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Thermo-responsive systems for controlled drug delivery

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Controlled drug delivery systems represent advanced systems that can be tightly modulated by stimuli in order to treat diseases in which sustained drug release is undesirable. Among the many different stimuli-sensitive delivery systems, temperature-sensitive drug delivery systems offer great potential over their counterparts due to their versatility in design, tunability of phase transition temperatures, passive targeting ability and *in situ* phase transitions. Thus, thermosensitive drug delivery systems can overcome many of the hurdles of conventional drug delivery systems in order to increase drug efficacies, drug targeting and decrease drug toxicities. In an effort to further control existing temperature-responsive systems, current innovative applications have combined temperature with other stimuli such as pH and light. The result has been the development of highly sophisticated systems, which demonstrate exquisite control over drug release and represent huge advances in biomedical research.

Keywords: drug delivery, stimuli-sensitive, temperature-sensitive

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1. Introduction: modulated delivery systems

Controlled drug delivery systems have evolved from traditional drug delivery systems in order to tailor drug release profiles to the physiological need for a particular drug. Such clinical conditions as diabetes, rhythmic heart disorder, hormone replacement therapy, birth control and chemotherapy require the release of the drug in response to biological rhythms (chronopharmacology) or at the onset of a certain disease condition [1]. Consequently, these controlled delivery systems are intended to deliver drugs in response to physiological requirements or the presence of certain biomolecular stimuli at predetermined time intervals. The most strategic approach would be to deliver the drug precisely upon physiological need to the delivery site via site-specific targeting at the proper times to ensure maximum drug efficacy. Such an approach requires an in-depth understanding of the pathophysiology of the disease so as to be able to design advanced drug delivery systems that can respond not only to the environment, but also to the intricate and sensitive homeostatic needs of the body.

In recent years, there has been significant progress towards the design of 'smart' delivery systems, which are designed to mimic normal physiological processes or to respond to the presence of specific stimuli. These systems can be broadly classified into either closed-loop or open-loop delivery systems, depending on the nature of drug release from the system. In closed-loop delivery systems, the drug is released in response to biochemical changes in the local environment, that is the presence or absence of specific molecules. Several research groups have designed delivery systems that facilitate the release of the drug in response to local changes in pH [2,3], glucose [4,5] and chloride ions [6]. In contrast, open-loop delivery systems involve the release of the drug in response to an external stimuli such as ultrasound [7], electric and magnetic fields [8,9], light [10], mechanical

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forces [11] and temperature. These open-loop delivery systems are commonly referred to as 'pulsatile' or 'externally regulated' systems and they are by far the most frequently explored systems for controlled drug release.

Temperature-sensitive drug delivery systems are the most widely explored class of environmentally sensitive polymers because of their ease of control and preparation, as well as for their practical applications. In particular, injectable thermo-responsive polymers can be introduced into the body in a minimally invasive manner prior to undergoing a phase transition to a solid or gel state, thereby circumventing the need for a surgical procedure for placement of the system. These systems can thus be molded into any desired shape *in situ* and can be formulated with therapeutic molecules by simple mixing. In addition, temperature-sensitive polymers used in all drug delivery systems are functional in the physiological range as well as the tumor-targeted range involving hyperthermia (42°C). Thus, temperature-sensitive systems are very promising stimuli-sensitive systems that have practical applications for controlled drug delivery.

This review will focus on the recent developments in drug delivery systems that are based on temperature-sensitive systems, which are able to undergo reversible volume phase transition and sol–gel phase transition in response to temperature. These drug delivery systems include poly(*N*-isopropylacrylamide) (PNIPAAm) copolymers; thermo-sensitive poly(ethylene glycol) (PEG) analogs; thermogelling systems; thermoresponsive elastin-like peptides (ELPs); and thermosensitive liposomes.

2. Poly(*N*-isopropylacrylamide)-based drug delivery systems

PNIPAAm polymer and its derivatives are some of the most extensively studied polymers in the field of drug delivery, due to its resilient lower critical solution temperatures (LCST), which are in the range of 25 – 32°C [12]. This range of LCST temperatures is highly attractive because of its close proximity to physiological temperatures that can trigger a reversible volume phase transition of the polymers without causing injury to surrounding tissues. The driving force for this phase separation is governed by the balance of hydrophilic and hydrophobic moieties along the polymer backbone and the free energy of mixing associated with enthalpy, entropy and the temperature of the system [13,14]. These energies are associated with the hydrogen bonding of the caged water molecules surrounding the hydrophobic groups on the polymer and the hydrophobic interactions between the hydrophobic groups on the polymer chains. At temperatures below the LCST, the polymer is in the most thermodynamically stable state, in which water molecules form a hydrated cage around the hydrophobic moieties along the polymer chain. However, at temperatures above the LCST, the hydrogen bonding between the polymer moieties and water molecules become thermodynamically unfavorable compared to polymer–polymer and water–water interactions, resulting in the desolvation of the hydrophobic moieties along the polymer chains. Thus, there is an increase in the entropy of the water molecules of the system as the temperature increases, resulting in the collapse of the polymer chains as the hydrophobic interactions increases [15-17]. In addition, the LCST can be modified by adjusting the ratio of hydrophobic or hydrophilic segments of the polymer chains in which an increase in hydrophilic groups increases the LCST and an increase in hydrophobic groups has the opposite effect [14]. Tantamount to this is the fact that the LCST of PNIPAAm is resilient to environmental conditions such as salt, pH and concentration effects. Moreover, PNIPAAm-based polymeric systems have demonstrated little or no cytotoxicity and good biocompatibility. PNIPAAm/acrylic acid hydrogels, when combined with isolated islets of Langerhans, showed no cytotoxicities at physiological temperatures [18]. Scaffolds consisting of PNIPAAm have supported the regeneration of nerve fibers in cell-delivery systems [19], enabled rapid wound healing [20] and limited foreign body reactions after implantation [21]. In addition, pH-responsive PNIPAAm polymeric micelle systems showed no cytotoxicity in EMT-6 cells and biodistribution studies revealed accumulation of the system in the liver, spleen and lungs [22], which is typical of macromolecular delivery systems. These properties of PNIPAAm and its ease of preparation have made this polymer very attractive for use in many types of drug delivery systems, including hydrogels, block copolymers and liposomes. The structure of PNIPAAm and some poly(*N*-substituted acrylamide) polymers are shown in **Figure 1**.

2.1 Temperature-triggered hydrogels

Hydrogels are a well-known class of biomaterials that have a broad range of applications in the biomedical [23,24], pharmaceutical [25-27] and tissue engineering fields [28,29]. These materials are three-dimensional polymeric networks formed by crosslinking the chains via covalent, physical or ionic interactions to yield a tissue-like scaffold that imbibes large amounts of water and can be neutral or ionic depending on the ionization of the pendant side chains. Temperature-sensitive hydrogels based on PNIPAAm have been widely explored in the field of drug delivery. However, one of the main disadvantages of PNIPAAm hydrogels in drug delivery is their intrinsic poor mechanical properties that lead to suboptimal drug release profiles, which are difficult to control. Thus, even though dehydrated crosslinked PNIPAAm hydrogels are hard and brittle, they become very fragile in their swollen state [30].

To overcome these hurdles, hydrogels composed of PNIPAAm have been modified via copolymerization in an effort to not only improve their mechanical properties, but also to develop more sophisticated devices that offer fine control of the system. Copolymers of *N*-isopropylacrylamide and butyl methacrylate (BMA) were used to prepare

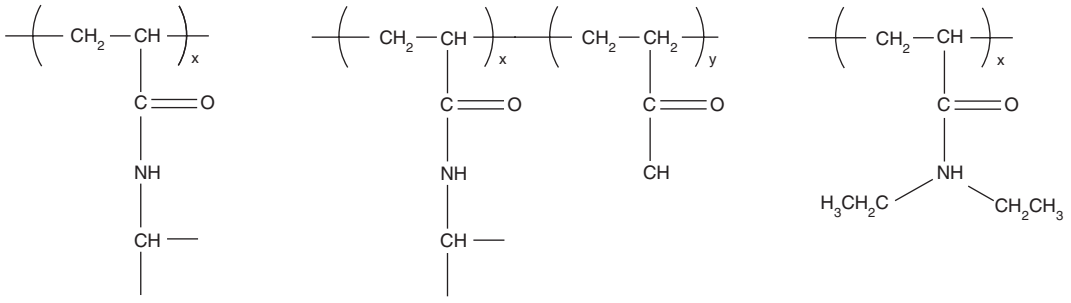

Figure 1. Chemical structures of PNIPAAm and some derivatives of poly(*N*-substituted acrylamide) polymers.

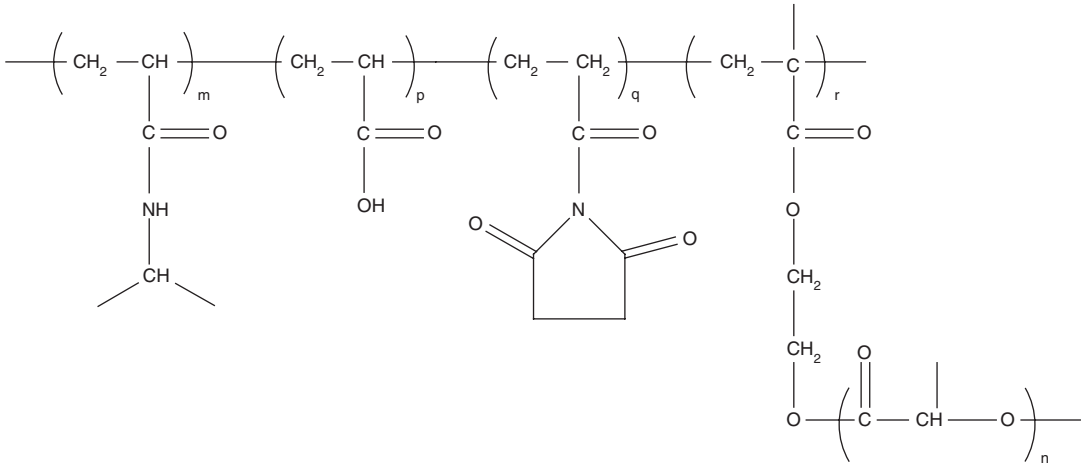

Figure 2. Chemical structure of synthesized copolymers of poly(NIPAAm-*co*-acrylic acid-co-*N*-acryloxysuccinimide-*co*-hydroxyethyl methacrylate-polylactide) (P(NIPAAm-*co*-AAc-*co*-NAS-*co*-HEMAPLA)).
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thermosensitive hydrogels with increased mechanical strength [31-34]. These hydrogels yielded on–off drug release profiles of the model drug indometacin, which showed pulsatile drug release as well as release profiles for insulin and glucose from the matrices as a function of temperature. The release profiles for these drugs followed pseudo zero-order or first-order release kinetics at low temperatures, while diffusion was inhibited at high temperatures. This on–off release was explained in terms of the formation of a polymer surface skin that not only localized a high water content inside of the polymer matrices, but also restricted the diffusion of the drug out of the matrices as the temperature increased. This modulated release of the matrix was also found to be dependent on the length of the methacrylate alkyl side chain. Guan *et al.* have synthesized copolymers of NIPAAm, hydroxyethyl methacrylate-polylactide (HEMA-PLA), acrylic acid (AAc) and *N*-acryloxysuccinimide (NAS) to form thermoresponsive hydrogels (**Figure 2**) that were readily injectable at low temperatures and would form gels with high tensile strength and distensibility at 37°C [35]. The macromer HEMAPLA was included in the copolymers so as to introduce biodegradable bonds in the hydrogels that would increase the biocompatibility of the hydrogels upon PLA hydrolysis. The AAc was added to increase the hydrophilicity of the hydrogels, and NAS was used to provide bioconjugation sites for biomolecular binding. In addition, the researchers added type I collagen into the hydrogels so as to improve the biocompatibility of the hydrogels. PNIPAAm is degraded *in vivo* into monomers by hepatic glutathione *S*-transferase into acrylamide that is known to be carcinogenic [36-38]. These hydrogels showed LCSTs above 40°C with high tensile strengths from 0.3 to 1.1 MPa and elongations at break from 344 – 1841% as a function of NIPAAM/HEMAPLA ratio, AAc content and PLA length. The biocompatibility of the hydrogels was comparable to the control surfaces following collagen incorporation. Fang *et al.* have also synthesized copolymers of PNIPAAm, chitosan (CPN) and hyaluronic acid (HA) that form

hydrogels in order to increase the biocompatibility and versatility of the PNIPAAm polymers [39]. The drug release profiles for nalbuphine, indometacin and a nalbuphine pro-drug were obtained from these hydrogel copolymers. The release rate of hydrophilic nalbuphine increased in the order of CPN < CPNHA < PNIPAAm and the release of the lipophilic drugs was opposite to that observed for nalbuphine. The configuration of the PNIPAAm copolymers in hydrogels has been modified to develop self-assembled gels with faster response times. Lin and Cheng have developed block and star block copolymers of PNIPAAm and PEG as injectable hydrogels of various architectures of types AB, $A(B)_2$, $A(B)_4$ and $A(B)_8$, which showed fast gelation kinetics and reversible thermal behavior [40]. Copolymers of PNIPAAm with acrylic acid (AA), polyamidoamine (PAMAM) dendrimers, vinyl pyrrolidone-acrylic acid (VP-AA) and poly(hydroxyethyl methacrylate) (PHEMA) have been designed to address the slow deswelling rates of PNIPAAm gels and to improve the release kinetics of the hydrogels [41-44], while sulfonamide and hydroethylacrylate (HEAc) monomers have been polymerized with PNIPAAm to combine pH and temperature stimuli to develop injectable systems that do not undergo phase transitions at the physiological LCST [45,46]. Bikram *et al.* have developed composite hydrogels of silica-gold (SiO_2-Au) nanoshells and NIPAAm copolymerized with acrylamide (NIPAAm-co-AAm) to form photothermal modulated drug delivery system in which near infrared (NIR) light can be used to induce the collapse of the polymeric matrix loaded with model drug molecules (**Figure 3A**) [47]. Nanoshells consist of a dielectric core surrounded by an ultrathin metal shell that impart tunable plasmon resonances within the NIR regions [48,49]. The release profiles for the model drug methylene blue and the proteins insulin and lysozyme from these composite hydrogels were found to be dependent on the concentration of the nanoshells and the fluence of the laser used to irradiate the gels, as well as the molecular weight of the drug molecules (**Figure 3**). The release of methylene blue from the composite hydrogels showed that there was little control of drug release from the hydrogel despite higher amounts of the model drug released at 18 and 24.5 min (**Figure 3B**). The data indicated that the low molecular weight drug was small enough to diffuse freely from the hydrogel, independent of pore size and the tortuosity of the hydrogel. In contrast, the data for insulin and lysozyme release from the hydrogels showed that the pore size and tortuosity of the hydrogel significantly affects the diffusion of higher molecular weight proteins (**Figure 3C** and **D**).

2.2 PNIPAAm block copolymers

In addition to random copolymers comprised of *N*-isopropylacrylamide and pH [50] or hydrophilic/hydrophobic [51] groups that have been developed to modulate the LCSTs, block copolymers consisting of PNIPAAm and other polymers have been developed as drug delivery systems. Block copolymers of the type AB typically consist of a hydrophobic block and a hydrophilic block that assemble into micellar structures in which the hydrophobic block is sequestered in the core of the micelle surrounded by the hydrophilic corona. This type of macromolecular structure represents the most thermodynamically stable structure that maintains the macromolecule in solution. Okano and researchers have extensively studied polymeric micelles based on PNIPAAm as thermoresponsive drug delivery systems [52-58]. P(NIPAAm-b-DL-lactide) (PNIPAAm-PLA) block copolymers were synthesized by ring-opening polymerization having LCSTs from 38 – 42°C and micelle formation was monitored with dynamic light scattering, which showed nanoparticles with diameters of ~ 40 nm between 20 – 30°C. Block copolymers of PNIPAAm and butyl methacrylate (PNIPAAm-b-PBMA) were used to load adriamycin into micelles, which showed reversible thermoresponsive on/off switching in response to its LCST. Neradovic *et al.* developed block copolymers of poly(ethylene glycol) (PEG) as a hydrophilic block and PNIPAAm or poly(NIPAAm-co-*N*-(2-hydroxypropyl) methacrylamide-dilactate) (poly(NIPAAm-co-HPMAm-dilactate)) as the thermosensitive block that could self-assemble into nanoparticles [59-61]. These copolymers formed a new type of thermosensitive micelle with a unique drug release mechanism. The incubation of these copolymers in aqueous solution resulted in an increased LCST of the polymers from 31 – 37°C, due to hydrolysis of the hydrophobic lactate ester side group, which consequently produced poly (NIPAAm-*co*-HEMA) with an increase in hydrophilicity. Thus, at temperatures above the LCST of 37°C, the particles were destabilized to release their cargo. The size of the nano-particles with PEG 2000 were found to be 50 – 70 nm at temperatures below the LCST of the polymer (26.5 – 27°C), but the size increased significantly to ≥ 200 nm above the LCST. This was attributed to the lack of stabilization of the nanoparticle by the lower molecular weight PEG. In contrast, block copolymers with PEG 5000 or PEG 10,000 produced small particles (≤ 200 nm), which was the result of an increased dehydration of the block copolymers and more collapse of the temperature-sensitive block, resulting in more condensed particles. You and Oupicky developed Y-shaped heterobifunctional block copolymers of PNIPAAm and PEG using reversible addition-fragmentation chain transfer (RAFT) polymerization as a means of synthesizing α, ω-functionalized polymers having low polydispersity indices (PDIs) (**Figure 4A**) [62]. The LCST of the copolymers was 32°C, in which the PNIPAAm chains collapse to form a nanoparticle core with a hydrated PEG shell surrounding the temperature-sensitive center (**Figure 4B**). The terminus of the PNIPAAm block was functionalized with biotin to facilitate surface presentation of ligands to the stimulus-sensitive polymer. In addition to block copolymers, triblock, multiblock and graft copolymers have also been prepared with PNIPAAm polymers so as to develop tightly controlled stimuli-sensitive drug delivery systems [63-65]. Recently, a polypeptide hybrid double hydrophilic diblock copolymer (DHBC) consisting of

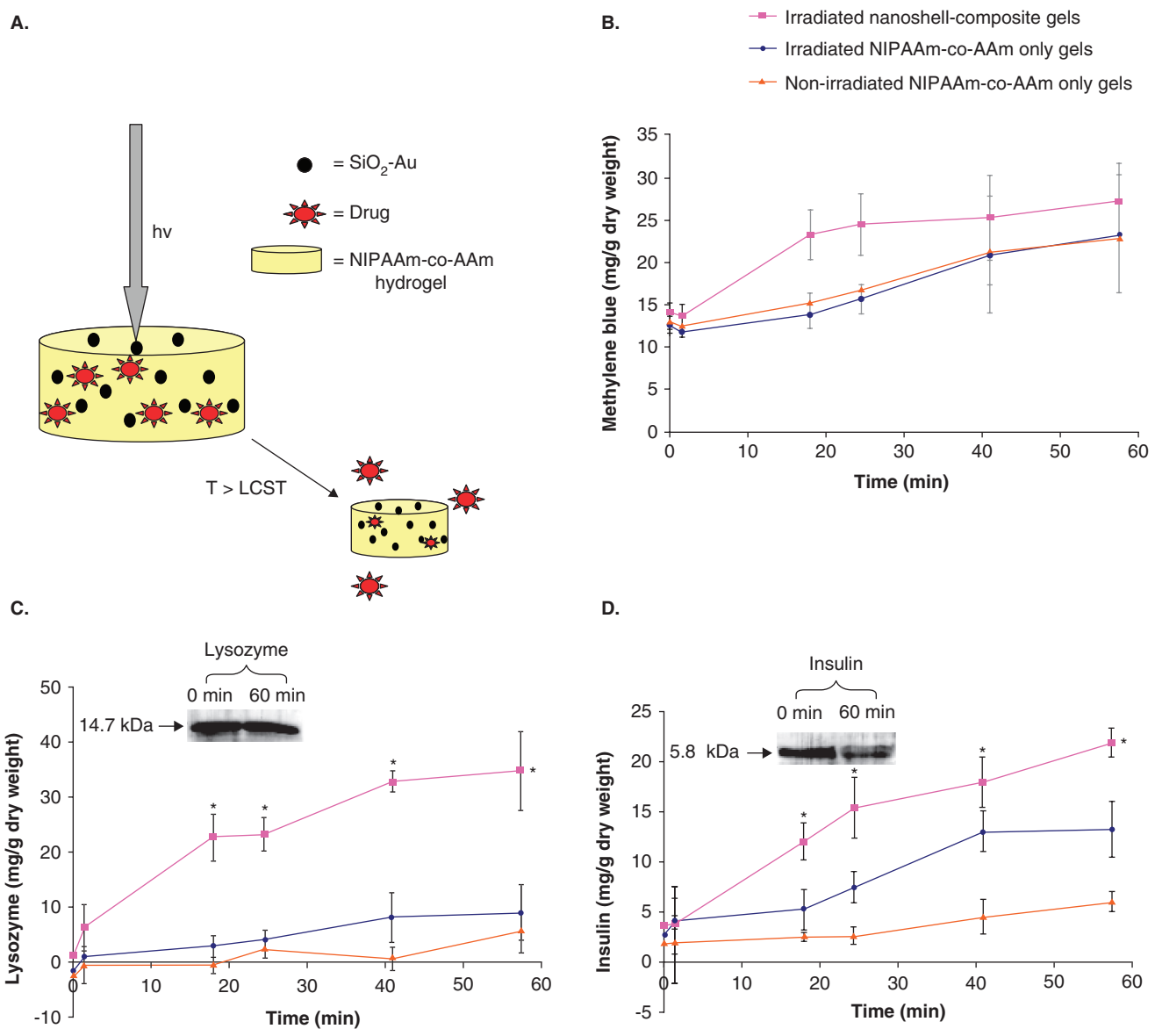

Figure 3. Drug delivery design of composite hydrogel and the release profiles of model drugs from fabricated hydrogels. (**A**) Schematic representation of laser irradiation of nanoshell-composite hydrogels loaded with drug molecules resulting in heating above the lower critical solution temperature (LCST) and subsequent collapse of the hydrogels, (**B**) drug release profile of methylene blue from composite hydrogels, (**C**) drug release profile of insulin protein from composite hydrogels, and (**D**) drug release profile of lysozyme protein from composite hydrogels as a function of time. Legend represents: irradiated nanoshell-composite hydrogels (square), irradiated NIPAAm-co-AAm hydrogels (diamond), and nonirradiated NIPAAm-co-AAm hydrogels (triangle).

Data reported as mean ± SD, n = 3.

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PNIPAAm-b-poly(L-glutamic acid) (PNIPAAm-b-PLGA) was developed by ring-opening polymerization as 'schizophrenic' micelles in which the polypeptide sequence of PNIPAAm-b-PLGA was located, whether within the micelle cores or the stabilizing coronas [66].

2.3 Liposomes

Liposomes are considered one of the cornerstones of drug delivery carriers in the pharmaceutical industry due to their versatility in being able to encapsulate both hydrophobic and hydrophilic drugs, their variations in size and their biocompatibility. Liposomes are vesicles that are spontaneously formed and are composed of a lipid membrane encapsulating an aqueous volume. These macromolecules were first discovered by Bangham in 1965 as models for studying cellular membranes [67]. Since then, liposomes have been extensively explored as drug carriers in which a drug can be loaded into the sequestered interior, thereby effectively

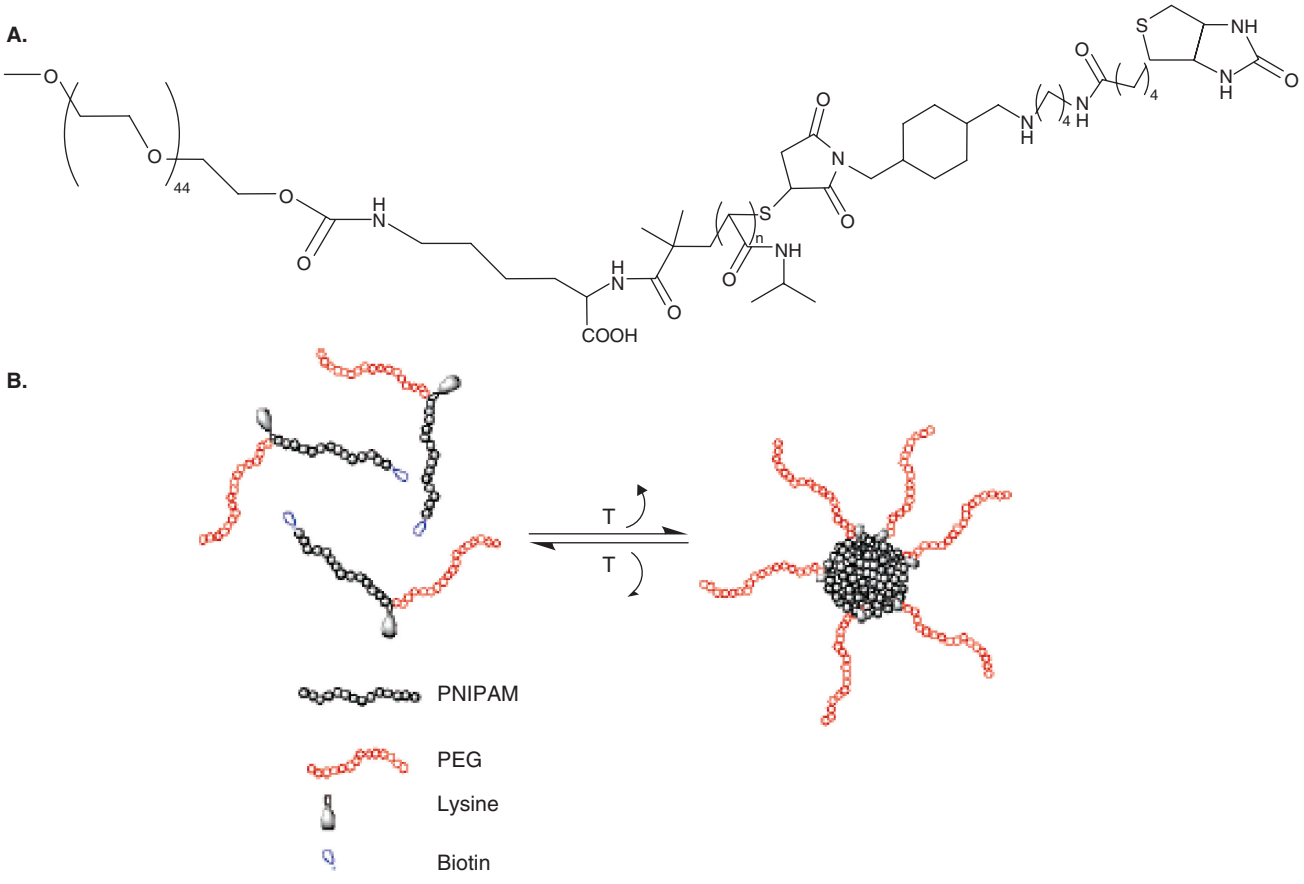

Figure 4. Chemical structure for the synthesized (A) heterobifunctional Y-shaped block copolymers monomethoxy poly(ethylene glycol)-lysyl-block-poly(N-isopropylacrylamide-biotin) (mPEG-Lys-block-PNIPAAm-biotin) and (B) the schematic representation of macromolecular temperature-induced association of mPEG-Lys-block-PNIPAAm-biotin copolymers at the LCST temperature.
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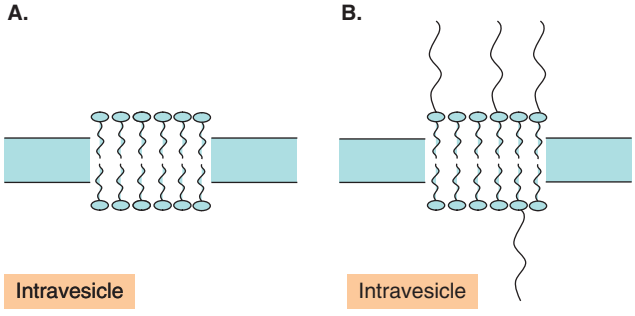

Figure 5. Schematic representation of (A) liposomes that consist solely of temperature-sensitive lipids and (B) liposomes that may or may not contain thermoresponsive lipids but have been modified with temperature-sensitive polymers.

solubilizing poorly soluble hydrophobic drugs, as well as to protect the drug cargo. However, the success of conventional liposomal drug delivery systems was affected by short circulation times and non-specific uptake of the liposomes by elements of the reticuloendothelial system (RES). To this end, the liposomes were modified on the surface with hydrophilic polymers such as PEG so as to produce stealth macromolecules that could evade the RES and thus increase the half-lifes by hours. Consequently, as the areas of controlled drug delivery evolved to encompass specific cellular targeting and triggered drug release, so did the need to modify liposomes so as to optimize their efficacy. Yatvin *et al.* introduced the concept of temperature-sensitive liposomes by proposing that the liposome would be stable at normal body temperature and permeable to the encapsulated drug at higher temperatures [68]. Hyperthermia has since been shown to increase the extravasation of liposomes from the tumor microvaculature into the tumor volume [69]. There are two main types of thermoresponsive liposomes: liposomes that consist of temperature-sensitive lipids (**Figure 5A**) (addressed below) and liposomes that may or may not contain thermoresponsive lipids but have been modified on their surface with temperature-sensitive polymers (**Figure 5B**).

2.3.1 *PNIPAAm-modified liposomes*

Temperature-sensitive liposomes that contain lipids such as dipalmitoylphosphatidylcholine have a gel-to-liquid crystalline

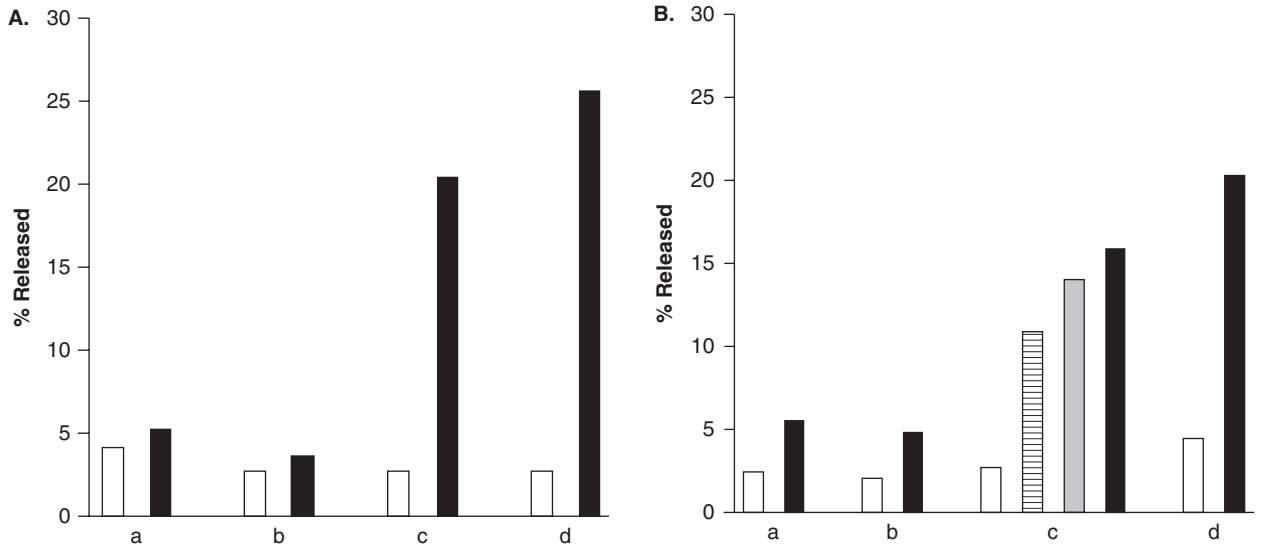

Figure 6. The drug release profile of (A) entrapped fluorescent marker from egg phosphatidylcholine (EPC) liposomes and (B) sterically stabilized liposomes after 5 min of incubation at 37°C as a function of pH. Bars represent: (a) control liposomes, (b) liposomes in the presence of poly(NIPAAm-*co*-methacrylic acid (MAA)): polymer/lipid = 0.28 (m/m), (c) liposomes in the presence of poly(NIPAAm-*co*-MAA-*co*-octadecyl acrylated (ODA): polymer/lipid = 0.28 (m/m), (d) liposomes in the presence of poly(NIPAAm-*co*-MAA-*co*-ODA: (polymer/lipid = 0.56 (m/m)).

The open bars: pH 7.2; Closed bars: pH 4.9; Hatched bars: pH 5.3; Gray bars: pH 5.5.

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phase transition temperature at ~ 42°C. Thus, the phospholipid bilayer becomes highly leaky at this temperature, which is also the upper temperature limit for hyperthermia. Thus, the liposomes are stable after they are introduced into the body and release their cargo at a specific site when the temperature is raised above 42°C. In an effort to develop temperature-sensitive liposomes that release their contents around physiological temperature, Kono *et al.* synthesized copolymers of dioleoylphosphatidylethanolamine (DOPE), PNIPAAm and *N*-acryloylpyrrolidine (APr) [70]. These liposomes released about 40 and 64% of the encapsulated calcein at 40 and 45°C respectively. PNIPAAm polymers were utilized in these liposomes because the polymer chains are hydrated at temperatures below its LCST, which serve to stabilize the liposomes. In contrast, the polymer chains become dehydrated above the LCST, which destabilizes the liposomes resulting in triggered release of the liposome cargo. Thus, PNIPAAm copolymers serve to stabilize as well as to confer thermoresponsive properties to liposomes to which they are attached. Apart from the development of temperature-sensitive systems, liposomes have undergone extensive modifications in an effort to develop targeted drug carriers so as to increase drug efficacy and decrease unwanted side effects [71,72]. The modification of liposomes with pH-sensitive moieties represents one strategy aimed at targeting the endocytic pathway whereby macromolecules are typically internalized. The end point of the endocytic pathway is the lysosome whose acidic environment triggers the activation of enzymes such as peptidases and hydrolases, which can degrade the contents of the liposomes. Therefore, to circumvent this problem, liposomes have been modified to be pH-responsive so as to release their contents under mild acidic conditions that resemble the endosomal compartment [73,74]. Typical pH-sensitive liposomes combine polymorphic lipids such as phosphatidylethanolamine (PE) with acidic amphiphiles, which act as membrane stabilizers at neutral pH [75]. However, these lipids are severely affected by loss of pH-sensitivity and instability in serum [76,77]. Meyer *et al.* were the first to develop pH-sensitive liposomes in which a copolymer of *N*-isopropylacrylamide was anchored onto the liposome membrane [78]. *N*-isopropylacrylamide-methacrylate acid with or without octadecyl acrylate (poly(NIPAAm-co-MAA-co-ODA)) was used to render stable liposomes pH-sensitive so that the liposomes released their contents at the LCST temperature of 37°C with the pH below the phase transition of the polymer. In addition, these liposomes released their contents at pH 5.5 – 4.9, which corresponded to the internal pH of endosomes and lysosomes (**Figure 6**). The pH-sensitivity of the PNIPAAm copolymers was a result of the introduction of ionizable MAA monomers into the structure of the polymer, which produced an LCST that was sensitive to pH. At neutral pH, the carboxylic moieties of MAA are ionized and the LCST of the PNIPAAm copolymers is increased above 37°C due to the hydrophilicity of the copolymers. However, at acidic pH, the MAA carboxylic groups are protonated and the lack of charge reduces the LCST below 37°C, resulting in precipitation of the polymer, which is driven by hydrophobic interactions between

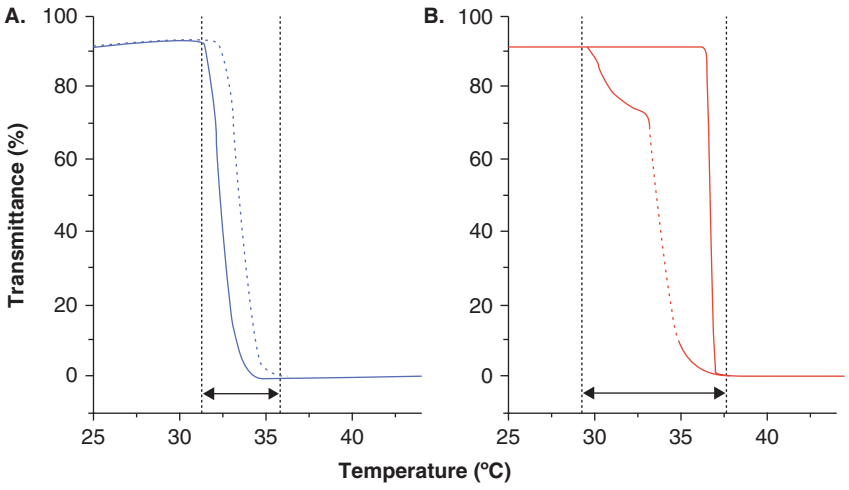

Figure 7. Graphs of transmittance as a function of temperature measured aqueous solution (3 mg/ml) of either (A) a copolymer P(MEO2MA-*co*-OEGMA) containing 5 mol % of OEGMA per chain (DP_n ~ 100; M_w/M_n = 1.34); or (B) a homopolymer of PNIPAM (DP_n ~ 100; M_w/M_n = 1.12): solid lines, heating cycles; dotted lines, cooling cycles.
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the isopropyl side chains of PNIPAAm. The ODA was incorporated into the copolymers to enable anchoring of the polymer onto the liposome and had no effect on the phase transition pH. Hence, the pH-triggered release was a result of a transient destabilization of the liposomal membrane due to the conformational change of the polymer upon acidification. The resulting charge neutralization that occurred rendered the polymer more hydrophobic and thus susceptible to interact with the liposome lipid bilayer, resulting in structural defects. Since this time, liposomes modified with NIPAAm copolymers have been extensively studied as a means of developing pH-sensitive liposomes for targeted drug delivery [79-85].

3. Thermosensitive PEG analogs

Despite the low cytotoxicities of PNIPAAm-based polymers utilized for drug delivery and tissue engineering applications, the by-products of degradation pose a concern for long-term administration and implantation. To this end, Lutz *et al.* have developed novel thermosensitive 2-(2-methoxyethoxy) ethyl methacrylate-*co*-oligo(ethylene glycol) methacrylate (MEO_2MA-*co*-OEGMA) copolymers as biocompatible alternatives to PNIPAAm polymers [86-88]. These copolymers are composed of oligo(ethylene glycol) segments identical to PEG monomers, in which linear PEG is known to be non-toxic and non-immunogenic. Thus, Lutz *et al.* developed nonlinear PEG analogs with tunable LCSTs between 26 and 90°C, depending on the comonomer composition. Copolymers of P(MEO_2MA-*co*-OEGMA) with comonomer feed ratios of 95% MEO_2MA and 5% OEGMA (comparable to PNIPAAm polymers exhibiting an LCST of 32°C) were prepared by atom transfer radical polymerization (ATRP). The phase transition of the P(MEO_2MA-*co*-OEGMA) copolymers showed uniform heating and cooling profiles (**Figure 7A**) as opposed to a sharp heating transition of the PNIPAAm copolymers, which showed a broad hysteresis upon cooling (**Figure 7B**). In addition, both polymers showed comparable cloud points in saline solution that were independent of their concentration in water. To further improve the biocompatibility of these polymers, Lutz *et al.* also introduced labile linkages into the backbone of these polymers without affecting the stimuli-responsiveness of the copolymers [87]. The monomer 5, 6-benzo-2-methylene-1,3-dioxepane (BMDO) was used as a comonomer to prepare biodegradable P(MEO_2MA-*co*-OEGMA-*co*-BMDO) copolymers with molecular weights of ~ 12,000 – 15,000 Da and narrow polydispersities of ~ 1.5 via a ring-opening mechanism. Moreover, the incorporation of the BMDO monomers did not affect the thermoresponsiveness or the biocompatibility of the copolymers as compared to poly(ethylene oxide) (PEO).

4. Thermogelling systems

In addition to thermosensitive hydrogels, there is another class of temperature-responsive polymeric system known as thermogelling systems, in which a hydrogel solution forms by a sol–gel transition in water without any chemical reaction. The sol phase is a flowing solution whereas the gel phase is a non-flowing solid in which the phase transition occurs at the critical gel concentration (CGC) of a polymer. Thermogelling polymers typically exist as a solution at room temperature and undergo a phase transition to form a gel at physiological temperature. Thus, these systems are highly attractive for the delivery of drugs since the polymer–drug solution can quickly become a drug depot upon injection to

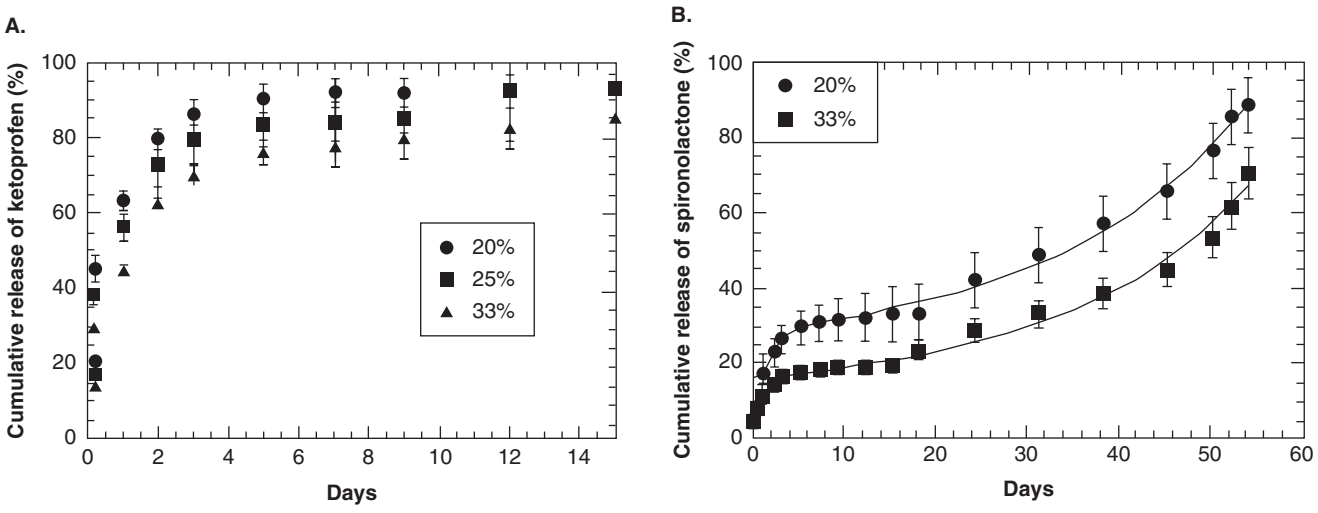

Figure 8. The release profile of (A) ketoprofen and (B) spironolactone from PEG-PLGA-PEG triblock copolymer hydrogels.
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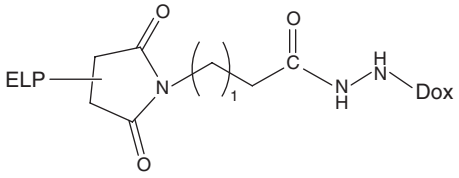
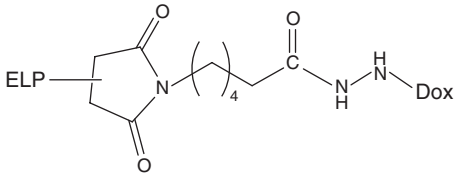
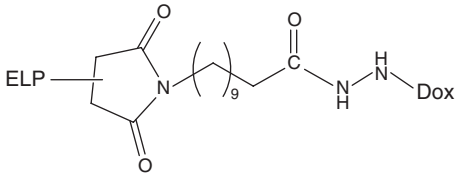
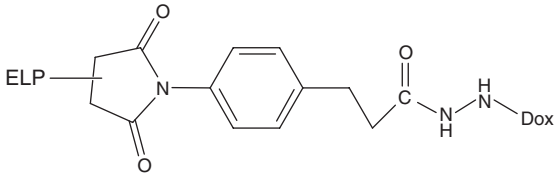
a target site in the body that then acts as a sustained drug delivery system. Typical thermogelling systems consist of non-ionic triblock copolymers of the type ABA and most are analogs of the commercial triblock copolymer: poly (ethylene oxide-b-propylene oxide-b-ethylene oxide) (PEO-PPO-PEO) that is also known as Pluronics (BASF) or Poloxamer (ICI) [89]. Jeong synthesized poly(ethylene oxide-b-L-lactide-b-ethylene oxide) (PEO-PLLA-PEO) as a thermosensitive biodegradable thermogelling system for sustained drug release that could circumvent the need to remove the delivery system after introduction into the body [90]. Aqueous solutions of the triblock formed micelles at low concentrations that were driven by hydrophobic interactions between PLLA blocks, whereas at high concentration levels, the aqueous polymer solutions formed a gel as a result of the association of the micelles. In addition, these block copolymers formed a gel at lower temperatures and a sol at higher temperature. Thus, the sol–gel transition temperature depended on both the concentration of the polymers as well as the composition of the copolymers. Poly(ethylene glycol-DL-lactic acid-co-glycolic acid-ethylene glycol) (PEG-PLGA-PEG) was also synthesized and the release of ketoprofen and spironolactone was studied from the triblock copolymer gels formed *in situ* by injection of the solution into an aqueous solution held at 37°C [27,91]. Ketoprofen, a hydrophilic drug, was found to be released over 2 weeks, as opposed to the hydrophobic drug spironolactone that was released over 2 months (**Figure 8A** and **B**). The hydrophilic drug was found to partition into the hydrophilic PEO domain, while spironolactone partitioned into the PLGA block at the core of the micelles in which diffusion was a limiting factor. Kim and researchers synthesized PLGA-PEG-PLGA triblock copolymers as thermosensitive, biodegradable polymers without the use of organic solvents that limit the bioactivity of protein drug molecules as well as poly(L-lactide-co-caprolactone)-b-poly(ethylene glycol)-b-poly(L-lactide-co-caprolactone) (PLLACL-PEG-PLLACL) for the development of modulated thermogelling properties [92,93]. The PLGA-PEG-PLGA triblock copolymers are currently available as ReGel™ (MacroMed, Inc., Salt Lake City, UT, USA), a free-flowing sol at room temperature, which forms a gel at body temperature. ReGel has been shown to be an effective depot for insulin release [94], for dipyridamole release [95] and for interleukin-2 (IL-2) release [96]. Since the development of these systems, there have been many other thermogelling systems synthesized including poly(ethylene glycol)-poly(caprolactone)-poly(ethylene glycol) (PEG-PCL-PEG) [97], poly(ε-caprolactone-co-lactide)-poly (ethylene glycol)-poly(ε-caprolactone-co-lactide) (PCLA-PEG-PCLA) [98] and triblock copolymers of methoxy poly (ethylene glycol) and poly(propylene fumarate) [99].

5. Thermoresponsive elastin-like peptides

Synthetic polymers are extensively used in drug delivery systems because of their versatility – they are able to be modified chemically; their hydrophilicity that can increase bioavailability *in vivo*; and their biocompatibility that circumvents the need to remove the delivery system following administration. However, one of the disadvantages of synthetic polymers is their polydispersity, which creates a significant hurdle with respect to batch-to-batch replication in the pharmaceutical industry. Elastin-like polypeptides (ELPs) are oligomeric repeats of the pentapeptide Val-Pro-Gly-Xaa-Gly (VPGXG) (where Xaa is any amino acid except proline), which is derived from the hydrophobic domain of tropoelastin [100]. These biopolymers are unique in that ELPs are soluble in aqueous solution at room temperature but as the temperature is raised above the inverse transition

Table 1. Elastin-like polypeptides-doxorubicin (ELP-Dox) conjugates with spacer arm length, degree of Dox conjugation to the ELP, hydrodynamic radius (R_h) determined by DLS, and the chemical structures.

ELP	Spacer arm length (A)	Conjugation ratio	R_h (nm)	T_t (°C)	ELP–Dox conjugate
B-Dox	8.1	0.7 – 0.8	12.5	39.1	
E-Dox	11.8	0.7	15.9	37.6	
K-Dox	19	1.2 – 1.3	17.6	39.1	
M-Dox	17.9	1.0 – 1.4	21.5	41.5	

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temperature (T_t) (also referred to as the LCST), the polymers become insoluble and precipitate. This phase transition is completely reversible and the T_t temperature can be tuned and designed to respond to different stimuli such as salts [101] and light [102], etc, by modulating the guest residue position in the polypeptide chain. Moreover, ELPs are also very attractive for use in drug delivery because they are produced as monodisperse polymers in *Escherichia coli* via genetic engineering. Therefore, these biopolymers can be produced in large reproducible quantities without cumbersome purification and they have been shown to be biocompatible [103]. Chilkoti and researchers were the first group to demonstrate that the T_t of ELPs was due to the fusion proteins and later developed the protein purification process termed inverse transition cycling (ITC), which is currently used today [104,105]. Since this time, Chilkoti *et al.* have designed thermally responsive ELPs with an inverse transition temperature of 40°C so as to target tumors via local hyperthermia [106-108]. Four different ELP-doxorubicin (ELP-Dox) conjugates were synthesized as macromolecular drug carriers in which the focus of the study was the design of the acid-labile linker to not only efficiently release the Dox molecules in the conjugates but to maintain the T_t, which was shown to be affected by the linker [109]. The 13-keto position of Dox was targeted with one of four heterobifunctional hydrazide linkers: MPBH(4-[4-*N*-maleimidophenyl] butyric acid hydrazide- HCl) (M-Dox), BMPH (*N*-(β-maleimidopropionic acid) hydrazide) (B-Dox), EMCH (*N*-(ε-maleimidocaproic acid) hydrazide) (E-Dox) and KMUH (*N*-(κ-maleimidoundecanoic acid) hydrazide) (K-Dox) (**Table 1**). The results showed that the selected linker structures and lengths in which the maleimide-activated Dox was conjugated to the thiol group of a unique cysteine in the ELP did not affect the T_t of the conjugates and could thus be used to modulate the release of Dox. In addition, conjugates with longer linkers (K-Dox and M-Dox) showed slower transition kinetics compared to the shorter linkers, which was related to the hydrophobicity of the linkers. Setton *et al.* have developed injectable *in situ* ELP biopolymers for sustained drug release and lower systemic exposure following perineural administration. The results showed that the aggregating ELP had a sevenfold longer perineural half-life compared with the soluble ELP, due to the formation of a depot from which slow resolubilization and clearance produced sustained local protein release [110]. Other applications of ELP biopolymers include the development of macromolecules for intra-articular

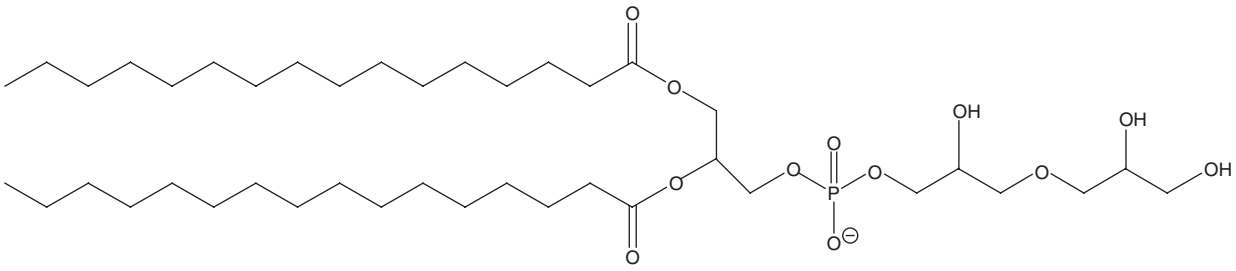

Figure 9. Chemical structure of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglyceroglycerol (DPPGOG).
Reproduced from [120] with permission.

drug delivery [111], the synthesis of a silk-elastin-like hydrogel for gene delivery [112] and the development of cell-penetrating peptides to ELPs for drug delivery [113].

6. Thermosensitive liposomes

Since Yatvin *et al.* and Weinstein *et al.* presented temperature-sensitive liposomes (TSLs) as controlled drug delivery systems, there have been many liposomal formulations developed to overcome the hurdles of poor targeting and low drug efficacy at target sites [68,114]. These liposomes exhibit a phase transition temperature (T_m) in which the lipids undergo a gel-to-liquid transition in water. Typical TSLs have been prepared from 1, 2-dipalimitoyl-*sn*-glycero-3-phosphocholine (DPPC) as the primary lipid, because its T_m occurs at 41.5°C [115,116]. The gel-to-liquid transition at the T_m is a result of a conformational change in the alky chains of the lipids, which leads to an increase in the volume occupied by the hydrocarbon chains in the membrane and thus an increase in the permeability of the lipid bilayer [117]. However, the rate of drug release and the amount of drug released from liposomes that consist of DPPC alone are relatively small [115,118]. Therefore, these liposomes are mixed with other lipids such as 1, 2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), which increases the transition temperature to 43 – 45°C [119]. Unfortunately, the clinical treatment of tumors using hyperthermia requires that the intratumoral temperature does not exceed 42°C. Lindner *et al.* have developed novel temperature-sensitive and long-circulating liposomes for tumor targeting using mild hyperthermic conditions of 41 – 42°C [120]. The researchers designed a novel lipid, 1, 2-dipalmitoyl-sn-glycero-3-phospho-glyceroglycerol (DPPGOG) as a long-circulating TSL and encapsulated carboxyfluorescein (CF) so as to monitor the drug release as a function of temperature (**Figure 9**). The half-life ($t_{1/2}$) for the encapsulated drug was 9.6 h in hamsters and 5.0 h in rats, which was a result of the increased hydrophilicity of the DPPGOG that contains a free hydroxyl group. In addition, there was a sixfold increase in drug accumulation in the heated tumor tissue after i.v. injection of the liposomes compared to the non-liposomal control, which was probably due to the intracellular localization of the CF. Other researchers have used lysolecithin-containing thermosensitive liposome (LTSL) encapsulating doxorubicin to affect tumor microcirculation [121], applied radiofrequency phased arrays to TSLs for treatment of ovarian cancer so as to modulate the T_m between 39.7 – 41.6°C [122], and DPPC has been combined with an acyl chain-matched lysolipid (MPPC) to form TSLs containing doxorubicin for mild hyperthermic temperatures of between 39 – 40°C [123].

7. Expert opinion

Temperature-sensitive drug delivery systems have great potential to revolutionize conventional means of drug delivery. However, there is a huge impasse between the development of these delivery systems and the ability to clinically translate these systems for patient use. Even though there are numerous research papers published with PNIPAAm polymers, the safety of the delivery system has to be ensured prior to advancement. These polymeric systems can be degraded within the body to produce toxic by-products, which can outweigh the benefits of controlled release. To deal with these issues of toxicities, the drug delivery system can be enclosed within an implant device so as to minimize biodegradation and localize possible by-products of hydrolysis. However, these devices would be implantable systems that would require surgical removal, which could be unattractive depending on the disease state or location of the implant device.

Polymers such as ELPs or thermosensitive lipids are biocompatible macromolecules that do not produce toxic by-products and these have the ability to gel *in situ*. Therefore, these systems have the benefits of not requiring surgical placement of the device and can be molded to fill a desired cavity or shape. The combination of thermoresponsive systems with pH-sensitive moieties enables tunability of the phase transition temperatures. This change in phase transition temperatures away from physiological temperatures overcomes the hurdles of polymer gelling at the injectable site prior to the administration of the drug and its delivery system. Moreover, combination stimuli-sensitive systems have been designed to enable tumor targeting via hyperthermia.

Thus, even though there remain many problems associated with bringing temperature-sensitive drug delivery systems to the bedside, the innovative approaches applied to current systems so as to tightly control drug delivery and thus limit toxicities promise to overcome these hurdles in the very near future. This advancement in thermosensitive drug delivery systems holds the promise of delivering drugs at therapeutic levels only when needed, with negligible side effects.

Declaration of interest

The authors state no conflict of interests and have received no payment in the preparation of this manuscript.

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