive Systems

Zhao and coworkers [120] reported the formation of vesicles with PAzo-*b*-P(tBA-AA) copolymers, where PAzo is a hydrophobic methacrylate-based azobenzene containing side-chain liquid crystalline polymer, and p(tBA-AA) stands for the weakly hydrophilic poly-(*tert*-butyl acrylate-co-acrylic acid) polymer. Upon UV-irradiation, the hydrophilicity switch of the PAzo block from hydrophobic to hydrophilic causes a change in the hydrophilic/hydrophobic balance of the copolymer, inducing vesicle dissociation.

Using the same chromophore, Lin et al. [121]. reported a novel photoresponsive polymersome, obtained by self-assembly of a copolymer composed of hydrophilic poly (ethylene oxide) (PEO) and hydrophobic azopyridine containing poly(methacrylate) (PAP). Upon UV-exposure, several morphology changes were observed, and were described as a cycle including transitions from initial vesicles to larger vesicles via fusion, disintegration and rearrangements. These transitions resulted from the deformation of the membrane structure due to the isomerization of azopyridine moieties disturbing the tight packing of the polymer chains in the membrane.

Recently, Mabrouk et al. [122]. reported on a very original light-responsive system. They fabricated polymeric vesicles in the micrometer-size range, with asymmetric membranes composed of inert poly(ethylene glycol)-*b*-polybutadiene (PEG-*b*-PBD), and a liquid crystal-based copolymer, PEG-*b*-PMAazo444 (PAzo). Upon self assembly, the PEG–PBD copolymers are segregated in the inner leaflet of the membrane, while the PAzo copolymers compose the outer leaflet of the membrane, hence forming an asymmetric membrane. When the azo moieties are in the *trans* form, the PAzo polymer adopts a rod-like structure in the membrane. When light is switched on, azo moieties are in the *cis* form, and the PAzo polymers undergo a conformational change to reach a coil conformation. Subsequently, the volume occupied by the PAzo chains increased, leading to a spontaneous change in curvature and to bursting of the giant vesicles by "curling" of the membrane.

3.4 Smart Surfaces: Surface-Supported Polymer Layers and Films

In all nanomedicine studies, a major challenge is determining how nanomaterials will interact with mucosa, tissues, and targeted cells. New modulation systems that control the surface properties or solubility of materials in response to an external signal are designed using the stimuli-responsive polymers on a material surface, or by modifying the surface with bioactive substances, such as enzymes. Indeed, smart surfaces that respond to specific chemical and biological species have been the basis for the fabrication of highly sensitive, reagent-less, re-usable biosensors [22].

Surface grafted polymers can be defined as long chain polymer molecules that are attached to a surface through one or a few anchor sites [123]. Two primary covalent attachment techniques, i.e. "grafting-to" and "grafting-from", have been reported to create polymer brushes. In the "grafting-to" technique, a pre-formed end-functionalised polymer in a solution reacts with a suitable substrate surface to form a tethered polymer brush. In the "grafting-from" method, also called the surface-initiated polymerization method, monomers are polymerised from surface-anchored initiators generally immobilised by the self-assembled monolayer technique (SAM) [124, 125]. SAMs offer ease of preparation and versatile surface chemistry, while polymer brushes can be produced by surface-initiated polymerization techniques with improved control of surface coverage, thickness and composition.

Stimuli-responsive polymer films can be prepared on substrate surfaces using several deposition techniques of differing complexities and applicability, such as spin coating, chemical vapour deposition, laser ablation, plasma deposition, and chemical or electrochemical reactions [126–128]. The choice of deposition methods depends on the physicochemical properties of the polymer material, the film quality requirements and the substrate being coated.

3.4.1 Temperature-Responsive Surfaces

The most widely studied temperature-controlled films are built from PNiPAAm, a thermo-responsive polymer that has an LCST of 32°C in aqueous solution [129]. PNiPAAm chains present a widespread hydrogen bonding network between the amide groups and water molecules. Above LCST, PNiPAAm films undergo a phase transition, from a hydrated swollen state to yield a collapsed morphology (solvent is forced out) [130–132]. The reversible volume phase transition of PNiPAAm films can be utilised to develop thermo-responsive culture media for cells [133–135].

Surface attached stimuli-responsive polymers do not aggregate to form a separate phase, but the conformational transition from the hydrophilic to hydrophobic state endows the surface with regulated hydrophobicity. For example, when PNiPAAm was end-grafted to solid substrates, it provided the surface with thermally controlled

wettability and thickness [136]. At low temperatures, the composition profiles are approximately parabolic and extend into the solvent, while at temperatures above the LCST, the polymer profiles are collapsed near the surface. Moreover, nano-patterned thermo-responsive poly(2-(2-methosyethosy)-ethyl methacrylate) brushes demonstrate switching of both the thickness and the topography under temperature stimuli [137].

3.4.2 Electro-Responsive Surfaces

Height changes of polyelectrolyte brushes in response to the presence of ions of different sizes and charge were recently actively explored. When polymer chains bond with counter ions, the swelling and the hydrophilic/hydrophobic properties of the polymer layer change, while patterned brushes with two oppositely charged polyelectrolytes provide reversible switching of wettability, charge, and topography in an inverse manner. For example, by employing the electrochemical reaction in which aromatic nitro (NO_2) groups can be chemically modified by a redox process to amino (NH_2) groups, a surface can be functionalized by site-selective and reaction-controlled immobilisation of DNA [138, 139], and protein [140]. Also, by using the electroactive *O*-silyl hydroquinone moiety to tether the RGD peptide ligand to the monolayer, electroactive functionalised surfaces based on the hydroquinone–quinone redox couple have been shown to allow real-time control of molecular interactions that mediate peptide attachment and consequently the adhesion of cells [141].

On the basis of reversible doping of conducting polymers, a variety of anions have been electrostatically entrapped in conducting polymer films and released by electrical stimulus in a controlled way. As an example of this, positive charged neurotransmitter dopamine was successfully released from a conducting composite polymer, poly(*N*-methyl pyrrolylium)/poly(styrene sulfonate), prepared by anodic polymerization [142]. In its reduced state, this film was able to bind dopamine cations, which were then released by oxidizing the polymer film. Another example is polypyrrole films that can reversibly change their oxidation state, and consequently their properties and surface binding characteristics [143].

3.4.3 Photo-Responsive Surfaces

As described previously, there are mainly two types of photo-responsive molecules that may be used for a photo-triggered response. Spiropyran derivatives can transform from a hydrophobic spiro conformation to a polar hydrophilic zwitterionic merocyanine conformation under UV light, and can reversibly change with visible light [144, 145]. This change from the hydrophobic to the hydrophilic state upon isomerisation has been applied to demonstrate UV light-induced modification of surfaces [145]. The second type is azobenzene molecules that can change from the stable *trans* form to the *cis* state under UV light irradiation (300–400 nm), and reverse the isomerisation by irradiation with visible light [146–148].

A photo-responsive copolymer monolayer combining PNiPAAm and spiropyran chromophores has been used to tailor cell-adhesion by switching light on or off [149]. Change in surface hydrophilicity was obtained by irradiation with 365 nm light and 'reset' by visible light irradiation (400–440 nm) [144]. Additionally, a surface that can be photo-activated for spatio-temporal control of cell adhesion has also been developed by the release of nitric oxide from 2-nitrobenzyl ester-terminated monolayer [150, 151]. The 2-nitrobenzyl groups were selectively removed and consequently the protein and polymer dissociated from the surface.

3.4.4 pH-Responsive Surfaces

Polyelectrolyte brushes are pH-responsive materials that undergo structural changes at interfaces when their chains are charged and/or discharged because of the protonation/dissociation of acid/base groups [152]. As a result, upon an alteration in pH, polyelectrolyte brushes transform from the swollen state to a shrunken state in which the polymer chains collapse [153]. For example, surfaces grafted with an Os-complex redox unit modified poly(4-vinyl pyridine) [154]. Another type of surface was obtained from a mixed polyelectrolyte brush consisting of poly(2-vinylpyridine) and poly(acrylic acid) that had switchable permeability for both anions and cations [155]. When the ambient pH was acidic ($pH < 3$), the poly(2-vinylpyridine) chains were positively charged and permeable to the anionic probe. However, the redox process for the cationic probe was prevented, resulting in a lack of transport for positively charged ions.

3.4.5 Dual-Stimuli Responsive Surfaces

A smart and stable polymer brush interface based on PNiPAAm, PAA and poly(*N*-isopropylacrylamide-co-acrylic acid) was able to reversibly respond to temperature, ionic strength and pH, independently or simultaneously [156]. The reversible change in hydrogen bonding between the two components (NIPAm and AAc) and water, and the ionization of carboxylate groups under different environmental condition resulted in the dual-stimuli response.

Chitosan based PNiPAAm films possessing both thermal and pH sensitivity were prepared by blending chitosan with

PNiPAAm and PEG [157]. The resulting film had an LCST at around 32°C, due to PNiPAAm, and showed pH responsiveness due to the amino groups of chitosan component. Poly(vinylidene fluoride) (PVDF) hydrophobic films grafted with PAA via radiation grafting demonstrated convective permeability that changed significantly with the pH and/or the salt concentration of the surrounding fluids [158].

3.5 Polymer–Protein and Polymer–Drug Conjugates

Polymers conjugated with therapeutic agents have been extensively investigated over the past 30 years. Conjugation of polymers to therapeutic molecules resulted in macromolecular systems that synergistically combined the individual properties of the components. Drug solubilization, protein efficacy and stability are increased by conjugation, while immunogenicity and toxicity are lowered.

3.5.1 Temperature-Responsive Conjugates

Azido-terminated poly(*N*-isopropylacrylamide) (PNiPAAm–N_3) was conjugated to bovine serum albumin (BSA) [159]. When the temperature increased above the PNiPAAm lower critical solution temperature (LCST), the PNiPAAm–BSA bioconjugates formed stable nanoparticles composed of dehydrated polymer and hydrophilic protein. As an alternative to this systems, protein–polymer conjugates are based on biocompatible polyethyleneglycol methacrylate (PEGMA) [160]. Hybrid polymer–protein (PEGMA–trypsin) conjugates are promising candidates for biomedical applications. The first hybrid (diblock conjugate) and the second hybrid (triblock) demonstrated behaviour depending on their architectures but also their enzymatic activities—hydrolysis of peptide and protein substrates were different for various hybrids. This is an example of polymer–protein conjugates with varied architectures, and it can be used to regulate the properties of the protein polymer hybrids in terms of stability and reactivity.

3.5.2 pH-Responsive Conjugates

A pH-sensitive polymeric carrier for drug release in cancer therapy made of poly(vinylpyrrolidone-co- dimethylmaleic anhydride) (PVD) was conjugated with the drug adriamycin (ADR) [161]. At pH 8.5 no release of the drug from the conjugate was observed. In contrast, at neutral pH (7.0) and slightly acidic pH (6.0), fully active drug in the native form was released.

Also, anticancer polymer [P(*N*-(2-hydroxypropyl)methacrylamide)] drug conjugates, containing doxorubicin (DOX) attached via a pH-responsive hydrolytically labile spacer susceptible to hydrolysis (hydrazone conjugates) showed stability in pH 7.4 buffer but released DOX in response to pH change (from 7.4 to 5.6) [162].

3.5.3 Glutathione-responsive conjugates

N-acetyl-L-cysteine (NAC) is an antioxidant and anti-inflammatory agent with significant potential for applications in the treatment of stroke, neuro-inflammation and cerebral palsy. However, NACs with free sulfhydryl groups display high plasma binding, resulting in low stability and reduced drug efficacy. Conjugates of NAC with thiol-terminated multiarm (6 and 8) poly(ethylene–glycol) (PEG) with disulfide linkages involving sulfhydryls of NAC released the drug at intracellular GSH levels [163]. At physiological extracellular glutathione concentration (2 μM), both conjugates were stable and release of the NAC was not observed. NAC was also conjugated to poly(amidoamine) (PAMAM) dendrimers [164, 165]. PAMAM dendrimers, G4–NH_2 and G3.5–COOH, all with cleavable disulfide linkages, were designed for intracellular delivery. Based on PEG, a dendritic system for intracellular peptide delivery was manufactured via cleavable disulfide bonds [166]. The variable quantity of the disulphide linker allowed the adjustment of the cleavage and release of the drug peptide. Disulphide bonds were also used for the preparation of triazine dendrimer-paclitaxel (PAX) conjugates, as was an ester bond [167]. *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer and TNP-470 ([*O*-(chloracetyl-carbomoyl) fumagillol]), an angiogenesis inhibitor, were covalently bound to GFLG (Gly-Phe-Leu-Gly) linker via an enzymatically degradable bond, ethylenediamine [168]. When the concentration of lysosomal cysteine proteases such as cathepsin B increased (this happens in many tumour endothelial cells), cleavage of the linker took place. This conjugate was studied further in vivo and in vitro and went to preclinical trials under the name caplostatin [169, 170].

3.5.4 Dual-Response Conjugates

Dual-response conjugates are also known. A biotin-terminated poly(*N*-isopropylacrylamide)-*b*-poly(acrylic acid) (PNiPAAm)-*b*-(PAA) was conjugated to streptavidin (SA) via the terminal biotin on the PNiPAAm block [171]. Interestingly, the usual aggregation and phase separation of PNiPAAm-SA following the thermally triggered collapse and dehydration of PNIPAAM (the lower critical solution temperature of PNiPAAm is 32°C in water) was prevented by the shielding of the PAA block. In addition, the aggregation properties of the [(PNiPAAm)-*b*-(PAA)]-SA conjugate were pH dependent. By varying temperature and pH, the sizes of these particles differed from 60 nm (pH 7.0, temperatures above the lower critical solution

temperature of PNiPAAm) to 218 nm (pH 5.5 and 20°C). This was explained by hydrogen bonding between the –COOH groups of PAA with other –COOH groups and also with the –CONH– groups of PNIPAAM. The aggregation properties of the block copolymer–streptavidin conjugate differ from those of the free block copolymer.

4 Applications of Stimuli-Responsive Polymers in Nanomedicine

The need for accurate and non-invasive diagnostic tools is essential for early intervention to prevent disease progression. In this regard, the development of nanodevices capable of detecting specific and meaningful analytes associated with syndromes, of visualizing the location and distribution of affected cells, and of reporting the activity of a therapeutic agent are highly desirable.

In therapy, the introduction of these agents into the body (regardless of the administration route employed) is confronted by a set of efficient biological barriers, constituting the body's system defenses. Building smart nanoscale systems that are able to circumvent such barriers is seen as a potential way to administer therapeutic agents in a safe, selective, and efficient manner.

As described previously, polymeric systems are available in a variety of forms and structures, from bulk to supramolecular assemblies. In addition, because of their unique properties, stimuli-responsive polymers offer many opportunities to introduce functionalities into nanostructures and allow the fabrication of various smart systems.

The exploitation of polymer responses to stimuli finds wide-ranging application in the biomedical field: smart systems are useful in imaging and sensing (diagnosis), controlled drug delivery and regenerative medicine (therapy), but also in bioseparation, gating valves, or transport and microfluidics [22, 104, 172–180].

In the next sections, we will highlight the most relevant applications of such polymers in several subfields of nanomedicine, and pay particular attention to the advantages and drawbacks associated with those techniques. We focus on systems exploiting the intrinsic properties of stimuli-responsive polymers, i.e. where the functioning of nanostructures is a direct result of polymer chain properties that change upon activation by a given stimulus. Therefore, stimuli such as a magnetic field and ultrasound fall beyond the scope of this review, because they are applied to nanoparticles found within a self-assembled system.

4.1 Diagnosis

Polymer sensors that respond to relevant biomolecules and analytes, as well as pH and temperature, may be very useful in the detection of diseases that are usually accompanied by a significant imbalance in chemicals or variations of physical variables in the environment. Because monitoring these changes and gradients is vital to the diagnosis of certain diseases, great efforts have been made in the field of polymeric biosensors. Another important feature of nanodevices used in biomedical applications is their ability to self-report effective functioning (delivery in a specific location for instance) with the use of imaging techniques.

4.1.1 Sensors

In the field of polymer sensors, the most relevant examples in literature make use of smart surfaces (either composed of self-assembled multilayers or thin polymer films) responding to a change in the conformation of polymer chains, smart polymer probes that respond to chemical modification of polymer chains, and self-immolative dendrimers [181]. In the next sections, these systems are reviewed and classified according to their specific applications.

4.1.1.1 Systems for the Detection of Physical Variables (pH and T) Several groups exploited the motion of particles, such as gold nanoparticles or quantum dots linked to responsive polymer brushes anchored to a surface, in order to design polymeric nanosensors [182–184]. In such devices, conformational changes of the polymer chains caused by a given stimulus induce a vertical motion to the nanoparticles which can be easily monitored using surface plasmon resonance spectroscopy (SPR). In one example of a pH nanosensor, poly(2-vinylpyridine) (P2VP) polymer brushes reversibly collapsed due to a pH switch from 2 to 5 [183]. Surfaces acting as nano-thermometers were developed using a similar approach with core/shell CdSe/ZnS quantum dots attached to PNiPAAm polymer brushes [185].

Another type of sensor, known as a fluorescent polymeric sensor, presents the advantage of being based solely on the intrinsic properties of polymers. In these systems, a combination of stimuli-sensitive monomers and polymerizable fluorescent dyes compose the segments of the copolymers. Because the dye fluorescence is strongly dependent on its environment, significant changes in the fluorescence signal are observed upon changes in polymer chain hydrophilicity induced by stimuli. Such a copolymer of PNiPAAm and benzofurazan dye-modified units was reported by Uchiyama et al. [186], and showed a clear and reversible response to temperature cycles, associated with PNiPAAm chain conformational changes and the polarity sensitivity of the benzofurazan moieties (Fig. 6A). The same group reported other polymers based on the same concept using a variety of dyes [187]. It should be

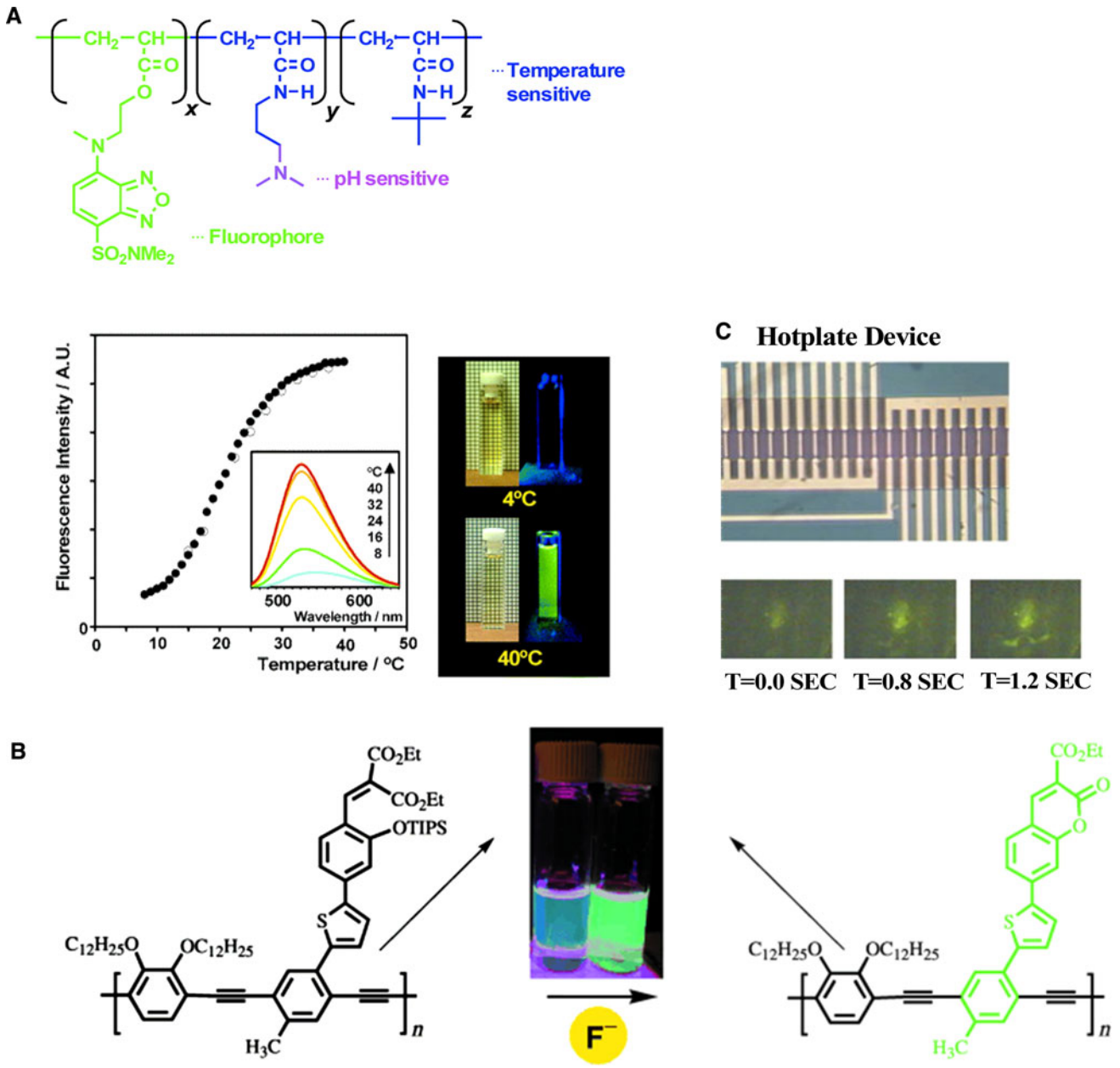

Fig. 6 **A** Fluorescent polymer sensor for temperature [186]. **B** Fluorescent polymer sensor for the detection of fluoride ions [194]. **C** Micrograph showing the microfluidic hot plate with gold hot lines and fluorescence microscopy images showing thermally triggered release of fluorescein-labeled myoglobin from the PNiPAAm surfaces [192]

mentioned that, in these systems, the temperature is correlated to the fluorescence intensity variations, which may be influenced by local concentration gradients, and that difficulties associated with measurement may occur (signal to noise ratio).

To address this drawback, devices from which the temperature (or other stimuli like pH) may be correlated to emissions at different wavelengths were proposed. In this regard, we describe here some examples using the fluorescence resonance energy transfer technique (FRET).

The transition from coil to globular conformation of responsive polymers was used in combination with FRET to produce pH and temperature sensors. As an example, a diblock copolymer of poly(ethylene glycol) and poly (sulfadimethoxine) (PEG–PSDM) was synthesized, with a FRET donor as a linker between the two chains, and a FRET acceptor as an end-group on the PSDM chain. When pH switches from 7.6 to 6.8 (values framing SDM pKa), the pH-responsive PSDM chains switch from coil to globular conformation. Consequently, the distance between

the FRET molecules varies as a function of pH, and the emission wavelength changes accordingly [188].

In recent work, Wu et al. [189] reported the fabrication of silica nanoparticles coated with PNiPAAm temperature-responsive polymer brushes labeled with FRET molecules. 4-(2-acryloyloxyethylamino)-7-nitro-2,1,3-benzoxadiazole (NBDAE), and 10-(2-methacryloxyethyl)-30,30-dimethyl-6-nitro-spiro(2H-1-benzo-pyran-2,20-indoline) (SPMA), were copolymerized with NiPAAm to yield P(NiPAAm-co-NBDAE)-*b*-P(NiPAAm-co-SPMA) copolymer brushes. According to the temperature variations that induce PNiPAAm collapse, specific emissions from FRET moieties were observed.

These two systems represent good examples of fluorescent pH- and thermo-meters.

4.1.1.2 Systems for the Detection of Small Analytes and Biomolecules Detectors based on SPR spectroscopy have also been used successfully for immunoassay devices based on the enzyme-catalyzed degradation of polymer films. Sumner et al. coated substrates with poly(ester amide) films sensitive to chymotrypsin, and poly(trimethylene) succinate films sensitive to lipase. The decrease in polymer film thickness resulting from the gradual degradation of the polymer chains activated by the enzymes and monitored with SPR was shown to be directly proportional to the enzyme concentration. Therefore, the sensor was proposed as a simplified alternative to ELISA tests [190].

Another array nanodevice based on a microfluidic hot plate grafted with PNiPAAm polymer was reported [191]. It was shown that, depending on the temperature of the hot line, the surface adsorbed and desorbed proteins within seconds (Fig. 6C) [192]. As competitive adsorption/desorption between two proteins occurs interdependent with heating time, the system can be used for selective analysis and separation of proteins.

Another type of detection based on the sensing of analytes via specific chemical reactions changing the properties of polymers has also been reported. An example of fluorescent amplification via enzymatic degradation of a polymer chain was reported recently by Tanaka et al. [193]. A polymer with a phosphate-caged fluorescein main chain was synthesized via polycondensation with diol linkers. Although the polymer obtained was not fluorescent, digestion of the backbone with alkaline phosphatase released highly fluorescent moieties, and the polymer was used to assess the enzymatic activity of a cell lysate.

Chemically induced response was also proposed by several groups to detect potentially toxic elements in drinking water. Although this application may not be core nanomedicine, we mention it in this review because it represents an improvement to prevent future complications and diseases. Kim et al. [194] synthesized a polymer with coumarin derivatives as side groups, able to detect fluoride ions (F^-). The structure of coumarin derivatives can be converted back to coumarin by fluoride ions, thus restoring their fluorescent properties (Fig. 6B). This represents a good example of a fluorescent polymeric sensor for F^-.

The detection of highly toxic mercury using fluorescent polymers was also reported, using a copolymer of poly(ethylene oxide)-*b*-poly(*N*-isopropylacrylamide-*co*-RhBHA) [PEO-*b*-P(NiPAAm-*co*-RhBHA)], where RhBHA is a rhodamine-based Hg^{2+}-sensitive dye [195]. Detection is based on the selective ring-opening of the RhBHA moieties by Hg^{2+} to yield fluorescent acyclic moieties. In this account, authors also investigated the effect of the thermo-induced self-assembly of the amphiphilic block copolymer on the fluorescence intensity and found that, upon formation of micelles, the fluorescent moieties were located inside the hydrophobic core, significantly enhancing the fluorescence. Many other systems exist for the detection of different analytes, such as metalloproteins and transition metals [196, 197].

The group of Sun developed several sensors based on wettability switching (i.e. a reversible transition from superhydrophilicity to superhydrophobicity) of surfaces grafted with PNiPAAm [178]. They synthesized block copolymers comprising PNiPAAm segments and blocks able to recognize different biomolecules. For instance, poly(*N*-isopropylacrylamide)-poly(phenyl boronic acid) (PNiPAAm-PBA) surfaces exhibiting a dramatic change in the presence of glucose, or PNiPAAm comprising oligopeptide units able to bind specific saccharide enantiomers based on chiral recognition, have been reported and used to monitor activity and concentration levels.

A novel class of recently developed molecules called self-immolative dendrimers showed promising use in different applications, including diagnostics and drug delivery. The self-immolative dendrimer molecules comprise a triggerable focal point, which initiates a cascade-like fragmentation of the structure into its building blocks upon activation. It is possible to design the building blocks as active molecules that can be detected once cleaved (these molecules being known as reporters). The release of these subunits can be seen as an amplification of the activation signal (physical, chemical or biological).

Using this approach, Danieli et al. [198] built dendrons with a phenylacetamide group as a point of focus, and two different probes as reporters. As the phenylacetamide group is a substrate of bacterial enzyme penicillin-*G*-amidase (PGA), the dendrimers readily degraded upon enzymatic activation, and subsequent detection of the two reporters allowed the evaluation of enzymatic activity. Because of the limitations of dendrimers, especially the limited number of building blocks due to steric hindrance, the concept was adapted to linear polymers, coined self-immolative polymers [199], to improve the amplification of

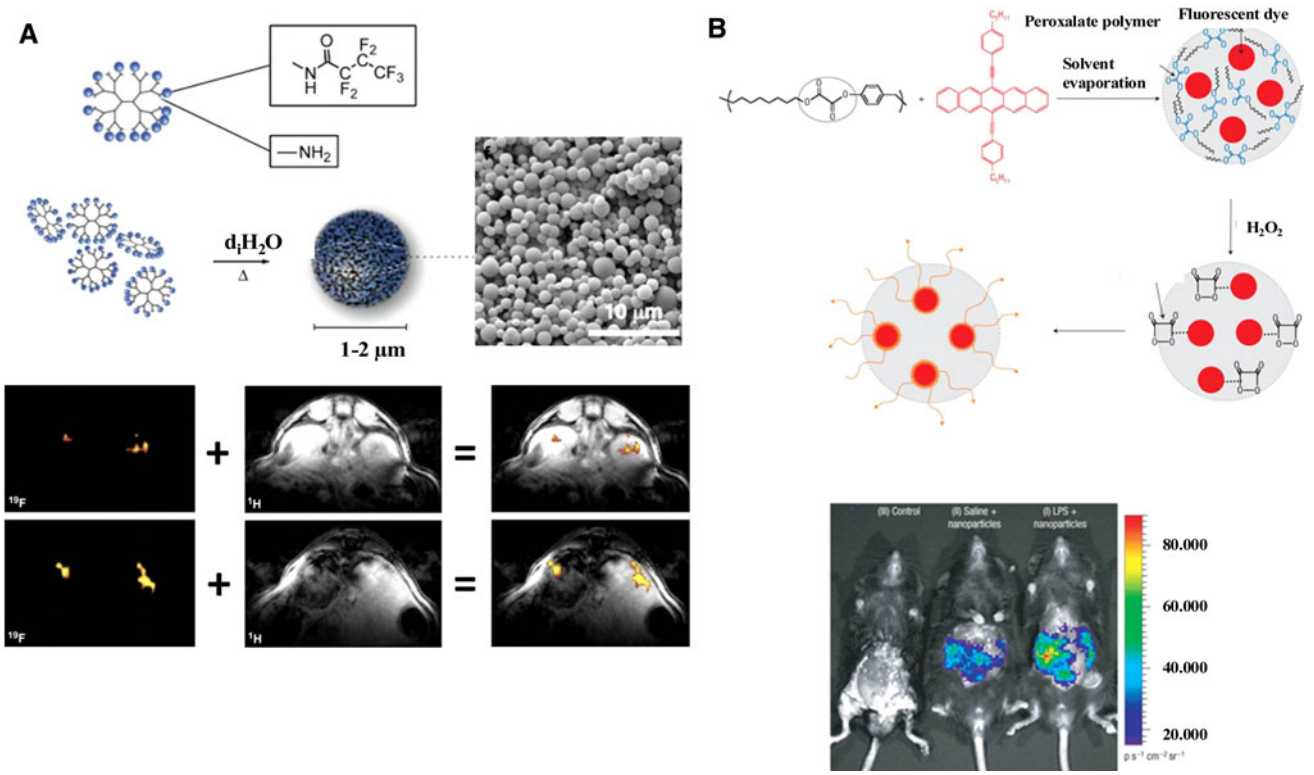

Fig. 7 **A** Self-assembly of fluorinated PAMAM dendrimers with fluorine groups for ^{19}F MRI imaging. ^{1}H and ^{19}F images showing accumulation in vivo after IV injection of the nanoparticles: Overlaid picture of showing localization of the particles in the renal vasculature and localization of the particles in the liver after efficient filtration. [202] **B** Peroxalate–pentacene nanoparticles and H_2O_2-induced reaction yielding fluorescence, and in vivo imaging of hydrogen peroxide production in the inflamed peritoneal cavities of mice [203]

the signal. One drawback of these self-immolative systems is that the chemistry used in the cascade-like degradation has been, until now, exclusively based on aromatic compounds and the toxicity of such cleaved compounds represents a potential issue in terms of biocompatibility [200].

4.1.2 Imaging

It is interesting to note that the concept of fluorescent polymeric sensors presented previously may be used reversibly, as an imaging technique for the detection of diseased tissues that show slightly elevated temperatures or acidic pH. A good example was reported using polymers comprising dyes sensitive to near infrared (NIR), which is the ideal wavelength range for biomedical applications, since it has superior depth penetration in tissue as opposed to other wavelengths. In this work, Lee et al. [201] made use of Pluronic triblock copolymers [poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO–PPO–PEO)] end-capped with a cyanine dye (Cy5.5). Contrary to PNiPAAm polymers showing an intrinsic responsive property, Pluronic block copolymers react to temperature via changes in their supramolecular interactions. Upon heating, the polymer chains evolve from a dissolved state to a micellar aggregation state. According to this work, the transition from dissolved chains to micelles is accompanied by fluorescence quenching of the Cy5.5 terminal dye. In turn, these structures can be used as NIR thermo-probes for imaging.

Another imaging system using stimuli-responsive dendrimers was developed by Criscione et al. [202]. They synthesized PAMAM dendrimers with fluorinated end groups that self-assembled into nano- and micro-particles. The system can deliver drugs under pH-induced disassembly, and the fluorine spins can be used for in vivo imaging using ^{19}F magnetic resonance imaging (^{19}F MRI). Experiments with mice show that the dendrimers can be tracked with non-invasive imaging (Fig. 7A). Interestingly, a shift in relaxation time was observed according to changes in environmental pH, meaning that the system can also be used as a powerful imaging technique for the localization of tumor, with acidic pH.

The detection of hydrogen peroxide is very desirable, as it is over-produced in a number of diseases. A smart system capable of imaging H_2O_2 in vivo was proposed by Lee et al. [203]. The nanoparticles were built from peroxalate polymers embedding a fluorescent dye, pentacene. The polymers reacted with hydrogen peroxide to form

dixioetanedione intermediates that, in turn, excited the fluorescent dye, leading to light emission in the 460–630 nm wavelength region. Imaging efficiency was investigated in vivo with mice injected with lipopolysaccharide, inducing an inflammatory response. As shown in Fig. 7B, the nanoparticles were capable of imaging H_2O_2 production in the peritoneal cavity of mice.

4.2 Therapy

In this section, the use of stimuli-responsive polymers is classified into two categories. The first deals with devices used as nanocarriers for the transport and delivery of therapeutic agents. As mentioned earlier, the delivery of compounds to a specific location of the body is subject to a variety of obstacles, known as biological barriers, including the reticulo-endothelial system, endothelial/epithelial membranes, complex networks of blood vessels, abnormal flow of blood, and interstitial pressure gradients and the blood–brain barrier [9]. According to the nature of the therapeutic agent, these barriers may simply reduce the efficacy of the treatment, or completely prevent or annihilate its effect. Therefore, one can easily understand the benefit of using a protective vehicle to avoid early screening or biodegradation of a given cargo, with the goal of improving pharmacodynamics and pharmacokinetics, and delivering an intact molecule to a specific target in a controlled manner.

In the second section, we present some interesting works dealing with the use of stimuli-responsive polymers in the field of regenerative medicine. Synthetic polymers have been used to produce scaffolds and supports for cell growth, and the functionalities offered by stimuli-responsive polymers have actually improved those systems a great deal in the direction of biomimetic materials.

4.2.1 Delivery Systems

Delivery applications of smart polymers constitute an overwhelming collection of articles, referring to virtually all polymeric nanostructures described previously. The most trivial structures used for the entrapment and subsequent release of small hydrophobic molecules are micelles. However, the use of classic micellar structures is limited to the encapsulation of hydrophobic drugs in the core, at a time when the demand for carriers able to encapsulate hydrophilic compounds is ever growing. Polymeric vesicles, or polymersomes, have the advantage of encapsulating hydrophobic and hydrophilic therapeutic agents. As reported by Onaca et al. [176], they find applications as nanocarriers for hydrophilic and hydrophobic low molecular weight drugs, proteins, enzymes, and genes. A number of other polymeric nanostructures have shown great potential in drug delivery, including dendrimers, smart surfaces, and in situ forming nanogels, and will be briefly addressed in this review.

Due to the length of the present review, we focused on the most relevant works, with very promising or demonstrated applications in nanomedicine. The examples described below pertain to the triggered delivery of low molecular weight drugs, proteins and enzymes, as well as genes. The stimulus used may be external (i.e. external application of localized light irradiation, ultrasound, or temperature) or internal (i.e. the system responds to local hyperthermia, elevated pH, or over-expression of proteins and enzymes in a tumor environment) [204].

4.2.1.1 Delivery of Low Molecular Weight Drugs Most of the low molecular weight drugs are hydrophobic molecules, and as such may be limited in their use due to solubility issues. Therefore, their pharmacodynamics and pharmacokinetics are greatly enhanced by solubilization in the hydrophobic domains of micelle cores, or dendrimers, or even the membranes of polymersomes, or by conjugation to polymers. Their release in the body can then be mediated by a number of different stimuli.

Doxorubicin (DOX), an anticancer hydrophobic drug, is perhaps most studied. However, many other small drugs have been used, including paclitaxel (PAX), camptothecin, cisplatine, dexamethasone, indomethacin, *N*-acetyl cysteine, …

As discussed previously, a number of systems exploit the pH differences found in the body, whether in the vicinity of a tumor, or in endosomes. In those systems, the pH effect may result in the cleavage of pH-sensitive bonds (hydrozone, acetal), as was shown with drug–polymer conjugates releasing doxorubicin, paclitaxel, indomethacin, and camptothecin, which were covalently attached to polymer blocks forming micelles via acid-labile linkages [108, 205–208]. As an example, polymer–DOX conjugates were designed with hydrazone or amide pH-sensitive bonds linking the drug to a poly(ethylene glycol)-poly(caprolactone) (PEG–PCL) diblock copolymer [208]. The pH-triggered release and cellular uptake were evaluated in vitro with MDA-435/LCC6^{WT} and MDA-435/LCC6^{MDR} cells. The therapeutic effect was also investigated in vivo on mice bearing tumors, and tumor regression was shown to be more significant for mice treated with the polymer–DOX micelles (Fig. 8A).

The liberation of DOX was also shown using a dendritic polyester with pH-sensitive linkers [209]. Dendrimers as drug delivery systems have advantages over classic polymers, due to their well-defined architecture (low polydispersity, specific morphology, high density of functional groups) [210]. Drugs can be entrapped in dendrimer structures via encapsulation, complexation through electrostatic interactions, or covalent attachment (conjugation) [210].

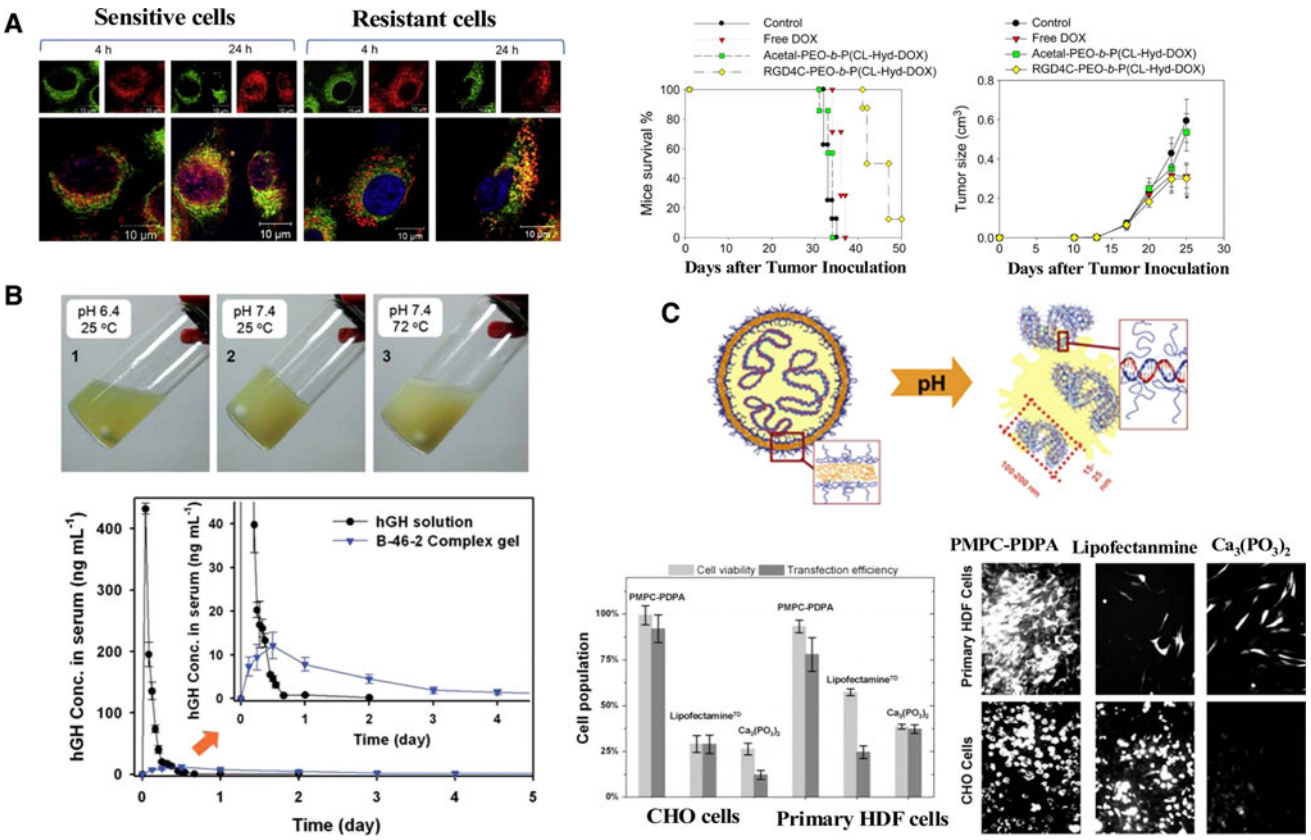

Fig. 8 **A** Mitochondrial, endosomal and nuclear distribution of DOX in MDA-435/LCC6^{WT} and MDA-435/LCC6^{MDR} cells after internalization of pH-sensitive DOX–polymer conjugates: *Pink color* shows localization of DOX (*red*) in nucleus (*blue*), while *yellow* color is an indication of localization of DOX (*red*) in mitochondria (*green*) or endo/lysosomes. *Curves* showing mice survival and tumor size evolution for mice treated with DOX–polymer conjugates versus other groups [208]. **B** Photographs of phase transitions of PAEU–PEG–PAEU copolymers with respect to pH or temperature, and hGH concentration in blood of SD rats after injection of hGH solution, and hGH-gel formulation [256]. **C** Scheme depicting concept of pH-responsive PMPC–PDPA vesicles used for gene transfection and the cell viability assay and enhanced GFP expression [234]

Drug–polymer conjugates are more attractive than drug–dendrimer complexes, because of their increased stability and higher payloads.

As reported by Ahmed et al., polymersomes have also been used as nanocarriers for smaller drugs. They reported on polymer vesicles capable of encapsulating a cocktail of anticancer drugs, PAX (hydrophobic, entrapped in the membrane) and DOX (DOX–HCl salt, hydrophilic, encapsulated in the inner pool) [211]. The contents can be released from polymersomes via poration in the membrane induced by pH-triggered degradation of the PLA blocks. The system was tested in vivo, and tumors in rats were shown to shrink significantly (by 50% in 5 days). The limitation of the system, and of biodegradable polyesters in general, is due to the rather slow rate of poly(lactic acid) hydrolysis.

The reducing intracellular environment, due to the presence of glutathione, or the action of enzymes (including NADH-oxidase and disulfide isomerase) was also used to trigger the release of smaller drugs via cleavage of reduction-sensitive linkages. As an example *N*-acetyl cysteine (anti-inflammatory agent) was conjugated to polyamidoamine (PAMAM) dendrimers via disulfide linkages, and released in the intracellular domain, in the presence of reducing agents (glutathione, cysteine) [164]. The efficacy of the system was assessed by measuring the reactive oxygen species level in microglial cells. After 72 h, up to a 125% reduction of H_2O_2 was observed for cells treated with the loaded dendrimers. The efficacy of micelles sensitive to a reducing environment was also demonstrated, with a system based on camptothecin–polymer conjugates [212].

Responsiveness to temperature was exploited as well. Most of the temperature-sensitive systems are based on PNiPAAm. Using a thermo-responsive block copolymer, PEO- PNiPAAm, Qin et al. [213]. prepared vesicles which can encapsulate doxorubicin, and sequester a hydrophobic dye in their membranes. Upon cooling to temperatures below PNiPAAm LCST, the membrane is dissolved, and both contents are released upon complete dissociation of the vesicles. Quan et al. [214] designed thermo-responsive micelles from a poly(*N*-acroyloxysuccinimide)-*b*-poly

(*N*-isopropylacrylamide)-*b*-poly(caprolactone) (PNAS-*b*-PNiPAAm-*b*-PCL) triblock copolymer for the delivery of DOX to HeLa cells. The micelles are internalized in HeLa cells, and above the LCST of PNIPAAM, i.e. at physiological temperature, 97% of the DOX payload is released.

In targeted drug delivery, it is also of interest to feature sensitivity towards a specific enzyme. A self-immolative dendrimer structure for the release of PAX activated by enzyme was reported [215]. The dendrimer was linked with an enzyme-responsive moiety to a *N*-(2-hydroxypropyl)-methacrylamide (HPMA) polymer for solubilization enhancement. Upon activation with cathepsin B (a lysosomal cystein protease), three PAX molecules were released. Cell growth inhibition assay using TRAMP-2 cells revealed a clear inhibition of cell proliferation when compared to controls. Polymeric micelles sensitive to lysosime were also reported [216].

Ionic interactions were used to deliver drugs, using the concept of PIC micelles, i.e. structures formed via electrostatic interactions between charged macromolecules and oppositely charged polymer chains. While conventional polymer micelles are mainly used for solubilization of hydrophobic drugs, hydrophilic, charged macromolecules (i.e. metal complexes, proteins, nucleic acids, and peptides) can be encapsulated in PIC micelles, and easily released via addition of counterions or pH switches [172, 217]. Cisplatine, a platinum complex-based anticancer drug, was bound to the carboxylic acids of poly(glutamic acid), which acted as ligands for Pt, and the complex was released upon ligand exchange with chloride ions in the body [218]. The micelles accumulated in tumor tissues of mice via EPR effect, leading to complete tumor regression.

Ionic interactions may also mediate the sol–gel transition of polyelectrolytes. Sol–gel polymers undergo a reversible gelation caused by a stimulus. They have application in drug delivery, where they can be formulated as a solution that embeds drugs, transforming into a gel when in contact with the body [179]. The drug is then released by diffusing through the gel, or upon gel degradation in the case of biodegradable polymers. As an example, alginate polymers containing pilocarpine (an alkaloid used in the treatment of glaucoma) undergo a sol–gel transition upon the addition of calcium ions, present in lachrymal fluid. Eye-drops of an alginate solution containing pilocarpine showed a significant decrease of intra-ocular pressure in rabbits over 10 h, due to the diffusion-controlled release of the drug [219]. Thermally induced gel formation was also reported in an ocular drug delivery system, with Pluronic and PNiPAAm based systems, for the delivery of pilocarpine and timolol maleate [220, 221].

4.2.1.2 Protein and Enzyme Delivery The release of proteins and enzymes is also very challenging. These biomacromolecules are often fragile and present net charges. Therefore, they need to be shielded from potentially harmful species in the body, either via encapsulation in the lumen of polymeric vesicles, or reversible association with polyelectrolytes to form PIC micelles.

It should be noted that, although the encapsulation (and subsequent release) of functional proteins into responsive polymersomes has been demonstrated [116, 222, 223], to the best of our knowledge the triggered release of a therapeutic protein with demonstrated biomedical applications has never been shown [174]. Therefore, although polymersomes represent an attractive nanocarrier for protein delivery, in vivo medical applications are yet to be reported.

As described previously, the dissociation of PIC micelles may be triggered through the use of different stimuli responsive polymers, either via a charge conversion induced by the addition of counterions or pH change, the degradation of chemical bonds via pH or a reducing condition, or via temperature changes [224]. Using such charge conversion, lysozyme was encapsulated in PIC micelles composed of poly(ethylene glycol)-poly[(*N*′-citraconyl-2-aminoethyl)aspartamide] (PEG-pAsp(EDACit)). The PIC micelles degraded in response to the endosomal pH and released lysozyme [225].

Another approach to controlled drug delivery of proteins is to use smart surfaces responsive to temperature, chemical stimuli, or electric stimulus. Polymer films grafted on surfaces are good candidates for drug delivery because they have high storage and high retention capability, and can uptake and release biomacromolecules on demand [22]. As an example, polypyrrole (PPy) offers an opportunity to build electrically responsive systems. Nerve growth factor (NGP) was loaded on a polypyrrole conductive film, and was released upon electrical activation [226]. A similar system was used to release adenosine triphosphate (ATP) [227].

Smart polymer films can also be used as stimuli-activated gates to control release of molecules. The use of thermo-responsive PNiPAAm as an on–off gate was reported by Yavuz et al. [228]. PNiPAAm was covalently attached to gold nanocages via thiolate linkage. Using the photothermal effect of the gold nanocages, PNiPAAm underwent reversible conformational changes resulting in an on–off gating of the pores. The controlled release of DOX and lysozyme was investigated, and in vitro experiments respectively showed significant decreases in cell viability after 5 min of irradiation with IR light, and 80% bioavailabilty of the enzyme.

In situ-forming polymer gels are another class of materials built of stimuli-responsive polymers and having great potential in drug delivery. As an example, poly(ethylene glycol) (PEG) and poly(*β*-amino ester urethane)

(PAEU) copolymer undergo pH- and temperature-induced gelation under physiological conditions [229]. These materials were used to deliver human growth hormone (hGH) to rats. Results show that the hGH concentration in the serum of rats was maintained at a higher level than in the control, due to the controlled release rate obtained with the gel (Fig. 8B).

4.2.1.3 Gene Delivery The delivery of genes, or gene therapy, was proven very effective in the treatment of several diseases. As with proteins and enzymes, the transport of DNA into a cell is a difficult process, because of the charge and size of such molecules. Therefore, the need for gene carriers that can safely and effectively administer these materials in vivo is growing.

A method of choice is to use PIC micelles. As described above, these structures can help the vectorization of charged macromolecules using polyelectrolytes. Plasmid DNA complexed with a α-lactosyl-poly(ethylene glycol)-poly(2-(dimethylamino)ethyl methacrylate) block copolymer (lactose–PEG–PAMA) was efficiently transfected to HepG2 cells [230]. Another example of PIC micelles was reported by Xiong et al. [231], where siRNA was delivered to metastatic human MDA435/LCC6 cancer cells, and efficient gene silencing was observed.

Recently, an example of a block copolymer for gene delivery bearing a pH-sensitive linkage between hydrophilic and hydrophobic segments was reported. The poly[(2-dimethylamino)ethyl methacrylate] (PDMAEMA) and PEG blocks are connected via an ortho-ester, which can be cleaved upon pH-triggering [232]. Transfection efficiency was proven with the encapsulation of luciferase and EGFP gene expression plasmids, and their pH-triggered release in the endosome of 293T cells.

An example of an instantaneously pH-responsive polymer vesicle was described by Armes and coworkers [233]. They developed a highly biocompatible and pH-sensitive block copolymer, poly[2-(diisopropylamino)ethyl methacrylate]-poly[2-(methacryloyloxy)ethyl phosphorylcholine] (PDPA-PMPC). The PDPA block is deprotonated and insoluble at pH above 7 (pKa around 5.8–6.6). Water-soluble doxorubicin was encapsulated within PDPA–PMPC vesicles, and released upon lowering the solution pH. The system also proved useful for the physical encapsulation and intracellular delivery of GFP-encoding DNA plasmid [234, 235]. As shown in Fig. 8C, superior GFP expression is obtained with the polymer vesicles when compared to Lipofectamine TD and calcium phosphate.

Polypeptide-based block copolymers also show temperature induced conformal changes, from α-helical to β-sheets structures. As an example, polymersomes built of PLL-*b*-PBLG-d7-*b*-PLL have been synthesized, where PLL and PBLG-d7 are poly(L-lysine hydrochloride) and poly(γ-benzyl-d7-L-glutamate), respectively [236]. In vitro encapsulation and release of plasmid DNA was shown.

In an example of structures similar to polymer–drug conjugates by Oishi et al. [237], micelles in which the corona-forming block itself is a therapeutic agent have been synthesized. The oligonucleotides, connected to the hydrophobic block with a pH-sensitive spacer, were released upon pH change.

As emphasized in several reviews, dendrimers are also very useful as transfection vectors, for different DNA molecules [210, 238, 239].

4.2.2 Regenerative Medicine

Stimuli-responsive polymers also find application in regenerative medicine. In this regard, they can be classified into polymers for the design of smart surfaces, and polymers that undergo sol–gel transitions for injectable implants. Smart surfaces may be used as supports or scaffolds, with excellent controllability of surfaces properties, that can, in turn, be used for adsorption and desorption of biomacromolecules and cells. It is known that cell behavior and attachment is greatly influenced by the wettability of a surface, and that biomacromolecules have higher affinity for hydrophobic surfaces. Therefore, depending on the application, stimuli-responsive polymers grafted on surfaces provide possibilities to design scaffolds for tissue engineering.

4.2.2.1 Smart surfaces for tissue engineering Cells in tissues grow in a rather complex fashion, surrounded by an extracellular matrix (ECM) that plays an essential role as a support. In addition, ECM elicits a wide range of biological signals and releases various biological factors, controlling both cell behavior and proliferation. In order to build viable cell sheets for tissue engineering, synthetic materials should mimic functionalities, similar to ECM. Thus, the use of stimuli-responsive polymers to design smart surfaces as ECM biomimetic materials to be used as scaffolds for the growth of new cells and tissue engineering is currently a fast growing research area. In order to advantageously replace other existing materials and allow the growth and proliferation of cell sheets, smart surfaces should display reversible changes in their affinity for biomolecules and their cell adhesion properties, as well as provide sustained release of biomacromolecules.

Although polymer substrates have been used previously in cell culture (with polystyrene, for instance), the use of stimuli-responsive polymers represents a gentler alternative to mechanical or enzymatic digestion (protease) for cell detachment procedures needed in these systems. It guarantees the collection of intact cell sheets using a non-invasive cell recovery method, and these cell sheets can

then be implanted in the body for tissue engineering applications.

As an example, thermo-responsive polymer films have been shown to be very useful in the control of cell recognition, adhesion and detachment. In this field, pioneering work was performed by Okano et al. [133, 240, 241] using PNiPAAm as the thermo-responsive polymer. Various cells, including hepatocytes, endothelial cells, fibroblasts, keratinocytes, epithelial cells, macrophages, and microglial cells, adhere and proliferate on such surfaces. When temperature is lowered under the LCST of PNiPAAm, the surface gradually switches from hydrophobic to hydrophilic, leading to cell desorption, without the need to use EDTA or trypsin [242].

In order to improve selective cell adhesion, biologically active moieties have been integrated into smart surfaces. As an example, dynamic surfaces controlling the presentation of recognition and regulatory signals were investigated [243]. In these systems, immobilized RGD sequences promote cell adhesion, and are shielded upon lowering temperature.

As mentioned earlier, the immobilization and programmed release of biologically active agents is desirable in order to promote cell adhesion and direct cell behavior. Such molecules can be hosted on smart surfaces via electrostatic interactions, conjugation, or encapsulation. Release of proteins was shown using ionic strength-sensitive [244] and thermo-responsive systems [245–250].

Even though temperature responsive surfaces based on PNiPAAm have been studied the most, other stimuli have also been investigated, such as light and electrical signals. Nerve regeneration is crucial, because it is very difficult to reconnect severed nerves by surgical means. The use of electro-responsive surfaces based on conductive polypyrol (PPy) was explored, and PC-12 as well as chicken sciatic nerve explants were shown to grow and proliferate preferentially on PPy surfaces submitted to an electric stimulus, when compared to controls [251].

Light was used with spiropyran-based polymers to efficiently detach cells from surfaces in a reversible manner [145]. Platelets and mesenchymal stem cells were shown to adhere to a poly(nitrobenzospiropyran)-poly(methyl methacrylate) copolymer, where the photo-sensitive groups are in a closed, non-polar spiropyran isomer conformation (hydrophobic surface). Upon UV irradiation, the spiropyran is converted to a zwitterionic merocyanine isomer, facilitating cell detachment (hydrophilic surface). Interestingly, light activated systems allow the manipulation of cell sheets, via the selective irradiation of a given region, thus creating patterns (Fig. 9A) [144].

Another application of smart surfaces is the controlled fabrication of biomimetic ceramics. Recently, a thermo-responsive surface built of PLA and Bioglass with grafted PNiPAAm showed an interesting application in biomineralization. The production of bonelike apatite is of prime interest for regeneration and tissue engineering, especially for orthopedic applications. In their work, Shi et al. [252] showed that calcification could be controlled by temperature, and yielded apatite material with bone-like structure.

4.2.2.2 Sol–Gel Transition Polymers as Injectable Implants Most of these systems are used exclusively for in vitro cell cultures, followed by cell desorption: for in vivo use, surgery must be performed to implant the cell sheets. To avoid this, a class of materials known as injectable implants is used. These systems are based on the gelation of a polymer solution upon injection into the body, and can promote cell delivery or other useful therapeutic agents such as growth factor.

The basis for using injectable polymers is that the matrix temporarily replaces damaged tissue, allowing proliferation and growth of cells until a new cell sheet or extracellular matrix is produced on site. Among the physiological stimuli used for gelling, temperature is the most studied and the most advantageous for in vivo application, due to its ease of use. Chitosan–PNiPAAm copolymer-forming gels have been employed as thermo-responsive injectable nanogels as scaffolds for tissue engineering [253, 254]. Mesenchymal stem cells embedded in the copolymer solution were able to differentiate into chondrocytes (cells found in cartilaginous matrix) in vitro (Fig. 9B). The cell–polymer mixture was injected into rabbit bladders, where the formation of new cartilage on the polymer matrix was detected [253]. Another thermo-responsive in situ forming gel based on chitosan and Pluronic polymers was shown to exhibit superior haemostatic properties [255].

5 Summary and Conclusions

Progress in medicine today relies on the advent of new systems and approaches that serve to detect pathological events in early stages, permit precise, safe surgery, and treat a specific region efficiently with minimal side effects. In this respect, stimuli-responsive systems are of particular interest. Stimuli-responsiveness represents a key property in medical applications because it serves to allow for controllable response from biological compartments, such as the release of an encapsulated/entrapped active compound, the triggering of a signaling process, or the detection of a specific biomolecule. A variety of systems that are intended to response to stimuli or a combination of stimuli has been developed based on polymers. There are two possible ways to obtain responsiveness: by using an SR polymer or by using a stimuli-responsive compound combined with a non-responsive polymer serving as a template.

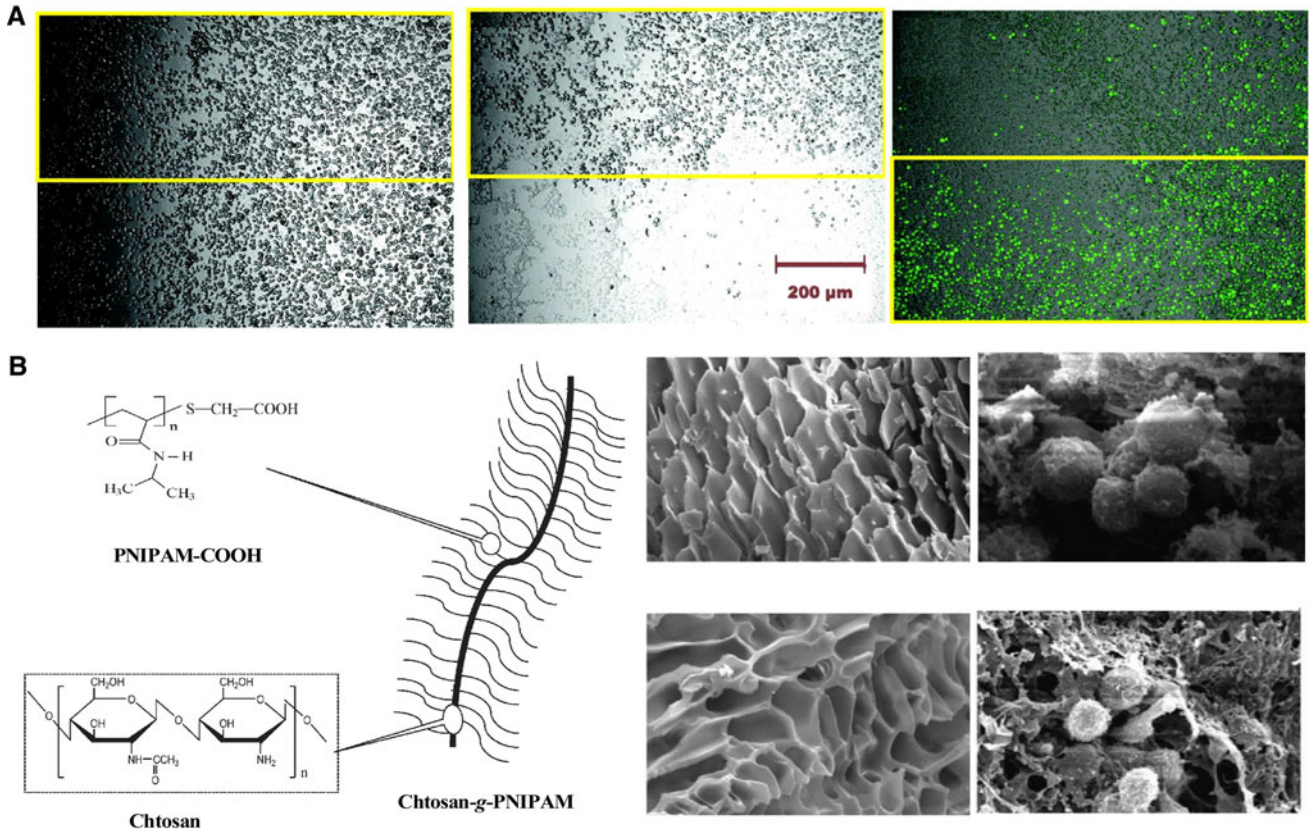

Fig. 9 **A** Manipulation of CHO-K1 cell sheets with UV irradiation and temperature: microscopic images of photoresponsive culture surface before (*left*) and after (*middle*) regional UV irradiation followed by the low-temperature washing, and after second regional UV irradiation followed by the low-temperature washing (*right*). *Yellow rectangles* indicate UV-irradiated regions [144]. **B** SEM pictures of injectable nanogels formed by chitosan–PNiPAAm copolymers (*left*): SEM micrographs of chitosan–PNiPAAm hydrogel scaffold and hydrogel scaffold after temperature cycling between 25 and 37°C 100 times (*up*), chondrocytes and meniscus cells cultured in chitosan–PNiPAAm hydrogel scaffolds for 21 days (*bottom*) [254]

Stimuli-responsive polymers represent a smart, synthetic way to mimic the behavior of biopolymers, such as proteins, that undergo drastic conformational change at a critical point while remaining stable over a wide range of environmental conditions. Here, we have focused on stimuli-responsive polymers and have indicated both the variety of changes to physical, chemical and biological stimuli, and the possible medical applications. The response of a given polymer is based either on a dramatic alteration of its structure or on a change in its properties, such as charge, solubility, or polarity. An alteration to the polymer structure takes place when the polymer is degraded by breaking chemical bonds in the backbone or at specific positions where cross-linking moieties are inserted in its structure for this purpose. The change in properties is achieved by introducing functional groups that support or even induce changes in chain dimension, secondary structure or supramolecular assembly architecture. Changes in properties are mediated by changes in intermolecular interactions, by undergoing a specific chemical reaction, or by the presence of modified physical conditions.

A large variety of SR polymer-based systems has been developed, both in solution and on solid support, to serve diagnostic and therapeutic purposes. In solution, various architectures have been introduced, ranging from dendrimers to supramolecular assemblies generated by the self-assembly of amphiphilic copolymers, such as micelles and vesicles. On solid support, polymer mono- and multilayers undergo a change in properties as a response to an external stimulus and thus generate smart, active surfaces—especially important in biosensing approaches. However, the multitude of polymer systems and assemblies is dramatically reduced when medical application is intended, due to the complex requirements related to use inside the body. In this respect only SR polymers that are biocompatible and biodegradable can be used without toxicity problems. In addition, size, charge, flexibility, and shape of supramolecular assemblies are properties that should be modulated so as to allow for an optimum administration route and simultaneous high efficacy. Multifunctionality is another key factor that serves to increase the potential of polymer systems in medical applications in terms of developing

targeting approaches, or theragnostic strategies. We have presented various medical applications here, in which SR polymer systems represent ideal candidate systems, starting with diagnostic approaches and extending to therapeutic treatment and tissue regeneration. However, using SR polymer systems/assemblies at the nanometer scale is an emerging field that will benefit greatly from more and extended studies on biodisposability, biodistribution, and toxicity in order to provide safe solutions and improve a patient's condition. The modulation of polymer properties for an efficient response to a stimulus represents an important parameter that must be adjusted in medical applications, but must always take into account the overall behavior of the system as it copes with the challenges presented under biological conditions, especially inside the body.

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