Injectable hydrogels as unique biomedical materials

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A concentrated fish soup could be gelled in the winter and re-solled upon heating. In contrast, some synthetic copolymers exhibit an inverse sol–gel transition with spontaneous physical gelation upon heating instead of cooling. If the transition in water takes place below the body temperature and the chemicals are biocompatible and biodegradable, such gelling behavior makes the associated physical gels injectable biomaterials with unique applications in drug delivery and tissue engineering *etc*. Various therapeutic agents or cells can be entrapped *in situ* and form a depot merely by a syringe injection of their aqueous solutions at target sites with minimal invasiveness and pain. This *tutorial review* summarizes and comments on this soft matter, especially thermogelling poly(ethylene glycol)–(biodegradable polyester) block copolymers. The main types of injectable hydrogels are also briefly introduced, including both physical gels and chemical gels.

1 Introduction

Hydrogels are three-dimensional polymeric networks absorbing a significant amount of water or biological fluids. These networks can be classified into two main categories according to the types of cross-linking. The network crosslinked by covalent bonds is the so-called chemical gel, while the formation of a physical gel takes place *via* physical association between polymeric chains or nanoparticles. In some cases, chemical and physical gellings might coexist in one hydrogel. Due to their capability of retaining water and other biomimetic properties, hydrogels constitute unique biomaterials applied in drug delivery, tissue engineering, and medical devices *etc*.[1–5]

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Among various biomaterials, injectable hydrogels formed by *in situ* chemical polymerization or by the sol–gel phase transition have recently been paid much attention.[6,7] These material systems are flowable aqueous solutions before administration, but once injected, rapidly gel under physiological conditions. The gel formation after injection brings about some advantages: an injectable matrix can be implanted in the human body with minimal surgical wounds, and bioactive molecules or cells can be incorporated simply by mixing before injection. Following gelation, these matrices become drug delivery deposits in pharmaceutics or cell-growing depots for tissue regeneration. Thermosensitive hydrogels are especially attractive as specific injectable biomaterials due to their spontaneous gelation with the employment of body temperature, free of any requirement of extra chemical treatment.

Sustained drug release not only reduces administration times and undesired side effects, but also improves the patients' compliance and comfort significantly. When applied in a drug delivery system, the injectable drug/polymer

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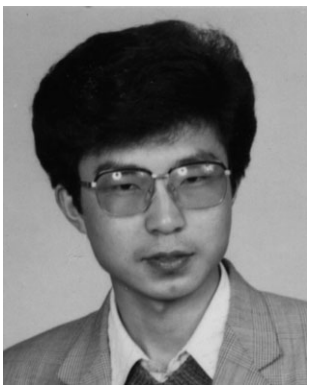

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formulation can be free of any organic solvent in the drug-loading process (an organic solvent might denature labile therapeutic agents like proteins). The rate of drug release is easily adjusted *via* altering the material properties. These hydrogel formulations are useful for parenteral and topical injection for a site-specific action.

Tissue engineering aims to develop biological substitutes that restore, maintain, or improve the lost or damaged tissues and organs. The typical tissue engineering paradigm depends on a scaffold that is utilized as a temporary support matrix for cell transplantation. Biocompatible and biodegradable poly (hydroxy ester)s such as polyglycolide (PGA), polylactide (PLA), poly(ε-caprolactone) (PCL), and their copolymers have been extensively investigated.[3,8,9] Conventionally, these materials should be prefabricated with a porous interior structure for cell loading and with a complicated exterior shape reminiscent of an organ. The surgical intervention in the implantation of such a preshaped porous scaffold is thus inevitable. An injectable hydrogel affords an alternative approach to encapsulate cells with minimal invasiveness. In addition, *in situ* cell immobilization is also beneficial for filling an irregular defect.

A schematic presentation of an injectable biomaterial is shown in Fig. 1. An ideal injectable medical hydrogel should meet the following requirements:

(1) In order to guarantee the injectability, the system should be, as usual, in a sol state before administration. The sol is desired to be of sufficiently low viscosity and thus allow a smaller pinhead in injection to alleviate the pain of a patient.

(2) Gelation *via* either chemical crosslinking or physical association starts to happen or is completed *after* injection.

(3) The gels should be biodegradable or gradually dissolvable, and the products should be bioresorbable.

(4) The polymer itself and the degradable products should be biocompatible. So are some necessary additives such as crosslinking agents in the case of *in situ* chemical gelling.

(5) Some specific requirements should be met, for instance, a sustained release profile for a drug delivery system, or cell-adhesive capability for tissue engineering.

To date, several reviews pertinent to injectable hydrogels have been published. For instance, Hoffman has given an introduction of medical hydrogels;[1] Ruel-Gariepy and Leroux have generally summarized *in situ* forming thermosensitive hydrogels including both natural and synthetic polymers;[7] Jeong *et al*. have reviewed thermosensitive sol–gel reversible hydrogels;[6] Kissel *et al*. have specifically commented on ABA-triblock copolymers as candidates for protein carriers.[2] The present review summarizes biodegradable injectable hydrogels including both *in situ* chemically-crosslinked hydrogels and physical gels, and their applications in drug delivery and tissue engineering. Particular attention will be paid to the recent developments of reversibly thermogelling synthetic polymers.

A well-known thermosensitive polymer, poly(*N*-isopropylacrylamide) (PNIPAM) is not emphasized in this review. This polymer has been fully or partially reviewed by other researchers.[6,7] The homopolymer exhibits a lower critical solution temperature (LCST) in water. The associated chemically-crosslinked network undergoes a volume phase transition, and an aqueous solution of copolymer of high molecular weight (MW) PNIPAM and poly(acrylic acid) shows a reversible sol–gel transition with the increase of temperature. The relatively weak introduction of PNIPAM in our review is due to its non-biodegradability unless a significant chemical modification is performed, and also due to the nerve toxicity of residual acrylamide-like monomers and the lack of sufficient *in vivo* evidence of the bioabsorbance of a high-MW PNIPAM so far. Although this polymer serves as a good model for physical and chemical studies of thermosensitive polymers and is applicable in some fields including medicine (for instance, as a smart substrate for *in vitro* preparation of tissue-engineering cell sheets[10]), it seems, in our opinion, rather hard to be commercialized as an implanted material *in vivo*.

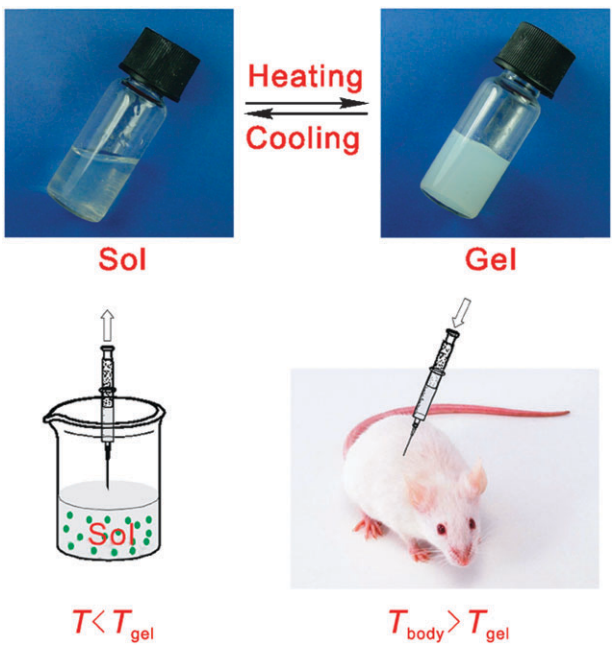

Fig. 1 A schematic presentation of an injectable hydrogel system exampled by a physically thermogelling material. T_{gel} is the sol–gel transition temperature. The polymers could be dissolved in water to form a sol at low temperatures. Bioactive molecules or cells indicated by the dots in the lower-left image can be incorporated by simple mixing with sols. The sols are injectable, and *in situ* gelling takes place after injection if the gelling temperature is lower than the body temperature T_{body}. As a result, the encapsulation of drugs or cells and the implantation of biomaterial are carried out with minimal surgical invasiveness.

2 Main types of injectable hydrogels

2.1 Chemically crosslinked hydrogels

In situ chemical cross-linking is a conventional approach to prepare a stable hydrogel. As an implanted biomaterial, biodegradability or bioabsorbability is also necessary. Here, we do not aim to give a full summary of chemical hydrogels, but just introduce a few biodegradable hydrogels that have had attention recently. One of the more interesting chemical gels is prepared based upon a macromer technique. The term "macromer" refers to a polymerizable monomer, but with a high MW. The most popular polymerization obeys the free

radical mechanism triggered *via* a photoinitiator or a thermal radical initiator. Hubbell *et al*. explored a photopolymerized hydrogel made from poly(ethylene glycol)-*b*-poly(α-hydroxy acid) diacrylate macromers.[11] Here, the introduction of oligo(aliphatic ester) makes the resulting gels biodegradable, and the hydrogel has been tried as a novel protein drug carrier.[11] Nevertheless, the capacity of light penetration in the body restricts the applications of the photoinitiated system to a certain extent.

In order to overcome the problem above, our group tried a water-soluble redox initiation system consisting of ammonium persulfate and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, and examined its efficacy in initiating macromers containing a biodegradable moiety and either a poly(ethylene glycol) (PEG) segment or a thermosensitive block copolymer composed of PEG and poly(propylene glycol) (PPG) to prepare biodegradable chemical hydrogels.[12,13] The degradation rate and gelation time were found to be well tuned. The *in vitro* cytotoxicity of the redox initiating system was also evaluated.[14] In addition, the corresponding computer modeling of gel formation *via* free radical polymerization of amphiphilic macromers has been performed.[15]

Mikos *et al*. exploited *in situ* crosslinked oligo(poly(ethylene glycol)fumarate) hydrogels. The hydrogel was formed by thermal free-radical polymerization under physiological conditions. These hydrogels are of good biocompatibility and biodegradability, and have been used for drug delivery and cell attachment.[16]

Some chemical crosslinking approaches free of initiators have also been suggested. An *in situ* hyaluronic acid (HA) hydrogel was prepared by a chemical crosslinking upon mixing of one HA derivative with a hydrazide moiety and another HA derivative with an aldehyde.[17] The crosslinked matrix showed good biocompatibility *in vitro* and *in vivo*, and has been used in the prevention of peritoneal adhesions in rabbit experiments.[17] The Michael addition between thiols and the associated electrophilic α,β-unsaturated agents offers another novel approach to obtain *in situ* forming hydrogels.[18,19] For instance, Hubbell *et al*. synthesized PEG hydrogels by the Michael addition between multi-thiol compounds and either multi-acrylate or multi-vinyl sulfone PEG chains.[18] A rapid reaction was achieved under physiological temperature and pH. The associated thiol-involved reaction was predominant over the possible Michael addition between the multi-functional PEGs and biological amines in proteins, and thus the adverse effect in protein encapsulation during cross-linking could be ignored. The incorporated human growth hormone was released sustainedly up to a few months and the integrity of the protein was preserved quite well.[18]

2.2 Physical hydrogels

Besides chemically crosslinked hydrogels, physical hydrogels constitute another injectable hydrogel. Physical gelation is free of any chemical reaction. The biocompatibility problems of residue initiators or monomers in some chemical gelations are also avoided in physical gelation.

Some polymers in water undergo reversible phase transition upon a modest change of environmental conditions like temperature, pH, electric field, salt, or ionic concentration *etc*. For example, alginates are a family of linear polysaccharides, and their aqueous solutions could, after addition of multivalent cations, be gelled due to the coulombic interaction. Calcium alginate has been successfully applied in tissue engineering of an autologous porcine cartilage.[3] This is an exceptional case of injectable physical hydrogels because the gel-inducing factor is added *before* injection. In this case, slow physical gelation is required in order to avoid syringe jam. To combat this, calcium ions were released slowly from the $CaSO_4$ powder after the powder was added to a sodium alginate aqueous solution.[20]

The hydrophobic association provides another driving force of physical gelation. For instance, HA derivatives modified with linear amines can form viscous physical gels in water and maintain long-lasting stability. A HA physical gel, a product of Fidia Advanced Biopolymers, has been used as an injectable matrix for reconstruction of soft tissues such as adipose in rats, and a scrutiny of inflammatory response has obtained a positive result for the HA physical gel as a tissue engineering material.[21] In contrast to the physical gels emphasized in the remaining text, the physical gelling of HA derivatives is usually neither reversible nor driven by body temperature.

In most cases, with injectable physical hydrogels, the gelation happens *after* injection. The most significant gel-inducing factors are physiological conditions such as body temperature. The favoured thermosensitive material might exhibit an *inverse* sol–gel transition. The term ''inverse'' here means that gelation occurs upon heating instead of cooling. For drug delivery systems, the low temperature used when mixing polymers and drugs is beneficial for protecting the drug from denaturation or aggregation; for tissue engineering, use of low temperatures when mixing cells with materials is beneficial for cell prevention.

Some typical biodegradable or bioabsorbable thermogelling polymers reported so far are shown in Fig. 2. According to the origin of materials, thermogelling hydrogels can be classified into natural (or seminatural) polymeric systems and synthetic polymeric systems. The polymers in the former system include cellulose, chitosan, xyloglucan, gelatin *etc*. and their derivatives, and some examples are presented in Fig. 2a and b. The polymers in the latter class include some polyethers, block copolymers of polyethers and biodegradable polyesters, and synthetic polypeptides *etc*. (Fig. 2c–i). The synthetic polymers have relatively easier to control MW and structures. Those thermogelling synthetic polymers, especially PEG–polyester copolymers will be highlighted in the next section.

3 Thermogelling synthetic copolymer hydrogels

3.1 PEG–PPG block copolymers

Triblock copolymers PEG–PPG–PEG (Fig. 2c), known as Pluronic (BASF) or Poloxamer (ICI), are commercialized non-ionic surfactants (the copolymer is also called poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (PEO–PPO–PEO)). The bulk Pluronics exhibit different states from liquid to paste to solid depending on MW and PEG–PPG block ratio. At normal temperatures, PEG blocks

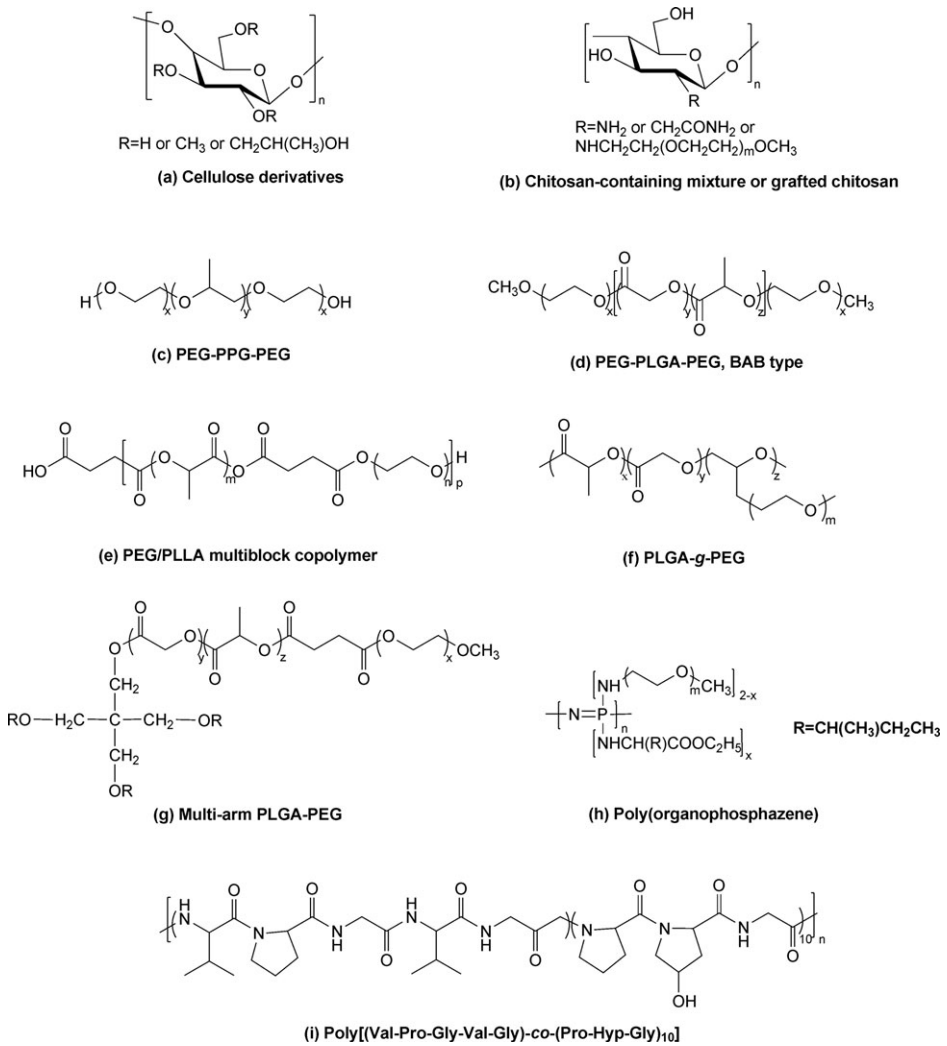

Fig. 2 Chemical formulae of some biodegradable or bioabsorbable polymers capable of thermogelling in water with an inverse sol–gel transition. (a) Methyl- or hydroxypropyl methyl-cellulose;[7] (b) chitosan derivatives (chitosan itself is also thermogellable after addition of polyol salts such as β-glycerophosphate);[7] (c) poly(ethylene glycol)-*b*-poly(propylene glycol)-*b*-poly(ethylene glycol) (PEG–PPG–PEG, Pluronic or Poloxamer);[6] (d) poly(ethylene glycol)-*b*-poly(D,L-lactic acid-*co*-glycolic acid)-*b*-poly(ethylene glycol) (PEG–PLGA–PEG, BAB-type);[22] (e) poly(ethylene glycol)–poly(L-lactic acid) (PEG–PLLA) multiblock copolymer;[23] (f) poly(D,L-lactic acid-*co*-glycolic acid)-*g*-poly(ethylene glycol) (PLGA-*g*-PEG);[24] (g) multi-arm poly(D,L-lactic acid-*co*-glycolic acid)-*b*-poly(ethylene glycol) (PLGA–PEG);[25] (h) poly(organophosphazene);[26] (i) poly[(valyl-prolyl-glycyl-valyl-glycyl)-*co*-(prolyl-*trans*-4-hydroxyprolyl-glycyl)$_{10}$] (poly[(Val-Pro-Gly-Val-Gly)-*co*-(Pro-Hyp-Gly)$_{10}$])[27].

are hydrophilic and PPG blocks are hydrophobic. The amphiphilic copolymers can be self-assembled into micelles in water above the critical micellization concentration (CMC). Some Pluronic aqueous solutions can form thermoreversible gels when the polymer concentration is above the critical gelation concentration (CGC).

In the past few decades, Pluronics perhaps represent the most intensively investigated thermogelling polymers for drug delivery carriers. Pluronics have also been tried in tissue engineering as injectable hydrogels.[20] However, Pluronic hydrogels are not considered as ideal implanted materials due to the non-biodegradability of the polymers, low mechanical strength and relatively rapid erosion of the gels at the injection site, although a Pluronic or Poloxamer with MW $<$ 13 000 is considered to be bioabsorbable (penetration out of blood in kidney).

In order to circumvent the drawbacks of common Pluronic gels, multiblock Pluronic copolymers *via* linking biodegradable carbonate, ester, disulfide, urea or urethane bonds were designed and synthesized;[28,29] a covalent linking of PEG and PPG chains to obtain a thermogelling PEG–PPG multiblock copolymer was also carried out recently using different synthetic pathways and diverse coupling agents such as phosgene and diacyl chlorides.[28,30] The degradation rates of the resulting multiblock copolymer hydrogels were controlled by adjusting the length and composition of biodegradable moieties such as aliphatic oligoesters, which are inserted into the backbones. But a relatively long hydrophobic oligoester in the copolymer might lead to the disappearance of the sol–gel transition.[29] These new materials also showed much enhanced rheological properties compared to the associated Pluronics.[28–30] A 30 wt% (F127)$_4$ hydrogel released, *in vitro*, RG-13577, an

anti-restenosis agent, for up to 40 days *versus* 7 days using Pluronic F127.[28]

3.2 PEG–PLGA block copolymers

While the nanoparticle formation of amphiphilic block copolymers in water is well known, physical gelling due to macroscopic self assembly of block copolymers is not trivial. In 1997, Kim and co-workers reported a temperature-induced spontaneous physical gelation of block copolymers composed of PEG and biodegradable polyester.[31] Considering further the biodegradability of these copolymers, the pioneering work of Kim's group exploited a new era of injectable biomaterials, which triggered further studies on the block copolymers of PEG and PLGA.

The initial block copolymers of PEG and poly(L-lactide) (PLLA) described in 1997 exhibited a normal gel–sol transition in water, namely, the gelation occurred upon a decrease of temperature.[31] The entrapment of drugs at an elevated temperature might lead to denaturation of bioactive agents such as proteins. Therefore, an inverse thermosensitive system was called for, leading to the invention of PEG–PLGA hydrogels.[22,24,32]

3.2.1 Synthesis of PEG–PLGA copolymers. Over the last decade, various thermogelling block copolymers have been synthesized and characterized, which have different macromolecular structures including diblock, triblock, multiblock, and graft architectures. Some examples are shown in Fig. 2d–g. All of these block copolymers were generated based on the principle of ring opening polymerization and a coupling reaction as usual. The synthesis route of a linear ABA type triblock copolymer, PLGA–PEG–PLGA could be performed in one step, as shown in Fig. 3. PEG–PLGA–PEG (BAB type) triblock copolymers was usually synthesized by two steps: firstly, ring-opening polymerization of lactide and glycolide in the presence of monomethoxy poly(ethylene glycol) (mPEG) to obtain a diblock copolymer; secondly, the covalent binding of the diblock copolymers using hexamethylene diisocyanate as a linker to prepare triblock copolymers.[22]

3.2.2 Parameters adjusting gelling behaviors. Fig. 4 is a typical phase diagram of a copolymer aqueous solution prepared by the present authors. The sol–gel transition temperature was measured *via* the test tube inverting method with a temperature increment of 1 °C per step. A gel was determined when no significant flow was observed 30 s after the vial was inverted. The gel state disappeared upon further heating, and the re-solled suspension eventually precipitated. The gel window could be further divided into two regions, referring to transparent gels and opaque gels.[33] Our group also found that the end-capping might lead to surprisingly subtle effects on

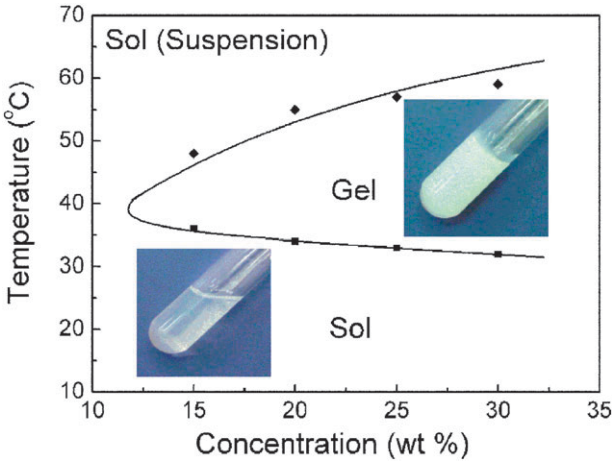

Fig. 4 The phase diagram of PLGA–PEG–PLGA triblock copolymer aqueous solutions.

macroscopic physical gelation—an addition or deletion of even a methylene group to the end of a PLGA–PEG–PLGA block copolymer within a certain composition region might determine the sol, gel or precipitation state of the aqueous system.[34]

The gelation properties including T_{gel}, CGC, and degradation rate could be modulated by several other factors such as MW of copolymer, composition of the hydrophobic block, and polymer concentration *etc*.[22,32–34] The BAB-type copolymers display a relative higher CGC and higher sol–gel transition temperature compared to the corresponding ABA-type copolymers.[22,32]

Some additives can also alter the phase transition temperatures of these thermogelling systems significantly. NaCl as a typical salt-out cosolute can shift the sol–gel transition boundary in the phase diagram to lower temperatures, while NaSCN as a typical salt-in cosolute causes an opposite effect.[22] Therefore, the gelling point in phosphate buffer saline (PBS) solution is different from that in pure water. Surprisingly, the addition of PEG homopolymers[22,35] or PEGylated drugs[35] was found to lower the sol–gel transition temperature. This phenomenon affords a practical technique to adjust the gelling temperature of the injectable material in medical applications. But the reason that an addition of hydrophilic polymers enhances the physical gelation of amphiphilic block copolymers is still unknown.

3.2.3 A hierarchy mechanism of physical gelation. A hierarchy mechanism has been suggested to interpret the physical gelation process of the PLGA–PEG–PLGA block copolymers in water: amphiphilic block copolymers are self assembled into micelles, and micelles are further percolated into a gel

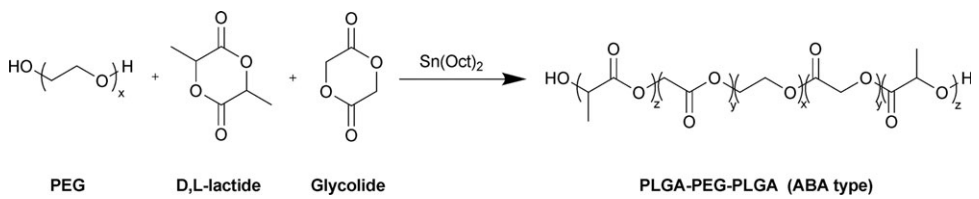

Fig. 3 The synthetic route of the PLGA–PEG–PLGA triblock copolymer.

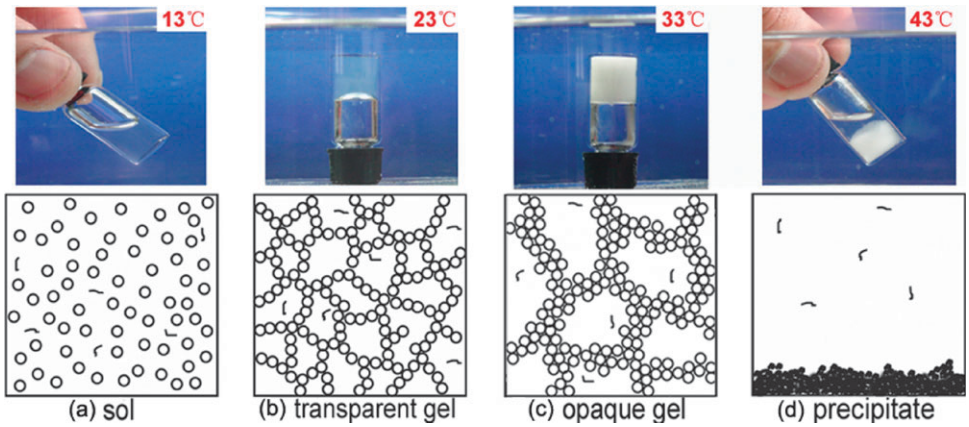

Fig. 5 A schematic presentation of the mechanism of a spontaneous thermogelling of the appropriate block copolymers in water *via* the formation of a "micelle-network". For simplicity, a micelle is denoted as a circle, although a micelle owns the core-corona structure and is deformable. The aqueous system takes on a sol-like suspension at a low temperature (a); the micelles are aggregated into a percolated micelle-network in which each micelle is still intact but micelle aggregation happens due to the hydrophobic interaction between micelles, and the solvent loses flowability, leading to the so-called sol–gel transition (b); the micelle-network is coarsened until the mesh size is in the order of wavelength of visible light, and the gel is thus opaque (c); the micelle structure is destroyed due to over-hydrophobicity of the sample at higher temperatures, eventually leading to macroscopic precipitation (d) (reprinted with permission from ref. 33, copyright 2006, Wiley-VCH).

network, as schematically presented in Fig. 5. The hydrophobic PLGA blocks occupy the cores of micelles, and the hydrophilic PEG blocks constitute the coronas. The micelle formation of such block copolymers in water has been confirmed by the hydrophobic dye solubilization method, ^{13}C NMR, dynamic laser scattering, transmission electronic microscopy and so on.[22,32–34] It is anticipated that the micelles are further associated to form a macroscopic gel as the temperature increases, and an intact micellar structure is maintained during the phase transition, which has been detected and confirmed in experiments.[22,33,34] The micelle-network might be coarsened by a further increase of temperature. Finally, the molecular motion of hydrophobic PLGA blocks is increased and the micellar structure is broken at higher temperatures due to the overhydrophobicity of block copolymers, resulting in the precipitation of copolymers (Fig. 5). Recently, an evidence of formation of a micelle-network or nanoparticle-network during thermogelling has been afforded in our group by achieving a thermosensitive physical gel with chemically crosslinked nanogels as the building blocks.[36]

The thermodynamic driving force of such physical gelation is the hydrophobic interaction.[33,34] Hence, the balance of hydrophobic and hydrophilic segments is critical to exhibit a thermoreversible phase transition in water for these copolymers. Generally, for the copolymers with the same PEG block length, a longer hydrophobic block leads to a lower T_{gel}, a lower CGC, and a wider gel window.

3.2.4 Medical applications of PEG–PLGA copolymer hydrogels. *In vitro* and *in vivo* studies have revealed that these thermogelling copolymers are of good biocompatibility and mechanical property. Hydrogels were rapidly formed once injected and no significant immune response was observed surrounding the injection sites.[6,37] In contrast to Pluronics, the PEG–PLGA copolymers are degradable and the gel state persists for a much longer time both *in vitro* and *in vivo*. Our lab-synthesized linear PLGA–PEG–PLGA copolymer and the associated physical hydrogel persisted over 3 weeks after subcutaneous injection into Sprague Dawley rats (Fig. 6). All of the animal experiments of the authors adhere to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985). According to Jeong *et al.*, a PLGA-*g*-PEG matrix lasted for more than 2 months *in vivo* while a PEG-*g*-PLGA matrix disappeared within one week; by mixing the two polymers with various ratios, the duration of the gel was able to be tailored from 1 week to 3 months.[24]

These PEG–PLGA copolymer hydrogels have been applied as drug delivery carriers. The *in vitro* releases of ketoprofen (a hydrophilic drug) and spironolactone (a hydrophobic drug) from PEG–PLGA–PEG hydrogels lasted for 2 weeks and 2 months, respectively.[6] A variety of other therapeutic agents have been encapsulated into and then released out of PLGA–PEG–PLGA copolymer hydrogels as well. These

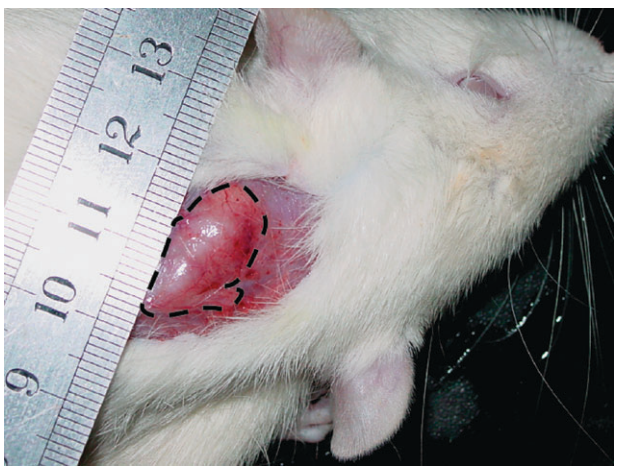

Fig. 6 A global observation of a physical gel formed underneath the skin of a rat. The image was taken 21 days after subcutaneous injection of an aqueous solution of PLGA–PEG–PLGA copolymer. The gel region is emphasized by the dashed line.

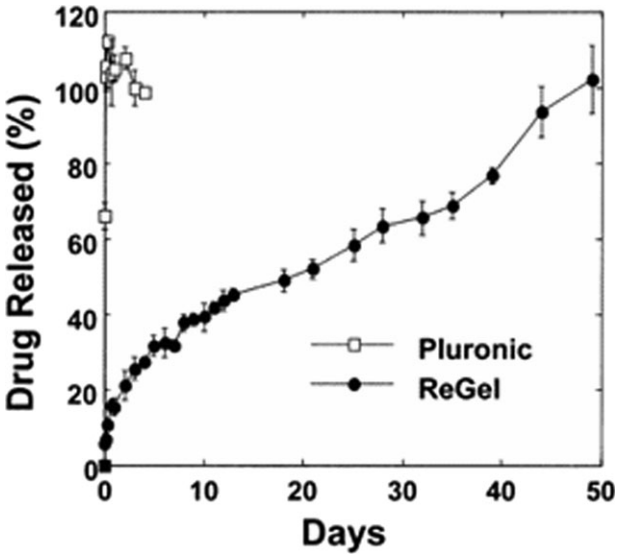

Fig. 7 *In vitro* release of paclitaxel from ReGel (PLGA–PEG–PLGA) as well as Pluronic (PEG–PPG–PEG) hydrogels (reprinted with permission from ref. 37, copyright 2001, Elsevier).

agents include paclitaxel, granulocyte colony-stimulating factor, porcine growth hormone, insulin, lysozyme, testosterone, *etc*.[35,37,38] Zentner *et al*. demonstrated that paclitaxel, an anticancer agent, was continuously released from 23 wt% PLGA–PEG–PLGA copolymer hydrogel (ReGel) *in vitro* for over 50 days *versus* 1 day in the case of the corresponding Pluronic F127 (Fig. 7).[37] Direct intratumoral injection of the above formulation revealed that the drug was slowly cleared from the injection site with minimal distribution into any other organs. Additionally, compared to the commercial paclitaxel product Taxol, the ReGel–paclitaxel formulation against human breast tumor xenografts showed higher efficiency and less drug-related adverse effects.[37] Now, this novel ReGel–paclitaxel formulation (OncoGeTM, a MacroMed's leading product) is in the advanced stages of clinical trials and is anticipated to come onto the market in the very near future. In order to treat diabetes mellitus, an ailment due to pancreatic beta cell dysfunction and insulin resistance, Kim's group evaluated the sustained release of insulin from ReGel formulation *in vitro* and *in vivo*.[38] A zero-order release profile was observed and the *in vitro* release lasted over 2 weeks. After a single injection in Zucker Diabetic Fatty rats, sustained insulin release maintained blood glucose levels in the euglycemic range for almost 2 weeks.[38] Additionally, the formation of micelles might increase the solubility of hydrophobic drugs, such as paclitaxel and cyclosporine A.[37]

Recently, our group examined the sustained release of a drug grafted with PEG (called PEGylated drug) *via* the thermogelling PLGA–PEG–PLGA copolymers.[35] This work was the first combination of the long-circulation technique of a PEGylated drug and the sustained release technique of an injectable hydrogel. The *in vitro* release of PEGylated camptothecin was sustained for one month. The release was thought to be diffusion-controlled at the first stage and controlled by both drug diffusion and hydrogel erosion at the late stage. *In vivo* anti-tumor tests in mice further demonstrated the feasibility of this combinatory technique.[35]

Wound healing and tissue repair are other potential biomedical applications for thermogelling PEG–PLGA copolymer matrices. In order to promote diabetic wound healing, PEG–PLGA–PEG hydrogels were used as a gene delivery vehicle of transforming growth factor β1 (TGF-β1), an important growth factor closely related to tissue repair.[39] The plasmid-loaded gel depot promoted reepithealization and enhanced cell proliferation in the wound bed. The efficacy was superior to Humatrix, a commercially available wound dressing, irrespective of whether it was plasmid-loaded or not.[39] Administration of the mixture of thermogelling PLGA-*g*-PEG aqueous solutions and chondrocyte suspensions into a cartilage-defect site was found to promote cartilage repair in rabbits.[24]

3.3 PEG–(other degradable polyesters)

PEG–PLA copolymers. Temperature-responsive PEG–PLA block copolymers have been investigated extensively as well. Both PEG–PLLA diblock copolymers and their triblock copolymers showed a gel–sol transition as temperature increased.[31,40] Vert's group reported that multiblock copolymers of PEG and poly(D,L-lactide) (PDLLA) also underwent a gel–sol transition.[41] Recently, Jeong *et al*. found that an alternating PEG–PLLA multiblock copolymer with short PEG and PLLA chains and relatively small total MW exhibited a sol–gel transition upon heating.[23]

PEG–PCL copolymers. Although the PLGA-based thermosensitive copolymers are quite attractive as drug delivery carriers *etc*., these polymers are a sticky paste at room temperature, and thus a bit difficult to handle. Jeong *et al*. exploited thermogelling poly(ethylene glycol)-*b*-poly(ε-caprolactone)-*b*-poly(ethylene glycol) (PEG–PCL–PEG) and PCL–PEG–PCL triblock copolymers to overcome this problem.[42] PCL–PEG–PCL triblock copolymers exhibited a wider gel window and higher gel modulus compared to PEG–PCL–PEG triblock copolymers. Both copolymers are in the powder morphology at ambient temperature, and thus convenient to weigh and transfer. The dissolution in water was also rather easy. However, the triblock copolymer aqueous solution can, although initially in a sol state, be transformed into a gel even at ambient temperature overnight. This phenomenon was due to the PCL crystallization in water. Subsequently, this group designed PEG–PCL multiblock copolymers.[42] These multiblock copolymers maintained a powder form at the bulk state and the temperature-dependent reversible sol–gel transition in water, and their solutions were stable as a sol state at room temperature.

Almost at the same time, another research group described a series of thermoresponsive MPEG–PCL diblock copolymers.[43,44] These polymer–water mixtures took on the normal[43] or inverse[44] sol–gel transition as a function of temperature depending upon the variation of MWs of PEG and PCL blocks. Moreover, CL as the degradation product of PCL exhibited weaker acidification than LA and GA. (Acidic degradable products could lower pH and cause a non-bacterial inflammation *in vivo*.) The phase transition of the diblock

copolymer solutions was attributed to their crystallization in water. *In vivo* studies illustrated that the injected gel depots maintained their original shapes over a month without inflammation.[44] It was found that the fluorescein isothiocyanate-labeled bovine serum albumin was able to be continuously released from these thermogelling copolymers more than 20 days *in vitro* and up to 30 days *in vivo*.[45] An *in vivo* osteogenic differentiation of rat bone marrow stromal cells was also found using this copolymer gel after 4 weeks.[44] These results indicated that MPEG–PCL diblock copolymers were a promising injectable biomaterial for both drug delivery and tissue engineering.

PHB-related copolymers. Recently, a group from Singapore explored novel biodegradable thermogelling poly(ether ester urethane)s consisting of poly-[(*R*)-3-hydroxybutyrate] (PHB), PEG and PPG blocks.[46,47] In contrast to PLA and PCL *etc*., PHB is a natural polyester generated in some bacteria. The crystallinity and hydrophobicity of PHB are usually higher than most synthetic biodegradable polyesters. The corresponding poly(ether ester urethane) showed a low CGC from 2 to 5 wt%.[46] The protein-loading formulation and copolymer composition were found to influence the release rate of proteins as well.[47]

Dual-responsive biodegradable polymers. Lee *et al*. developed a biodegradable hydrogel responsive to both temperature and pH.[48] The copolymer was prepared by capping a pH responsive moiety to the end of a temperature-responsive copolymer. The thermosensitivity comes from the block copolymer poly(ε-caprolactone-*co*-D,L-lactic acid)-*b*-poly(ethylene glycol)-*b*-poly(ε-caprolactone-*co*-D,L-lactic acid) (PCLA–PEG–PCLA), while the pH sensitivity comes from sulfamethazine oligomers (SMO). The resulting SMO–PCLA–PEG–PCLA–SMO formed a stable gel under physiological conditions (37 °C and pH 7.4). The dual-response is very helpful for avoiding gelation during syringe injection. The injected site presented a typical acute inflammation within 2 weeks, but no chronic inflammation was observed during the whole *in vivo* degradation period for 6 weeks.[48] The hydrogel formulation containing paclitaxel exhibited good anticancer efficacy for 2 weeks after subcutaneous injection into tumor-bearing mice.[49]

PEG–other polyester copolymers. Under appropriate conditions, some other block copolymers composed of PEG and polyesters have also been found to exhibit the phase transition from gel to sol or sol to gel in response to an increase of temperature. These polyesters include poly(δ-valerolactone) (PVL),[43] poly(trimethylene carbonate) (PTMC),[50] poly(ε-carprolactone-*co*-trimethylene carbonate) (PCL-*co*-PTMC),[51] poly(ε-carprolactone-*co*-1,4-dioxan-2-one) (PCL-*co*-PDO),[51] and so on.

3.4 Other thermogelling polymers

Biodegradable thermogelling poly(organophosphazene)s[26] (Fig. 3h) and polypeptides (or poly(amino acid)s)[27] (Fig. 3i) constitute alternative injectable biomedical materials. The gelation of poly(organophosphazene)s can be controlled by the composition of substituents, the chain length of hydrophilic side groups (α-amino-ω-methoxy-poly(ethylene glycol) (AMPEG)), the type of hydrophobic side groups (amino acid esters), the concentration of the polymeric aqueous solution *etc*. The thermally induced physical gelation of the polymers seemed to be driven by the hydrophobic packing of the side chains.[26] *In vitro* studies revealed that both hydrophilic and hydrophobic drugs could be released sustainedly from the gels.[52] Even spheroidal hepatocytes with enhanced liver-specific functions were successfully cultured in an injectable poly(organophosphazene) hydrogel as a bioreactor.[53]

Tanihara *et al*. have reported a thermogelling random copolypeptide composed solely of amino acid residues with a sol–gel transition near body temperature.[27] One building block in such a random coupling is an elastin-related pentapeptide valyl-prolyl-glycyl-valyl-glycyl (Val-Pro-Gly-Val-Gly), and the other building block is an oligomer of a collagen-derived tripeptide prolyl-*trans*-4-hydroxyprolyl-glycyl (Pro-Hyp-Gly), $(Pro\text{-}Hyp\text{-}Gly)_{10}$. Poly(Val-Pro-Gly-Val-Gly) in water exhibits an LCST behavior and a transition from an extended conformation to a β-spiral with a rise in temperature. Poly(Pro-Hyp-Gly) chains in water have a triple-helix conformation and are always hydrophilic in the examined temperature range. The copolypeptide of Val-Pro-Gly-Val-Gly and $(Pro\text{-}Hyp\text{-}Gly)_{10}$ is thus an amphiphilic copolymer. These polypeptides exhibit, under an appropriate composition, a low CGC and an inverse sol–gel transition above the CGC.[27] Unlike thermally reversible PEG–polyester hydrogels, these polypeptides do not show a gel–resol transition at higher temperatures according to the reports so far. The underlying gelation might be related to temperature-induced conformational change of polypeptides.

4 Summary and perspectives

The past decade has witnessed a wide array of novel injectable hydrogels. An injectable system is a low viscous aqueous solution or suspension before administration, but is semi-solidified or percolated inside the body *via* either chemical crosslinking or physical association. Cells or pharmaceutical agents are thus able to be *in situ* entrapped simply by syringe injection of their aqueous solutions at target sites with minimal invasiveness. Chemical hydrogels have relatively strong and stable mechanical properties. But *in vivo* chemical reaction is potentially harmful for human beings. With this in mind, a physical gelation is beneficial. Thermogelling copolymer hydrogels are especially attractive due to the convenience of their operation. An inverse themosensitivity is meaningful for biomedical applications because the protection of encapsulated proteins or cells before injection is important. Among these novel materials, PEG–polyester copolymer hydrogels have potential. They are biocompatible and biodegradable with tunable degradation rates. The gelling behaviors could be modulated by altering MW, composition (including end groups) and concentration of block copolymers, and also additives. The amphiphilicity of the PEG–polyester block copolymers is a key inherent factor for induction of thermosensitivity. Macroscopic self assembly driven by hydrophobic interaction is responsible for thermogelling.

With the rapid development in regenerative medicine, the demand for controlled drug or cell deliveries is increasing.

Compared with traditional drug carriers and tissue engineering scaffolds, biodegradable and injectable synthetic hydrogels offer alternative materials. Besides biocompatibility, an appropriate biodegradation rate of an implant matrix should be kept in mind for any specific application as a drug or cell carrier. As far as a drug vehicle is concerned, the penetrability of various drugs and precise control of their release profiles should also be considered; as a tissue engineering material, the enhancement of cell adhesion and cell responses to gel softness are two challenging topics. The detailed mechanism of physical gelation, especially inverse thermosensitivity, is an amazing topic. It is also worth noting that the phase-transition behavior of a copolymer system might be altered after the addition of drugs, cells or even a cell culture medium. Further fundamental investigations of injectable hydrogels are thus called for.

It is unlikely that any one hydrogel can fulfil the requirements of all biomedical applications. Hence, novel injectable materials will have to be tailored to specific applications in the future. The integration of material properties, instead of using just one, should be taken into consideration for potential applications. Although much progress has been made in the fundamental research of injectable hydrogels, including thermogelling block copolymers, the rich physics of this soft matter or wet material will undoubtedly make injectable hydrogels an important topic in both chemistry and material sciences in the next decade.

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