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Review

In situ gelling pH- and temperature-sensitive biodegradable block copolymer hydrogels for drug delivery

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ABSTRACT

Stimuli-sensitive injectable polymeric hydrogels have been extensively investigated during the past decade as bioactive agent delivery vehicles and for tissue engineering applications. An aqueous solution of these polymers undergoes a sol-to-gel phase transition in response to external stimuli such as pH, temperature, salt, light, biomolecules, electromagnetic field, *etc*. Bioactive molecules or cells can be mixed into the low-viscosity state of the polymer solution and injected into the body at a target site, forming an *in situ* hydrogel depot, which can then serve as bioactive-molecule-releasing carriers or a cell-growing microenvironment. This review systematically summarizes the recent progress in biodegradable and injectable block copolymer hydrogels, giving special attention to the novel and promising pH- and temperature-sensitive injectable block copolymer hydrogels for biomedical applications. The gelation mechanism, formation of ionic complexes, and biodegradation are highlighted as key factors responsible for controlled protein/drug delivery. The advantages and perspectives of pH- and temperature-sensitive injectable block copolymer hydrogels are also highlighted.

Contents

1. Introduction

In recent years, controlled drug release systems based on hydrogels have been highlighted as therapeutic carriers to deliver drugs at targeted sites within a specific time frame [1–6]. Hydrogels are basically three-dimensional hydrophilic or amphiphilic polymer networks formed by chemical or physical crosslinking that are capable of retaining large amounts of water or biological fluids yet remaining insoluble in physiological conditions [7,8]. Due to their high water content, soft nature, good biocompatibility, flexibility in fabrication, desirable physical characteristics (similar to physiological conditions), they provide an ideal microenvironment that mimics *in vivo* tissues for the functional reconstruction of soft tissues and smart building blocks in regenerative medicine [9–14]. They have a wide range of biomedical applications, including therapeutic compound delivery [15–18], 3D-scaffold platforms for tissue engineering, carriers for cell encapsulation [19], and adhesive or barriers between tissue and material surfaces [20,21].

However, the administration of preformed hydrogels through surgical intervention is rather costly and inconvenient for the patient, and therefore, attention has been focused on injectable hydrogels that can be administered in a minimally invasive manner, as well as drugs, proteins, DNAs, and cells that can also be easily mixed into polymer solutions prior to administration [22]. *In situ* forming hydrogels can have the form of clear polymer solutions prior to administration, which turn into a viscoelastic system (gel) in response to changes in external stimuli such as pH [22,23] and temperature, or by means of chemical crosslinking with enzymes [24–29], Schiff base [30–35], Michael addition [36–42], photo-polymerization, *etc*. [43–45], at the site of administration. In comparison with the permanent polymer networks

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crosslinked by covalent bonds, physically crosslinked injectable hydrogels are reversible networks and can be developed by simply varying the environmental stimuli, exhibiting a sol–gel transition without a significant volume change. Due to their unique advantages, such as minimal invasion, lack of organic solvent and photo-initiator, site-specificity, reduced systematic toxicity, and ability to deliver hydrophobic/hydrophilic drugs and bioactive molecules, stimuli-sensitive injectable hydrogels have received extensive attention for drug delivery in the past decade [23]. For instance, drugs, bioactive molecules, or cells can be encapsulated with aqueous polymer solutions at low temperature, and the mixed solutions rapidly form *in situ* hydrogels after injection into the body. Although the ideal properties of stimuli-sensitive injectable hydrogels depend basically on their intended application, the design of injectable hydrogels for drug delivery applications must fulfill some important requirements: (a) aqueous polymer solution viscosity must be low (free flowing) enough to facilitate the easier subcutaneous injection, (b) fast gelation is needed to minimize the initial burst release; (c) the hydrogel must be biocompatible and biodegradable, and its degradation product must be non-cytotoxic; and (d) efficient drug loading and controlled drug release over a range of time periods are required.

In this review, we summarize the recent progress on injectable block copolymer hydrogels used for the release of pharmaceutical drugs/and biomolecules, giving special attention to the novel and promising pH- and temperature-sensitive injectable hydrogels of our lab. The discussion covers temperature-sensitive hydrogels such as polyesters, polyphosphazenes, and polypeptides, and key materials developed in our laboratory based on sulfamethazine-, poly(β-amino ester)-, poly(amino urethane)-, poly (amino urea urethane)-, and poly(amidoamine)-, poly(amino ester urethane)-, dually ionic (cationic and anionic) poly(urethane amino sulfamethazine)- for the construction of biodegradable pH- and temperature-sensitive hydrogels. The limitations of temperature-sensitive injectable hydrogels such as burst release and needle clogging are discussed, and approaches in using pH- and temperature-sensitive hydrogels are provided in detail. Finally, the advantages and perspectives of pH- and temperature-sensitive hydrogels for biomedical application are emphasized.

2. Injectable temperature-sensitive block copolymer hydrogels

Temperature is the easiest stimulus to manipulate in environmentally responsive hydrogels. The aqueous solutions of temperature-responsive polymers display sol–gel transitions at higher temperatures, where self-assembly of the polymer chains occur due to hydrophobic interactions. Pluronics® or poloxamers, which are aqueous solutions of triblock copolymers poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) (Fig. 1), can self-assemble in a certain concentration range to form micelles and micellar association as temperature is increased, resulting in a temperature-sensitive sol-to-gel transition [46–52].

At room temperature, the aqueous solutions of Pluronics® are in the sol state, which facilitate the incorporation of bioactive molecules. When they are injected into the subcutaneous layer of a warm-blooded animal, they form gel at body temperature, thus allowing them to serve as a drug-delivery carrier. However, the formed gel has undesired characteristics including short residence time, weak mechanical properties, and high permeability, so it has only been applied as a short-term implantable system for pharmaceutical agents

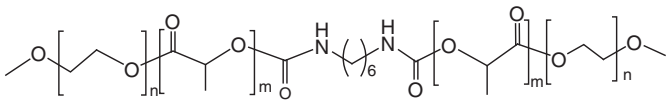


Fig. 2. Chemical structure of PEG–PLLA–PEG copolymer hydrogel.

or formulation excipients to solubilize hydrophobic drugs [53–56]. Apart from these drawbacks, another shortcoming of Pluronics® is that their repeating unit consists of ether functional groups, which are non-biodegradable in humans [53]. Therefore, there is an urgent need for biodegradable injectable hydrogel systems with controlled drug release kinetics and tunable biodegradation within a desirable time frame.

In the ground breaking work of Kim et al. on biodegradable and biocompatible triblock copolymer PEG–poly(L-lactic acid)–PEG (PEG–PLLA–PEG) hydrogels (Fig. 2) [57], a novel class of ABA-type thermosensitive matrices, was synthesized by ring-opening polymerization of L-lactide (L-LA) using monomethoxy PEG (MPEG) as a macroinitiator to form MPEG–PLLA diblock copolymers. Subsequently, PEG–PLLA–PEG triblock copolymers were obtained by coupling the resulting diblock MPEG–PLLA using hexamethylene diisocyanate (HMDI).

These polymers exhibited upper critical solution temperature (UCST) behavior, meaning that the gel-to-sol phase transition occurs with an increase of temperature, which can be strongly controlled by varying the biodegradable PLLA block length, hydrophobic/hydrophilic ratios, and stereoregularity of the hydrophobic block [57]. Hydrogels loaded with bioactive molecules or cells must be prepared at high temperature, then gelation is induced by lowering the temperature to less than 37 °C. Unfortunately, at high temperatures, the structure and activity of labile biomolecules were severely affected and the injection of copolymer solutions at temperatures higher than body temperature may be uncomfortable for patients.

To address these problems, efforts have led to the development of a novel class of triblock copolymer PEG–poly(D,L-lactide-co-glycolide)–PEG (PEG–PLGA–PEG) hydrogels (Fig. 3) [58,59]. These materials displayed both lower critical solution temperature (LCST) and upper critical solution temperature (UCST) behavior and were processable without the use of high temperatures to dissolve the polymer. An aqueous solution of PEG–PLGA–PEG with low molecular weight PEG (<1000 Da) was in a sol state at low temperature and converted to a very durable thermogelling system at body temperature [58,60].

The sol–gel phase transition of PEG–PLGA–PEG was controlled by varying the molecular weights of PEG and PLGA and the ratio of lactic acid to glycolic acid [57]. The mechanism of gel formation of PEG–PLGA–PEG hydrogel was governed by the increase in molecular attraction and micellar aggregation accompanying significant percolation among the crew-cut micelles with short chain PEGs, whereas Pluronics® formed a gel by packing the micelles [61,62]. It was shown that upon subcutaneous injection into an *in vivo* rat model, the hydrogels were stable for one month [60]. It has been possible to vary the duration of PLGA-based thermosensitive block copolymer hydrogels over a time span of 1–2 months by adjusting the triblock topology of PEG and PLGA (PEG–PLGA–PEG or PLGA–PEG–PLGA) [60,63]. These PEG–PLGA–PEG hydrogels were investigated for sustained released of the synthetic hydrophilic drugs ketoprofen and hydrophobic spirolactone, where hydrophilic ketoprofen was released by diffusion control and hydrophobic spironolactone was released through diffusion followed by degradation [63]. These hydrogels were also used as a reservoir for the controlled release of TGF-β1 for wound healing purposes,

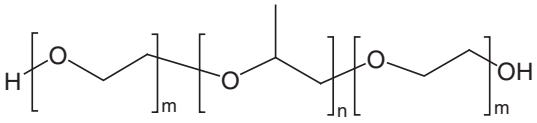


Fig. 1. Chemical structure of Pluronics® copolymer hydrogel.

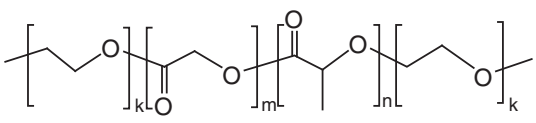


Fig. 3. Chemical structure of PEG–PLGA–PEG copolymer.

demonstrating high levels of re-epithelialization, cell proliferation, and collagen organization [64]. In addition, the release of insulin, porcine growth hormone, and glycosylated granulocyte colony stimulating factor using these hydrogel systems (*in vitro* and *in vivo*) have also been reported [65,66]. Overall, the short gel duration as well as biodegradation problems of Pluronics® were solved by the use of PEG–PLGA–PEG, where the PPG of Pluronics® was replaced by PLGA. However, tuning of the degradation of thermogels is desirable according to the particular application, and some systems need a short-term (1 week) delivery system, while others need more than 2-months. For this issue, grafting of PEG to PLGA or PLGA in a PEG system has been evaluated. The thermogel of PEG-g-PLGA showed a 1-week duration as a gel, whereas PLGA-g-PEG showed a 3-month duration as a gel, instead of having a similar sol–gel phase transition diagram [67,68]. In the case of PEG-g-PLGA, the degradation of the pendant PLGA made the remaining PEG-g-PLGA more hydrophilic, causing fast degradation, while degradation occurred along the backbone of PLGA by the preferential mass loss of the PEG-rich moieties in PLGA-g-PEG, which slowed the degradation of hydrogel. PLGA-g-PEG hydrogels capable of encapsulating chondrocytes were shown to improve articular cartilage defects [67–70].

Developed PLGA-based thermosensitive copolymer hydrogels as drug delivery carriers became a milestone in the field of pharmaceutical research. However, PLGA-based thermosensitive copolymers are sticky paste at room temperature and require long dissolution times to prepare aqueous solution. Thermo-sensitive copolymers of PEG with caprolactone (PCL) were designed to overcome these problems [71,72].

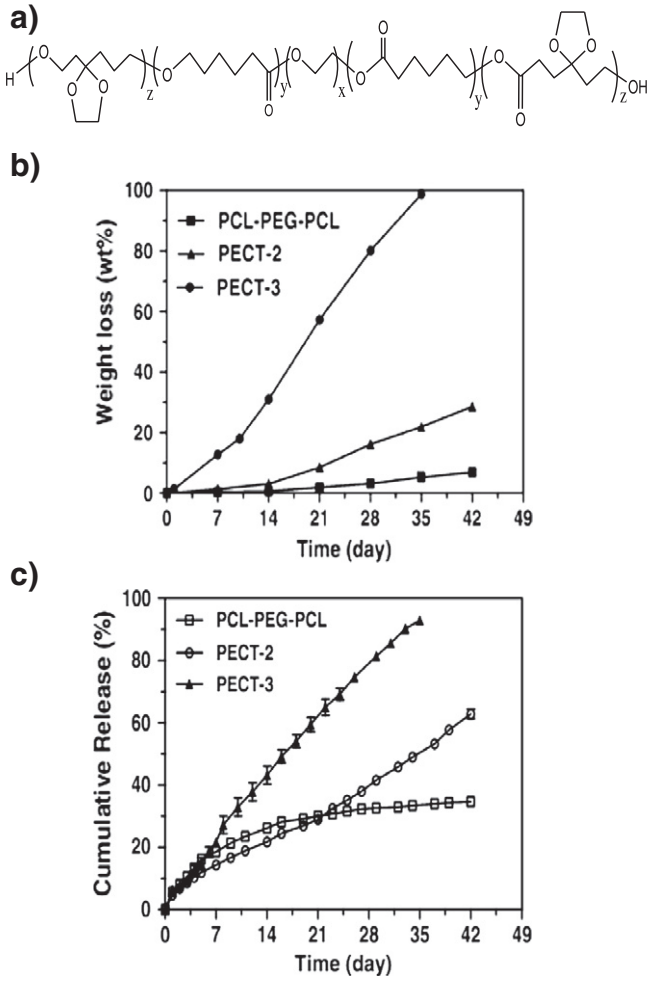


Fig. 4. (a) Structure of PECT. (b) Weight loss of triblock copolymer hydrogel during *in vitro* degradation at 37 °C in 0.01 M PBS, pH 7.4. (c) *In vitro* release of paclitaxel from the PCL–PEG–PCL, PECT-2, and PECT-3 hydrogels [85]. Copyright 2012 Elsevier.

Biodegradable thermogelling poly(ethylene glycol)-b-poly(-caprolactone)-b-poly(ethylene glycol) (PEG–PCL–PEG) and PCL–PEG–PCL are in powder form at ambient temperature and easily dissolve in water within a very short time. The applicability of these copolymers to form an injectable drug delivery vehicle was investigated for hydrophilic drug (Vitamin B12), hydrophobic drugs (honokiol and lidocaine), and protein (bovine serum albumin) [73–75]. Several other thermosensitive copolymers of PEG with aliphatic polyesters were synthesized and utilized for drug delivery [76–84]. Recently, injectable thermosensitive hydrogels were formed from PCL–PEG–PCL amphiphilic-co-polymers with 1,4,8-trioxa[4.6]spiro-9-undecanone (TOSUO) moieties incorporated in PCL blocks, *i.e.* poly(ϵ-caprolactone-co-1,4,8-trioxa[4.6] spiro-9-undecanone)-poly(ethylene glycol)-poly(ϵ-caprolactone-co-1,4,8-trioxa [4.6]spiro-9-undecanone) (PECT) (Fig. 4a) to provide a route to tailor degradation and drug release behavior [85] (Fig. 4b&c).

Thermosensitive hydrogels based on polyphosphazenes are also paid special attention to as emerging biomaterials because of their non-toxic degradation products (i.e. parent amino acids, ethanol, phosphates, and ammonia); a mixture that results in a buffer system of near neutral pH. Polyphosphazenes consist of a hydrophilic PEG block and hydrophobic amino acids or a peptide block [L-isoleucine ethyl ester (IleOEt), D,L-leucine ethyl ester(LeuOEt), L-valine ethyl ester (ValOEt)], or di-, tri-, and oligopeptides in the side groups [86–89]. Intermolecular association of hydrophobic oligopeptides were responsible for the formation of hydrogels and higher mechanical strength compared to PEG–IleOEt polymer gels. Polyphosphazenes containing depsipeptide (GlyGlycOEt) degrade faster because of the generation of carboxylic acid groups that makes the polymers more hydrophilic and susceptible to the degradation of hydrophobic amino acid groups. The application of these hydrogels to bovine serum albumin (BSA), human growth hormone (hGH) proteins as *in vitro* and *in vivo* release carriers (Fig. 5a&b) [90,91] and extracellular matrix for an artificial pancreas were investigated (Fig. 5c) [92].

Recently, stimuli-sensitive *in situ* gelling hydrogels developed based on polypeptides were found to be stable in phosphate buffer (PBS) and enzymatically degradable *in vivo* (Fig. 6) [93,94]. Polypeptides composed of poly(amino acid)s linked by peptide bonds, are attracting attention as biomaterials because of their unique biodegradation and biocompatibility. Synthetic polypeptides not only form a variety of secondary structures (*i.e.* α-helix, β-sheet, and random coils) due to cooperative hydrogen bonding, but also have a broad spectrum of hydrophobic, hydrophilic, ionic, and non-ionic amino acid building blocks, leading to unique self-assembled structures which exhibit sol–gel phase transition in response to temperature [53,95–98]. Drugs and bioactive molecules such as DNAs, RNAs, and proteins can be easily incorporated in polypeptides, and these form a strong ionic complex with the ionizable side groups of polypeptides. Therefore, the mentioned properties of polypeptide-based hydrogels have attracted increasing attention for their great potential in biomedical and drug delivery applications [53]. Jeong et al. developed poly(ethylene glycol)-b-poly(L/DL-alanine) (PEG–L/DL–PA) with similar block length *in situ* forming polypeptide hydrogels [99].

Aqueous solutions of PEG-L-PA (8 wt.%) with β-sheet secondary structures exhibited sol-to-gel transitions with increasing temperature in the range of 20–40 °C. On the other hand, PEG-b-poly(D,L-alanine) (PEG–DL–PA) diblock copolymers with random coil secondary structure only formed hydrogels at higher temperatures (>60 °C) and polymer concentrations (≥16 wt.%) than those of PEG–L–PA, suggesting that the secondary conformation of the L–PA blocks played an important role in the thermo-induced gelation. Sol–gel behavior was controlled by adjusting the block sequence and molecular weight of the block copolymers [100]. PEG–L–PAs hydrogels were investigated for their potential as a three-dimensional (3D) culture matrix of adipose-tissue-derived stem cells (ADSCs) for *in vitro*/*in vivo* differentiation [101]. Very recently, poly(ethylene glycol)-block-poly(γ-propargyl-L-glutamate) (PEG–PPLG) with pendent alkynyl groups were reported

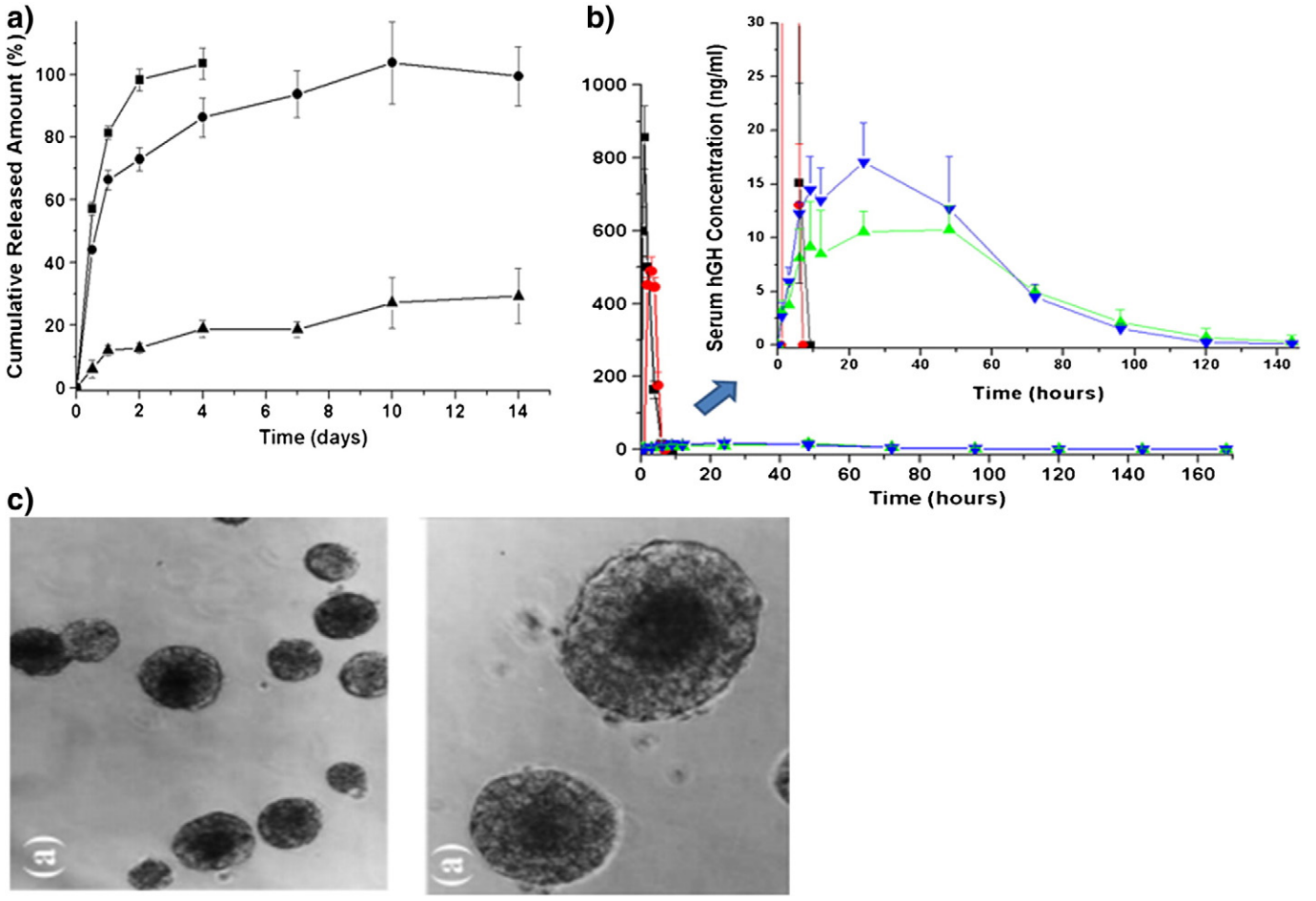


Fig. 5. (a) *In vitro* hGH release from poly-L-arginine (PLA)/Zn-hGH complexes loaded polyphosphazene polymer 1 hydrogel (■: Zn-hGH only,●: PLA/Zn-hGH = 1:1, ▲: PLA/Zn-hGH = 10:1). (b) Effect of zinc on the *in vivo* hGH release behavior (■: hGH alone, ●: Zn-hGH alone, ▲: polymer 1 hydrogel/PLA/hGH = 200:10:1,▼: polymer 1 hydrogel/PLA/Zn-hGH = 200:10:1). (c) Morphology of rat islets entrapped in poly(organophosphazene) hydrogel for 4 weeks [90–92]. Copyright 2010 Elsevier and 2005 VSP publication.

and their aqueous solution underwent sol–gel transition with changing temperature[102]. The resulting copolymers, which contained alkynyl groups, were functionalized with various bioactive molecules such as biotin and galactose, and retained sol–gel phase transition properties near body temperature [102]. PEG-L-PA-co-L-Phe diblock copolymer hydrogels were also studied, showing thermoinduced sol-gel transition [103]. The hydrogels were stable *in vitro* without enzymes, showing significant *in vivo* degradation. *In vitro* insulin release was observed over 16 days with an initial burst effect [103]. Apart from these, thermo-gelling block copolymers based on poloxamer flanked by two polyalanine blocks (i.e. poly((DL/L)alanine)-poloxamers-(DL/L)-poly(alanine) ((DL/L)-PA-PLX-(DL/L)-PA)) were also highlighted [104]. Aqueous solutions of these polymers displayed sol-gel transition due to partial dehydration of poloxamer (PLX), strengthening the β-sheet structure as temperature increased. Their sol–gel transition was strongly influenced by the polypeptide block length and the L-alanine content within the polypeptide block. Very recently, Heise et al. synthesized a range of PEG-conjugated tyrosine-based amphiphiles with the ability to display thermo-induced sol–gel transition at a very low concentration range (0.25–3.0 wt.%) within a temperature range of 25 to 50 °C [105]. It was found that hydrogel formation was driven by amphiphilic balance between PEG and tyrosine blocks, and hydrogen bonding by β-sheet conformation and the phenolic group. *In vitro* biocompatibility and biodegradability of the hydrogels were verified with the sustained release of a small model drug, pro-angiogenic desferrioxamine (DFO).

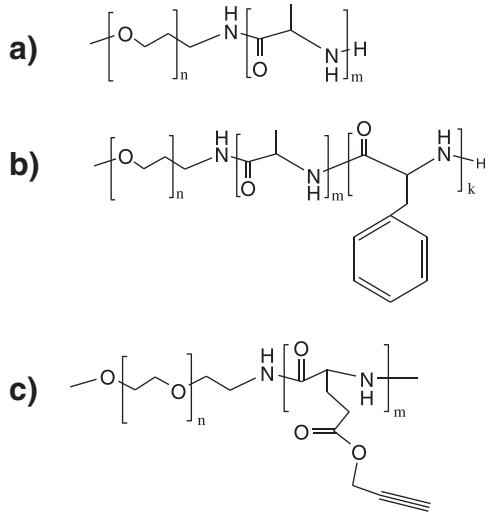


Fig. 6. Chemical structures of representative thermogelling polypeptide-based block copolymers.

3. Injectable pH- and temperature-sensitive block copolymer hydrogels

Injectable pH- and temperature-sensitive hydrogels that respond to change in pH and temperature, have unique advantages over

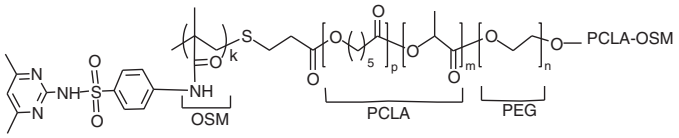


Fig. 7. pH- and temperature-sensitive anionic pentablock copolymer (OSM–PCLA–PEG–PCLA–OSMs).

temperature-sensitive hydrogels such as to prevent the gelation within the injection needle and form a better ionic complex with therapeutic agents. Injectable pH- and temperature-sensitive hydrogels are biocompatible and biodegradable, and their drug release behavior can be triggered by changing the chemical structure of the pH- and temperature-sensitive polymers, leading to enhanced specificity of drug delivery and less side effects. Recently, biodegradable pH- and temperature-sensitive anionic pentablock copolymers (OSMs–PCLA–PEG–PCLA–OSMs) were reported for the first time [106–112] (Fig. 7).

Pentablock copolymers were synthesized by the coupling of acidic sulfamethazine oligomers (OSMs) with thermosensitive poly(-CL-co-LA)-PEG-poly-(-CL-co-LA) triblock copolymers [106]. An aqueous solution of this polymer showed a reversible sol-gel transition by a small pH change in the range of 7.4–8.0, as well as temperature change in the region of body temperature, forming a gel at 37 °C and pH 7.4 (Fig. 8a). The block copolymer OSM–PCLA–PEG–PCLA did not form a gel at pH 8.0 in the range of 4–60 °C because the sulfonamide groups of the OSM blocks were ionized, and when the pH was decreased, the OSM blocks were deionized, resulting in the enhancement of the hydrophobic interaction between PCLA–OSM blocks and the formation of gel (Fig. 8b). It has been found that by changing both pH and thermosensitive functionalities of the polymer, it was possible to broaden the gelation window and obtain a sol between 10 and 70 °C at pH 8.0. A solution of the polymer can be easily injected without concern for premature gelation in the needle, and it rapidly forms a strong gel once at physiological body pH/and temperature, showing slow *in vivo* degradation (six weeks) [107,108]. These hydrogels have been used for the sustained delivery of Paclitaxel (PTX), showing antitumor efficacy for up to 2 weeks after one injection into tumor-bearing mice [109].

Anionic pentablock copolymer hydrogels were also demonstrated as scaffolds for autologous bone tissue engineering. At physiological condition, hydrogels easily encapsulated human mesenchymal stem cells (hMSCs) as well as recombinant human bone morphogenetic protein-2 (rhBMP-2) with encapsulating efficiencies of about 90% and 85% respectively. After subcutaneous injection of pentablock copolymer solutions containing hMSCs and rhBMP-2 in mice, hMSCs differentiation was observed for 7 days [110]. Even though this pentablock polymer hydrogel is anionic in nature, it did not form an improved ionic complex with anionic drug and biomolecules in comparison to cationic hydrogel. For this issue, our group further synthesized a series of cationic biodegradable hydrogels based on a basic poly(β-amino ester) (PAE), which can form an ionic complex with anionic drugs and biomolecules.

Initially, we prepared a MPEG–PCL–PAE diblock copolymer hydrogel by coupling PAE with MPEG and PCL [113]. The copolymer shows a pH- and temperature-responsive sol–gel transition diagram due to ionization of PAE blocks. The sol–gel phase diagrams of the block copolymer solutions were optimized by changing the ratio of hydrophobic to hydrophilic blocks within the block copolymer and PEG lengths to cover the *in vivo* conditions (37 °C, pH 7.4). Furthermore, our group also synthesized dual responsive PAE–PCL–PEG–PCL–PAE pentablock copolymer by Michael addition polymerization of 4,4-trimethylene dipiperidine (TMDP), PCL–PEG–PCL diacrylate, and butane-1,4-diol diacrylate (BDA) (Fig. 9a) [114,115].

The PAE–PCL–PEG–PCL–PAE copolymer solution undergoes a sol-to-gel transition as a function of both pH and temperature (Fig. 9b), and its gel window can be modulated by varying the PEG molecular weight, PAE block lengths, PCL/PEG ratio, and concentration [114–116]. Insulin loaded into PAE–PCL–PEG–PCL–PAE lowered its LCST due to the formation of ionic complexes with degradable PAE blocks, and acted as a physical crosslinker (Fig. 10), which was confirmed by the

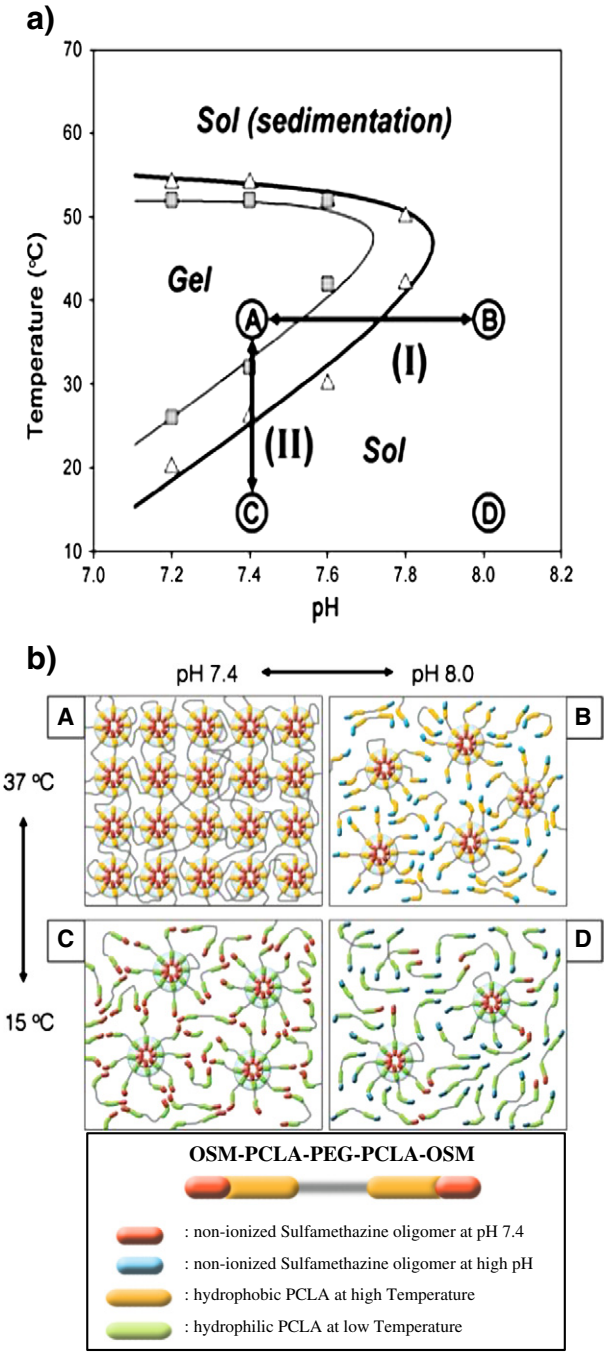


Fig. 8. (a) Phase diagram and (b) schematic representation of the sol–gel mechanism of the pH- and temperature-sensitive block OSMs–PCLA–PEG–PCLA–OSMs copolymer solution: (A) pH 7.4, 37 °C; (B) pH 8.0, 37 °C; (C) pH 7.4, 15 °C; (D) pH 8.0, 15 °C. Reprinted with permission from Ref. [106]. Copyright 2005 American Chemical Society.

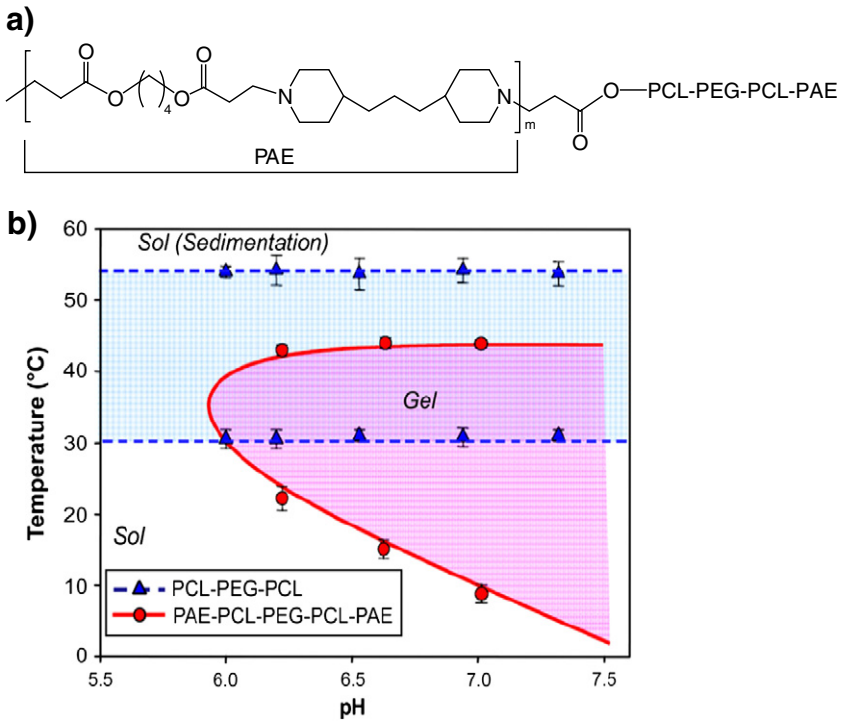


Fig. 9. (a) Chemical structure, (b) sol–gel phase transition diagram of 20 wt.% pentablock PAE–PCL–PEG–PCL–PAE copolymer solutions [114]. Copyright 2008 Elsevier.

longer stability of the protein-loaded hydrogels compared with thermosensitive hydrogels [114].

The sustained release of insulin from PAE-based hydrogels heavily depended on an ionic complex between insulin and PAE segments, as well as degradation kinetics of the copolymer complexes. Complete release of insulin was obtained in about 30 days [114,115]. In addition, the degradation rate and gelation window of hydrogels can be tuned by changing the hydrophobic PCL blocks with less hydrophobic PCLA [117] as well as graft copolymers of (PAE-g-PCL) PEG (PAE-g-PCL) [118], respectively. Apart from these, an effort has been made to synthesize PAE-based hydrogels without hydrophobic blocks, *i.e.* PAE–PEG–PAE or PEG $(PAE)_4$ (Fig. 11), which can be easily dissolved in water at relatively low pH and undergo a gel-to-sol phase transition with increasing pH and temperature, respectively [119,120]. These PAE–PEG–PAE hydrogels have shown a bioadhesive nature and controlled release of lidocaine *in vitro* [119].

Recently, pH- and temperature-sensitive multiblock poly(ester amino urethane)s were synthesized by coupling non-biodegradable poly(amino urethane) (PAU) with biodegradable PCL–PEG–PCL triblock copolymers through a condensation reaction to obtain biodegradable pH- and temperature-sensitive multiblock copolymers $(PCL–PEG–PCL–PAU)_x$ [121]. The presence of the ionizable PAU segments with tertiary amine groups is responsible for pH sensitivity in the copolymer (Fig. 12a). At pH values below 7.0, the piperazine groups of the poly(amino urethane) (PAU) segments are ionized, and the resulting copolymer solution (20 wt.%) existed in a sol state at up to 80 °C due to the electrostatic repulsion of the piperazine groups (Fig. 12b).

In contrast, at a physiological pH of 7.4, the copolymer solution displays a sol–gel transition upon increasing the temperature to 37 °C, which strongly depends on the hydrophobic/hydrophilic balance and block lengths. The mechanism of gel formation in multiblock copolymer is governed by interconnected micelles. After subcutaneous injection of 20 wt.% multiblock copolymer solutions into mice, hydrogels were formed *in vivo* in a short time (Fig. 12c). The *in vitro* sustained release of paclitaxel from the multiblock copolymer hydrogels has been observed for up to 1 month under physiological conditions [121].

Although $(PCL–PEG–PCL–PAU)_x$ multiblock copolymers are biodegradable and have ionizable PAU segments, which could form better ionic complexes with proteins and DNAs, the major drawback of these multiblock copolymers is that they are not easily dissolvable in PBS, even at low pH, due to the presence of hydrophobic PCL moieties. To overcome this, a class of pH- and temperature-sensitive multiblock copolymers without hydrophobic segments $[PEG–PAU]_x$ were synthesized by polyaddition reaction between PEG 1,4-bis(hydroxyethyl) piperazine (HEP) and 1,6-diisocyanato hexamethylene (HDI), dissolved completely in PBS at low pH has been introduced [122] (Fig. 13a). The concentrated $[PEG–PAU]_x$ solutions displayed thermo-induced sol–gel phase transition in physiological conditions. The formation of a transparent and soft gel was assessed *in vivo*. *In vitro* chlorambucil was released for 2 weeks using $[PEG–PAU]_x$ copolymer hydrogel as a drug carrier [122,123].

In addition, a novel copolymer based on poly(amino urea urethane) $[PEG–PAUU]_x$ was also designed without hydrophobic block, which dissolved at mildly acidic pH (20 °C, pH 5.5). The copolymer was suitable for mixing with bioactive molecules to avoid denaturation, and exhibited a pH- and temperature-dependent sol–gel transition (Fig. 13b) [124]. Due to the presence of urea groups in the copolymer, the gelation was achieved with a low concentration of the copolymer, which may decrease the possibility of systematic cytotoxicity of the implanted biomaterials. Gels formed *in vivo* or *in vitro* have excellent mechanical properties and long-term stability, and prolong the sustained release of protein (FITC-BSA) for 6 weeks (Fig. 14) [124].

However, $[PEG–PAU]_x$ and $[PEG–PAUU]_x$ have a disadvantage in regard to controlling the molecular weight of PAU and PAUU segments. Therefore, we further designed injectable poly(amidoamine)-poly(ethylene glycol)-poly(amidoamine) PAA–PEG–PAA triblock copolymer hydrogels exhibiting pH and temperature sensitivity for bioadhesive applications (Fig. 15a). It has been possible to precisely control the composition of triblock PAA–PEG–PAA compared to

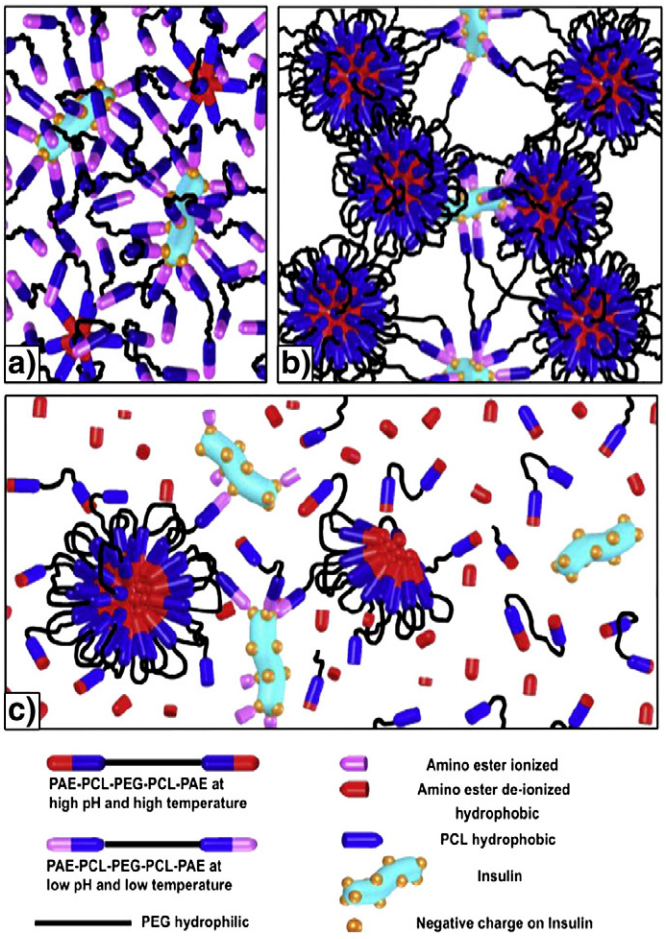


Fig. 10. Representative mechanism of insulin loading and release. (a) The polymer solution in sol state at 10 °C and pH 7.0 with the ionic complex between insulin and PAE–PCL–PEG–PC–PAE. (b) The gel formed by insulin free PAE–PCL–PEG–PCL–PAE after injection to human body (37 °C and pH 7.4). (c) Insulin release from gel by polymer degradation [114]. Copyright 2008 Elsevier.

$[PEG–PAU]_x$ and $[PEG–PAUU]_x$. The presence of poly(amidoamine) (PAA) blocks in triblock polymer was mainly responsible for the dual sensitivity (pH, temperature), which was converted from a hydrophilic state to a hydrophobic state upon increasing pH and/or temperature [125,126]. The sol-to-gel transition of this copolymer in an aqueous medium (12.5 wt.%) is illustrated as a function of pH and temperature in Fig. 15b. At low pH (~6.8), the polymer was soluble due to the presence of ionized PAA blocks and exhibited a sol state at up to 60 °C. In contrast, at pH above 7.0, the high degree of deionization of the PAA blocks and the association of the bridged micelles were responsible for the formation of gel.

Further, at a higher temperature (37 °C), the hydrophobicity of PAA blocks was enhanced and the tightly ordered packing of bridged micelles, and a strong gel was formed. The sol–gel behavior was controlled by adjusting the molecular weights of the PEG and PAA blocks, and changing the PAA–PEG–PAA concentration. *In vivo* experiments showed that upon subcutaneous injection of the copolymer solution into rat, a gel was immediately formed. Copolymer hydrogels demonstrated strong bioadhesive properties (Fig. 15c) and the controlled release of flubiprofen [125,126]. These copolymer hydrogels ([PEG–PAU]x, PAA–PEG–PAA) have gained great potential for use as drugs/proteins delivery carriers because of their ability to form hydrogen bonds and ionic interactions with negatively charged drugs/biomolecules, and because of their non-cytotoxicity.

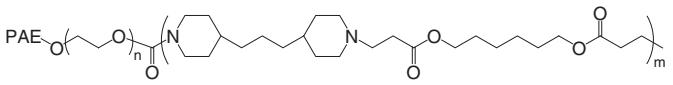


Fig. 11. Chemical structure of PAE–PEG–PAE block copolymer.

The use of degradable polymeric biomaterials is desirable, especially for controlled drug delivery and avoiding the need for the surgical removal of polymers. Efforts have been made to design a biodegradable PAU-based polymer without hydrophobic blocks, which led to the recent development of poly(β-amino ester urethane)-based copolymers (PAEU) (Fig. 16) [127–129].

Multiblock copolymers $[PEG–PAEU]_x$ were synthesized by the polyaddition of isocyanate groups of HDI and hydroxyl groups at the ends of PEG and monomer amino ester dihydroxyl (HPB) in the presence of DBTL as a catalyst [127]. These copolymers have a urethane and tertiary amine groups, which make strong hydrogen bonds and ionic interactions with anionic drugs and proteins. Aqueous solutions of $[PEG–PAEU]_x$ copolymers displayed a sol-to-gel phase transition with increasing pH and temperature (Figs. 17a & b). At low pH and temperature (pH 6.0, 20 °C), the copolymers existed in a sol state due to the electrostatic repulsion between the ionized PAEU blocks [127,128].

In contrast, in physiological conditions (pH 7.4, 37 °C), the PAEU blocks were deionized by hydrophobic interactions and hydrogen bonding between the deionized PAEU blocks, which led to the formation of bridged micelles, resulting in gel formation. It was also found that the gel window could be controlled by varying the PEG/PAEU ratio, copolymer concentration, and PEG molecular weight. The gel formation, biodegradability, and non-cytotoxicity of $[PEG–PAEU]_x$ copolymers were assessed *in vivo* and *in vitro*. The controlled release of human growth hormone (hGH) for 5 days *in vitro* and 3 days *in vivo* from the triblock PAEU–PEG–PAEU hydrogel was investigated without an initial burst effect. The

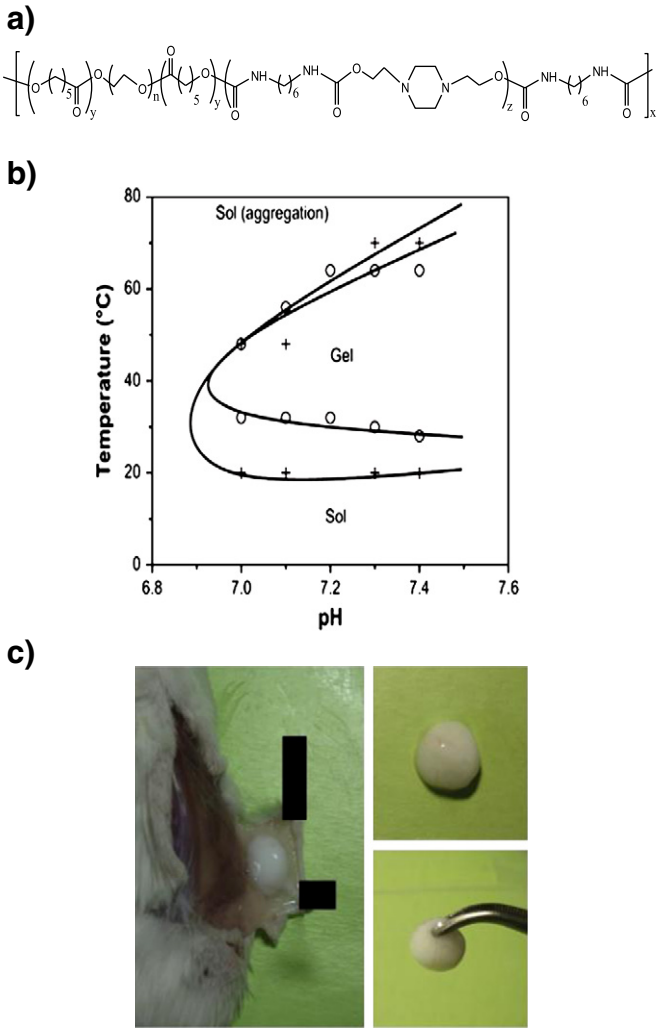


Fig. 12. (a) Chemical structure, (b) pH- and temperature-dependent sol–gel phase diagrams, and (c) *in vivo* gelation of the 20 wt.% $(PCL–PEG–PCL–PAU)_x$ multiblock copolymer [121]. Copyright 2008 Elsevier.

controlled release of hGH from the complex hydrogel was attributed to the formation of ionic complex between the anionic hGH and cationic triblock PAEU–PEG–PAEU hydrogels (Fig. 18). Multiblock $[PEG–PAEU]_x$ hydrogel systems also showed the sustained release of doxorubicin for more than 5 weeks.

However, the developed pH- and temperature-sensitive hydrogels are either cationic or anionic types that can make better interactions with only cationic or anionic drugs or biomolecules. A novel class of amphoteric, biodegradable, and biocompatible copolymers, poly(urethane amino sulfamethazine)-based block-copolymers (PUASM), were designed, which produced both cationic and anionic hydrogels with respect to changes in pH and temperature (Fig. 19) [129]. The PUASM block copolymers were synthesized through the polyaddition of 1,6-diisocyanatohexamethylene (HDI) with hydroxyl groups of a synthesized dihydroxyl amino sulfamethazine monomer (DHASM) and of triblock poly(-caprolactone-lactide)-poly(ethylene glycol)-poly-(-caprolactone-lactide) (PCLA–PEG–PCLA) copolymers in the presence of dibutyl tin dilaurate (DBTL) as a catalyst. Dual ionic properties of the PUASM block copolymers were confirmed by zeta potential measurement. Copolymers displayed special closed-loop reversible sol–gel–sol phase transition due to the presence of anionic sulphonamide and cationic tertiary amine pH-sensitive groups in the poly(amino sulfamethazine) (PASM) blocks. The ionization of tertiary amine in slightly acidic conditions (pH 6.5) and that of sulfonamide groups in basic conditions (pH 8.5) were responsible for the sol state of the copolymer in the temperature range of 0–65 °C.

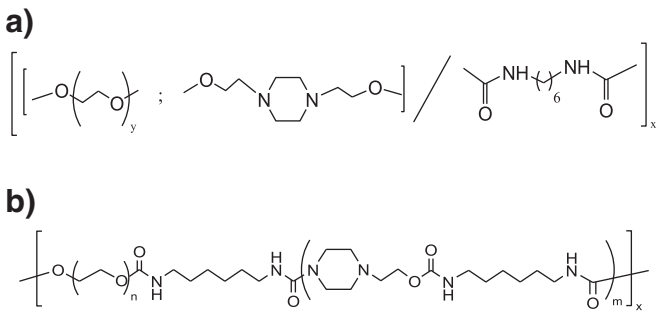


Fig. 13. The chemical structure of (a) poly(amino urethane) $[PEG–PAU]_x$ and (b) poly(amino ureaurethane) $[PEG–PAUU]_x$.

In contrast, in the pH range of 6.8–8.2, gel was formed as the temperature increased to body temperature due to the enhancement of the hydrophobic nature of both PASM and PCLA segments. Upon increasing the temperature further, a gel-to-sol transition appeared because of the partial dehydration of PEG segments and the breakage of hydrophobic interactions (Figs. 20a & b). *In vivo* gel formation was observed after subcutaneous injection of both acidic (pH 6.8) and basic pH (pH 8.5)

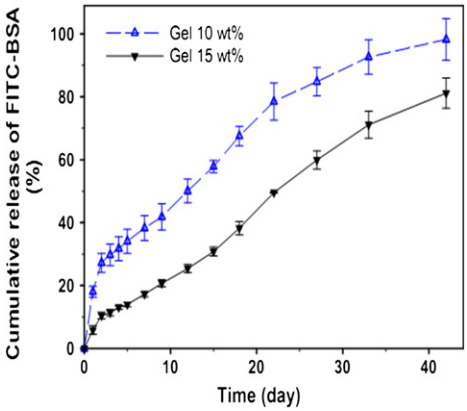


Fig. 14. *In vitro* sustained release of FITC-BSA from $[PEG–PAUU]_x$ hydrogel [124]. Copyright 2012 Elsevier.

solutions in Sprague-Dawley (SD) rats (Fig. 20c). An *in vivo* release of hGH from the PUASM hydrogels was demonstrated for more than 3 days with minimal initial burst (Fig. 21). Controlled release of hGH protein from hydrogels resulted from ionic interaction between the anionic hGH and cationic moieties in the PUASM copolymers [129].

In contrast to block copolymers, whose gelation mechanism was attributed to the formation of bridged micelle networks, we introduced another new class of pH- and temperature-responsive gelators based on oligo(amido amine)s (OAAs) and oligo-(β-amino ester urethane)

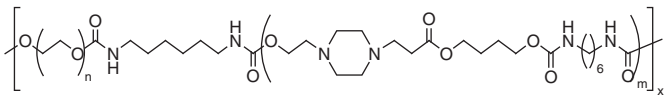
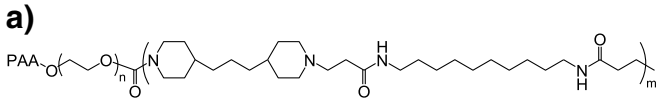
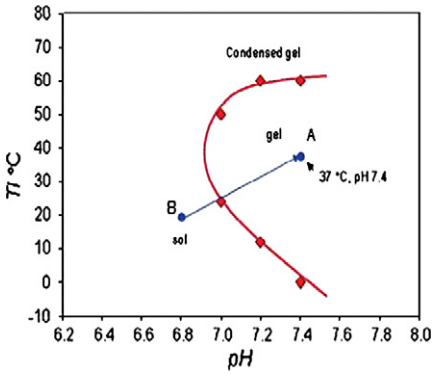


Fig. 16. Poly(β-amino ester urethane)-based multiblock copolymers $[PEG–PAEU]_x$.

(OAEU), where hydrogel formation resulted from non-covalent forces, such as hydrophobic interactions or hydrogen bonding, providing a structure-tuning method to modulate the elasticity of the supramolecular gels and easy injection into the body using a syringe in physiological conditions [130,131]. A series of oligo(amidoamine)s (OAAs) that can be reversibly switched between sol and gel phases upon changes in pH and temperature have been synthesized and characterized (Figs. 22a & b).

The gel properties of (OAAs) were strongly dependent on the methylene group lengths of diacryl amide segments. With increasing methylene group lengths, the sol–gel phase diagram was shifted to a lower pH value, presumably due to increased hydrophobicity. A scanning electron micrograph (SEM) of the OAA hydrogel showed that the molecules assembled into a three-dimensional interconnecting network (Fig. 22c), and intermolecular hydrogen bonding between the amide moieties contributed to the stability of the gels, as confirmed by FTIR spectra. However, these hydrogels were degraded in more than six months, which limited their pharmaceutical applications. Further, we introduced a modification of OAA gelator with oligo-(β-amino esters) OAE, a biodegradable thermosensitive and pH-sensitive hydrogel oligo-(amidoamin/β-amino esters) (OAAAE) developed for protein delivery applications [132]. OAAAE was synthesized in one step through





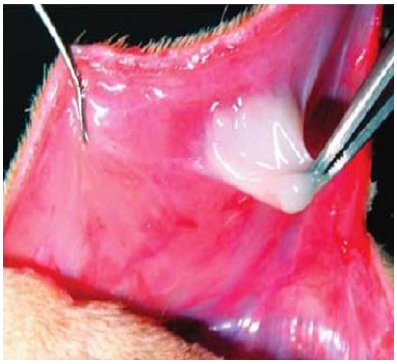


Fig. 15. (a) Chemical structure, (b) pH and temperature phase diagram of the 12.5 wt.% PAA–PEG–PAA triblock copolymer in an aqueous medium. (c) *In vivo* hydrogel was found to be adhered to the SD rat tissue after only 1 min, showing bioadhesive properties with respect to SD rat tissue [125]. Copyright 2009 American Chemical Society.

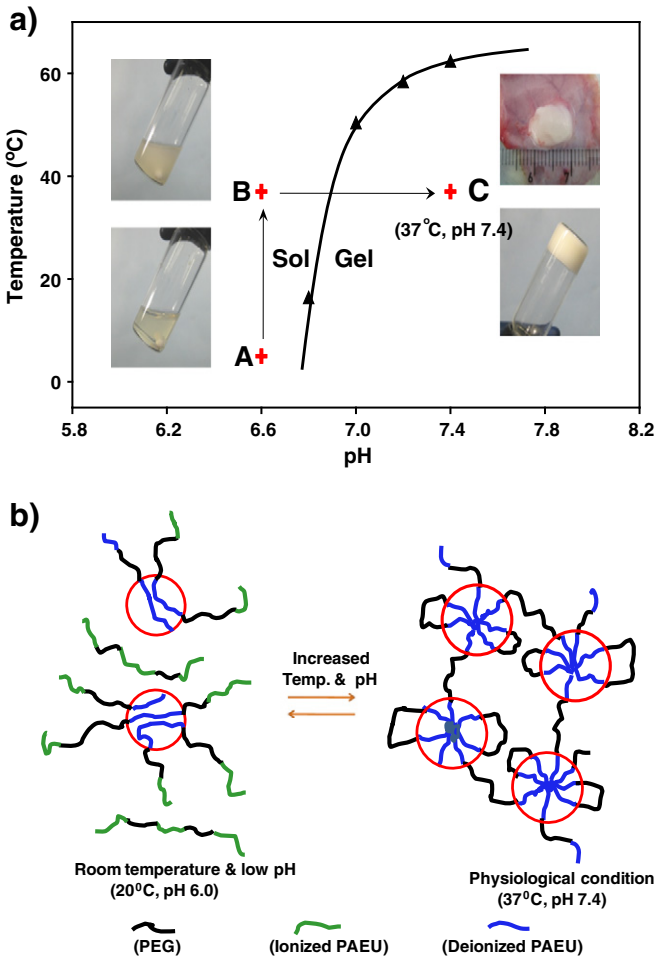


Fig. 17. (a) Sol–gel phase diagram, (b) representative mechanism of gel formation of $[PEG–PAEU]_x$ multiblock copolymer hydrogels (20 wt.%) [127]. Copyright 2011 from the Royal Society of Chemistry.

Michael addition of the secondary amine groups of 4,4-trimethylene dipiperidine (TMDP) with the vinyl groups of 1,8-octylene diacrylamide (ODA) and 1,6-hexane dioldiacrylate (HDA). At a low pH of 6.6, OAAAE was hydrophilic in nature and existed in a sol state in the temperature range of 0–70 °C. However, at a physiological pH of 7.4, OAAAE becomes hydrophobic due to the deprotonation of tertiary amines, resulting in the formation of gel. Upon injecting OAAAE polymer solution (20%, pH 6.6) subcutaneously in rats, gel formation occurred at physiological pH and temperature. The applicability of this hydrogel for prolonged insulin release was observed without the initial burst *in vitro* and *in vivo* [132].

Therefore, we developed a wide range of pH- and temperature-sensitive hydrogels based on anionic, cationic, and dually cationic and anionic behaviors, which have the advantages of making a good ionic complex, bio-adhesive nature, and a desired range of biodegradation (1 week to several months) with excellent mechanical properties. Recently, research has been focused mainly on the use of these developed hydrogel systems for various challenging biomedical applications, like protein therapeutics have gained significant attention over small-molecule drugs, because of their advantageous properties. However, the major challenges associated with protein delivery are achieving

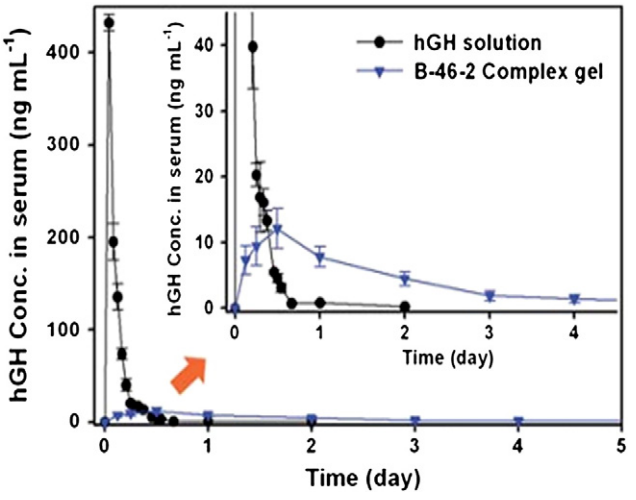


Fig. 18. Human growth hormone (hGH) concentration in the plasma of SD rats at different points in time after the administration of 200 μL of a hGH solution (10 mg mL^{-1}) and 200 μL of the hGH-loaded PAEU–PEG–PAEU hydrogel complex (hGH 10 mg mL^{-1}, 20 wt.% copolymer B-46-2) [128]. Copyright 2011 from the Royal Society of Chemistry.

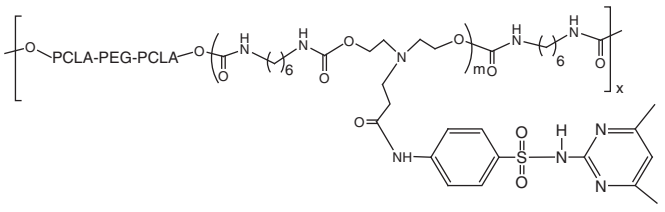


Fig. 19. Chemical structure of poly(urethane amino sulfamethazine)-based block copolymers (PUASM).

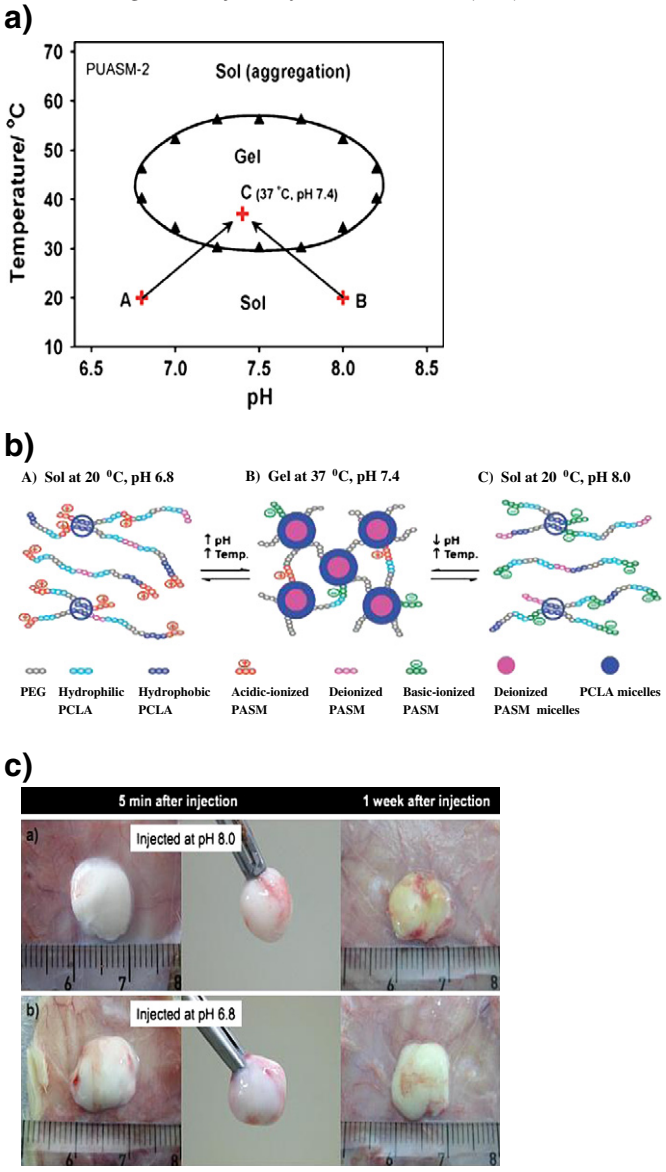


Fig. 20. (a) Closed-loop sol–gel phase diagram (25 wt.%) and (b) schematic representation of mechanism of sol–gel–sol phase transition of PUASM hydrogels with changing temperature and pH. Ionization of basic amine groups in the PASM at slightly acidic pH (A) and acidic sulfonamide groups in the PASM at slightly basic pH (B), resulting in a sol state in water. In contrast, relative deionization of both basic and acidic portions of the PASM and increases in hydrophobicity of the PCLA under physiological conditions led to formation and interconnection of nanostructured polymeric micelles, resulting in gelation (C). (c) Optical micrographs of *in vivo* PUASM-2 (25 wt.% hydrogels) at 5 min and one week after the copolymer solutions were injected into SD rats at room temperature and at pH 8.0 and pH 6.8 [129]. Copyright 2012 from the Royal Society of Chemistry.

sustained long-term release with minimal burst and maintaining their delicate three-dimensional stability. Presently, researchers are trying to develop sustained and prolonged protein delivery carriers using the concept of nanotechnology. The introduction of protein-intercalated 2-D nano-fillers into the pH- and temperature-sensitive block copolymer hydrogels to produce nano-biohybrid hydrogels has a dramatic effect on the delivery of proteins without affecting its stability. In addition, our groups are working to generate long-term injectable biodegradable X-ray markers using pH- and temperature-sensitive hydrogels with gold nanoparticles and $BaSO_4$ to improve upon the present limitations associated with X-ray markers for cancer diagnosis and therapy. Apart from these, hepatocellular carcinoma (HCC) has been recognized as one of the top causes of cancer-related death. For this issue, research has been focused on chemo-embolization therapy for the treatment of HCC using a catheter to deliver a pH- and temperature-sensitive hydrogel mixture of an imaging agent and a therapeutic agent (doxorubicin) through hepatic arteries into tumor sites. Our group is developing novel injectable anionic hydrogel systems that can form gel in liver tumor conditions (low pH) and deliver mixtures of DOX and imaging agent through catheters for transcatheter arterial chemo-embolization therapy of HCC.

4. Conclusion and perspectives

Stimuli-sensitive hydrogels have enormous potential in biomedical applications for site-specific controlled drug delivery. In this review, the characteristics, sol–gel mechanisms, and biomedical applications (such as drugs/proteins delivery and tissue engineering) of temperature- and pH-/and temperature-sensitive hydrogels were highlighted. Initially, the developed thermoresponsive copolymers (poloxamers) suffer from a serious problem related to its biodegradability and toxicity. PEG-based thermoresponsive PEG/polyesters triblock copolymers have overcome the problem associated with poloxamers, making it the most suitable and effective *in situ* gelling agent for delivery. However, the acid degradation product of PEG/polyesters may limit their applications. For this concern, polyphasphazenes and polypeptides were developed. The potential

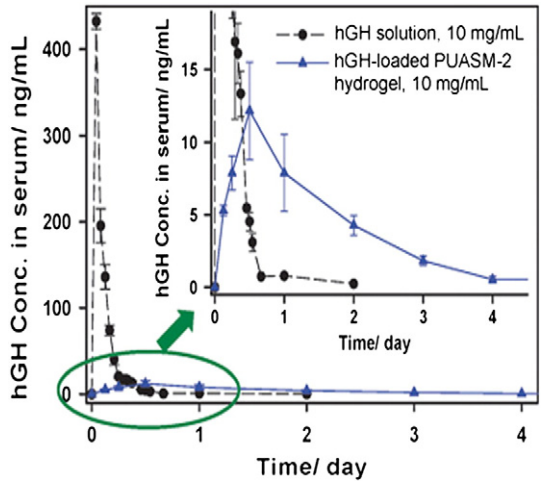


Fig. 21. Concentration of hGH in the plasma of SD rats after injecting 200 μL of the hGH solutions (10 mg mL^{-1}) and 200 μL of the hGH-loaded PUASM-2 (25 wt.%) solutions (hGH 10 mg mL^{-1}) [129]. Copyright 2012 from the Royal Society of Chemistry.

applications of thermosensitive injectable hydrogels are limited due to several issues, including needle clogging, acidic degradation products, and complicated burst release. Currently, pH- and temperature-sensitive block copolymer hydrogels (anionic, cationic, and both dually cationic/anionic) have been developed to solve clogging during injection, which allow facile injection into deep sites in the body and minimize the acidic degradation products and burst release. These pH- and temperature-sensitive cationic/anionic hydrogels formed a better ionic complex with anionic/cationic biomolecules, in body conditions, resulting in sustained release. Smart dually ionic injectable hydrogels have been recently introduced to deliver both anionic and cationic types of drugs/proteins, and have great advantages over cationic/anionic injectable hydrogels, with which only one type of drug can be delivered. The discussions suggest that the combination of pH- and temperature-sensitive block copolymer hydrogels open a new perspective in biomedical applications with tunable sol-gel transition, morphological and biodegradation behavior. Although considerable achievements have been attained in pH- and temperature-sensitive injectable block copolymer hydrogels, there are still several unsolved challenges for clinical applications. For example, the developed pH- and temperature-sensitive block copolymers do not contain simple chemical structures for approval from the Food and Drug Administration (FDA). Thus, the development of a simple, well-defined chemical structure of pH- and temperature-sensitive block copolymer hydrogels is an important challenge. From the point of view of the living body, issues such as biocompatibility, biointegration, and medical safety are key considerations. The developed pH- and temperature-sensitive block copolymers like poly(amidoamine) injectable hydrogel containing cytotoxic moieties may cause inflammation at the site of injection. Thus, optimizing and modulating the structure of copolymers represent a major scientific challenge. In addition, for particular tissue engineering applications, the development of injectable block copolymer hydrogels with desired mechanical and degradation properties to foster cell adhesion, proliferation, and differentiation should also be major issues.

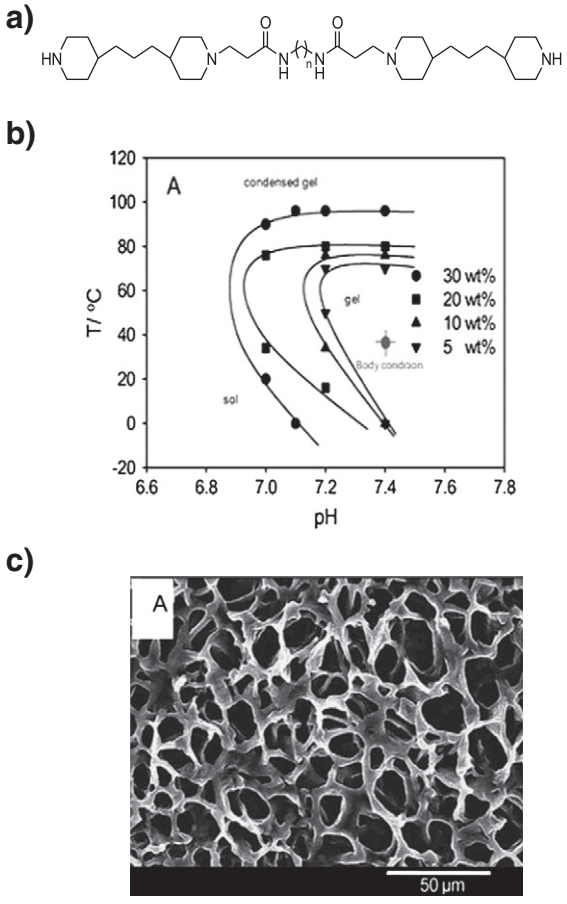


Fig. 22. (a) Chemical structure, (b) sol–gel phase diagram, and (c) SEM micrograph of oligo(amido amine)s (OAAs). Copyright 2010 from the Royal Society of Chemistry.

Acknowledgment

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