Stimuli responsive polymers for biomedical applications

Carolina de las Heras Alarcón, Sivanand Pennadam and Cameron Alexander*

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Polymers that can respond to external stimuli are of great interest in medicine, especially as controlled drug release vehicles. In this *critical review*, we consider the types of stimulus response used in therapeutic applications and the main classes of responsive materials developed to date. Particular emphasis is placed on the wide-ranging possibilities for the biomedical use of these polymers, ranging from drug delivery systems and cell adhesion mediators to controllers of enzyme function and gene expression (134 references).

Introduction

The functions of living cells are regulated by macromolecules that respond to changes in local environment and these biopolymers form the basis around which all major natural processes are controlled. Many synthetic polymers that exhibit environmentally responsive behaviour can thus be considered as biomimetic and their development is central to emerging 'smart' applications in biology and medicine.[1] Of especial interest are synthetic or modified biological materials that can undergo conformational or phase changes in response to variations in temperature and/or pH. Polymers of this type are being developed for uses in fields as diverse as bulk engineering and microscale medicine, while specific examples range from microfluidic devices,[2] pulsatile drug release systems,[3–6] bioadhesion mediators[7–9] and motors/actuators.[10,11] Responsive polymers are also a major focus in emerging nanoscale technologies.[12–15]

In all these cases the key parameter defining the responsive or 'smart' behaviour of the polymers is a non-linear response to an external signal. Although there are many responsive elements that can be incorporated in synthetic materials or engineered/modified biopolymers, much of the research to date has involved pH, temperature or light as the stimulus. As in nature, the bulk response of the polymer is usually due to multiple co-operative interactions such as progressive ionisation or loss of H-bonding, that, although individually small, ultimately evoke a large structural change in the material when summed over the whole polymer. This behaviour intrinsically lends itself to biomedical applications and in this review the aim is to highlight selected yet diverse recent research showing the potential for bringing these classes of materials into therapeutic use.

*cameron.alexander@port.ac.uk

Carolina de las Heras Alarcón

Carolina de las Heras completed her first Degree in Chemistry at Universidad Complutense, Madrid, Spain, where she specialised in Biochemistry and graduated in 1999. Following a year in research in the departments of Interfaces and Inorganic Chemistry at the University of Utrecht, Netherlands, working on catalysis and AFM characterisation of sublimed oximes, Carolina joined the School of Pharmacy and Biomedical Sciences at the University of Portsmouth, where she has recently received her PhD following a thesis on Responsive Polymers for Biomedical Applications under the guidance of Dr Cameron Alexander.

Synthetic polymers responsive to temperature and/or pH changes

The most studied synthetic responsive polymer is poly(*N*-isopropylacrylamide) (PNIPAm), which undergoes a sharp coil–globule transition in water at 32 °C, changing from a hydrophilic state below this temperature to a hydrophobic state above it.[14] The phase transition, as shown schematically in Fig. 1, and hence the origin of the 'smart' behaviour, arises from the entropic gain as water molecules associated with the side-chain isopropyl moieties are released into the bulk aqueous phase as the temperature increases past a critical point. The temperature at which this occurs (the Lower Critical Solution Temperature or LCST) corresponds to the region in the phase diagram at which the enthalpic contribution of water hydrogen-bonded to the polymer chain becomes less than the entropic gain of the system as a whole and thus is largely dependent on the hydrogen-bonding capabilities of the constituent monomer units. Accordingly, the LCST of a given polymer can be ''tuned'' as desired by variation in hydrophilic

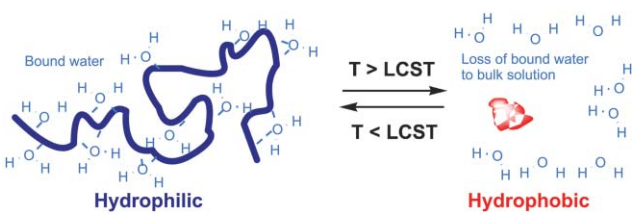

Fig. 1 Schematic of 'smart' polymer response with temperature.

or hydrophobic co-monomer content: materials based on co-polymers of *N*-isopropylacrylamide with a wide range of phase transition temperatures have now been reported.

The fact that the LCST of PNIPAm homopolymer lies close to body temperature and can be increased above and below 37 °C by incorporation of co-monomer units renders PNIPAm-based materials particularly suitable for biomedical applications.[15–19] The LCST phenomenon itself is quite widespread for polymers containing H-bonding sites for water molecules and the related homopolymer *N*,*N*-diethylacrylamide (DEAAm) also exhibits an LCST although with a broader range of 25–32 °C. Amongst the other important polymers in this class (Fig. 2) are poly(*N*-vinylcaprolactam) (PVCL), poly(ethylene glycol) (PEG) or poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO).

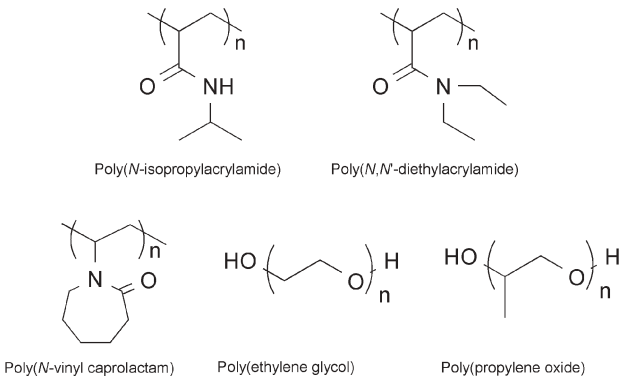

Fig. 2 Structures of commonly used responsive polymer systems.

PVCL is hydrophilic and water soluble at room temperature, gradually becoming hydrophobic and insoluble from 25 °C to 35 °C.[20] PEO polymers are highly soluble in water up to temperatures of ~85 °C, while PPO itself is hydrophobic, but co-polymers of these materials can be prepared with a very wide range of solubilities and phase transition behaviour. PEO–PPO co-polymers are of especial interest for their reverse thermal gelation (RTG) behaviour, which arises from the effect of the LCST-mediated transition on solution viscosity.[21–24] Solutions of these polymers in water exhibit a dramatic viscosity increase with temperature, forming semi-solid gels when heated above LCST. A large variety of PEO and PPO block co-polymers known as Pluronics, Poloxamers and Tetronics are commercially available and exhibit phase transitions varying from 20 °C to 85 °C.[25] These materials are already used in the pharmaceutical industry as surfactants and their ability to change from a low viscosity solution state to a semi-solid gelled structure when raised to body temperature makes them very suitable for application as injectable drug-dosing forms.

The incorporation of ionisable monomer units into polymer backbones enables phase transitions and solubility changes dependent on pH to take place. Poly(acrylic acid) (PAAc) and poly(methacrylic acid) (PMAc) based materials have been investigated for therapeutic use on account of their ability to swell reversibly with changes in pH.[26,27] In addition, the low cost of acrylic polymers and their adhesion to biological surfaces when partially protonated have also contributed to making this class of polymers of long-standing interest in pharmaceutical applications.[28,29] Combinations of temperature responsive polymers with pH and/or light sensitive components offer further control over polymer phase

Sivanand Pennadam

Dr Sivanand Pennadam received BS and MSc degrees in Chemistry from Bharathidasan University, Trichy, India in 1994 and then moved to the Indian Institute of Science, Bangalore where he conducted research into the synthesis of disulfide antibiotics. Dr Pennadam completed a PhD in polymer sensor technology at the University of the West of England under the supervision of Professor Norman Ratcliffe in January 2002 and joined the School of Pharmacy and Biomedical Sciences at Portsmouth in October 2002. Dr Pennadam's research interests range from organic and polymer synthesis to enzymology and gene delivery and he is currently working on polymer–biopolymer conjugates in collaboration with Drs Dariusz Górecki and Cameron Alexander in a project funded by the Wellcome Trust SHOWCASE scheme.

Cameron Alexander

Cameron Alexander graduated in Chemistry from the University of Durham, UK in 1987 and stayed on at Durham to undertake post-graduate research under the supervision of Professor W James Feast, FRS, receiving his PhD in Organic and Polymer Chemistry in 1990. He then moved to the Melville Laboratory for Polymer Synthesis, University of Cambridge (1991–1992) to work with Professor Anselm Griffin, before taking up Higher Scientific Officer (1992–1994) and Senior Scientific Officer positions (1994–1999) in the Macromolecular Science Department of the BBSRC Institute of Food Research. In January 2000 he was appointed to a Senior Lectureship at the School of Pharmacy and Biomedical Sciences at the University of Portsmouth and in October 2000 commenced an EPSRC Advanced Research Fellowship to investigate 'The Rational Design of Templated Surfaces'. Research interests range from polymer chemistry to medicine, with particular emphasis on the development of novel polymer systems for directing chemical and biological processes at surfaces and interfaces. Current projects are focusing on 'responsive' and imprinted polymers for control of bioadhesion, molecular recognition and targeted delivery of drugs and biopolymers.

behaviour, enabling a very diverse set of 'smart' materials to be prepared.[30–33]

Responsive polymer micelles

The combination of hydrophilic, hydrophobic and charged groupings on single polymer chains, coupled with the ability to interchange these properties *via* temperature or pH switching has given rise to materials with elaborate solution structures that strongly resemble biological entities. Poly(alkylene oxide)s combined with poly(styrene) and poly(4-vinylpyridine) forms permanent nanoparticles in water arising from the self-organisation of the amphiphilic AB diblock copolymer into responsive micelles, described as Shell Cross Linked (SCL) particles, as shown in Fig. 3.[34,35]

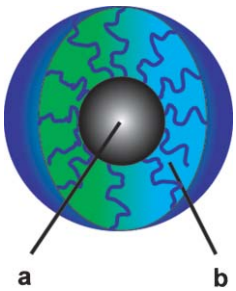

Fig. 3 Shell cross-linked micelle illustrating (a) hydrophobic polystyrene micelle core and (b) hydrophilic cross-linked shell or corona.

Complex multi-block responsive micellar materials have been described by the research groups of Laschewsky,[36] Wooley[37] and of Armes *et al.*[38] Liu and Armes[39] prepared triblock copolymers containing poly(propylene oxide) (PPO), as the hydrophobic component with poly(2-(dimethylamino)ethylmethacrylate) (PDMAEMA), as a cross-linkable unit and poly(oligoethyleneglycolmethacrylate) (POEGMA), as a solubilising block. These materials were shown to form structured permanent nanoparticles following a thermally induced structural change and cross-linking of the structured PDMAEMA inner layer. The key to the formation of these membrane-mimetic particles was the self-association of the PPO blocks in response to a temperature increase and the consequent ordering of the triblocks into micellar architecture. The highly hydrophilic POEGMA blocks maintained the overall solubility of the particles and also acted as a steric stabilising layer preventing micellar fusion during cross-linking to form the ''onion-like'' particles (Fig. 4).

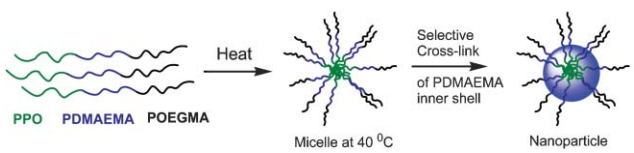

Fig. 4 Schematic drawing of aqueous solution of molecularly dissolved triblock copolymer at 5 °C (a); and formation of micelles at 40 °C (b); selective cross-linking of inner-shell permanent nanoparticle (c).

The equivalent pH sensitive triblock co-polymer micelles were generated from poly [(ethylene oxide)-*block*-glycerol monomethacrylate-*block*-2- (diethylamino)ethylmethacrylate (PEO–GMA–DEA) and poly[(ethylene oxide)-*block*-2-hydroxyethyl methacrylate-*block*- 2-(diethylamino)ethyl methacrylate] (PEO–HEMA–DEA) materials.[40] The tri-blocks were synthesised *via* atom transfer radical polymerisation of GMA or HEMA followed by DEA monomers using a PEO-based macroinitiator. Full solubility was exhibited in aqueous solution at low pH but deprotonation of the DEA layers above pH 8 led to micellisation and the formation of the tri-layer micelles as before. In this case, at pH 8 the micelles contained DEA cores with GMA or HEMA inner shells, and PEO chains as the outer surface layer (corona). Selective cross-linking of the hydroxy-functional inner shell was carried out with divinyl sulfone [DVS] under alkaline conditions retaining the DEA at the core of the micelle. The resulting SCL micelles exhibited reversible pH dependent swelling behaviour upon protonation of the DEA cores at low pH.

The same group also prepared diblock copolymers that formed two types of micelles in aqueous solution depending on pH.[41–43] These resulting states were described as 'schizophrenic' since by changing external pH, temperature or ionic strength the more hydrophilic block could be transformed to a hydrophobic state in order to form the core of a micelle. By altering pH again, the second block became hydrophobic, effectively switching the micelle. The key to this behaviour was in choosing the correct polymer block components (Fig. 5): the use of poly(4-vinylbenzoic acid)

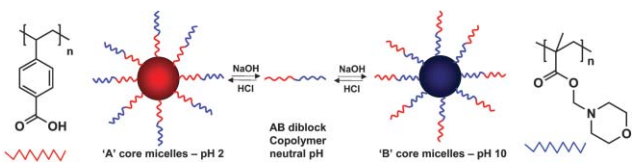

Fig. 5 Control of micellar states dependent on pH.

(pK_a = 7.1) as one block and poly(2-*N*-(morpholino)ethyl-methacrylate) (pK_a of the conjugate acid = 4.9) ensured that precipitation did not occur during pH variation across the isoelectric point.

The close resemblance between these multi-layer structures and biological membranes suggests that these materials could act as carriers for therapeutics or biomolecules or even behave as artificial cells. Amphiphilic block copolymers of this type are already of major interest for drug delivery as 'dual-triggered' release systems, and the increasing degree of sophistication in their responses arising from control over their structures through synthesis offers further medical benefits. Control over micelle size is therapeutically important, as it has been observed that particles of between 20 and 100 nm diameter are effective in avoiding renal exclusion and reticuloendothelial uptake.[44–48] Furthermore, particles in this size range can be selectively taken up by tumours because of the higher vascular permeability of these cells compared to normal tissue.[49–51]

Responsive polymer hydrogels as drug release matrices

Devices for controlled delivery of drugs are a particularly important application that exploits the reversible collapse and expansion of responsive polymers. Incorporation of poly(*N*-isopropylacrylamide) into a cross-linked polymer gel generates a matrix that can exhibit thermally-reversible shrinkage or collapse above the LCST of the homopolymer. The change in the matrix structure (the lower gel collapse point) is accompanied by loss of water and any co-solutes, such as a therapeutic agent. Swollen PNIPAm hydrogels kept in drug solutions at low temperatures have been shown to display rapid initial drug release when transferred to a medium at temperatures well above the gel collapse point, as a result of fast matrix contraction. Drug expulsion and water loss takes place in the initial stage of gel collapse, followed by a slower release as the drug diffuses from the shrunken and physically compacted gel. Pulsatile release of the prostaglandin H2 synthase-1 inhibitor, indomethacin,[52] and the sodium salt of salicylic acid[53] have been achieved in this way. Combination hydrogels can also be utilised, for example the group of Okano *et al*. reported a hydrogel with grafted oligomers of NIPAm on a cross-linked PVCL backbone and showed that the hydrogel decreased in volume above the LCST of PNIPAm and also experienced a second volume phase transition as the PVCL backbone passed through its LCST.[54]

If the therapeutic is incorporated in a responsive gel when the polymer is in the collapsed state, the *swelling* of the gel can be exploited as a release mechanism as the diffusivity and porosity of the matrix changes as a consequence of polymer expansion. The controlled release of budesonide, a steroid used for treatment of allergic rhinitis, has been demonstrated by Nakamura *et al*.[55] using a polymer gel composed of poly(methacrylic acid) grafted with poly(ethylene glycol) (P(MAA-*g*-EG)). In this case, the co-nonsolvency properties of ethanol–water mixtures were used to collapse the polymer gel in the presence of the drug thus entrapping the therapeutic. Administration of the particles into the nasal cavity (pH $\approx$ 7.2) of rabbits resulted in rapid initial release of budesonide followed by a more sustained biodistribution compared to intravenous injections of the drug.[55]

Smart polymer hydrogels have the potential to be used in a variety of drug-loading and release formats, and their release characteristics can be tailored to a range of target environments. Although the detailed kinetics of drug release from these systems are complex,[56–59] to a first approximation correlations between gel collapse point, matrix structure and drug release can be obtained. Appropriate synthesis then allows delivery systems to be prepared that will respond at a pre-designated pH and/or temperature to released a therapeutic. For drug delivery applications polymer response should be non-linear, *i.e.* with distinct 'on' and 'off' states and there is a drive to develop materials that display very sharp transitions for a small stimulus or change in environment. One way to accomplish this is by further elaboration of hydrogel structures at the micro- and nano-scale. Grafting of linear PNIPAm oligomers to existing cross-linked hydrogels has enhanced the rate of total gel collapse (20 min compared to 1 day) as a result

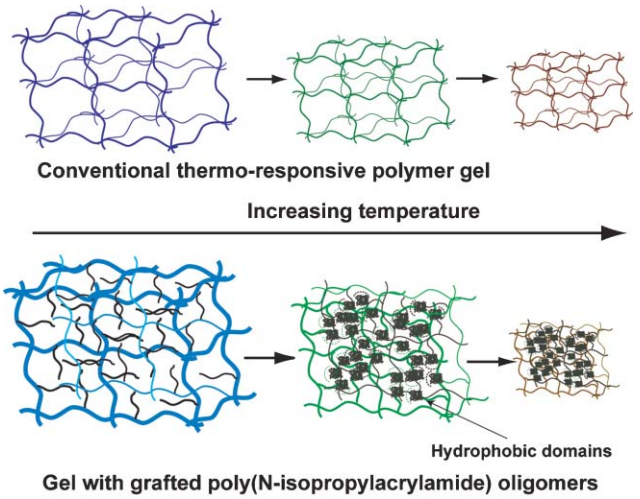

Fig. 6 Architectures of responsive hydrogels.

of the rapid aggregation of the non-cross-linked oligomers, which then act as 'hydrophobic nuclei' to which the rest of the network can more quickly associate, as shown in Fig. 6.[54,60]

The encapsulated therapeutic can, in theory, be of almost any type and since collapsed hydrogels are essentially impermeable to high molecular weight species, these systems are of interest for controlled release of biomacromolecules, especially peptides and proteins. Much of the reported literature centres on insulin release for feedback regulated treatment of diabetes, wherein pH responsive systems as well as temperature response have been evaluated.[61–65] One example of an insulin delivery system was a hydrogel comprising an insulin-containing reservoir within a poly(methacrylic acid-*graft*-poly[ethylene glycol]) (P(MAA-*g*-EG)) copolymer in which glucose oxidase was immobilised.[66,67] The surface of the polymer contained a series of molecular 'entrances' which opened and released insulin dependent on glucose concentration. Ingress of glucose through the polymer layer to the entrapped glucose oxidase resulted in a pH drop as glucose was oxidised to gluconic acid, and the released protons caused the pendent PMAA chains of the hydrogel to contract, thus opening the gates to allow insulin transport (see Fig. 7). An additional feature of this system was the cross-linked polyethylene glycol graft component, which in the expanded state of the gel was able to adhere to specific regions in the upper intestine. In this way, delivery of insulin could be targeted to preferred locations in the body.

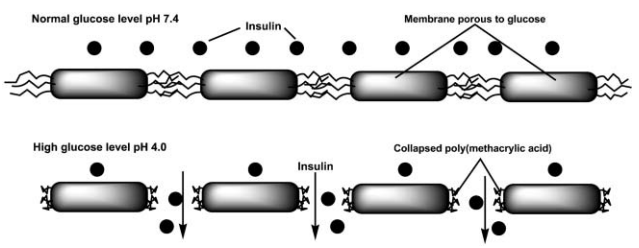

Fig. 7 P(MAA-*g*-EG) responsive hydrogel system for controlled insulin release.

It is now possible to produce large numbers and relatively high quantities of therapeutic peptides and proteins *via* biotechnology approaches, but to date these materials have been under-used due to their very poor bioavailabilities from conventional drug formulations. 'Smart' hydrogels that can enhance peptide or protein protection during *in vivo* transit, but which can improve subsequent release, thus have a very promising future in the pharmaceutical industry, especially if the response mechanism of the gel can include a biodegradation step. Proteins that have been incorporated in polymer hydrogels for controlled release already include calcitonin,[68] interleukin-2,[69] lysozyme[70]and LHRH analogues.[71]

Responsive hydrogel systems also offer the possibility of controlling the *activity* of biopolymers as well as behaving as devices that modulate release of a therapeutic. Biomolecules can be encapsulated by physical entrapment at temperatures around the lower critical gel collapse temperature, and exposure of the biopolymer to its surroundings can be controlled by swelling or collapse of the responsive hydrogel. The kinetics of encapsulated enzyme catalysed reactions can accordingly be modified, as shown for α-amylase by Sun *et al.*[72] while Park and Hoffman reported that the activity of β-galactosidase immobilised in a responsive gel could be controlled over several thermal cycles by matrix collapse or expanse.[73]

There are disadvantages when using cross-linked gels to control the release or modulate the activity of biopolymers. Highly cross-linked materials are difficult to prepare with predetermined 3-D structure and architecture, while, as noted above, the response times of many gels can be too long for therapeutic applications. The two problems are interconnected—lack of control in gel synthesis leads to large heterogeneous cross-linked matrices, which require multiple coupled individual phase transitions in order to exhibit a bulk response. There is much interest in preparing micro-or nanoscale gels[74] with smaller numbers of closely associated responsive components in order to give a much more rapid response to external stimuli. These microgel systems are closely analogous to biological macromolecules, since they are essentially single molecules and so do not suffer from slow diffusion processes or protracted chain relaxation phenomena. Micro- and nano-gels of PNIPAm with ultra-fast responses and fascinating rheological properties have recently been reported[57,75–80] but these systems are only just beginning to be developed for encapsulating biopolymers or modifying their activity.[81]

Responsive polymer–biopolymer conjugates

Control over the function of a therapeutic biopolymer can be effected by direct attachment to a synthetic polymer, especially if the attached polymer is responsive and anchored close to an active site. The basic principle of polymer–biopolymer conjugate chemistry is already widely exploited in pharmaceutical development: for example, PEG chains attached to therapeutic proteins have been shown to stabilize a great variety of proteins while maintaining their biological activity.[82–87]

Responsive polymer–biopolymer conjugates have been extensively studied by Hoffman, Stayton and co-workers. In order to attach the responsive polymer at specific locations on proteins, site-directed mutagenesis approaches have been used to introduce cysteine residues close to the active centres. This has allowed thiol-reactive termini on the responsive polymers to be targeted, ensuring single point attachment and minimisation of non-specific steric hindrance at enzyme binding sites. For example maleimide-terminated PNIPAm was reacted with a streptavidin mutant engineered to contain thiol functionality through introduction of a cysteine residue close to the biotin recognition site. Biotin bound strongly to the polymer–streptavidin conjugate below 32 °C in accordance with the normal high affinity of this interaction, but above the LCST no binding was observed, as collapse of the polymer blocked the recognition site. The switching behaviour was reversible across a number of temperature cycles, indicating that the regulation of binding was due to the reversible coil–globule transition of the attached responsive polymer.[88] This approach has proved to be very versatile and the Hoffmann group have utilised this method to prepare responsive oligomer/polymer conjugates with trypsin, dextran, and IgG antibodies.[89] In the case of the trypsin conjugates, the enzymes were engineered to contain a number of cysteine residues, and perhaps surprisingly, these conjugates increased in enzymatic activity as more responsive oligomers were conjugated to the native trypsin. Trypsin active sites in the conjugates were still accessible to large molecules, including a natural trypsin binding agent, soybean trypsin inhibitor with a molecular weight of 21.5 kDa. The enzyme conjugates were also more stable than native trypsin, both in solution and when precipitated above the polymer phase transition. The Hoffmann and Stayton groups have also prepared a temperature and photochemically switchable endoglucanase by this methodology, which displayed varying and opposite activities depending on whether temperature or UV–Vis illumination was used as the switch.[90] The polymer component and a putative conjugate structure are shown in Fig. 8.

The polymer–endoglucanase conjugate was tested for glycoside hydrolase activity against *o*-nitrophenyl-D-cellobioside (ONPC), as a model substrate. The engineered cysteine containing endoglucanase mutant EG 12A displayed very similar activity to the wild-type enzyme, whereas its conjugate with a responsive ((*N*,*N*′-dimethylacrylamide)- *co*-4-phenylazophenyl acrylate) polymer was active under UV irradiation at 350 nm but inactive for glycoside hydrolysis under light of 420 nm. A related polymer–enzyme conjugate, poly((*N*,*N*′-dimethylacrylamide)-*co*-4-phenylazophenyl acrylamide)-*graft*-endoglucanase EG 12A was active under 420 nm light but inactive under irradiation at 350 nm, which in this case correlated with the inverse photoinduced phase transition of the 4-phenylazophenyl acrylamide containing polymer compared to the *co*-4-phenylazophenyl acrylate based conjugate. The differential responses of the two polymers were most likely a consequence of the changes in dipole moments following photo-induced *trans–cis* isomerisation of the azobenzene group. As a consequence, the free volumes of the polymers varied dependent on the differential absorptions of the amide-linked azobenzene compared to the ester-linked

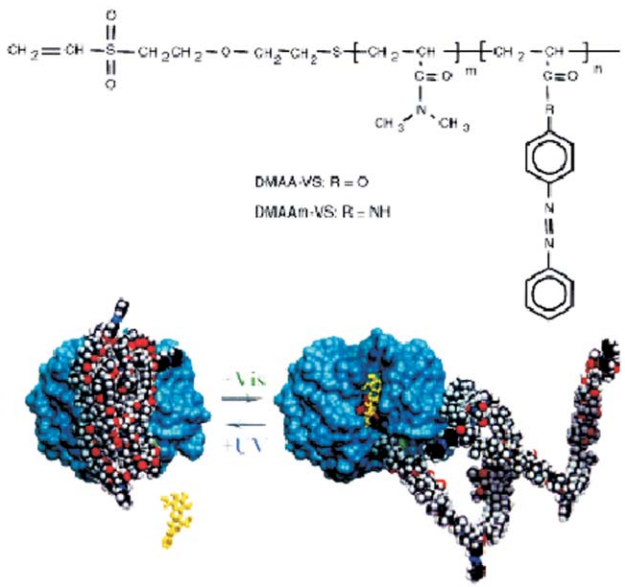

Fig. 8 Schematic of photoresponsive switchable endoglucanase. Copolymer compositions shown with end-modified vinyl sulfone terminus for cysteine thiol-specific conjugation. 3D model of EG 12A displays relative locations of position 55 (green, with schematic polymer coil attached) and catalytic active site residues D99, E116, and E200 (red). Substrate (ONPC) shown schematically to show orientation of active site groove. Polymer coil shown as a 10 kDa chain with a distribution of nine dimethylacrylamide monomers to one azobenzene monomer. See ref. 90. Copyright 2002 National Academy of Sciences, U.S.A.

azobenzene. Kinetic analysis showed that K_m values for glycoside hydrolysis were dependent on the conformational states of the attached responsive polymers but observed k_{cat} values were little changed across the various switching regimes. This suggested that large changes in polymer hydrodynamic volume between the extended and collapsed states were exerting a steric blocking effect on enzymatic activity following the photochemical switching step.

An emerging medical field for responsive polymer–biopolymer conjugates is the targeting of gene expression *via* switchable polymers that display antisense nucleotide binding behaviour,[91,92] as depicted in Fig. 9. PNIPAm conjugates with pendent oligodeoxynucleotides (ODNs) were prepared *via* direct co-polymerisation of NIPAm with a methacryloyl-terminated ODN. The conjugate exhibited the expected temperature-induced coil–globule transition at 33 °C in physiological-like buffers (pH 7.4, 100 mM NaCl). The activity of the conjugate, which contained the antisense sequence for the ribosomal binding site of mRNA encoding enhanced green fluorescent protein (EGFP), was assessed in *E. coli*. Translation of a plasmid encoding EGFP was suppressed in a dose-dependent fashion by the PNIPAm–antisense ODN conjugates, whereas no translational repression was observed for PNIPAm alone. GFP expression was reduced by up to 74% by the conjugates even though the theoretical loading of the oligonucleotide in the polymer was very low (~4000 : 1 PNIPAm : ODN), indicating the extremely high affinity of the antisense sections to their complementary nucleic acid strands

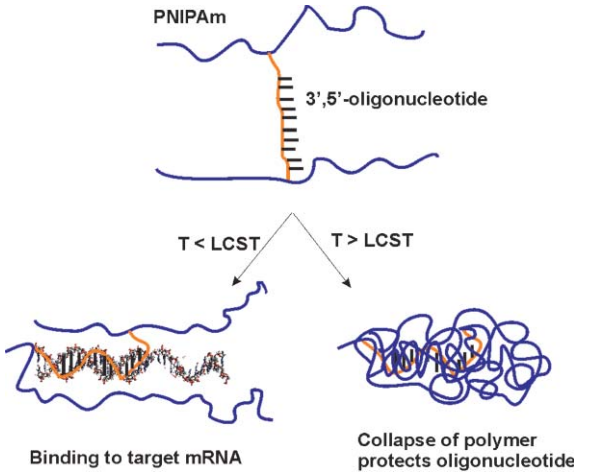

Fig. 9 Schematic of responsive polymer–oligonucleotide conjugate for antisense regulation of gene expression.

and the lack of interference by the polymer chains below the LCST.

An important further advantage in this antisense strategy was the stability of the PNIPAm–ODN co-polymer to nuclease degradation, which is a major problem to delivery of conventional antisense oligonucleotides. Incubation of the 3′,5′-modified ODN–PNIPAm conjugate with endonuclease S1 at 27 °C (*i.e.* below polymer LCST) did result in partial degradation of the oligonucleotide, but this was suppressed by ~50% compared to the free ODN. However, the same experiment conducted at 37 °C did not result in any degradation of the ODN at all. This indicated that the coil–globule transition of the polymer effectively protected the oligonucleotide against nuclease attack, most probably due to steric shielding of the ODN by the collapsed polymer chains. Further elaboration of polymer–DNA conjugates in this way may enable the regulation of gene expression as a specific 'knockdown' strategy. Alternatively, control of gene regulation through nucleic acid delivery is possible, and while this field is in itself too large to feature in this review, a number of papers have indicated that DNA binding and release can be controlled by complexation with responsive cationic polymers.[93–96]

Responsive polymers for control of cell adhesion

The use of synthetic materials as medical implants or as supports for tissue growth/regeneration requires surfaces that either resist attachment of certain cells while binding others, or that are capable of binding a biological moiety under one set of conditions but which can be switched in order to become non-adhesive.[97] Surface modification of materials can be used to control and modulate cellular interaction with implant biomaterials, to promote bone and skin cell interaction with the implant, and to prevent the adhesion of unwanted cells. The group of Okano and co-workers have extensively used thermo-responsive PNIPAm-based polymers as surface mediators of biopolymer and cell attachment.[9,15,98–102] For example, blood platelet contact activation and inactivation was shown to vary with hydrophilic/hydrophobic switching of

polystyrene tissue culture dishes plasma-grafted with PNIPAm, whereas blood platelet activation at poly(ethylene glycol)-grafted surfaces was not temperature dependent. Platelets on PNIPAm-grafted surfaces below polymer LCST maintained a rounded shape identical with those on PEG-grafted surfaces, whereas above the LCST the cells attached and spread on the hydrophobic PNIPAm-grafts in a similar fashion to platelet growth on tissue culture polystyrene.[103] These results revealed the ability to modulate cell activation state by the temperature-induced change in the hydration state of a responsive polymer surface.

For tissue engineering, it is important to be able to grow cells at a surface and then to detach the cells at an appropriate stage to be harvested, ideally without a biochemical or chemical reagent step. PNIPAm grafted surfaces were shown to support growth of cells as diverse as bovine endothelia and rat hepatocytes when PNIPAm-grafted tissue culture surfaces were above polymer LCST and to allow recovery of the cells when the temperature was reduced below the phase transition temperature. The kinetics of the cell recovery process following initial culture is also important: rapid recovery of cell sheets is an essential pre-requisite for the practical assembly of tissue-mimicking structures. Kwon *et al*. showed that the time required to detach cell sheets from PNIPAm-grafted porous membranes was lower than from PNIPAm-grafted tissue culture polystyrene (TCPS) surfaces as well as lower than from TCPS or PEG surfaces.[104] The rapid detachment of the cell sheets was ascribed to a rapid hydration of the grafted PNIPAm layer on the porous membranes because the water can access the PNIPAm-grafted surface both from underneath and from the lateral periphery of the attached cell sheet. Further accelerations in cell detachment following temperature-induced surface phase transitions were apparent from PNIPAm co-grafted with PEG onto porous culture membranes.[105]

Human skin fibroblasts have been shown to attach to and proliferate at the surface of thermoresponsive hydrogels of ethylene glycol vinyl ether and butyl vinyl ether co-polymers. Cultured cells were readily detached from the polymer surface by lowering the incubation temperature from 37 °C to 10 °C for 30 min. Incorporation of Arg-Gly-Asp (RGD) peptides at the surfaces resulted in higher values of cell proliferation in the initial stage.[106] This concept was extended by Stile and Healy, who prepared PNIPAm–RGD conjugates for characterising and controlling osteoblast adhesion.[107,108] Other studies exploiting the coil-globule transition of PNIPAm at surfaces include the demonstration of reversible attachment of human retinal pigmented epithelia.[109]

The use of PNIPAm co-polymers as mediators of prokaryotic cell adhesion was first reported by Lopez and co-workers.[7,16,110,111] LCST mediated transitions of surface-grafted PNIPAm homopolymer materials resulted in reversible changes in the number of adsorbed bacteria (*Staphylococcus epidermidis* and *Halomonas marina*) dependent on the inherent preferences of these bacterial strains for hydrophilic or hydrophobic substrates. Cunliffe and co-workers assessed end-grafted PNIPAm homo- and co-polymers as potential surface 'passivators' for prevention of attachment of the foodborne pathogen *Listeria monocytogenes*, and found that adsorption of this organism decreased above polymer LCST, indicating the lower ability of *Listeria* spp. to colonise hydrophobic surfaces.[112] Further bioadhesion studies were carried out to probe both protein and bacterial attachment,[8] as in clinical settings extracellular proteins and polysaccharides generally adsorb more quickly than cells to substrates immersed in biological media. The attachment of model proteins (Cytochrome C and Bovine Serum Albumin) was greater when the surfaces were switched to a hydrophobic state, in accord with thermodynamic predictions based on minimisation of interfacial free energy. The same pattern of behaviour was shown by *Salmonella typhimurium* and *Bacillus cereus*, with both strains attaching in higher numbers to PNIPAm and PNIPAm co-polymers above the phase transition temperatures. These surfaces reversibility adsorbed and desorbed both types of cells over sub-24 h timescales. Furthermore, correlations between LCST-mediated changes in Lewis basicity and short-term bioadhesion were obtained, demonstrating that protein and cell attachment at synthetic surfaces is not a simple function of hydrophilicity and/or hydrohobicity but is strongly dependent on local lone pair donor capacity and the presence of tightly bound water.

Responsive biopolymers

Molecular biology strategies have been adopted to generate switchable materials based on natural temperature- and pH-responsive polypeptides.[113,114] Tirrell and co-workers have incorporated unnatural monomers into peptidic backbones such that the resultant materials exhibited the normal attributes of related natural proteins but with additional functional properties.[115–117] Polypeptides based on the fibrous protein elastin, which exhibits a natural phase transition, were generated by substitution of valine at position 4 in the pentapeptide repeat unit of the protein by both natural and unnatural amino acids. Incorporation of the hydrophobic amino acid isoleucine rather than valine at position 4 reduced the LCST to below ambient temperatures whereas incorporation of more hydrophilic lysine raised the LCST from 12–27 °C without affecting the narrow temperature range (2–3 °C) of the response.[118] A number of other examples have been reported that illustrate the range of materials accessible *via* this method, including engineered polypeptides with phenylalanine analogue insertion,[119] azide incorporation,[120] and aryl bromide functionality.[121]

Engineered elastin-like polypeptides have also been extensively investigated by the groups of Urry,[122–125] Chilkoti[126–129] and Ghandehari[130–133] as smart drug delivery and targeting systems. Chilkoti *et al*. prepared materials based on the elastin pentapeptide repeat Val-Pro-Gly-Xaa-Gly (Xaa = any natural amino acids except proline), but engineered to exhibit LCST behaviour around 40 °C by modification of repeat sequences and insertion of oligoalanine and oligoglycine residues. The transition temperatures of these materials were designed such that particles might form upon ultrasound induction of hyperthermia as in this way local targeting of drugs conjugated to an ELP backbone might be possible. The thermal transitions for homopolypeptides occurred over a narrow range and were fully reversible: for a $(Val_5\text{-}Ala_2\text{-}Gly_3)_{150}$

polypeptide, the LCST onset was at 40 °C and complete by 42 °C. Block co-polymers were also prepared, but the thermal transitions were more complex indicating a range of intermediate species formed as differential blocks aggregated. For both homo-and co-polymers, particles of 40–100 nm were produced above the LCST suggesting advantageous use in cancer therapies owing to the accumulation of particles of this size in tumour tissues. Recently, the same group has reported the use of ELP–doxorubicin conjugates for temperature-mediated tumour suppression.[134] The conjugates were taken in to squamous cell carcinoma cells (FaDu) and trafficked into lysosomes *via* endocytosis, but there were no differences in the *in vitro* cytotoxicity of free doxorubicin and the ELP–doxorubicin conjugates, even though their subcellular localisation was significantly different. Further work is undoubtedly necessary to optimize these materials, however, the local accumulation of the ELP-conjugates in tumour cells is a promising first step in drug targeting by responsive synthetic polypeptides.

Conclusions

In this review it has been possible to feature only a small proportion of the literature in this growing research field, yet it is clear that responsive polymers will find many uses in biomedical applications. To date, most of the research in this area has focused on temperature-responsive polymers, as temperature-induced phase transitions are perhaps the best understood in both theoretical and practical terms. In addition, the accessibility of *N*-alkylacrylamide monomers and PEO–PPO block co-polymers has given researchers a large 'tool-kit' with which to carry out fundamental investigations into responsive polymer behaviour. These model studies are beginning to lead to clinical applications as, for example, some PEO-based materials are already approved for pharmaceutical formulations. However, polymers that respond to temperature alone are unlikely to find widespread medical use owing to the difficulties in making changes in local temperature *in vivo*: materials that can respond to a specific biochemical stimulus, such as production of cytokines or inflammatory response signals, are obviously of greater medical relevance. However, in order to make materials of this sort it is first necessary to exhibit very fine control over polymer synthesis such that functionality capable of inducing the desired biological response can be introduced exactly where it is needed. New synthetic techniques mean that it is now possible to produce polymers with defined molecular weight, architecture, (co)monomer content and block distribution, while functional group tolerance of polymerisation catalysts/reagents is allowing nearly all biologically important 'building blocks' to be incorporated into polymer structures. This will lead to materials wherein a variety of responsive elements can be combined, spatially distributed or entirely decoupled, in a way hitherto not possible. Polymer chains can be prepared with individual segments that respond to pH, temperature, ionic strength, UV irradiation and electric fields, affording truly multifunctional materials. 'Chemically-responsive' systems, such as the glucose-sensitive polymers, are also becoming accessible. Structure–function relationships previously only obtainable for biomacromolecules can now be deduced for wholly synthetic materials owing to the degree of control accessible through living polymerisation methodologies, while biopolymer synthesis and activity can be manipulated through molecular biology approaches. This convergence of synthetic and natural macromolecular chemistry inherently leads to biomedical applications, as the ability to control polymer structure leads to the ability to manipulate functionality. Polymer–biopolymer interactions can increasingly be designed as well as selected, and so intervention in cellular dysfunctions may be possible leading to much more powerful, specific and potent therapies.

Carolina de las Heras Alarcón, Sivanand Pennadam and Cameron Alexander*
School of Pharmacy and Biomedical Sciences, University of Portsmouth, St. Michael's Building, White Swan Road, Portsmouth, UK PO1 2DT. E-mail: cameron.alexander@port.ac.uk; Fax: +44 (0) 23 92 84 35 65; Tel: +44 (0) 23 92 84 35 98

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