Thermo- and pH-responsive polymers in drug delivery ☆

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Abstract

Stimuli-responsive polymers show a sharp change in properties upon a small or modest change in environmental condition, e.g. temperature, light, salt concentration or pH. This behaviour can be utilised for the preparation of so-called 'smart' drug delivery systems, which mimic biological response behaviour to a certain extent. The possible environmental conditions to use for this purpose are limited due to the biomedical setting of drug delivery as application. Different organs, tissues and cellular compartments may have large differences in pH, which makes the pH a suitable stimulus. Therefore the majority of examples, discussed in this paper, deal with pH-responsive drug delivery system. Thermo-responsive polymer is also covered to a large extent, as well as double-responsive system. The physico-chemical behaviour underlying the phase transition will be discussed in brief. Then selected examples of applications are described.
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1. Introduction

Synthetic polymers are of increasing interest in drug delivery as therapeutic agent. Polymers show usually an improved pharmacokinetics compared to small molecule drugs with longer circulation time and the potential for tissue targeting. Synthetic polymers are being used as drug delivery systems as a polymeric drug itself or in combination with small molecule drugs or with biomacromolecules such as proteins and poly(nucleic acids). The classification is not complete without mentioning hybrid materials such as modified biopolymers (e.g. modified chitosans) or modified synthetic polymers (e.g. polymer peptide conjugates). The field is usually characterised by the terms 'polymer therapeutics' or 'nanomedicines'. Polymer therapeutics are here divided into 5 subclasses: polymeric drugs, polymer-drug conjugates, polymer–protein conjugates, polymeric micelles and polyplexes (complexes of polymers and poly(nucleic acids)) [1,2]. The area of polymer therapeutics can then be considered as a subcategory in the field of nanomedicines, which encompasses analytical tools and diagnostics, imaging techniques and innovative drug delivery systems, therapeutics and systems for tissue regeneration and repair. All of these are materials within the nanometer size range [3–6]. Therefore any kind of polymers, including stimuli-responsive polymers, falls within this category.

There is an extensive list of criteria a polymer has to fulfil, in order to be applied safely as a polymer therapeutics or as an agent in tissue regeneration and repair. If the polymer is not a drug itself, it often provides a passive function as a drug carrier, reducing immunogenicity, toxicity or degradation, whilst improving circulation time and potentially a passive targeting function. In this case the polymer has to be water-soluble, non-toxic, non-immunogenic and it needs to be safe at all stages of the drug delivery process (e.g. before and after the drug has been released) including a safe excretion. If the polymer is non-degradable (e.g. poly(meth)acrylates), the size needs to be below the renal threshold ensuring that it is not accumulated in the body. If the polymer is degradable (e.g. polyesters), the toxicity and/or immune response of the degradation products have to be considered as well. Polymer therapeutics are new chemical entities, which means that they have to be assessed as such. Besides its application in a passive fashion, synthetic polymers often adopt a more active role such as releasing a drug molecule, peptide or oligo/poly(nucleic acid) upon an external stimulus. In this case, we consider these polymers as stimuli-responsive polymers.

Stimuli-responsive polymers mimic biological systems in a crude way where an external stimulus (e.g. change in pH or temperature) results in a change in properties. This can be a change in conformation, change in solubility, alteration of the hydrophilic/hydrophobic balance or release of a bioactive molecule (e.g. drug molecule). This also includes a combination of several responses at the same time.

In medicine, stimuli-responsive polymers and hydrogels have to show their response properties
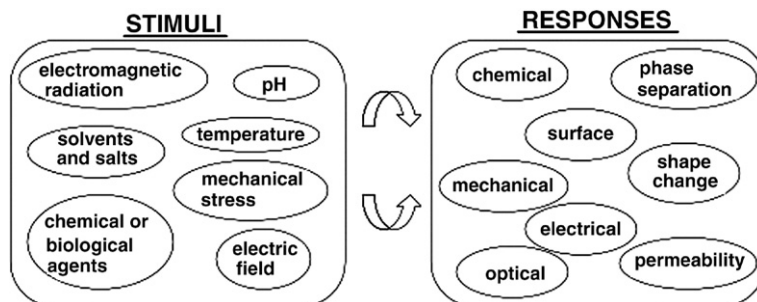
STIMULI

RESPONSES

Fig. 1. Potential stimuli and responses of synthetic polymers.

within the setting of biological conditions, hence there is a large variety of different approaches and only a few selected examples will be discussed here. Further examples are discussed in several review articles [7,8]. Typical stimuli are temperature [9–12], pH [13,14], electric field [15], light [16,17], magnetic field [18], concentration of e.g. electrolytes or glucose. And the responses can also be manifold: dissolution/precipitation, degradation, drug release, change in hydration state, swelling/collapsing, hydrophilic/hydrophobic surface, change in shape, conformational change and micellisation (Fig. 1). The most important stimuli are pH, temperature, ionic strength, light and redox potential [19,20]. This article will focus on temperature and pH as external stimuli, since these systems are predominantly studied. The temperature has to be altered externally in most cases except maybe hyperthermia therapy within narrow limits. But the pH changes within the body and it can therefore be used to direct the response to a certain tissue or cellular compartment (Table 1).

The obvious change in pH along the GI tract [21] from acidic in the stomach (pH=2) to basic in the intestine (pH=5–8) has to be considered for oral delivery of any kind of drug, but there are also more subtle changes within different tissue. Certain cancers as well as inflamed or wound tissue exhibit a pH different from 7.4 as it is in circulation. For example, chronic wounds have been reported to have pH values between 7.4 and 5.4 [22] and cancer tissue is also reported to be acidic extracellularly [23,24]. The same is valid for different cellular compartments [25,26]. Polymers are usually taken up into cells by fluid-phase pinocytosis or receptor-mediated endocytosis. Within the early endosome towards the lysosomes (via late

endosomes) the pH drops from 6.2 to 5.0 giving a large change in proton concentration inside these compartments. The drop in pH (as well as lysosomal enzymes) have been utilised in order to release drug molecules from the lysomes to the cytosol [1]. Intracellular delivery of oligo/poly(nucleic acids) usually uses cationic polymers, which complex the negatively charged nucleic acids. These cationic polymers are then deprotonated within the endosomes, which triggers endosome membrane disruption and release into the cytosol before reaching lysosome with its hydrolytic enzymes [27]. Thus, tailoring the protonation/deprotonation by altering the polymer structure can largely allow fine-tuning of the response in a specific compartment.

In addition, the combination of a pH-responsive system with a thermo-responsive polymer can further alter the hydrophilic/hydrophobic balance. This allows a polymer to become membrane active at a specific temperature [28] and/or a specific pH [29]. In conclusion, this allows a wide range of polymer properties to be used in order to e.g. a) bind to a cell surface, b) disrupt cellular or compartamental mem-

Table 1
pH in various tissues and cellular compartments [21,25,26]

Tissue/cellular compartment	pH
Blood	7.35–7.45
Stomach	1.0–3.0
Duodenum	4.8–8.2
Colon	7.0–7.5
Early endosome	6.0–6.5
Late endosome	5.0–6.0
Lysosome	4.5–5.0
Golgi	6.4
Tumour, extracellular	7.2–6.5

branes or c) release a bioactive compound. Even though we mostly assume that the environmental properties of cellular compartments are constant, one has to note that the polymers themselves can influence the pH of a compartment for example. This is especially the case when the ratio of charges on the polymer to the compartment volume is large, thus the buffering capacity is not sufficient.

Biological systems consist largely of regulation systems; these natural feedback regulation systems are very important to stabilize such non-equilibrium systems like a living organism. One example is release of hormones from secretory cells, which is regulated by physiological cycles or by specific input signals. It is not surprising that also regenerative medicine and drug delivery are utilizing similar responsive strategies in a biomimetic fashion.

Stimuli-responsive polymers have been used in a large variety of applications [30–33]. Even though there are systems, which show a linear response to an external stimulus, it is more interesting to study those polymers with a non-linear behaviour, because biological systems also accomplish specific settings of environmental conditions in different parts of the body (Table 1). This means that a polymer exhibits a large change in properties (response) as a result of a small change in environmental condition (stimulus), which is in addition often reversible. The lower critical solution temperature (LCST) behaviour, as discussed in the next section, is a classical example of a non-linear behaviour. The response can be a reversible collapse/expansion of a polymer chain, a bulk hydrogel or a surface-immobilised hydrogel (Fig. 2).

This article will review advances in stimuli-responsive polymers in drug delivery. It will show how polymers can be used in a smart fashion potentially leading to multiple responses at the desired point of action. A description of the physical basis behind these effects will be provided and the most important types of polymers used will be reviewed. A selection of examples in drug delivery is given and a brief outlook into future aspects is added at the end of this article. Some polymers, which have not been studied within the settings of drug delivery but show potential therefore, will also be discussed. There is a vast number of publications available on this topic, therefore only a selection of examples will be discussed.

2. Thermo-responsive polymers in drug delivery

2.1. Volume phase transitions

Ilmain et al. have classified volume phase transitions according to the nature of the intermolecular forces for hydrogels for example [34]. The phase transitions in terms of the biologically relevant intermolecular forces can rely on several different interactions. (1) Van-der-Waals interaction: Van-der-Waals interaction causes a phase transition in hydrophilic gels in mixed solvents, such as an acrylamide gel in an acetone–water mixture. The non-polar solvent is needed to decrease the dieletric constant of the solvent. (2) Hydrophobic interaction: hydrophobic gels, such as N-isopropylacrylamide (NIPAM) gels, undergo a phase transition in pure water, from a swollen state at low temperature to a collapsed state at high temperature. (3) Hydrogen bonding with change in ionic interaction: gels with cooperative hydrogen bonding, such as an interpenetrating polymer network (IPN) of poly(acrylic acid) and poly(acrylamide), undergo a phase transition in pure water (the swollen state at high temperatures). The repulsive ionic interaction determines the transition temperature and the volume change at the transition [35]. (4) Attractive ionic interaction: the attractive ionic interaction is responsible for the pH-driven phase transition, such as in acrylamide-sodium acrylate/methacrylamidopropyltrimethyl ammonium chloride gels.
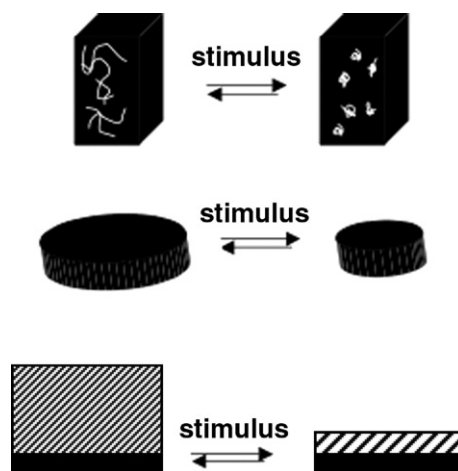

Fig. 2. Collapse/expansion of a polymer chain, a bulk hydrogel or a surface-immobilised hydrogel as response to an external stimulus.

2.2. Thermo-responsive polymers

Temperature-responsive polymers and hydrogels exhibit a volume phase transition at a certain temperature, which causes a sudden change in the solvation state. Polymers, which become insoluble upon heating, have a so-called lower critical solution temperature (LCST). Systems, which become soluble upon heating, have an upper critical solution temperature (UCST). LCST and UCST systems are not restricted to an aqueous solvent environment, but only the aqueous systems are of interest for biomedical applications. The change in the hydration state, which causes the volume phase transition, reflects competing hydrogen bonding properties, where intra- and inter-molecular hydrogen bonding of the polymer molecules are favoured compared to a solubilisation by water. Thermodynamics can explain this with a balance between entropic effects due to the dissolution process itself and due to the ordered state of water molecules in the vicinity of the polymer. Enthalpic effects are due to the balance between intra- and intermolecular forces and due to solvation, e.g. hydrogen bonding and hydrophobic interaction. The transition is then accompanied by coil-to-globule transition. There are also systems, which exhibit both LCST and UCST behaviour, but that is usually not occurring within the setting of the intended biomedical applications. The corresponding hydrogels have similar transitions, the so-called lower gel transition temperature (LGTT) or upper gel transition temperature (UGTT).

Typical LCST polymers are based on N-isopropylacrylamide (NIPAM) [36,37], N,N-diethylacrylamide (DEAM) [38], methylvinylether (MVE) [39,40], and N-vinylcaprolactam (NVCl) [41,42] as monomers (Fig. 3). A typical UCST system is based on a combination of acrylamide (AAm) and acrylic acid (AAc) [43]. PEO-b-PPO block copolymers, PEO-b-PPO-b-PEO and PEG-b-PLGA-b-PEG will not be discussed in detail even though there are some interesting applications [44–46].

The combination of a thermo-responsive monomer like NIPAM with one of a pH-responsive monomer yields double-responsive copolymers [47].

Most applications use the change from e.g. room temperature to body temperature in order to induce a change in the physical properties for e.g. gelation, especially in topical applications and in injectable biodegradable scaffolds. In-vitro applications in cell culture are also using the stimulated swelling and collapsing of hydrogels with their change in surface properties.
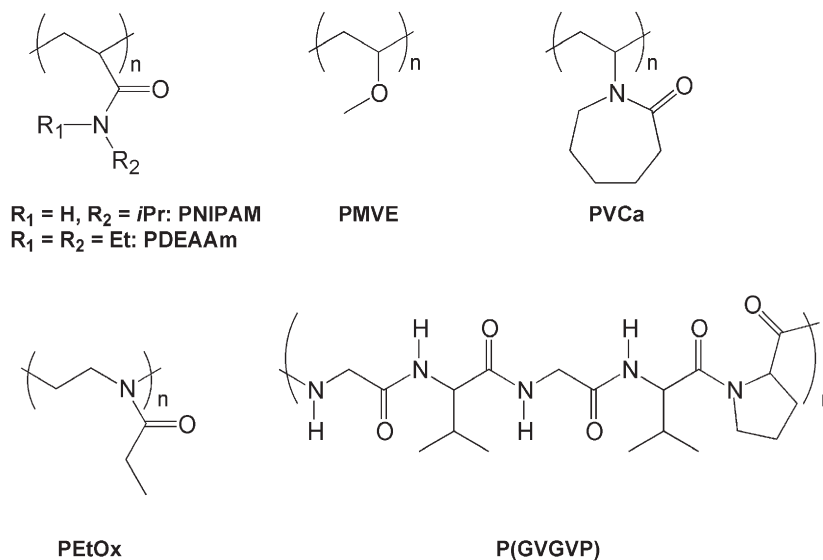

R₁ = H, R₂ = iPr: PNIPAM
R₁ = R₂ = Et: PDEAAm

PMVE

PVCa

PEtOx

P(GVGVP)

Fig. 3. Chemical structure of selected LCST polymers.

2.3. LCST and UCST behaviour

The solubility of a polymer in aqueous solution is dependent on various factors such as molecular weight, temperature or addition of a co-solvent or additive. If the phase diagram of a polymer/solvent mixture vs. temperature shows both a one-phase and a two-phase region, one can identify the critical solution temperature: the UCST or LCST. Often the terms UCST and LCST are used in a misleading fashion, therefore, it has to be noted that they should only be used, if the phase diagram has been determined. Then it is the maximum (UCST) or the minimum (LCST), respectively, of the phase diagram. Any other transition from soluble to insoluble or vice versa (at a given concentration) should be denoted as transition temperature T_{tr}. However, some polymers like PNI-PAM exhibit a phase transition, which is almost independent of the concentration or molecular weight. Then the T_{tr} at any given concentration is almost identical to the LCST. Table 2 gives a selection of polymers with either LCST or UCST behaviour in aqueous solution. These polymers have the transition temperature in the temperature region, which is interesting for biomedical applications (~ 20–40 °C). It has to be noted that the transition temperature can be strongly dependent on factors such as solvent quality, salt concentration, etc. (besides molecular weight and

concentration). Obviously, the transition temperature has to be determined for the setting of the intended application.

One example of a pseudo-natural polymer will be discussed as well. It is the elastin-like polypeptide poly (GVGP), which is usually prepared by genetic engineering [48].

2.4. Influence of the salt concentration, surfactants or co-solvents on the transition temperature

Since the thermo-responsive behaviour depends on the solvent interaction with the polymer and the hydrophilic/hydrophobic balance within the polymer molecules, it is not surprising that additives to polymer/solvent system can influence the position of the volume phase transition. Three interesting "additives" are salts, surfactants and a co-solvent, because all of them relate to the biomedical applications discussed in a later chapter, either as additive in a potential drug formulation or as molecules present in an in-vivo environment. All additives can alter the solvent quality and therefore can alter the polymer–solvent (+additive) interactions. Surfactants are as amphiphiles of particular interest, because as soon as a surfactant absorbs to a polymer molecule it substantially alters the hydrophilic/hydrophobic balance. Therefore, the transition temperature can be shifted to a large extent or it can even disappear. Other aggregation forms such as micellisation can also occur (in contrast to a coil-to-globule transition) [42].

PNIPAM and PVCa differ in their response to addition of a surfactant. Where PNIPAM shows a monotonous increase in the hydrodynamic radius r_H upon addition of an ionic surfactant like sodium dodecyl sulfate (SDS), r_H of PVCa is initially decreasing when adding SDS. In both cases the transition temperature increases with increasing surfactant concentration until it levels out at a certain surfactant concentration [42,49].

2.5. Selected thermo-responsive polymer classes

2.5.1. Poly(N-alkylacrylamide)s

Poly(NIPAM) is the most prominent candidate a thermo-responsive polymer even though a second polymer in this class has a nearly identical transition temperature: poly(N,N-diethylacrylamide) (PDEAM)

Table 2
Selected polymers with LCST or UCST behaviour in the temperature region interesting for biomedical applications

Polymer	Phase transition temperature in aqueous solution	References
LCST behaviour:		
PNIPAM	30–34 °C	[36,37]
Poly(N,N-diethylacrylamide)	32–34 °C	[38]
Poly(methyl vinyl ether)	37 °C	[40]
Poly(N-vinylcaprolactam)	30–50 °C (a)	[41,42]
PEO-b-PPO (b)	20–85 °C	[44]
Poly(GVGVP)	28–30 °C	[48]
UCST behaviour:		
PAAm/PAAc IPN	25 °C	[43]

(a) Strongly dependent on
  MW and concentration
(b) Pluronics, tetronics,
  poloxamer

[36]. However, the transition temperature of PDEAM depends on the tacticity of the polymer, which is in contrast to PNIPAM. Its biocompatibility and the position of the LCST at 32–33 °C makes PNIPAM a very interesting material, e.g. for controlled release application. The LCST of PNIPAM is independent of the molecular weight and the concentration [50], but it can be changed upon shifting the hydrophilic/hydrophobic balance. This can be achieved by copolymerisation with a second monomer. Hydrophobic comonomers increase the LCST, whereas hydrophilic comonomers have the opposite effect [51,52].

Wang et al. have investigated the coil-to-globule transition of PNIPAM in water [53]. They found a hysteresis between the radius of gyration during the heating and the cooling curve and they observed two intermediate states, which gives in total four different, thermodynamically stable states: coil, crumpled coil, molten globule, globule. The fully collapsed globule still contains ∼66% water in its hydrodynamic volume. A change in the solvent quality can alter the volume phase transitions, e.g. by replacing water by deuterated water, which causes an increase in the LCST by 1–2 K indicating that D_2O is a better solvent than water [54].

2.5.2. PNIPAM in drug delivery

PNIPAM copolymers have been mainly studied for the oral delivery of calcitonin and insulin. The peptide or hormone is immobilised in polymeric beads, which stay stable while passing through the stomach. Then in the alkaline intestine the beads disintegrate and the drug is released. Serres et al. [55] and Ramkisson-Ganorkar et al. [56] synthesised P(NIPAM-*co*-BMA-*co*-AAc) for the intestinal delivery of human calcitonin. Kim et al. investigated the delivery of insulin [57]. In all cases the combination of the hydrophobic BMA moiety (butylmethacrylate) and the acrylic acid (AAc), which is non-ionised at low pH, prevents disintegration of the beads in the acidic environment of the stomach. At elevated pH the beads disintegrated due to the solubilisation by the now ionised AAc. In this case the thermal stimulus from the PNIPAM is not needed for the delivery but it is utilised for the preparation of the loaded bead. Similar systems based on NIPAM and AAc have also been used in the form of hydrogels [58]. Again it was aimed at controlled delivery to the intestine.

2.5.3. Poly(methyl vinyl ether) PMVE

Poly(methyl vinyl ether) has a transition temperature exactly at 37 °C, which makes it very interesting for biomedical application. The polymer exhibits a typical type III demixing behaviour, which is in contrast to the thermal behaviour of PNIPAM [59]. Similar to the case of PEtOx (0), PMVE has to be synthesised by cationic polymerisation using inert condition. Nucleophiles like alcohol or amino groups cannot be tolerated during the synthesis, which limits the potential of PMVE.

2.5.4. Poly(N-vinyl caprolactam) PVCa

Poly(*N*-vinyl caprolactam) PVCa has not been studied as intensively as e.g. PNIPAM, but it also possesses very interesting properties for medical and biotechnological applications, e.g. solubility in water and organic solvents, biocompatibility, high absorption ability and a transition temperature within the settings of these applications (33 °C) [42].

2.5.5. Poly(N-ethyl oxazoline) PEtOx

Poly(*N*-ethyl oxazoline)s have a transition temperature around 62 °C, which is too high for any drug delivery application. However we recently prepared a double thermo-responsive system by graft polymerisation of EtOx onto a modified PNIPAM backbone [60]. Currently these systems are explored for their potential in drug delivery, because they tend to aggregate micellise above the LCST. Unfortunately the poly(oxazoline) chemistry has the disadvantage that it is not very tolerant against unprotected functionalities.

2.5.6. Elastin-like oligo- and polypeptides

Polypeptides can also show LCST behaviour, when hydrophilic and hydrophobic residues are balanced well. A polymer made out of the pentapeptide GVGVP as repeating unit exhibits a volume phase transition at 30 °C, which is the hydrophobic folding and assembling transition. Below the phase transition, water molecules are structured around the polymer molecule; the attractive forces weaken upon heating and they finally go into the bulk phase. Above their phase transition temperature, there is the stabilization of secondary supramolecular structure, i.e. a twisted filament structure of β-spirals, which have type II β-turns [61]. The phase transition of these protein-

based polymers can be described in terms of an increase in order. It occurs due to hydrophobic folding and assembly.

The field of elastin-like polypeptides has recently been reviewed by Rodriguez-Cabello et al. [62]. Therefore only one example in drug delivery should be mentioned. Chilkoti et al. have designed a double-responsive doxorubicin-polypeptide conjugate for cancer therapy [63,64]. Since the conjugate is a polymer it can be utilised for passive targeting by the EPR effect [65]. The LCST behaviour of these polymers is tailored in a way that the slightly higher temperature of the tumour is enough to undergo a phase transition, which means that the conjugate becomes insoluble once it reached the targeted tumour. The second responsive is the release of doxorubicin (Dox) via an acid-labile linker. So far the LCST and the linker chemistry has been optimised for Dox release at low pH with in vivo studies to come.

2.5.7. Poly(acrylic acid-co-acrylamide)

An interpenetrating network of poly(acrylic acid) and polyacrylamide is one of the few examples of a system with UCST behaviour within the biomedical setting. The transition temperature is at 25 °C [43]. The UCST behaviour is caused by the cooperative effects coming from the hydrogen bonding between AAc and AAm units.

A similar situation is found for 1:1 copolymers of acrylic acid and acrylamide. So far these systems have been studied as hydrogels including the determination of diffusion coefficients for drug molecules in and out of the hydrogel [66].

3. pH-responsive polymers in drug delivery

The pH is an important signal, which can be addressed through pH-responsive materials. The physiological pH changes have been mentioned earlier (Table 1). Ionisable polymers with a pK_a value between 3 and 10 are candidates for pH-responsive systems [67]. Weak acids and bases like carboxylic acids, phosporic acid and amines, respectively, exhibit a change in the ionisation state upon variation of the pH. This leads to a conformational change for the soluble polymers and a change in the swelling behaviour of the hydrogels when these ionisable groups are linked to the polymer structure.

Classical monomers are acrylic acid (AAc), methacrylic acid (MAAc), maleic anhydride (MA), and N,N-dimethylaminoethyl methacrylate (DMAEMA). But also polymers containing phosphoric acid derivatives have been reported [68,69].

The pH-responsive swelling and collapsing behaviour has been used to induce controlled release of model compounds like caffeine [70], drugs like indomethacin [47], or cationic proteins like lysozome [70,71].

The poly(amidoamine)s designed by Duncan et al. are slightly different since they combine positive and negative charges within the polymer backbone (ISA23: Fig. 8) [72]. On the one side a very unique profile in size changes upon protonation/deprotonation was found with neutron scattering and NMR experiments [73]. The amphoteric backbone yields an expanded shape at low pH, which slowly collapsed when neutral pH is approached. This seems to be the reason that these polymers exhibit endosomolytic properties, which makes them very interesting candidates in cancer therapy, e.g. by delivery of non-permeant toxins like gelonin.

3.1. Polycations in non-viral gene therapy

Cationic polymers are also used in non-viral gene therapy [2]. The polycations can complex nucleotides through electrostatic interaction. The responsive character of the polymer is important when the pH drops during cellular uptake as the polymer becomes more and more charged and triggers osmotic, endosomolytic or other events subsequently. Various amine-based polymers are currently under investigation (Fig. 4), however, there is no clear solution available. So far, transfection efficiency is still below that of viral vectors. In addition, the currently investigated polycations are still too toxic. Hence the search is still on for the right synthetic vector with high transfection efficiency whilst having a tolerable toxicity. Yet, the current studies provide some understanding of the underlying mechanism.

Poly(ethylene imine) (PEI) is still the golden standard against which every new polymer is being tested [74,75], even though a large number of investigated polymers perform better in terms of cytotoxicity and transfection efficiency. Some other candidates are PAMAM and other dendrimers [76–79], poly(N,N-dimethylaminoethyl
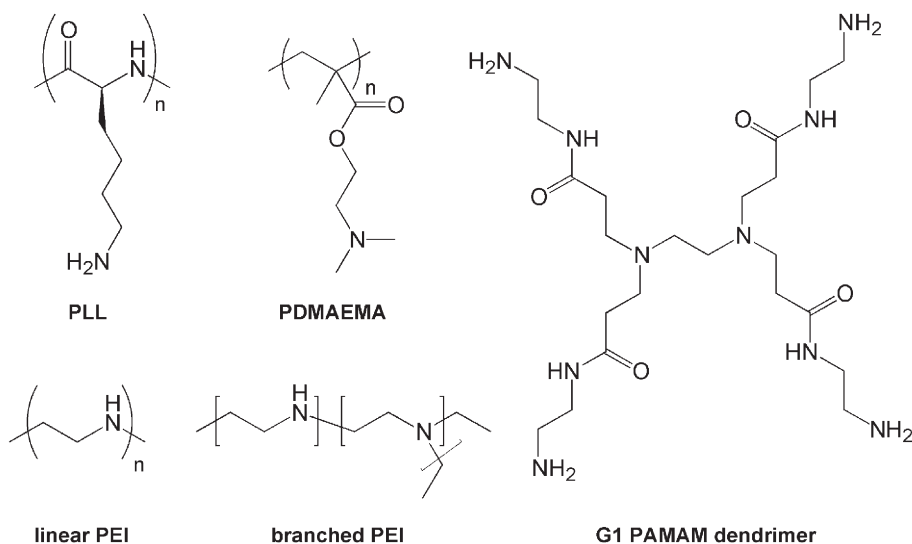
Fig. 4. Chemical structure of some candidates for non-viral gene delivery.

methacrylate) (PDMAEMA) [80,81], poly(amido amine)s [82,83], poly(L-lysine) (PLL) [84] or modified chitosan [85]. Furthermore, the group of Langer has screened a library of different poly(amidoamine) chemistries in order to find molecular parameters, which support efficient transfection [83,86]. Even though some lead compounds where identified, there was no clear direction towards which chemical make-up improves transfection. However not only the chemistry needs to be considered, other factor like charge density, polymer architecture and molecular weight need to be studied as well. For example, there are changes in cellular trafficking between linear PEI and branched PEI [87]. Thanou et al. studied trimethylated chitosan in relation to molecular weight and degree of trimethylation. Besides the fact that transfection efficiency was improved compared to PEI, the study showed that cytotoxicity decrease with decreasing molecular weight and decreasing degree of trimethylation [85]. Langer et al. studied a series of dendritic-linear hybrid polymer based on PAMAM dendrimers and poly(ethylene glycol) (PEG), which also contain a targeting moiety for a cell surface receptor [88]. These hybrid polymers self-assemble together with DNA to nanoparticles of around 200 nm diameter, which have a PEGylated outer shell bearing cell surface receptor targeting moieties (Fig. 5). Compared to PEI, the results show an improved transfection with lower toxicity. This example is interesting, because

it also demonstrates a certain relationship between transfection efficiency and size and polymer architecture. This indicates that there is potentially a multitude of factors responsible for an efficient cellular uptake with low toxicity. Furthermore, one should not forget that there are also differences depending on the choice of cellular or tissue model.

A non-viral gene delivery agent needs to fulfil a whole set of requirements to become successful (Fig. 6). The first step is the condensation of the polycation with the anionic oligo/polynucleotide. This polyion complex (or polyplex) is usually very compact with a size of tens
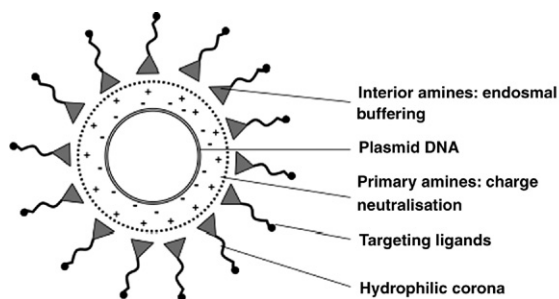

Fig. 5. Self-assembly of PAMAM-PEG-mannose dendritic-linear hybrid polymer with mannose targeting moiety; the system contains primary amines buffering (pK$_a$=6.9) for complexation of plasmid DNA and tertiary amines (pK$_a$=3.9) for endosomal buffering, PEG stabilises the polyplex.
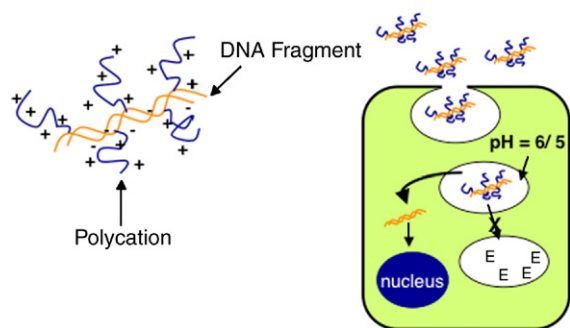
Fig. 6. Simplified view on cellular uptake of polyplexes.

to hundreds of nanometers. The preparation method has a large influence on the structure and shape of the polyplex [89]. There is slight excess of positive charges for protection of the nucleotides from destruction by nucleases, but also to effectively bind to the negatively charged cell surface. Once the target tissue is reached, the complex needs to be taken up by the cells. This happens usually through fluid-phase pinocytosis or receptor-mediated endocytosis. The endosome is the first cellular compartment, however with ongoing time the early endosome matures towards a late endosome and finally it becomes a lysosome. This mean the environment becomes more and more hostile for the polynucleotides, because the pH continuously drops and hydrolytic enzymes are added to the mixture. Therefore the complex or the dissociated polynucleotides needs to escape the endosome into the cytosol. Here the pH-responsiveness of the polycation comes into play, because as soon as the cell tries to acidify the late endosome, the polycation can buffer the system and keep the pH higher than normally expected. This either increases the osmotic pressure in such a way that the endosome burst and releases its content into the cytosol (proton-sponge effect [90]). This is happening for example with PEI upon cellular uptake. Otherwise the complex translocates from the endosome by another mechanism. Once inside the cytosol the polynucleotides still needs to find its target. Entering the nucleus is a particular bottleneck. Usually this is enhanced during mitosis when the nuclear membrane is temporarily absent [91].

After successful gene delivery, the encoded protein needs to be expressed efficiently. There is still ongoing debate about a suitable method and target due to the currently low transfection efficiencies.

3.2. Acid triggered drug release in cancer targeting

A change in pH can be utilised in two ways in order to have controlled and triggered release of a drug load. First, in the extracellular tissue: tumour tissue has extracellularly a pH_e of 6.5–7.2, thus slightly lower than the normal pH of 7.4 [92]. A second is used after cellular uptake when the drug conjugate reaches the lysosomes with a pH of 4.5–5.0. In the latter case, hydrolytic enzymes, such as cathepsin B, are also frequently utilised to release a drug load [1]. The nanometer size of the drug conjugates or micellar structures allows passive targeting by the EPR effect both cases [65].

Bae et al. investigated the weak acid sulfonamide (SD) as trigger for extracellular delivery of doxorubicin [92]. Micelles with system are kept in solution due to the partial charges at the SD. If SD (as a weak acid) is further deprotonated upon acidification, the micelle collapses and is no longer solvated. This means collapsed nanoparticles with doxorubicin accumulate in the tumour tissue and are subsequently taken up the cells. Poly(L-histidine)-b-PEG in combination with PLLA-b-PEG and adriamycin as drug was also studied for an extracellular tumour targeting. The system shows a very sharp transition from non-ionised (non-protonated) and hydrophobic at pH 7.4, where the mixed micelles are stable, to ionised and micelle-destabilising at ca. pH 6.6. Adriamycin is rapidly released from the micelles at this pH value [93].

Drug molecules conjugated to a polymer are usually inactive. Therefore these conjugates are called prodrug. This is an advantage for cytotoxic drugs, e.g. in cancer therapy, because the incorporation of a targeting system can avoid or at least minimise adverse side reactions due to non-specific toxicity. However, only an efficient release of the drug at the site of action gives these prodrugs the full advantage. Aside from the aforementioned pH drop in the extracellular cancer tissue, the delivery of the polymer inside the cell to the lysosome after cellular uptake can be used for a release mechanism a) through hydrolytic enzymes present in the lysosome (e.g. cathepsin B) and b) through the acidic environment; both paths have been used [1,94]. Most prominent acid-labile linkers, which have been used in a pH-triggered release mechanisms, are cis-aconityl acid, Schiff's base derivatives (Fig. 7).

For example adriamycin has been conjugated to IgM via a *cis*-aconityl linker [95].

3.3. Polyanions and amphoteric polymers for endosomolytic delivery

If a polyanion is slowly acidified, the carboxylate will be increasingly protonated and the polymer backbone becomes increasingly hydrophobic. At a certain point the deprotonated polymer becomes membrane active. This is accompanied by a coil-to-globule transition of the polymer [92]. The pH, at which this is happening, depends on the hydrophilic/hydrophobic balance, e.g. poly(acrylic acid) is protonated at ca. pH 3. Addition of more hydrophobic alkyl residue to the backbone shifts the transition to higher values (Fig. 8). This opens an avenue towards endosomolytic delivery, if the drug must not reach the lysosome. If the transition happens at endosomal pH (ca. pH 6.0), the drug load can escape to the cytosol and exert its action. Several polymers have been proposed and studied (Fig. 8). First experiments are usually carried out with liposomes or vesicles, sometimes a haemolysis test is conducted.

However, conclusions can be misleading because the lipid composition of the model systems is different from that of endosomes.

As mentioned earlier amphoteric poly(amido amine)s (PAA) show endosomolytic activity. Again the adjustment of the pK_a and the spatial position of the charges seem to have an influence on the transition point [82,96]. One example of such an ampholytic PAA is ISA23 (Fig. 8). A second class are the poly(L-histidine)s with a pK_a of 6.0. These were successfully
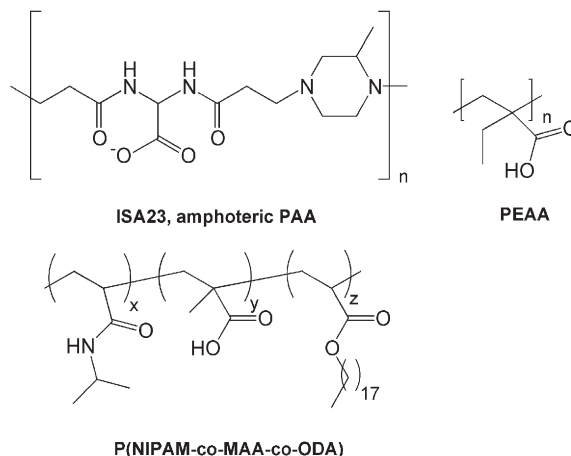
Fig. 8. Chemical structure of endosomolytic polymers and potential candidates, PEAA = poly(2-ethylacrylic acid), MAA = methacrylic acid, ODA = octadecylacrylate.

used in gene delivery in combination with poly(L-lysine) [97].

4. Expansion of concepts of stimuli-responsiveness

4.1. Double-responsive systems and micelles with stimuli-responsive behaviour

A series of double-responsive system, some of which assemble into micelles, have been reported. Double- or multi-responsive systems can be distinguished generally based on the polymer architecture. Random copolymers are used to tailor the transition point depending on two independent parameters, e.g. pH and temperature. In contrast block copolymer tend to self-assemble reversibly and form micelles depending on the environmental conditions.

The micelles are then either stabilised through strong non-covalent interaction (e.g. ionic) or fixed through subsequent crosslinking. In both cases one is receiving a nano-object, which can be utilised as a micellar responsive drug delivery system, but it can also mimic biological entities like e.g. vesicles [98].

Armes et al. produced shell crosslinked micelles by polyelectrolyte complexation by ATRP polymerisation [99]. The controlled polymerisation technique leads to uniform di- and triblock copolymers, which assemble into micelles with pH dependent size: 25–30 nm in acidic solution and 35–50 nm in alkali solution
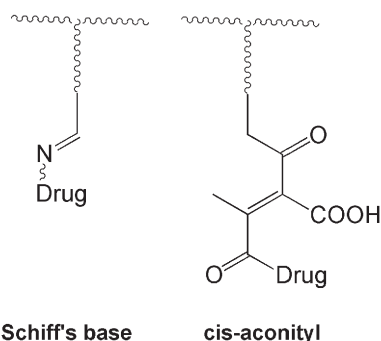
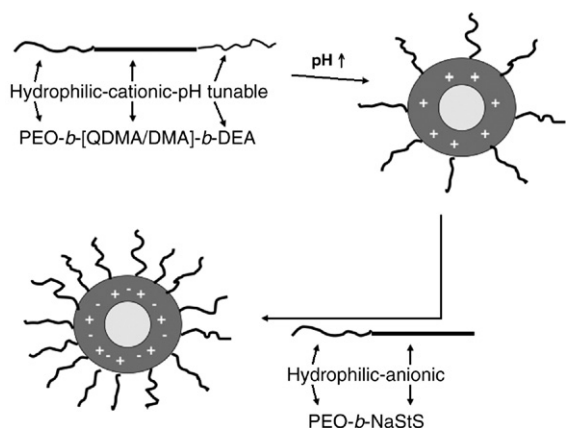Fig. 7. Acid-labile linker for drug conjugation.

Fig. 9. Formation of shell crosslinked micelles by polyelectrolyte complexation. The cationic triblock copolymer PEO-*b*-[QDMA/DMA]-*b*-DEA has a pH tunable PDEA block, which causes micelle formation at high pH; addition of the anionic diblock copolymer PEO-*b*-NaStS stabilises the micelle through ion complex formation.

(Fig. 9). These micelles remain stable between pH 3 and 10 even though they are only non-covalently bound. This is due to the pH-responsive behaviour of the PDEA block in the centre of the micelle.

Other examples have been reported by Wooley et al. [100] and Laschewski et al. [101] They used different crosslinking mechanism and various types of responsive behaviour for the formation and stabilisation of the micelles.
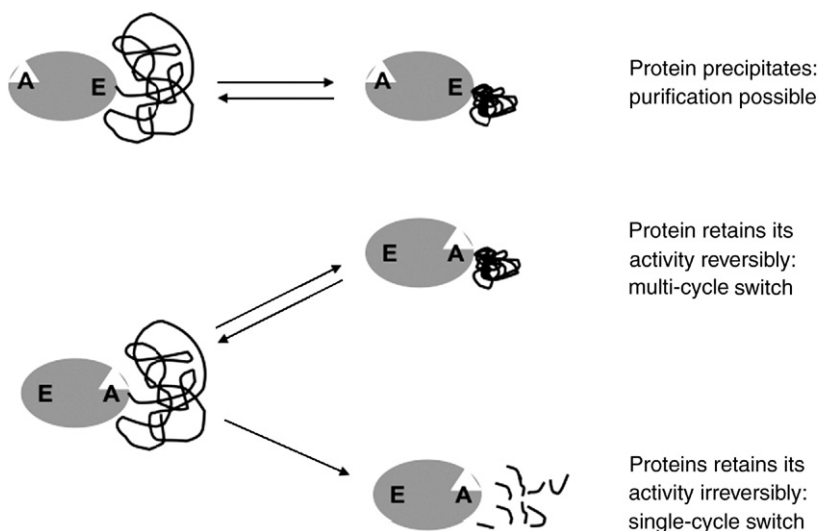

Fig. 11. Controlled cell adhesion and detachment through thermoresponsive hydrogels. PNIPAM based hydrogels are non-adherent below the LCST and adherent above the LCST; at high temperature bioactive molecule can be entrapped and subsequently released upon lowering the temperature.

4.2. Responsive polymer–protein conjugates

Responsive polymer–protein conjugates have been designed in various ways. Three important strategies will be highlighted briefly (Fig. 10). Usually a polymer is conjugated to a protein to influence the accessibility of the active site of e.g. an enzyme or a receptor binding-site. One strategy seeks to attenuate this accessibility (and therefore the proteins activity) in a reversible fashion. Thus by changing the environmental condition, it is possible to alter e.g. an enzyme activity. Hoffman et al. developed both thermo- and photoresponsive polymer–protein conjugates [102,103]. They synthesized a photoresponsive PNIPAM-endoglucanase conjugate by site-specific conjugation. The endoglucanase becomes inactive for glycoside hydrolysis under irradiation at 350 nm [103].

Fig. 10. Responsive polymer–protein conjugates. An enzyme E with an active site A is conjugated to a polymer; the activity changes depending on the position of the attachment and the kind of switch.
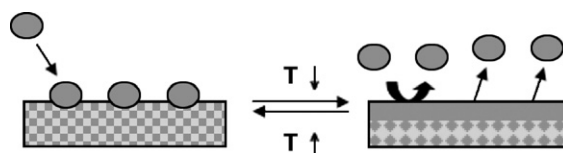
A second strategy is masking potentially toxic protein or avoiding adverse immune response before the protein reaches its target tissue. Once the target tissue is reached, a trigger is used to irreversibly remove the masking polymer and release the unmasked protein, which in an ideal situation retains its full activity at the site of action.

Duncan et al. studied polymer-phospholipase conjugates, where the enzyme activity can be tuned by conjugation of the polymer to the enzyme [104]. They further conjugated various poly(amido amine)s to melittin and were able to mask the haemolytic activity of melittin almost completely [105]. This opens the possibility of melittin delivery as an anti-cancer agent.

The third strategy is simply to make the polymer–protein conjugate insoluble when the polymer goes through the phase transition. This allows an easy purification step by centrifugation while the product stays in the supernatant. This particular application is interesting in biotechnology, where often the only alternative is to immobilise the protein on a solid substrate (e.g. beads) with severe loss in activity. Hoffmann et al. demonstrated this strategy with the conjugation of PNIPAM to trypsin [106]. The conjugate was stable over multiple cycles and retained 95% of the native activity throughout.

4.3. Responsive hydrogels

A lot of work has been carried out on stimuli-responsive hydrogels, especially temperature-sensitive hydrogels using PNIPAM as the thermo-responsive unit. Various groups [107–110] work on cell culture carrier with or without the option of immobilising bioactive molecules and subsequently releasing them (Fig. 11). This technique may be applied e.g. in the transplantation of retinal pigment epithelial cell sheets, which can be recovered without any defects [111].

In contrast, there are also studies on micro- or nanogels, which can again be used in drug delivery. Especially the immobilisation of hydrolytically sensitive molecules like peptides and proteins has been accomplished, e.g. lysozyme was immobilised in PLGA [112], and Peppas et al. prepared anionic pH-sensitive hydrogels for calcitonin entrapment [113]. These proteins can then be released upon a thermal stimulus since they are only physically entrapped.

Current developments go towards the synthesis of fast responding PNIPAM hydrogels avoiding the skin layer formation upon rapid temperature change. This has been achieved by grafting the thermo-responsive PNIPAM arms onto an inert hydrogel matrix [58].

5. Conclusion

Stimuli-responsive polymers offer great advantages in drug delivery. Instead of acting passively as pure drug carriers, they will interact and respond to the environmental setting. This allows us to aim further for tailor-made drug delivery with superior pharmacokinetics while having all safety questions addressed. Unfortunately, we often do not know the basic parameters in order to establish where, how and when our drug delivery system reaches a particular tissue or cellular compartment. The many open questions e.g. around gene delivery indicate that much more need to be understood to synthesize the most suitable vector or polymer therapeutic.

The discussion of targeting extracellular cancer tissue indicates that we are often operating within narrow margins. Within these limits, we wish to have ideally an on–off response. This leads to further effort on the synthesis of the responsive polymer. Similar to the ongoing trend towards site-specific protein conjugation, we should aim for (nearly) monodisperse sample with all reaction sites known. Besides the better control over the purity of our samples, this would lead to more reliable structure–property relationships and potentially increases the patient's safety.

Relatively new synthetic strategies like dendrimer synthesis and controlled polymerisation techniques are now quite well established for achieving these goals.

Acknowledgements

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